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Physical Basis for Altered Stem Elongation Rates in Internode Length Mutants of *Pisum*[1]

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ABSTRACT

Biophysical parameters related to gibberellin (GA)-dependent stem elongation were examined in dark-grown stem-length genotypes of *Pisum sativum* L. The rate of internode expansion in these genotypes is altered due to recessive mutations which affect either the endogenous levels of, or response to, GA. The GA deficient dwarf L181 (*ls*), two GA insensitive semierectoides dwarfs NGB5865 and NGB5862 (*lka* and *lkb*, respectively) and the 'slender' line L197 (*la cry*^{s}), which is tall regardless of GA content, were compared to the wild-type tall cultivar, Torsdag. Osmotic pressure, estimated by vapor pressure osmometry, and turgor pressure, measured directly with a pressure probe, did not correlate with the differences in growth rate among the genotypes. Mechanical wall properties of frozen-thawed tissue were measured using a constant force assay. GA deficiency resulted in increased wall stiffness judged both on the basis of plastic compliance and plastic extensibility normalized for equal stem circumference. Plastic compliance was not reduced in the GA insensitive dwarfs, though *lka* reduced circumference-normalized plasticity. In contrast, *in vivo* wall relaxation, determined by the pressure-block technique, differed among genotypes in a manner which did correlate with extension rates. The wall yield threshold was 1 bar or less in the tall lines, but ranged from 3 to 6 bars in the dwarf genotypes. The results with the *ls* mutant indicate that GA enhances stem elongation by both decreasing the wall yield threshold and increasing the wall yield coefficient. In the GA-insensitive mutants, *lka* and *lkb*, the wall yield threshold is substantially elevated. Plants possessing *lka* may also possess a reduced wall yield coefficient.

A range of stem-length mutants has been described for the garden pea, *Pisum sativum* L. (for review, see ref. 24). The altered internode length in most of these mutants results from either a change in the level of GA_1,[3] or a change in some aspect of GA sensitivity. The basis of the dramatic effects of altered GA physiology on stem elongation in these genotypes has not been examined in terms of the biophysical events related to extension growth. Consequently four mutant genotypes, representing the available range of GA related mutants, were chosen to examine several biophysical parameters related to extension growth.

Three dwarf genotypes caused by different recessive mutations on the WT tall Torsdag background were chosen for these experiments. These genotypes were selected because they arise from mutations at three different loci and because they possess the most extreme dwarf phenotypes that are amenable to analysis by the methods we used. Two mutations, *lka* and *lkb*, result in reduced sensitivity to GA_1 (26), while the *ls* mutation results in a deficiency of GA_1 (25) by introducing a block in GA biosynthesis prior to the formation of ent-kaurene (9). The concentration of GA_1 has been shown to correlate directly with internode length in *Pisum* (10, 27) as well as in other species which possess a predominant early 13-hydroxylation pathway for GA biosynthesis (18). While dwarfed to a similar extent as *ls* plants, *lka* and *lkb* plants display characteristics associated with the erectoides phenotype, including brittle stems with an increased diameter (22).

The fourth mutant examined has the opposite phenotype to the dwarfs. It results in extreme stem elongation, irrespective of the endogenous GA content (19, 21). This phenotype, conferred by the duplicate gene combination *la cry*^{s}, has been named 'slender' due to the extremely tall and spindly plant that results from these mutations.

The biophysical mechanism of GA-dependent growth has been the subject of numerous investigations with a variety of plants but the results have been contradictory. For example, it has been proposed that GA increases elongation rates by increasing turgor in cucumber hypocotyls (5, 12). This conclusion has been contradicted recently (29). Investigators have also reported that wall extensibility, as measured by the Instron technique or similar mechanical methods, increases as a result of GA application in oat internodes (1), cucumber hypocotyls (29), lettuce hypocotyls (11, 13, 28), and pea epicotyls (15, 16). However, evidence for an increase in extensibility in peas has been contradicted at least twice (8, 30). Recently the pressure-block technique, which allows the analysis of *in vivo* wall relaxation (7), has been applied to the study of GA-dependent growth. This technique has shown that in both cucumber hypocotyls (29) and pea epicotyls (8), GA alters the yield threshold and the wall yield coefficient as defined in Lockhart's growth equation (20):

$$G = \phi(P - Y) \quad (1)$$

where G represents volumetric growth rate, ϕ represents the

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[3] Abbreviations: GA, gibberellin; WT, wild type.

coefficient of wall expansion, P represents turgor pressure and Y represents the wall yield threshold.

In the present paper several biophysical parameters related to the rate of extension growth were measured in the above stem-length genotypes with the hope of clarifying the physical basis of endogenous GA action, at least in *Pisum*. Stem-length mutations have been shown to be expressed in dark-grown plants (21, 23) and therefore plants were grown in the dark to provide material that was more easily analyzed than that from light grown plants. We measured osmotic pressure and turgor pressure to assess the role of a turgor-related mechanism in GA-dependent growth. Wall mechanical properties were evaluated by a constant stress assay and *in vivo* stress relaxation was analyzed by the pressure-block technique. This work expands on the work of Cosgrove and Sovonick-Dunford (8) by analyzing plants in which extension rate has been manipulated by genetic means as opposed to chemical means (*e.g.* GA biosynthesis inhibitor and GA applications).

MATERIALS AND METHODS

Plant Material

The pure lines of *Pisum sativum* L. used in this study are held in the collection at Hobart, Australia (Department of Plant Science, University of Tasmania). The genotypes are listed in Table I. Lines NGB5865, NGB5862, and L181 are isogenic with Torsdag (9, 26). The slender gene combination (*la cry*^{s}) in L197 is on a different genetic background. All lines are homozygous for the mutant alleles and homozygous dominant for the other genes recognized as influencing internode length, namely *Le*, *Lw*, *Lk*, *Lm*, *Lv*, *Lh*, and *Na*.

Plants were grown in moist vermiculite and manipulated under dim green safe lights throughout the experiments (8). Plants for growth zone analysis and extensibility determinations were grown at 20 to 22°C, while those used for osmotic and turgor pressure measurements and for *in vivo* wall relaxation were grown at 26 to 28°C. Differences among the lines appeared similar at these two different temperatures. Plants were selected for experiments when the third internode (*i.e.* between the second scale leaf and the first true foliage leaf) was approximately 25 to 40% expanded.

Growth Analysis

The growth profile along the epicotyl was measured by applying India ink at 2 mm intervals over the upper 30 mm of the epicotyl with a stamp. After 6 to 8 h, the markings on the stems were copied onto paper under a dissecting microscope. Extension growth between the marks was measured and provided a measurement of overall extension growth and the location of growth along the epicotyl axis. To determine the growth profile, the percent increase in length per hour for each 2 mm interval was calculated.

Stem circumferences were measured from photomicrographs of 150 μm thick transverse sections taken 5 to 6 mm from the top of the epicotyl. Stem perimeters were digitized from tracings of enlarged photomicrographs and the circumferences determined by computer (17).

Osmotic Pressure and Turgor Pressure

Osmotic pressure and turgor pressure were determined in the same plants for cells lying within 3 to 9 mm of the hook or apical bud (the dwarf lines had poorly formed hooks at the time of analysis). We measured this zone from the point at which the epicotyl became straight and began to swell. To determine osmotic pressure, bulk cell sap was expressed from segments excised at 3 to 9 mm below the hook, loaded on a paper disk and measured with a vapor pressure osmometer

Table I. *Genotype, Phenotype, and Values for Elongation Rates, Dry Weight, and Circumference of Expanding Third Internodes of Etiolated Seedlings of the Stem-Length Lines Used in This Study*

Elongation rates, dry weights per 6 mm, and circumferences are means ± standard errors. Sample sizes are included in parentheses.

Line	Genotype Phenotype	Elongation Rate[a]	Dry Weight per 6 mm[b]	Circumference[c]
		mm hr^{-1}	*mg*	*mm*
Torsdag	*Ls La Cry*^{s} *Lka Lkb* Tall	1.5 ± 0.09 (7)	1.1 ± 0.04 (7)	6.91 ± 0.11 (6)
L197	*Ls la cry*^{s} *Lka Lkb* Slender	1.2 ± 0.07 (5)	1.0 ± 0.04 (11)	6.06 ± 0.18 (6)
L181	*ls La Cry*^{s} *Lka Lkb* GA-deficient dwarf	0.2 ± 0.02 (8)	1.5 ± 0.05 (12)	8.04 ± 0.14 (7)
NGB5865	*Ls La Cry*^{s} *lka Lkb* Semi-erectoides dwarf	0.3 ± 0.02 (7)	2.0 ± 0.11 (9)	8.13 ± 0.16 (4)
NGB5862	*Ls La Cry*^{s} *Lka lkb* Semierectoides dwarf	0.3 ± 0.04 (9)	2.2 ± 0.13 (7)	9.03 ± 0.28 (6)

[a] Calculated as the average rate of extension over a 6 to 8 h period. Third internodes were approximately 25 to 40% expanded. [b] Average dry weights of sections of stems analyzed for extensibility and compliance. This portion of the stem is located from 3 to 9 mm below the top of the epicotyl. [c] Determined from digitized photomicrographs of stem cross sections taken 5 to 6 mm from the top of the epicotyl.

(model 5500, Wescor, Logan, UT). Osmolality was divided by 41 mOsmol kg^{-1} bar^{-1} to obtain osmotic pressure at 20°C.

Turgor pressures of 8 to 20 cortical cells per plant were measured by the pressure probe technique (6, 8) in intact plants that were free to transpire. The measured cells were typically located 3 to 15 cells beneath the epidermis and 5 to 7 mm below the apical hook. Green light (550 nm interference filter) was used to illuminate the microcapillary of the pressure probe during the measurement, which typically required 5 to 10 min per plant.

Wall Mechanical Properties

Apical 12 mm sections of epicotyls were harvested and frozen at −80°C. At the time of assay the segments were thawed and gently pressed between glass plates with 400 g to remove the bulk of cellular fluid. This prevented hydrostatic forces from interfering with wall extension during the extensibility assay. A constant stress technique was used to measure extensibility. The middle 6 mm of the section was clamped and a tension of 13 g was applied for 1 min. The extension of the stem was recorded with an angular position transducer (Gould Electronics, Pittsburgh, PA) interfaced to a microcomputer. The differences in section length before the application of stress, prior to the release of the stress and 1 min after release of the stress were used to calculate plastic and elastic extensibility. The 6 mm sections were then dried for 2 d at room temperature and weighed to the nearest 10 μg. The weights were used to calculate plastic and elastic compliances as described by Cleland (3). A second correction of extensibility values was performed by multiplying the extensibility values by the mean stem circumference for each genotype.

In Vivo Wall Relaxation

Wall stress relaxation in the intact, growing stems was measured with the pressure-block technique (see ref. 7 for details). In short, the upper portion of the stem (approximately 1 cm) was connected to a linear displacement transducer and sealed into the pressure-block chamber. After the seedling recovered steady growth, pressurization of the chamber was used to induce wall relaxation. The chamber was pressurized just sufficiently to prevent further elongation of the stem. As the wall relaxes, further pressure must be applied to keep the stem from elongating. The pressure versus time curve was then taken as a record of wall relaxation. In this technique, pressure is both a means of inducing relaxation and a measure of relaxation.

RESULTS

Growth

The effects of the mutant recessive alleles at the *Ls*, *La*, *Cry*, *Lka*, and *Lkb* loci on growth in dark grown seedlings are illustrated in Figure 1. Quantitative comparisons of the genotypes are presented in Table I. The reduction in the rate of extension growth by the dwarfing alleles is 70 to 80%. The extension rate in slender is high, though somewhat lower than in the WT. Changes in dry weight of the growing zone accompany changes in elongation growth. This results primarily from an increase in diameter of the epicotyl in the dwarf lines.

When growth is plotted as a function of position along the epicotyl, the distribution of growth along the stem can be compared between the genotypes (Fig. 2). The more rapidly growing WT and *la cry*^{s} plants have growing zones extending roughly 3 cm from the hook of the epicotyl. In the dwarf lines the growth zone is approximately two thirds that of the WT and growth rate never exceeds that of the tall lines at any distance from the apical hook. Hence, at the morphological level, dwarfism in *ls*, *lka*, and *lkb* plants is the result of both a decreased rate of extension and a smaller region of expansion. The upper 10 mm in all lines, however, remained the zone of highest elongation and this was the region of the epicotyl used for measurement of osmotic pressure, turgor pressure and wall properties.

Osmotic and Turgor Pressure

We measured osmotic and turgor pressure to determine whether changes in these parameters were related to the altered elongation rates. There were significant differences in osmotic pressure between the genotypes but the differences were not in a pattern which suggested that lower extension rates were a consequence of reduced solute concentrations, and hence lower turgor pressure, within the growing cells (Table II). For example, WT plants and the *ls* dwarf had a similar osmotic pressure, while the rapidly growing *la cry*^{s} and dwarf *lkb* plants both had a significantly greater osmotic pressure than did WT. The second semierectoides line, *lka*, also had a high osmotic pressure though it was not significantly different from WT with the sample size analyzed.

Turgor pressure among the genotypes fell into two groups (Table II). In WT, *la cry*^{s}, and *ls* plants, turgor pressure measured about 5.0 bars. These values are comparable to values measured previously in dark-grown peas (7). Turgor pressure in both *lka* and *lkb* plants was significantly higher than in the WT, measuring approximately 6.8 bars. This correlates with the morphology of the semierectoides phenotype, in which the stems appear to be highly swollen and tend to fissure horizontally in the outer cell layers when the plants are well watered. It is possible that this fissuring results from the high turgor breaking a resistant outer wall. In any event, the high turgor in the *lka* and *lkb* dwarfs indicates that reduced stem elongation is not a consequence of reduced turgor in these plants.

Wall Mechanical Properties

Wall material was assayed for extensibility to determine whether the stem-length mutations resulted in changes in mechanical properties of the cell walls. Plastic extensibility was lower in the three dwarf genotypes than in either of the tall genotypes (Fig. 3). Elastic extensibility did not correlate with elongation growth. However, dry weight per unit length of stem and stem circumference are greatly different among these genetic lines (Table I). To correct for the possible differences in actual wall stress in the different genotypes, the

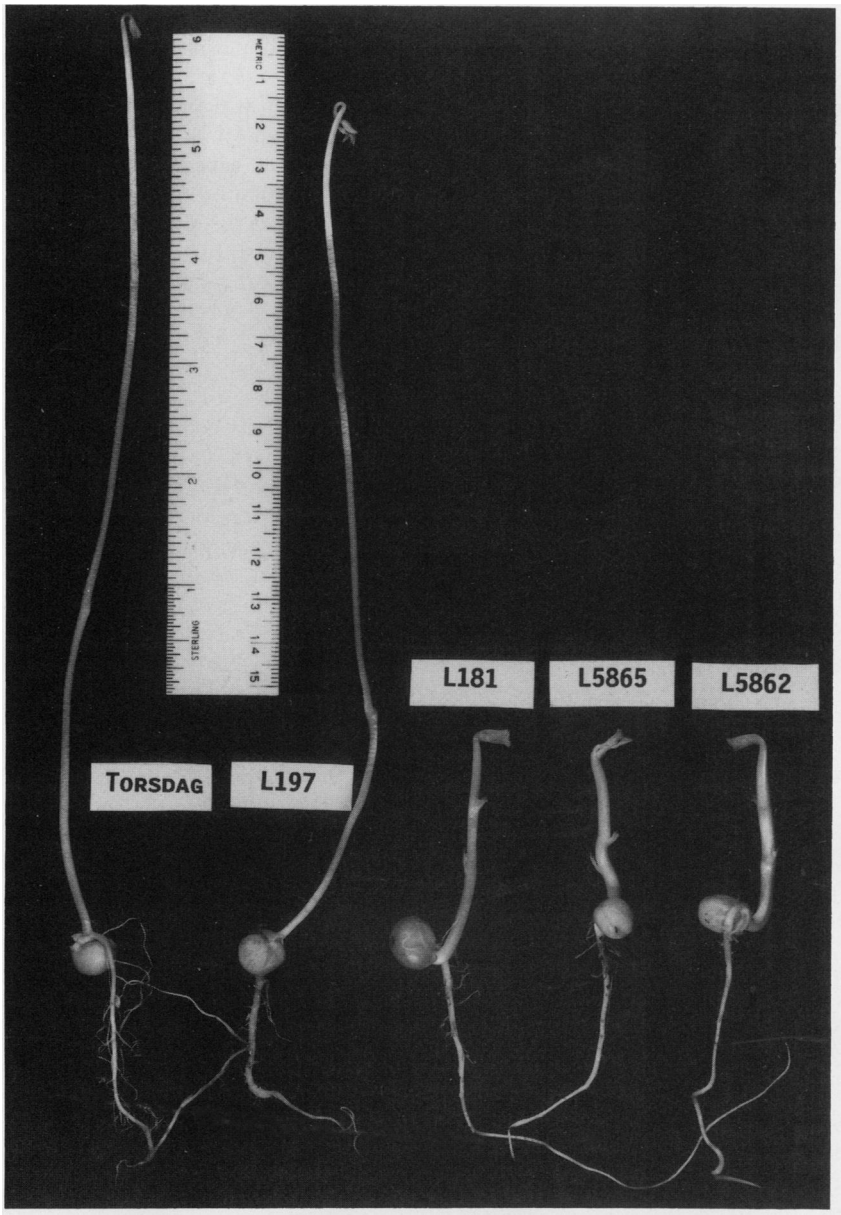

Figure 1. The WT Torsdag cultivar and the four stem-length mutants at the stage of growth at which the measurements in this study were made. Measured from the time of planting, Torsdag and L197(*la cry*^{s}) are 7 d old, L181 (*ls*) is 8 d old, and NGB5865 (*lka*) and NGB5862 (*lkb*) are 6 d old.

extensibility data were normalized in two ways. In the first, compliances were calculated based on dry weight per unit length and in the second extensibility was normalized for equal stem circumference. On the basis of plastic compliance both *lka* and *lkb* dwarfs do not differ significantly from WT or *la cry*^{s} plants (Fig. 3), in spite of the markedly different elongation rates. However, plastic compliance is significantly lower in *ls* plants compared to WT ($P < 0.05$, Dunnett's procedure). Judged on the basis of plastic extensibility values normalized for equal stem circumference, both the *ls* dwarf and the *lka* semierectoides line are significantly less extensible than the tall lines (Fig. 3). By any measure employed, elastic wall properties correlate poorly with elongation rate.

In Vivo Wall Relaxation

The pressure-block technique was used to measure *in vivo* wall relaxation in the genotypes (7, 8). Two components of the relaxation curve (Fig. 4) were analyzed, namely the initial rate of relaxation and the maximum pressure obtained in the experiment (Table III). The initial relaxation rate of the dwarf *lkb* line was about one-third the value of the WT peas, but in the other dwarf lines was not statistically different from the WT. The most marked difference between tall and dwarf genotypes was in the total amount of relaxation, here expressed as the maximum pressure attained in the first hour of relaxation (Fig. 4; Table III). This is the parameter measured in this study which best correlated with growth rate among all five genotypes. Both fast growing plants, WT and *la cry*^{s}, required 4.1 to 4.4 bars of pressure before the relaxation process was complete. The dwarf genotypes on the other hand needed much lower average final pressures (1.1–2.3 bars) than the tall plants to stop wall relaxation.

The maximum pressure obtained in the pressure-block

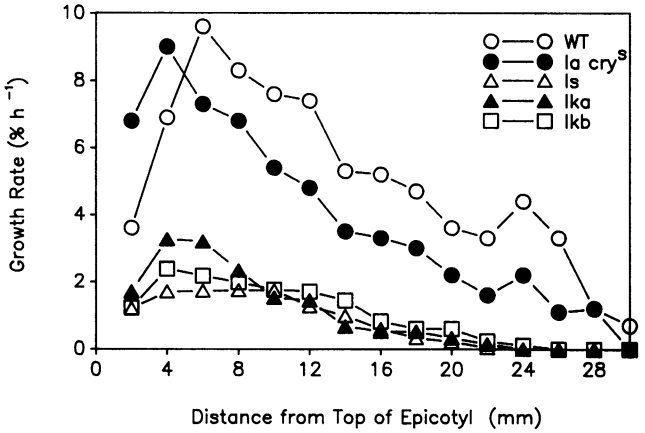

Figure 2. Distribution of growth along the epicotyl of etiolated seedlings. Growth was determined by measuring the displacement of ink marks over 6 to 8 h. Data points are averages of five to nine plants.

experiment can be taken as an approximation of the turgor pressure in excess of the yield threshold (7). We subtracted the average maximum pressure from the turgor values reported in Table II to derive an estimate of Y in the five genotypes (Table III). It can be seen that the calculated Y in WT and *la cry*^{s} is low (0.4 and 1.1 bars, respectively) while Y in the three dwarfs is much greater than in WT, ranging from 2.7 to 5.7 bars. We also calculated values for ϕ for each genotype by dividing growth rates (Fig. 2) by our estimates of $P - Y$ (Table III). These calculations suggest that ϕ is reduced by approximately half in *ls* and *lka* plants, but is not affected in the *lkb* genotype. These estimates of ϕ should be viewed with caution, however, because they were derived by combining data from sets of plants grown at slightly different temperatures.

DISCUSSION

We employed a range of stem-length mutants of *P. sativum* to analyze biophysical parameters related to stem elongation and found that only one parameter, wall yield threshold (Y), correlated with the rate of epicotyl extension in the five genotypes examined. Since the altered rates of stem elongation in these genotypes are a consequence of alteration in GA levels or response, these data demonstrate that GA regulates growth, at least in part, by influencing the yield threshold of the wall.

In theory at least, a more rapid growth rate could be a consequence of greater osmotic pressure of the growing cells. The rise in osmotic pressure would then generate a greater turgor pressure and drive wall extension at a greater rate (5, 12). Our studies show that differences in osmotic pressure or turgor pressure do not account for differences in extension rate in the five genotypes examined here (Table II). This agrees with recent studies on GA-mediated growth in pea (8) and cucumber (29). Thus, genotypes growing slower than WT have comparable (*ls* plants), if not higher (*lka* and *lkb* plants), osmotic and turgor pressures.

The pattern of growth rates and osmotic pressures in these different pea genotypes suggests to us that GA may affect two processes, namely translocation of solutes to the growth zone and wall relaxation in this zone. To a first approximation (20), the osmotic pressure in a growing region reflects a dynamic balance between the rates of solute import (tending to increase osmotic pressure) and cell expansion (tending to decrease osmotic pressure through dilution). The high turgor and osmotic pressure in the *lka* and *lkb* plants may result from a lesion in the wall relaxation process, without a concomitant effect on the solute transport process. By contrast, the lack of GA in the *ls* plants may result in coordinated reduction in solute transport and wall relaxation, such that osmotic pressure is unchanged in these dwarfs, despite the lower growth rate. These differences require further attention.

The lack of a positive correlation of osmotic and turgor pressure with growth rate implies that wall properties are in some way different between the tall and dwarf genotypes. We found a good correlation between extension rate and plastic extensibility (Fig. 3), measured by a constant force assay. However, such a correlation may be misleading because stem diameter differs greatly among the genotypes. Hence the lower plastic extensibilities observed in the dwarf may in large part be due to the greater cross sectional wall area (and thus lower stress) in these lines relative to WT. As a first approximation to correct for differences in cross sectional wall area, the dry weight per unit length was used to convert extensibility values into compliances. This calculation assumes that the dry weight per unit length is related directly to the cross sectional area of the stem bearing the imposed stress and that this relationship is constant among the genotypes. We are unable to comment in detail on how potential differences in anatomy may bias the compliance calculations except to say that there were no obvious differences in starch and lignin content (judged by staining stem sections with potassium iodide solution or phloroglucinol, data not shown) and that the distribution of wall material between longitudinal and transverse walls may indeed be different between the genotypes as the dwarfing mutations have been shown to affect cell length (21, 26). With these caveats in mind, we suggest that compliances represent the mechanical properties of the wall more accurately than

Table II. *Osmotic and Turgor Pressure of the Growing Regions in the WT and the Four Stem-Length Mutants*

Data are means ± standard error. Sample sizes are indicated in parentheses. The stem-length mutants were tested for differences from the WT using Dunnett's procedure.

Genotype	Osmotic Pressure[a]	Turgor Pressure[b]
	bars	
WT	8.2 ± 0.25 (7)	4.8 ± 0.11 (7)
la cry^s	10.9 ± 0.69 (5)**	5.2 ± 0.21 (6)
ls	7.9 ± 0.22 (7)	4.9 ± 0.08 (7)
lka	9.4 ± 0.26 (6)	6.8 ± 0.36 (6)**
lkb	9.6 ± 0.29 (7)*	6.8 ± 0.14 (7)**

[a] Determined by measuring the vapor pressure of bulk cell sap expressed from the growing region of the epicotyl. [b] Determined from pressure probe measurements of cortical cells within the growing region of intact plants. * $P < 0.05$; ** $P < 0.01$.

uncorrected extensibility values. A second correction based on stem circumference[4] has also been presented (Fig. 3) since it has been suggested that the outer cell layers of the stem limit growth (see Ref. 2 and references therein) and that therefore the load imposed in assays of mechanical strength may be disproportionately borne by walls of the epidermal cells.

With the *ls* dwarf we observed decreased plastic deformation, regardless of the basis by which plasticity is analyzed. Since dwarfism in the *ls* mutant is clearly a consequence of reduced GA levels (9), these observations support the view that GA acts to reduce the mechanical stiffness of walls (1, 11, 13–16, 28, 29). With the semierectoides dwarfs conclusions regarding the effect of *lka* or *lkb* on wall strength are less clear. If the method by which plastic compliance was determined results in an accurate correction for stress *versus* strain, then the implications are that *lka* and *lkb* block cell expansion through a lesion which affects wall expansion but not wall strength. If the outer cell layers are primarily load-bearing, then the circumference-normalized extensibility values would suggest that the *lka* mutant has a stiffening effect on the outer cell layers whereas the effect of *lkb* is not significant.

In comparison with the foregoing stress/strain analyses, the pressure-block experiments showed striking differences between tall and short genotypes (Fig. 4; Table III). Tall lines exhibited large relaxations (more than 4 bars), whereas short mutants showed little relaxation (1–2 bars). These measurements provide estimates of $P - Y$, and let us calculate that the tall lines have low values for Y (1 bar or less) and that the dwarfs have high Y values (3–6 bars). Calculations indicate that the *ls* genotype also reduces ϕ, whereas the *lkb* genotype has no apparent effect on ϕ. The *lka* genotype also appears to reduce ϕ, though the large variability and low sample number in the relaxation experiments make this conclusion less certain.

[4] Normalization by circumference presumes that the mechanical properties of stem samples are determined solely by the epidermal layer and that the epidermal walls are of equivalent thicknesses in the genotypes compared here. These assumptions remain unproven.

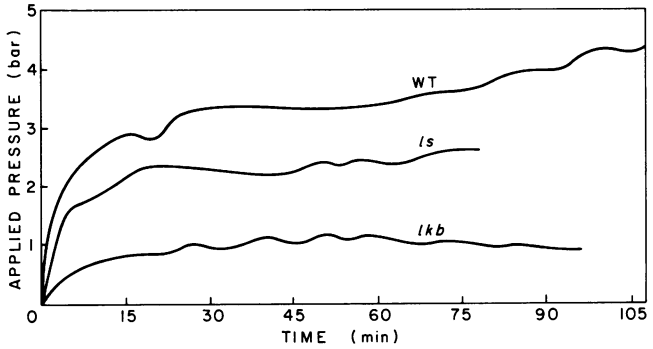

Figure 4. Representative relaxation curves from pressure-block analysis of wall relaxation in three seedlings, each representing a different stem-length type. The relaxation curve for WT in this experiment plateaus at an exceptionally low pressure but illustrates an unusual feature of both WT and *la cry*^{s} plants, namely additional slow relaxation beginning once the initial relaxation is largely complete. Relaxation curves for *ls* and *lkb* are typical of the dwarfs.

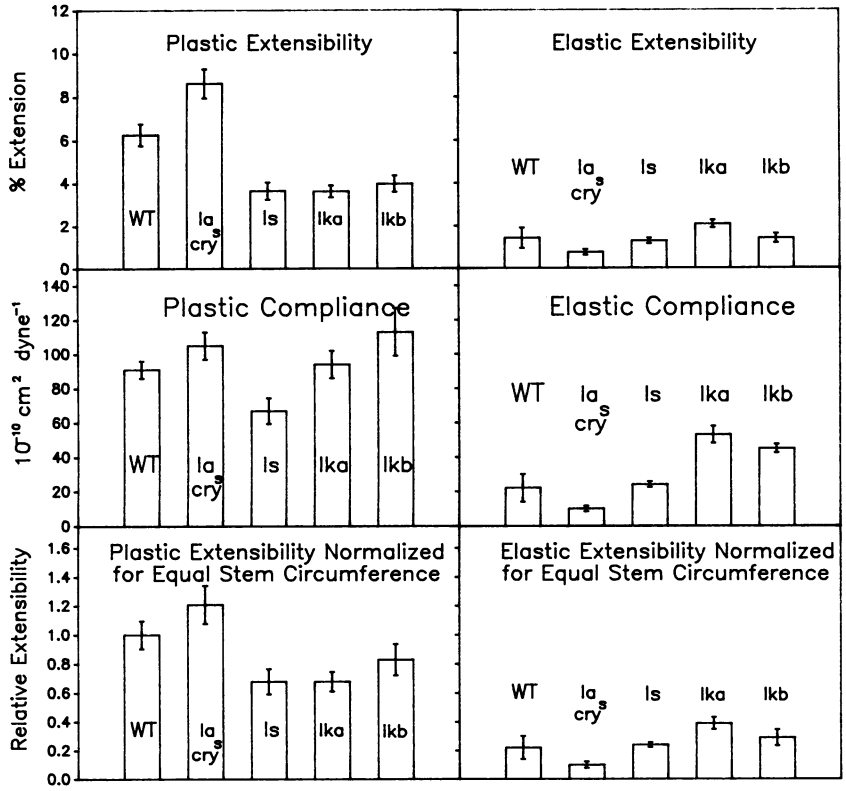

Figure 3. Mechanical properties of cell walls of the five genotypes as determined by a constant stress assay. Frozen/thawed sections, harvested from the upper 12 mm of the epicotyl, were squeezed to remove water and the central 6 mm of the sections extended with a force equivalent to 13 g for 1 min. Plastic extensibility was taken as the difference between the original length of the segment and the length of the section at one min after the release of the stress. Elastic extensibility was taken as the difference in length after 1 min of stress and 1 min after the release of stress. Compliances were derived by multiplying extensibility values by the dry weight per unit length (3). Circumference normalized extensibility was obtained by multiplying extensibility values by stem circumferences and is expressed relative to circumference normalized plastic extensibility of the WT. Means and SE are plotted. Sample sizes are: WT, 7; *la cry*^{s}, 11; *ls*, 12; *lka*, 9; *lkb*, 7.

Table III. *Summary of in Vivo Wall Relaxation as Measured by the Pressure-Block Technique*

Data are means ± standard error. Sample sizes are indicated in parentheses. The stem-length mutants were tested for differences with WT using Dunnett's procedure.

Genotype	Initial Relaxation[a]	Maximum Pressure[b]	Yield Threshold[c]	Yield Coefficient[d]
	bars hr^{-1}	*bars*		*bar*$^{-1}$ *hr*$^{-1}$
WT	10.1 ± 1.56 (9)	4.4 ± 0.38 (9)	0.4	0.019
la cry^{s}	8.3 ± 3.36 (3)	4.1 ± 1.13 (3)	1.1	0.016
ls	7.3 ± 2.07 (5)	2.2 ± 0.36 (5)**	2.7	0.008
lka	8.9 ± 1.36 (4)	2.3 ± 0.90 (3)*	4.5	0.010
lkb	3.6 ± 0.96 (5)*	1.1 ± 0.05 (5)**	5.7	0.018

[a] Determined from the slope of the relaxation curve at 5 min by fitting a cubic polynomial to the data points between 3 and 7 min after the start of relaxation. [b] The maximum pressure recorded or the pressure after 1 hr of wall relaxation, whichever came first. [c] Y was calculated by subtracting the maximum pressure values from turgor pressure determined by the pressure probe (Table II). [d] Calculated by dividing the average growth rates in the region from 4 to 10 mm below the hook (Fig. 2.) by $(P - Y)$, estimated from maximum chamber pressure, column 3. * $P < 0.05$; ** $P < 0.01$.

One curious anomaly is that the initial rates of relaxation (Table III) were not statistically different for the various genotypes, except for *lkb* which relaxed slower than the rest. Assuming wall relaxation were a simple process, the initial relaxation rate should be proportional to the growth rate and should be given by (6):

$$dP/dt = \phi\epsilon(P - Y) \quad (2)$$

where ϵ is the volumetric elastic modulus and all parameters except P are constants. In principle we could use dP/dt to calculate a second estimate of ϕ by Equation 2. We have not made this calculation partly because of the technical problems in obtaining ϵ in growing tissues (relaxation itself interferes with the measurement). More importantly, relaxation kinetics in intact plants are often more complicated than can be accounted for by Equation 2. and its derivatives, which means that the assumptions that the parameters of the equation remain constant during relaxation may not be generally valid (8). This problem is under current study (D. J. Cosgrove, manuscript in preparation).

When we compare wall analysis techniques (stress/strain *versus* relaxation), we see that the pressure-block method has a number of advantages. First, the pressure block technique can be used with intact plants, so excision artifacts are avoided. Second, there is a theoretical basis for quantitatively relating relaxation behavior to growth behavior (6, 7). Third, relaxation methods detect changes in wall growth properties that are undetected or obscured by the mechanical technique (*e.g.* increase in Y in *lkb*). These differences reflect the fundamentally different nature of the two techniques. Mechanical assays measure some average viscoelastic behavior of the wall, which depends on the overall structure and bonding between wall polymers at a given instant in time, whereas *in vivo* relaxation techniques measure the rate of wall modifications specifically giving rise to yielding or slippage of load bearing polymers in the wall. Because such yielding may come about from bond exchange between wall polymers without a net change in the number of bonds, a mechanical weakening of the wall is not an essential step for wall relaxation and expansion.

When bond exchange of the type described above is dependent on a chemical reaction in the wall, it is termed a chemorheological process (8, 20). Whereas mechanical assays generally measure only the viscoelastic properties of the wall, relaxation assays *in vivo* can detect the combined effects of changes in wall viscoelasticity and chemorheology. The GA deficiency in the *ls* mutant evidently affects both viscoelastic and chemorheological properties of the wall, whereas the *lka* and *lkb* genes seem to affect principally the chemorheological properties.

Our results with the GA-deficient *ls* genotype are in general agreement with the results of Cosgrove and Sovonick-Dunford (8) who employed the GA biosynthesis inhibitor uniconizol to study the effects of GA in pea epicotyl elongation. In both studies, the growth inhibition by GA deficiency was correlated with a decrease in ϕ and an increase in Y, and not to a reduction in turgor pressure. Additionally, plastic compliance was reduced by a small but significant amount by both chemical and genetic blockage of GA synthesis. We note, however, that initial *in vivo* relaxation rates were much lower in the uniconizol-treated seedlings than in the *ls* dwarf in comparison to GA-recovered and WT seedlings, respectively. This difference deserves attention.

In summary, our results point to a striking inhibition in wall relaxation in the dwarf and semierectoides genotypes in comparison with the WT and slender genotypes, largely as a consequence of an increase in Y in the short lines. There are, however, subtler differences between the GA-deficient dwarf (*ls*) and the GA-insensitive dwarfs (*lka*, *lkb*), suggesting different sites of action. For example, calculations suggest that *ls* and *lka* reduced ϕ whereas *lkb* did not. Regarding mechanical wall properties, we observed that *ls* reduced all three different measurements of plasticity but that *lka* and *lkb* did not. Also both GA-insensitive mutants showed increases in turgor pressure lacking in the *ls* line and, between the two semierectoides lines, P-Y (the effective turgor driving cell expansion) was half as great in *lkb* as in *lka*. It thus appears that the *lka* and *lkb* lesions may specifically block one or more GA-stimulated processes that simultaneously control Y and stem elongation, but that other aspects of GA response (increase in ϕ and solute translocation) are not necessarily affected by these genes. By showing that the *lka* and *lkb* genes affect wall relaxation via an increase in Y, our results add considerable detail to the earlier conclusion that these genes affect elongation by acting in the apical region of the shoot (26). The biochemical basis for the decrease in Y under the influence of these genes or GA-synthesis inhibitors needs to be addressed. These mutants may be useful in future studies to dissect the mechanisms of GA action and to analyze wall relaxation processes.

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