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Stress Relaxation Property of the Cell Wall and Auxin-Induced Cell Elongation

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A stress-relaxation method has been developed to measure the mechanical property of the plant cell wall, as a physically defined terms. In the method, the stress relaxation property of the cell wall is simulated with a Maxwell viscoelastic model whose character is represented by four parameters: the minimum relaxation time, To, the relaxation rate, b, the maximum relaxation time, Tm and the residual stress, c. Thus, the mechanical property of the cell wall is represented by the four parameters. Physical and physiological meanings of the parameters are discussed. Auxin effects on the parameters were also studied.

The cell elongation is simply thought to be extension of the cell wall under a force. The extension of the cell wall can be simulated by the mechanical property of the cell wall. However, the calculated extension was found to be incomparable to the real cell growth, indicating that there has to be other factors limiting the rate of cell growth. Major factors governing cell growth are discussed to be the cell wall mechanical property, the osmotic potential and water movement in the apoplast. A possibility to predict cell expansion with the three factors was discussed and a novel equation representing cell growth was obtained:

$$1/R = 1/R_w + 1/R_p$$

where R is the rate of cell elongation, R_w is the rate of cell wall extension due to the osmotic pressure and R_p is the rate of cell elongation determined by water conductivity.

Key words: Cell wall — Growth — Osmotic potential — Stress relaxation — Water conductivity

Cell elongation is an increase in the cell volume and thus the cell wall is needed to extend. Cell elongation is caused by water uptake into a cell from its ambience. The driving force of water uptake is provided by the osmotic water potential or the osmotic pressure of the cell sap. If cell elongation is controlled only by water uptake driven by the osmotic pressure, elongation would continue without restriction. Actual cell elongation in higher plants does not proceed in such a manner, since the cell wall restricts water absorption. Thus, the cell wall, as well as the osmotic water potential, plays an important role in cell elongation.

When the cell takes up water from its ambience, the cell wall is to be stretched. When the cell wall is stretched, stress is produced in the cell wall, suppressing cell elongation. The larger the stress, the smaller cell elongation, and the smaller the stress, the larger cell elongation. The rate of cell elongation is thus thought to be determined by a balance between a suction force provided by the osmotic pressure of the cell sap and the stress produced by the cell wall. Cell elongation would be caused by an increase in the osmotic pressure of the cell sap and/or a decrease in the cell wall stress. In general, cell elongation is controlled by plant hormones. Some plant hormones enhance cell elongation via the regulation of the osmotic pressure of the cell sap and some others cause changes in the mechanical property of the cell wall or both to regulate cell elongation.

When a plant hormone, auxin induces cell elongation, it decreases the cell wall stress (Heyn and van Overbeek 1931, Cleland 1967, Masuda 1969, Yamamoto *et al.* 1970) but does not increase the osmotic pressure of the cell sap but even decrease it (Yamamoto and Masuda 1984). The decrease in the cell wall stress is thought to be a cause of cell elongation induced by auxin. The change in the mechanical property of the cell wall resulting in enhancement of the cell elongation is referred to as "loosening" of the cell wall (Cleland 1958). Auxin causes the cell wall loosening. On the other hand, there are contradictory reports on gibberellin effects on the cell wall (Yoda and Ashida 1960, Nakamura *et al.* 1975, Katsu and Kamisaka 1983, Miyamoto and Kamisaka 1988). Recently gibberellin is reported to enhance cell elongation through an increase in the osmotic concentration of the cell sap or a suppression of its decrease during water absorption (Miyamoto and Kamisaka 1988).

Cell wall loosening can be measured as a change in the mechanical property of the cell wall. The mechanical property is defined as the relationship between the stress and the strain or the load and the extension. In the case of a spring or a rubber band, the extension is proportional to the load irrespective of the time. This kind of the mechanical property is elastic. The mechanical property of an ordinary substance is not just elastic but dependent on time, being viscoelastic. The mechanical property of the cell wall is viscoelastic.

The mechanical property of the cell wall has been measured by a variety of methods (Heyn and van Overbeek 1931, Olson *et al.* 1965, Cleland 1967, 1971, Masuda 1969, Yamamoto *et al.* 1970, Yamamoto and Masuda 1971, Cleland and Haughton 1971, Cosgrove *et al.* 1984). These methods

are designed to obtain mechanical parameters correlated well with cell wall loosening and essentially divided into three types: (1) a constant extension is given to a specimen and the change in the stress is measured, e.g. the stress-relaxation measurement (Yamamoto *et al.* 1970, Cleland and Haughton 1971), (2) a constant load is given to a specimen and the change in the extension is measured, e.g. the creep measurement (Hager *et al.* 1971, Cleland 1971, Jaccard and Pilet 1975) and (3) when the load or extension given to the specimen is changed, the extension or load is measured, e.g. the load extension and the resonance measurements (Virgin 1955, Olson *et al.* 1965, Cleland 1967, 1971, Masuda 1969). The situation in the third type of measurements is rather complex since both the stress and the strain change. The stress-relaxation and the creep measurements, in which only one of the stress and the strain changes, appear to have advantages in analyzing the mechanical property of the cell wall.

The creep which is the extension under a constant force mimics the cell elongation process (Cleland 1971). An apparatus equipped with a differential transducer has been generally used to measure the creep of the cell wall of higher plants. However, the measurement of the creep process with the plant cell wall seems to be impractical because it is hard to avoid the shock on a specimen when a load is applied to the specimen, as pointed out (Yamamoto and Sakurai 1990). Occasionally, application of a load causes an overshoot and a swing in the extension. A tensile tester which has been employed to measure the load extension relation of the cell wall (Olson *et al.* 1967, Cleland 1967, Masuda 1969) has been employed to measure the stress relaxation of the cell wall (Yamamoto *et al.* 1970, Cleland and Haughton 1971). With this technique there is little chance to have errors derived from the shock due to the load application during the operation of the apparatus.

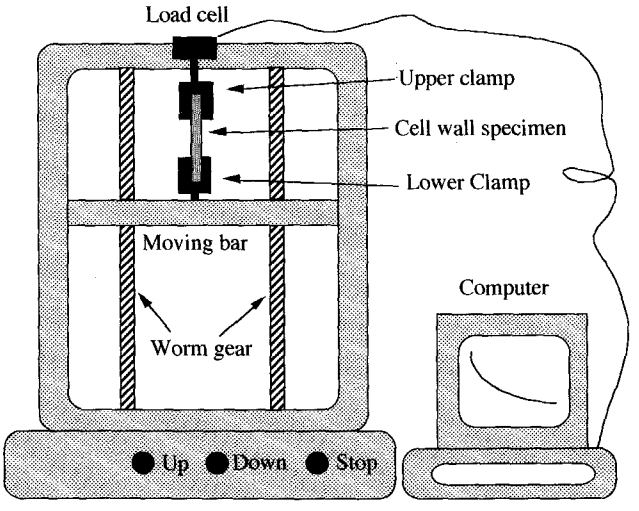

Fig. 1. A tensile tester. A cell wall specimen was fixed between the upper and lower clamps of the tensile tester. The upper clamp is connected to the load cell which is fixed on the top of the tester. When the down button is pushed, the worm gear moves down the moving bar to stretch the cell wall specimen. When the specimen produces a certain amount of the initial stress, the stop button is pushed to stop the moving bar and decay of the stress detected by the load cell is recorded by the computer.

Yamamoto *et al.* (1970, 1974) have developed a stress-relaxation method to measure the mechanical property of the plant cell wall and auxin effect on the parameters obtained as a physically defined terms. In the principle of the stress-relaxation measurement, the specimen is supposed to be extended instantaneously and thereafter the decay of the stress is measured. Although an application of an instantaneous extension is not practical, a high rate of extension can be given to the specimen with the tensile tester (Fig. 1).

The Stress-Relaxation Analysis

The physically defined way to deal with the mechanical property of the cell wall includes a mathematical analysis. The first step to the way is to create a physical model representing the stress-relaxation phenomenon in the cell wall. The model should be appropriate for explaining the phenomenon from the physical point of view. A simple model is good to understand phenomena although it does not necessarily mean that a good model is simple. In addition, it is a matter of course that a complex model fits well the actual phenomenon irrespective of whether or not it explains the phenomenon well.

The followings are examples of the stress-relaxation measurement of the plant cell wall (Yamamoto *et al.* 1970). Oat seedlings are grown in the dark for four days and segments are excised from coleoptiles. Segments are fixed with boiling methanol for 5 min and stored in fresh methanol until use. Stored segments are rehydrated and washed with water several times. A tensile tester is employed to measure the stress relaxation. A rehydrated segment is fixed between two clamps of the tensile tester (Fig. 1). The upper clamp is connected to the load cell to detect the load produced by the specimen and the lower clamp is quickly lowered (20 mm/min) to extend the specimen. The "down" button of the tensile tester is pushed to lower the lower clamp and stretch the specimen to produce a certain amount of load. The "stop" bottom is pushed to stop the lower clamp when the load produced by the specimen reaches 10 g or the strain reaches 10%, for example. Then the load produced by the specimen then quickly decreases. This is the way to observe stress relaxation of the wall specimen. This technique is applicable to the cell wall of shoots such as stems and coleoptiles. However, this stretching method seems to be hardly applicable to the cell wall of organs such as fruits and tubers (Yamamoto *et al.* 1981). The stress relaxation analysis by a compression method has been applied to measure firmness or ripeness of fruits (Kojima *et al.* 1991, 1992).

The stress relaxation has been simulated with Maxwell viscoelastic elements consisting of elastic element (Fig. 2a) and viscosity element (Fig. 2b) connected in series. The stress relaxation of an ordinary matter is not simulated by

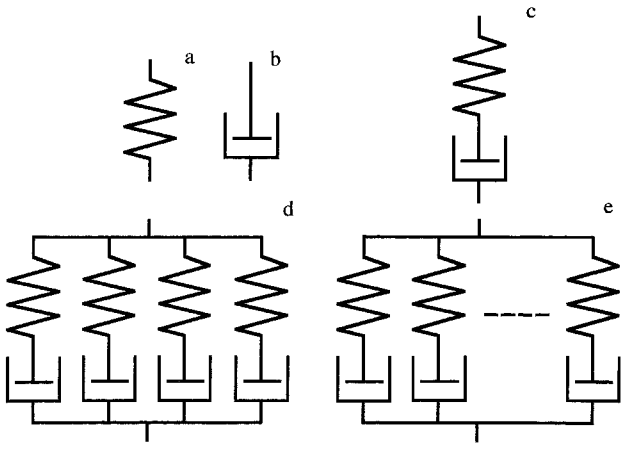

Fig. 2. Maxwell viscoelastic models. a: elastic element, b: viscosity element, c: a Maxwell element, d: a discrete Maxwell viscoelastic model and e: a generalized Maxwell viscoelastic model.

only one Maxwell element (Fig. 2c) but a Maxwell model composed of some number of Maxwell elements connected in parallel (Fig. 2d) is used to simulate the stress relaxation process. A generalized Maxwell model with an infinite number of Maxwell elements connected in parallel is also used more commonly (Fig. 2e). The density function of Maxwell elements in the model expresses the character of the mechanical property of the cell wall. The function is called the spectrum. The stress relaxation is expressed by Laplace's transformation of the spectrum.

The stress-relaxation equation is a physical model itself. The stress-relaxation curve of the cell wall appears to be expressed by an exponentially decreasing function and therefore may be represented by the following equation:

$$S=\alpha\cdot\exp(-\beta t) \qquad (I)$$

where S is stress, t is time and α and β are constants. This equation fails to represent the stress relaxation of the cell wall irrespective of the value of α and β. As mentioned above, the Maxwell model composed of only one Maxwell element is not enough to represent the stress relaxation of the wall specimen. At least four elements are needed to fit the model to the stress relaxation as represented by the following equation (Yamamoto *et al.* 1970):

$$S=\sum_{i=1}^{4}\varepsilon\cdot G_i\cdot\exp(-t/\tau_i) \qquad (II)$$

where ε is the strain. G_i and τ_i are the elastic value and the relaxation time of the i-th element of the Maxwell model, respectively. The stress relaxation parameters of the cell wall are obtained by the least square method. A plant hormone, auxin is found to cause a decrease in the value of τ_i when $\tau_1<\tau_2<\tau_3<\tau_4$ (Yamamoto *et al.* 1970).

The equation (II) contains eight parameters. It can be said that the model is rather complex and then a simpler model with a smaller number of parameters should be sought. For this purpose, the stress relaxation curve is numerically differentiated with respect to time and an apparent hyperbolic curve is obtained. The reciprocal value of the stress plotted against time gives a line represented by the equation:

$$y=c\cdot(t+To) \qquad (III)$$

An integration of equation (III) with respect to time gives

$$S=a-b\cdot\ln(t+To) \qquad (IV)$$

where S is stress, t is time and a, b and To are constants. Plotting the stress relaxation curve against logarithmic time gives a straight line. Equation (IV) fits well the stress relaxation of the plant cell wall. A generalized Maxwell model which is helpful to analyze the stress relaxation is characterized with parameters in equation (IV). The stress relaxation of a generalized Maxwell model is represented by the following equation:

$$S=\int_0^{\infty}G(\tau)\cdot\varepsilon\cdot\exp(-t/\tau)\cdot d\tau \qquad (V)$$

where t is time, τ is relaxation time, $G(\tau)$ is the density function, that is, the relaxation time spectrum of elasticity. Equation (IV) is an empirical equation of the stress relaxation and equation (V) is the one representing the stress relaxation of the Maxwell model. Equation (IV) is set to equal equation (V) as

$$a-b\cdot\ln(t+To)=\int_0^{\infty}G(\tau)\cdot\varepsilon\exp(-t/\tau)\cdot d\tau \qquad (VI)$$

$G(\tau)$ is obtained from equation (VI):

$$G(\tau)=b/\varepsilon/\tau\cdot\{\exp(-To/\tau)-\exp(-Tm/\tau)\} \qquad (VII)$$

From equation (VII), an equation representing the stress relaxation is obtained as (Yamamoto *et al.* 1970):

$$S(t)=b\cdot\ln\{(t+Tm)\,/\,(t+To)\} \qquad (VIII)$$

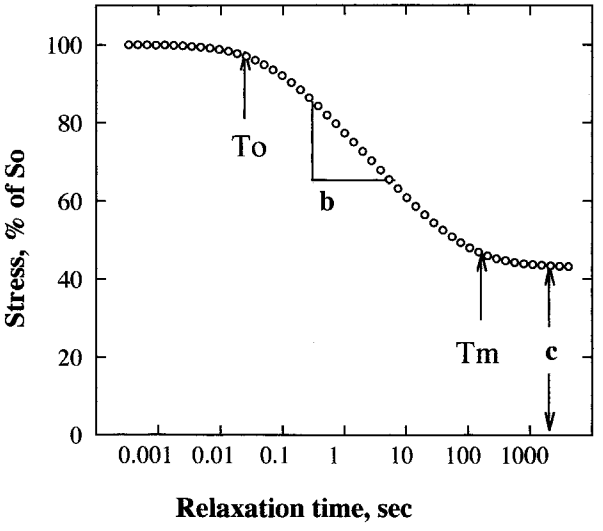

Fig. 3. A typical stress relaxation curve of the plant cell wall plotted against logarithmic time.

Plotting the actual stress relaxation curve against logarithmic time reveals that a residual stress, *c* remains after long time period and thus the following stress relaxation equation is obtained (Yamamoto *et al.* 1974):

$$S(t)=b\cdot\ln\{(t+Tm)/(t+To)\}+c \qquad \text{(IX)}$$

The Maxwell model simulating the stress relaxation of the plant cell wall is characterized by the parameters in equation (IX) such as *b, To, Tm* and *c*.

The mechanical property of the cell wall is characterized by the shape of the $G(\tau)$ spectrum. The spectrum obtained for the cell wall lies on a long time scale from 1 msec to 1,000 sec. When the spectrum is drawn on the logarithmic time scale, $H(\tau)$ spectrum is used. $H(\tau)$ equals $G(\tau)\tau$. The stress-relaxation property over the wide time range is characterized by the shape of the $H(\tau)$ spectrum.

Figure 3 shows the stress relaxation curve of the plant cell wall plotted against logarithmic time. The stress starts to decrease at the time, *To.* This time is referred to as the minimum stress relaxation time when relaxation starts. Afterwards the stress decreases linearly until the time, *Tm* which is referred to as the maximum stress relaxation time when relaxation stops. The slope of the line, *b* between *To* and *Tm* is referred to as the stress relaxation rate. The residual stress is c.

Meanings of the Parameters

The mechanical property of the cell wall is analyzed with the stress relaxation curve and thus the four parameters, *b, To, Tm* and c which can be calculated by the non-linear least square method. Cell wall loosening is defined as a change in the cell wall leading to the cell elongation. There are four parameters in the generalized Maxwell viscoelastic model. A question arises as to which parameter is correlated well with cell wall loosening. In addition, it is physiologically and chemically important to know the relationship between the parameters and cell wall components.

The cell wall is prepared from plant organs which are excised from plants grown under different conditions or treated with various agents. The cell wall is then subjected to stress relaxation measurement and the analysis of the cell wall components. The cell wall is mainly composed of polysaccharides such as cellulose, hemicellulose and pectin. The *To* value is found to be correlated well with the amount of hemicellulose in the cell wall (Kawamura *et al.* 1976). Using segments excised from oat coleoptiles or pea epicotyls, auxin is found to cause a decrease in *To* of the cell wall (Yamamoto *et al.* 1970, 1974, Masuda *et al.* 1974). These facts indicate that hemicellulose plays an important role in cell wall loosening leading to cell elongation.

Applying Eyring's theory of the reaction rate to the nature of viscosity, viscous flow or deformation is based on a movement of flowing units crossing over an energy barrier (Eyring 1936). According to the theory, *b* in equation (IV) or (IX) represents the concentration of flowing units in the viscous material (Tobolsky and Eyring 1943). Therefore, in the case of the present material, *b* in the equations is supposed to represent stress relaxation units in the cell wall (Yamamoto *et al.* 1981). The value of *b* is found to be correlated well with the amount of hemicelluloses in the cell wall (Kawamura *et al.* 1976) and with the growth ability (Sakurai *et al.* 1982). The main component of the cell wall supporting the stress relaxation is thus hemicelluloses.

The stress relaxation curve of synthetic fibers with different molecular weights is shifted horizontally along the logarithmic time axis and the shifting factor is found to be 3.3~3.4 times the logarithmic time of the mean molecular weight (Mark and Tobolsky 1950). The stress relaxation time is thus proportional to the 3.3~3.4 power of the molecular weight. Shifting of $H(\tau)$ spectrum of the cell wall toward right and left represents an increase and a decrease in molecular weights of hemicelluloses, respectively. The values of *To* and *Tm* are the lower and upper limits of the $H(\tau)$ spectrum. Their change is a reflection of changing molecular weights of the cell wall components such as hemicelluloses as suggested previously (Yamamoto *et al.* 1970).

Auxin treatment of excised plant organs such as segments of oat coleoptiles or pea epicotyls causes a decrease in *To*, suggesting that auxin causes a decrease in the molecular weight of hemicelluloses of the cell wall. In fact, auxin causes the degradation of a component of hemicelluloses (Loescher and Nevins 1972, 1973) and enzymes which are able to degrade the cell wall induce cell elongation and cell wall loosening (Masuda and Wada 1967, Masuda 1968, Yamamoto and Nevins 1981). Hemicelluloses which are extracted from plant organ segments treated with auxin are attempted to be analyzed by gel filtration chromatography. The elusion pattern of hemicelluloses from gel chromatography, that is, the molecular weight distribution is shifted toward lower molecular weights by auxin, indicating that auxin causes a decrease in the mean molecular weight of polysaccharides in hemicelluloses (Sakurai *et al.* 1979, Nishitani and Masuda 1982, Inouhe *et al.* 1984). Judging from these facts, it is likely that auxin-induced cell wall loosening is at least partly due to the degradation of hemicelluloses. Cell wall loosening may be caused by enzyme reactions conducting modifications of cell wall components, such as a degradation or molecular weight lowering of hemicelluloses. It is also likely that the modification of hemicelluloses is carried out by an enzyme conducting transglycosylation between hemicellulose molecules. An enzyme preparation which is able to induce cell elongation contained a transglucosylase activity which produces cellulose from cello-oligosaccharides (Yamamoto and Nevins 1979, Tanaka *et al.* 1982, Yamamoto *et al.* 1983). Xyloglucan molecules of the cell wall of higher plants are rearranged by a transglycosylase which is suggested to be involved in cell elongation and cell wall construction (Nishitani and Tominaga 1992, Nishitani 1995). The enzyme is extractable from the apoplast by centrifugation of organ segments (Nishitani 1992, Okazawa *et al.* 1993, Nishitani and Tominaga 1992), suggesting that it acts in the cell wall.

Mechanism of Elongation Growth

Cell elongation has been thought to be equivalent to cell wall extension under a constant load. The turgor pressure in the cell has been understood to act on the wall as a load. If we consider a protoplast without the cell wall, it can be imagined that no pressure is produced inside the protoplasts even if they absorb water. Thus, water uptake by protoplasts does not cause turgor pressure to be produced. Hence the turgor pressure is clearly due to the resistance of the cell wall against water uptake to enlarge the cell and extend the cell wall. It is therefore hard to understand that the turgor pressure is a driving force for cell wall extension. When the turgor pressure is balanced with the osmotic pressure of the cell, the cell is turgid and the net water uptake does not occur. The stress relaxation of the cell wall results in the turgor pressure decrease. Water uptake is driven by the force due to the difference between the osmotic pressure and the turgor pressure which is equal to the cell wall pressure in value; the decrease in the turgor pressure increases water uptake into the cell. As soon as water is taken up, then the turgor pressure is restored and the turgor pressure is balanced with the osmotic pressure. Therefore, water uptake is caused by the decrease in the turgor pressure. Thus it can be said that the primary cause for cell extension is the stress relaxation of the cell wall but not turgor pressure increase.

If an elongating cell absorbs water through an active mechanism, the corresponding pressure with active water absorption is to be produced. The active water absorption has been suggested to be carried out by active solute absorption (Katou and Furumoto 1986). The active water absorption is caused by energy consumption and thus dependent on energy production, that is, metabolism. If a cell is cooled or put in nitrogen atmosphere to suppress energy production, the portion of the turgor pressure due to active water absorption is canceled and the cell has to shrink. In fact, pea epicotyl segments cooled with ice are found to shrink (Yamamoto and Sakurai 1992b), suggesting that a portion of the force for water uptake is produced by active water uptake. Water channels (Bondy *et al.* 1993, Chrispeels and Agre 1994) may exist in plasma membrane and play some role in the maintenance of the turgor pressure. Water channels whose density in the plasmalemma and tonoplast limits the rate of water uptake may play a role in production of the turgor pressure. Auxin does not increase the osmotic pressure of the cell sap when it enhances cell elongation. On the contrary, it often decreases the osmotic concentration of the cell sap (Yamamoto and Masuda 1984). Let water uptake driven by the osmotic pressure of the cell sap be referred to as passive water uptake, although the osmotic pressure of the cell sap is maintained by active energy-dependent processes in the cell. Let water uptake coupled directly with an active process such as ion uptake driven by ion pumps be referred to as active water uptake. Active water uptake will be quickly inhibited by energy production blocked, while passive one will not. On the basis of this assumption, we can measure active water uptake and passive one, separately. If auxin enhances cell elongation through its action on active water uptake, the ratio of active water uptake to passive one has to be increased by auxin. When segments of pea epicotyls treated with or without auxin are cooled with ice and their lengths are measured, the ratio does not appear to be affected by auxin in pea epicotyl segments (Yamamoto and Sakurai 1992b). Active and passive water uptake are affected to the same extent by auxin. Since auxin does not enhance the osmotic pressure, supporting passive water uptake, active one is concluded to be unaffected by auxin in pea epicotyls.

Cell elongation enhanced by auxin is not due to an increase in the osmotic pressure or active water uptake. Auxin causes cell wall loosening to enhance cell elongation and hypotheses have been proposed to explain the auxin action on cell wall extension to enhance cell elongation. One of them is acid growth theory (Hager *et al.* 1971). According to the theory, auxin is to cause hydrogen ion secretion from the plasma membrane to bring about cell wall loosening. When segments of plant organs are treated with auxin, pH value of the ambience solution is observed to decrease. Auxin possibly causes hydrogen ion secretion with its acting directly on the plasma membrane as suggested by Hager (1971). Protons are thought to acidify the cell wall to cause cell wall loosening. There is another explanation of the proton action that solutes cotransported with protons, which are secreted by energy-dependent proton pumps, contribute to an increase in the osmotic pressure to enhance water uptake. On the other hand, galactose has been reported to suppress auxin-induced cell elongation in coleoptile segments of gramineous plants by inhibited synthesis of UDPG (Yamamoto *et al.* 1984, Inouhe *et al.* 1986, 1987). In *Vigna* epicotyl segments whose cell elongation is not inhibited by galactose, galactose inhibits the auxin-induced decrease in the pH value of the ambience solution as well as glucose (Kokubo *et al.* 1990). These sugars are speculated to be incorporated into cells together with hydrogen ions and thus hinder the auxin-induced decrease in pH of the ambience solution. Auxin may bring about an efflux of hydrogen ions to the ambience to enhance cotransport of substances such as sugars with hydrogen ions. Solute uptake suppresses a decrease in the osmotic pressure due to water uptake and maintains the ability of cell elongation.

Simulation of Cell Elongation

If we know the mechanical property of some material and the force to extend it, extension can be described with physical laws. Therefore, extension of the cell wall can be mathematically obtained because we know the viscoelastic property of the cell wall and the osmotic pressure which is the driving force of water uptake. Cell elongation is thought to be equivalent to cell wall extension caused by a force provided by the difference in the osmotic pressures between the cell sap and the apoplast. One of so-called Lockhart's equations has been empirically introduced to express the rate of cell elongation :

$$1/V \cdot V/dt = m \cdot (P - Y) \quad \text{(X)}$$

where V is the cell volume, $1/V \cdot V/dt$ is the rate of cell elongation, m is the extensibility of the cell wall, P is the turgor pressure and Y is the yield stress of the cell wall (Lockhart 1965, Green *et al.* 1971, Taiz 1984, Boyer 1985, Cosgrove 1986, Okamoto *et al.* 1989, 1990). The term, $P-Y$ is occasionally called as an effective turgor pressure because it is apparently the force to drive cell elongation according to equation (X) (Okamoto *et al.* 1989).

Lockhart (1965) assumed without having proof that the nature of the mechanical property of the cell wall is linear and introduced equations to express the rate of cell elongation. The linear relation of the rate with the force is viscosity. Therefore, Lockhart's assumption of equation (X) was that the mechanical property of the cell wall is simply viscous. The relation of the actual material between the rate and the force does not appear to be just proportional but appears to include a yield value in the force like equation (X). Equation (X) is equivalent to the one expressing the Bingham's viscosity. Such a bias is observed with many substances. However, the mechanical property of the cell wall does not obey such a simple law. The cell wall shows viscoelastic nature.

The other Lockhart's equation represents also the rate of cell elongation:

$$1/V \cdot V/dt = \phi \cdot (\Delta\Pi - P) \quad \text{(XI)}$$

where ϕ is water conductivity and $\Delta\Pi$ is difference between the osmotic pressures of the inside and outside of the cell. Solution of equations (X) and (XI) as simultaneous equations gives us the values the elongation rate and the turgor pressure in static cell elongation (Katou and Furumoto 1986).

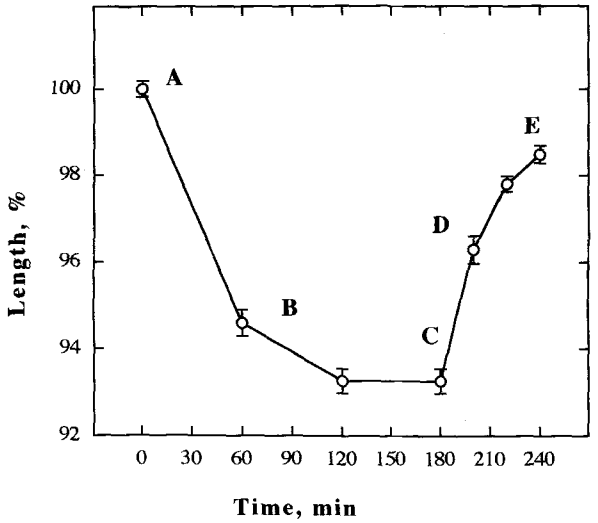

Fig. 4. Osmotic shrinkage and expansion of okra hypocotyl segments. Hypocotyl segments were excised from light-grown okra plants and incubated with 0.4 M sorbitol for 2 hr (A, B and C). Segments were then transferred to buffer solution containing no sorbitol (C, D and E).

Osmotic shrinkage and expansion of plant organ segments have been examined, for example with 0.4 M sorbitol as shown in Fig. 4. Segments are shrinking due to sorbitol (Fig. 4 A, B and C) and during this period the turgor pressure is supposed to be decreasing. Segments are then transferred to buffer solution containing no sorbitol to allow expansion (Fig. 4 C, D and E). At C of Fig. 4, segments begin to absorb water quickly because of the small turgor pressure and to elongate at a high rate. Around D, the rate of cell elongation is decreasing and the turgor pressure is supposed to be increased because of water absorption. At E, expansion is almost accomplished and the turgor pressure reaches the highest value. There may be another explanation of this phenomena; When segments are incubated in sorbitol solution with a high pressure, the cell wall is extensive. When segments are transferred to buffer solution with a lower osmotic pressure and the cell wall is extended (Fig. 4 D), the cell wall becomes less extensive and the rate of extension decreases. In this explanation, the extensibility of the cell wall is assumed to change with extension of the cell wall.

According to equation (X), the rate of cell elongation should increase when the turgor pressure increases. It is, however, not the case. In addition, all the terms in equation (X) are solely the functions of the property of the cell wall. The m is the wall extensibility, P is the turgor pressure which is equivalent to the wall pressure and Y is obviously a function of the wall. In other words, all the terms in the equation are functions of one single factor, namely the mechanical property of the cell wall as pointed out previously (Yamamoto and Sakurai 1992a).

The coefficient, m in equation (X) may not represent just the mechanical property of the cell wall. Taking $m \cdot \phi/(m+\phi)=M$, equation (X) gives

$$1/\cdot dV/dt \cdot (1/M - 1/\phi) = P - Y \quad \text{(XII)}.$$

Substitution of equation (XI) into equation (XII) gives

$$1/V \cdot dV/dt = M \cdot (\Delta\Pi - Y) \quad \text{(XIII)}.$$

This equation may show that the wall extension is driven by $\Delta\Pi$, the difference between the osmotic pressures of the inside and outside of the cell but not the turgor pressure. This equation agrees with the fact that the dependency of extension rate of the cell wall on the tensile force is linear (Hejnowicz and Sievers 1992). The physical and physiological meaning of M may have to be further studied.

Equations Representing Cell Elongation

The stress relaxation analysis is one of the ways to express the mechanical property of the cell wall by physically defined terms. The mechanical behaviors, such as the stress relaxation, the creep and the load-extension are interchangeable each other with differential or integral equations (Gross 1950). Using the stress relaxation parameters, the load-extension behavior of the cell wall can be reproduced (Fujihara *et al.* 1978, 1979). However, a similar type of the problem in the creep has not allowed a closed -form solution. A digital computer with a compiler language

integrates differential equations representing a continuous Maxwell model. The calculation based on integration is programmed with a computer language. The program advances time in the model step by step in a small increment to construct a series of sets of time and strain. This series describes the creep process which fits well the observed creep of the cell wall (Yamamoto and Sakurai 1990).

Creep, which is extension of an isolated cell wall specimen under a constant load, is thought to be equivalent to cell elongation. Cell elongation has to be predicted by the stress relaxation parameters. The creep process of an isolated cell wall was found to be much faster than the actual cell expansion (Hager *et al.* 1971, Cleland 1971, Yamamoto and Sakurai 1990). Therefore, cell elongation is not just equal to wall extension under constant force and could not be successfully predicted. There has to be another factor that limits the rate of cell expansion. Therefore cell elongation in organ segments incubated in an aqueous solution is caused by water uptake and water is taken up from the ambience by cells through the cuticle layer of the epidermis, the cell wall and the membrane. The apoplast has been suggested to limit water movement (Westgate and Steudle 1985, Steudle and Boyer 1985, Yamamoto and Sakurai 1992b).

The membrane permeability to tritium-labeled water was reported to be presumably large (Dowler *et al.* 1974, Radice *et al.* 1977, Palta and Stadelmann 1977, 1980). Tritium-labeled water penetrates little through the lateral side and mainly through the cut ends into cells of the segment when plant organ segments were incubated with tritium-labeled water (Dowler *et al.* 1974), indicating that the cuticle in stems or coleoptiles is a potent barrier to tritium-labeled water. However, the cell wall has been little discussed with regard to its participation in water permeability. Tepfer and Taylor (1981), Carpita (1982) and Baron-Epel *et al.* (1988) have reported that the cell wall acts as a barrier for passing of molecules. The cell wall may affect water movement from the ambience to cells through it and thus be one of candidates for additional factors limiting cell expansion, although the permeability of the membrane and cuticle should be considered to be main barriers for water movement (Dowler *et al.* 1974, Radice *et al.* 1977, Palta and Stadelmann 1977, 1980). When plant organ segments are incubated in hypertonic solution, they shrink. Water passes out through the membrane, the cell wall and the cuticle to the ambient solution. The rate of water movement is proportional to the pressure and inversely proportional to the water viscosity (Yamamoto 1995). Thus, water conductivity is a factor limiting cell elongation as well as the mechanical property of the cell wall. Water movement into the cell leading to cell elongation undergoes a resistance due to passing of water through the membrane, the cell wall and/or the cuticle. The force causing cell wall extension is decreased by the amount of the resistance. In other words, the force provided by the osmotic pressure of the cell sap minus the resistance due to water passing is the force causing cell wall extension. Roughly speaking, the resistance plus the cell wall stress equals the osmotic pressure.

According to equations (XI) and (XIII), the forces driving the water conductivity and cell wall extension are $\Delta\Pi - P$ and $\Delta\Pi - Y$, respectively. The sum of the forces gives the total force, that is, $\Delta\Pi$. *Thus,* $\Delta\Pi = 2 \cdot \Delta\Pi - P - Y$ giving the relation of

$$\Delta\Pi = P + Y \qquad \text{(XIV)}$$

This equation may indicate that P is the pressure creating the cell wall extension and Y is the pressure for water conductivity. Substitution of equation (XIV) into equation (XI) gives

$$1/V \cdot dV/dt = \phi \cdot Y \qquad \text{(XV)}$$

and that into equation (XIII) gives

$$1/V \cdot dV/dt = M \cdot P \qquad \text{(XVI)}.$$

Taking $M \cdot \Delta\Pi = R_w$ and $\phi \cdot \Delta\Pi = R_p$, the rate of cell elongation ($R = 1/V \cdot dV/dt$) is obtained from equations (XIV), (XV) and (XVI) as

$$1/R = 1/R_w + 1/R_p \qquad \text{(XVII)}$$

R_w is the maximum rate of cell wall extension due to the osmotic pressure, $\Delta\Pi$ and R_p is the maximum rate of cell elongation limited by water conductivity. According to equation (XVII), R does not become greater than either R_w or R_p irrespective of the values of R_w and R_p. In this sense, the mechanical property of the cell wall and water conductivity can be limiting factors in cell elongation. If other factors are involved in limiting cell elongation, equation (XVII) grows to the generalized form:

$$1/R = \sum_{i=1}^{N} 1/R_i \qquad \text{(XVIII)}$$

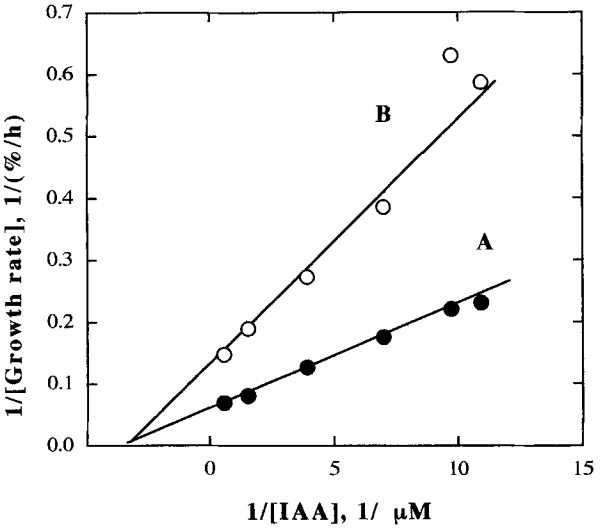

Fig. 5. Double reciprocal plots of elongation rates of oat coleoptile segments. Coleoptile segments were incubated with 2% sucrose solution for 5 hr in the presence of various concentrations of indole-3-acetic acid and 1 μM cycloheximide. The reciprocal values of elongation rates were plotted against the reciprocal values of the concentrations of indole-3-acetic acid. A: no cycloheximide, B: plus cycloheximide.

where n is the total number of factors and R_i is the rate determined by the i-th factor. This type of law appears in physiological phenomena (McRae and Bonner 1953, Yamamoto 1973) as shown in Fig. 5, ecological phenomena (Shinozaki and Kira 1956), enzyme reactions and others. Laws including limiting factors, such as the law of minimum may be represented by this type of equation.

In conclusion based on experimental results we have obtained and upon our theoretical consideration, the cell wall extension and thus cell elongation are primarily caused by cell wall relaxation.

References

Baron-Epel, O., Gharyal, P.K. and Schindler, M. 1988. Pectins as mediators of wall porosity in soybean cells. Planta **17**: 389-395.

Bondy, C., Chin, E., Smith, B.L., Preston, G.M. and Agre, P. 1993. Developmental gene expression and tissue distribution of the chip 28 water-channel protein. Proc. Natl. Acad. Sci. USA **90**: 4500-4504.

Boyer, J.S. 1985. Water transport. Ann. Rev. Plant Physiol. **36**: 473-516.

Carpita, N. 1982. Limiting diameters of pores and the surface structure of plant cell walls. Science **218**: 813-814.

Chrispeels, M.J. and Agre, P. 1994. Aquaporins: Water channel proteins of plant and animal cells. Trends Biochem. Sci. **19**: 421-425.

Cleland, R. 1958. A separation of auxin-induced cell wall loosening into its plastic and elastic components. Physiol. Plant. **11**: 599-609.

Cleland, R. 1967. Auxin and the mechanical properties of the cell wall. Proc. N.Y. Acad. Sci. **144**: 3-8.

Cleland, R. 1971. The mechanical behavior of isolated *Avena* coleoptile walls subjected to constant stress. Plant Physiol. **47**: 805-811.

Cleland, R. and Haughton, P.M. 1971. The effect of auxin on stress-relaxation in isolated *Avena* coleoptiles. Plant Physiol. **47**: 812-815.

Cosgrove, D.J. 1986. Biophysical control of plant cell growth. Ann. Rev. Plant Physiol. **37**: 377-405.

Cosgrove, D.J., VAN Volkenburgh, E. and Cleland, R.E. 1984. Stress-relaxation of cell walls and the yield threshold for growth: Demonstration and measurement by micro-pressure prove and psychrometer technique. Planta **162**: 46-54.

Dowler, M.J., Rayle, D.L., Cande, W.Z., Ray, P.M., Durand, H. and Zenk, M.H. 1974. Auxin does not alter the permeability of pea segments tritium-labeled water. Plant. Physiol. **53**: 229-232.

Eyring, H. 1936. Viscosity, plasticity, and diffusion as exam ples of absolute reaction rates. J. Chem. Phys. **4**: 283-291.

Finkelstein, A. 1987. Water Movement Through Lipid Bilayers, Pores and Plasma Membranes. Theory and Reality. Wiley-Interscience Pub, New York ISBN 0-471-84787-9.

Fujihara, S., Yamamoto, R. and Masuda Y. 1978. Viscoelastic properties of plant cell walls. III. Hysteresis loop in the stress-strain curve at constant strain rate. Biorheology **15**: 87-97.

Fujihara, S., Yamamoto, R. and Masuda Y. 1979. Viscoelastic properties of plant cell walls. IV. Physical meaning of parameter K in mathematical formulation for stress-relaxation and load extension process. Biorheology **16**: 387-396.

Green, P.B., Erickson, R.O. and Buggy, J. 1971. Metabolic and physical control of cell elongation rate. In vivo studies in *Nitella*. Plant Physiol. **47**: 423-430.

Gross, B. 1950. Relation between creep function and relaxation function. J. Polymer Sci. **6**:123-126.

Hager A., Menzel, H. and Krauss, A. 1971. Versuche und Hypothese des Auxins beim Streckungswachstum. Planta **100**: 47-75.

Hejnowicz, Z. and Sievers, A. 1992. Tissue tension — A factor in the regulation of cell wall growth. *In* Y. Masuda ed., Plant Cell Walls as Biopolymers with Physiological Functions. Yamada Sci. Foundation, Osaka, ISBN 4-906117-01-1, pp. 67-72.

Heyn, A.N.J. and van Overbeek, J. 1931. Weiteres Versuchsmaterial zur plastischen und elastischen Dehnbarrkeit der Zellmembrane. Proc. Kon. Akad. Wetensch. Amsterdam **34**: 1190-1195.

Inouhe, M., Yamamoto, R. and Masuda, Y. 1984. Auxin-induced changes in the molecular weight distribution of cell wall xyloglucans in *Avena* coleoptiles. Plant Cell Physiol. **25**: 1341-1351.

Inouhe, M., Yamamoto, R. and Masuda, Y. 1986. Inhibition of IAA-induced cell elongation in *Avena* coleoptile segments by galactose. its effect on UDP-glucose formation. Physiol. Plant. **66**: 370-376.

Inouhe, M., Yamamoto, R. and Masuda, Y. 1987. UDP-Glucose level as a limiting factor for IAA-induced cell elongation in *Avena* coleoptile segments. Physiol. Plant. **69**: 49-54.

Jaccard, M. and Pilet, P.E. 1975. Extensibility and rheology of collenchyma: I. Creep relaxation and viscoelasticity of young and senescent cells. Plant Cell Physiol. **16**: 113-120.

Katou, K. and Furumoto, M. 1986. A mechanism of respiration-dependent water uptake in higher plants. Protoplasma **130**: 80-82.

Katsu, N. and Kamisaka, S. 1983. Quantitative and qualitative changes in cell wall polysaccharides in relation to growth and cell wall loosening in *Lactuca sativa*. Physiol. Plant. **58**: 33-40.

Kawamura, H., Kamisaka, S. and Masuda, Y. 1976. Regulation of lettuce hypocotyl elongation by gibberellic acid. Correlation between cell elongation, stress-relaxation properties of the cell wall and cell wall polysaccharide content. Plant Cell Physiol. **17**: 23-34.

Kojima, K., Sakurai, N., Kuraishi, S., Yamamoto, R. and Nevins, D.J. 1991. Novel technique for measuring tissue firmness within tomato (*Lycopersicon esculentum* Mill.) fruit. Plant Physiol. **96**: 545-550.

Kojima, K., Sakurai, N., Kuraishi, S., Yamamoto, R. and Inaba, A. 1992. Physical measurement of firmness of banana fruit pulp: determination of optimum condition for measurement. Postharvest Biology and Technology **2**: 41-49.

Kokubo, A., Yamamoto, R. and Masuda, Y. 1993. Galactose prevents proton excretion but not IAA-induced growth in segments of azuki bean epicotyls. Planta **190**: 284-287.

Lockhart, J.A. 1965. An analysis of irreversible plant cell elongation. J. Theoret. Biol. **8**: 264-275.

Loescher, W.H. and Nevins, D.J. 1972. Auxin-induced changes in *Avena* coleoptile cell wall composition. Plant Physiol. **50**: 556-563.

Loescher, W.H. and Nevins, D.J. 1973. Turgor-dependent changes in *Avena* coleoptile cell wall composition. Plant Physiol. **52**: 248-251.

Mark, H. and Tobolsky, A.V. 1950. In Physical Chemistry of High Polymeric System, Interscience, New York, pp. 343.

Masuda, Y. 1968. Role of cell-wall-degrading enzymes in cell-wall loosening in oat coleoptiles. Planta **83**: 171-184.

Masuda, Y. 1969. Auxin-induced expansion in relation to cell wall extensibility. Plant Cell Physiol. **10**: 1-9.

Masuda, Y., Kawamura, H. and Yamamoto, R. 1974. Stress-growth conditions. Plant Cell Physiol. **15**: 1083-1092.

Masuda, Y. and Wada, S. 1967. Effect of β-1, 3-glucanases activity in Avena coleoptile. Bot. Mag. Tokyo **80**: 100-102.

McRae, D.H. and Bonner, J. 1953. Chemical structure and antiauxin activity. Physiol. Plant. **6**: 485-510.

Miyamoto, K. and Kamisaka, S. 1988. Stimulation of *Pisum sativum* hormones on osmoregulation and cell walls. Physiol. Plant. **74**: 457-466.

Nakamura, M., Sekine, S., Arai, K. and Takahashi, N. 1975. Effect of gibberellic acid and indole-3-acetic acid on stress-relaxation properties of pea hook cell wall. Plant Cell Physiol. **16**: 127-138.

Nishitani, K. 1992. A new method for detection of endo-xyloglucan transferase. Plant Cell Physiol. **33**: 1159-1164.

Nishitani, K. 1995. Endo-xyloglucan transferase, a new class of transferase involved in cell wall construction. J. Plant Res. **108**: 137-148.

Nishitani, K. and Masuda, Y. 1982. Auxin-induced changes in the cell structure: Changes in the sugar compositions, intrinsic viscosity and molecular weight distribution of matrix polysaccharides of the epicotyl cell wall of *Vigna angularis*. Physiol. Plant. **52**: 482-494.

Nishitani, K. and Tominaga, R. 1992. Endo-xyloglucan transferase, a novel class of glycosyltransferase that catalyzes transfer of a segment of xyloglucan molecule to another xyloglucan molecule. J. Biol. Chem. **267**: 21058-21064.

Okamoto, H., Liu, Q.L. and Katou, K. 1989. A pressure-jump method as a new tool in growth physiology for monitoring physiological wall extensibility and effective turgor. Plant Cell Physiol. **30**: 979-985.

Okamoto, H., Miwa, C., Masuda, T., Nakahori, K. and Katou, K. 1990. Effects of auxin and anoxia on the cell wall yield threshold determination by negative pressure jumps in segments of cowpea hypocotyl. Plant Cell Physiol. **31**: 783-788.

Okazawa, K., Sato, Y., Nakagawa, T., Asada, K., Kato, I., Tomita, E. and Nishitani, K. 1993. Molecular cloning and cDNA sequencing of endo-xyloglucan transferase, a novel class of glycosyltransferase that mediates molecular grafting between matrix polysaccharides in plant cell walls. J. Biol. Chem. **268**: 25364-25368.

Olson, A.C., Bonner, J. and Morre, D.J. 1965. Force extension analysis of *Avena* coleoptile cell wall. Planta **66**: 126-134.

Palta, J.P. and Stadelmann, E.J. 1977. Effect of turgor pressure on water permeability of *Allium cepa* epidermis cell membranes. J. Membrane. Biol. **33**: 231-247.

Palta, J.P. and Stadelmann, E.J. 1980. On simultaneous transport of water and solute through plant cell membranes: Evidence for the absence solvent drag effect and insensitivity of the reflection coefficient. Physiol. Plant. **50**: 83-90.

Radice, M., Martinotti, P., Lado, P. and Marre, E. 1977. Lack of effect of fusicoccin on passive transport in living plant tissues and in artificial phospholipid membranes. Plant Sci. Lett. **10**: 75-83.

Sakurai, N., Fujihara, S., Yamamoto, R. and Masuda, Y. 1982. A stress-relaxation parameter *b* of the oat coleoptile cell wall and its implication in cell wall loosening. J. Plant Growth Regul. **1**: 75-83.

Sakurai, N., Nishitani, K. and Masuda, Y. 1979. Auxin-induced changes in molecular weight of hemicellulosic polysaccharides of *Avena* coleoptile cell wall. Plant Cell Physiol. **20**: 1349-1357.

Shinozaki, K. and Kira, T. 1956. Intraspecific competition among higher plants VII. Logistic theory of the C-D effect. J. Inst. Polythech. Osaka City Univ. **D7**: 35-72.

Steudle, E. and Boyer, J.S. 1985. Hydraulic resistance to radial water flow in growing hypocotyl of soybeans measured by a new pressure perfusion technique. Planta **164**: 189-200.

Taiz, L. 1984. Plant cell expansion: Regulation of cell wall mechanical properties. Ann. Rev. Plant Physiol. **35**: 585-657.

Tanaka, T., Yamamoto, R., Oi, S. and Nevins, D.J. 1982. Purification and some properties of β-transglucosylases of *Sclerotinia libertiana*, having the ability to synthesize higher cello-oligosaccharides from cellotriose or cellotetraose. Carbohydr. Res. **106**: 131-142.

Tepfer, M. and Taylor, I.E.P. 1981. The permeability of plant cell walls as measured by gel filtration chromatography. Science **213**: 761-763.

Tobolsky, A. and Eyring, H. 1943. Mechanical properties of polymeric materials. J. Chem. Phys. **11**: 125-134.

Virgin, H.I. 1955. A new method for the determination of the turgor of plant tissues. Physiol. Plant. **8**: 954-962.

Palta, J.P. and Stadelmann, E.J. 1977. Effect of turgor pressure on water permeability of *Allium cepa* epidermis cell membranes. J. Membrane. Biol. **33**: 231-247.

Westgate, T.E. and Steudle, T.E. 1985. Water transport in the midrib tissue of maize leaves. Plant Physiol. **78**: 183-191.

Yamamoto, R. 1995. Dependency of water conductivity on pressure and temperature in plant stems. Biorheol. **32**: 421-430.

Yamamoto, R., Fujihara, S. and Masuda, Y. 1974. Measurement of stress-relaxation properties of plant cell walls. *In* Plant Growth Substances, 1973, Hirokawa Pub,

Tokyo, pp. 788-795.

Yamamoto, R., Fujihara, S. and Masuda, Y. 1981. Compression method for measurement of stress relaxation properties of plant cell walls with reference to plant hormone actions. Biorheology **18**: 643-652.

Yamamoto, R. and Masuda, Y. 1971. Stress-relaxation properties of the *Avena* cell wall. Physiol. Plant. **25**: 330-335.

Yamamoto, R. and Masuda ,Y. 1984. Galactose inhibition of auxin-induced cell elongation in oat coleoptile segments. Physiol. Plant. **61**: 321-326.

Yamamoto, R., Nagano, M., Masuda, Y. 1973. A kinetic study of the auxin-induced elongation of *Avena* coleoptile segments. Plant Cell Physiol. **14**: 397-408.

Yamamoto, R. and Nevins, D.J. 1979. A transglucosylase from *Sclerotinia libertiana*. Plant Physiol. **64**:193-196.

Yamamoto, R. and Nevins, D.J. 1981. Coleoptile growth- i nducing capacities of exo-β-(1->3)-glucanases from fungi. Physiol. Plant. **51**: 118-122.

Yamamoto, R., Nevins, D.J., Tanaka, T. and Oi, S. 1983. Studies on the linkage specificity of a β-transglucosylase of *Sclerotinia libertiana*. Carbohydr. Res. **119**: 292-299.

Yamamoto, R. and Sakurai, N. 1990. A computer simulation of the creep process of the cell wall using stress relaxation parameters. Biorheology **27**: 759-768.

Yamamoto, R. and Sakurai, N. 1992a. Computer simulation of cell wall expansion and auxin action. *In* Y. Masuda, ed., Plant Cell Walls as Biopolymers with Physiological Functions. Yamada Sci. Foundation, Osaka, ISBN 4-906117-01-1, pp. 139-145.

Yamamoto, R. and Sakurai, N. 1992b. Major factors governing growth in pea epicotyl segments: the cell wall mechanical property, the osmotic potential and solute movement in the apoplast. Proc. Japan Acad. **68B**: 100-105.

Yamamoto, R., Shinozaki, K. and Masuda, Y. 1970. Stress-relaxation properties of plant cell walls with special reference to auxin action. Plant Cell Physiol. **11**: 947-956.

Yoda, S. and Ashida, J. 1960. Effects of gibberellin and auxin on the extensibility of the pea stem. Plant Cell Physiol. **1**: 99-105.

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