On the YOUNG's Modulus during Growth of Pine Seedlings*

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Abstract—The value of apparent Young's modulus of the hypocotyl of pine seedling increased with growing duration. Both calculated Young's moduli of cell wall substances and of crystalline rich cell wall also increased.

Because the increasing pattern of α -cellulose, lignin and crystalline region were not similar to that of the moduli, a distinct linear correlation between these structural parameters and the modulus could not be found. Contributions of these constituents to mechanical characteristics of the growing hypocotyls are discussed.

Introduction

A previous paper¹⁾ has shown that the tensile stress relaxation curves of living hypocotyls excised just before exposure of cotyledons were always lower than those of the hypocotyls excised at about two weeks after exposure of cotyledons. This was partly attributed to the difference of crystallinity and conformation of cellulose structure in the cell wall. This investigation was carried out to establish the reason for the difference of stress relaxation behaviors of pine hypocotyls in short time region, which were water cultured for two different growing durations.

Since stress relaxation behavior of materials in short time region is supposed to be characterised by elastic modulus determined by static tests, static tensile tests of water cultured hypocotyls for different growing durations have been carried out in the present investigation. The relationships between YOUNG's modulus and amounts of two main chemical constituents, cellulose and lignin, and cellulose crystalline region have further been obtained.

Experimental

a) Samples of pine hypocotyls

Seeds of Japanese black pine (*Pinus Thumbergii* PARL.) were germinated and grown in a chamber conditioned as reported previously¹⁾. Hypocotyl samples A, B, C, D, E and F were taken out at 2, 7, 14, 21, 28 and 50 days after germination, respectively. Twenty millimeter segments of hypocotyls were excised at 5 mm below the cotyledons and used.

b) Tensile tests

Soon after excision these samples were subjected to tensile tests in their longitudinal direction by a TOM-5000X universal testing machine (Shinkoh Communication Industry Co.) with jaw-to-jaw distance of 5 mm giving strain by lowering the bottom jaw with the speed of 0.5 mm/min. All the tests were carried out in a room conditioned at 20°C and relative humidity 45%. Load-deformation curves were traced automatically by a X-Y recorder. Apparent YOUNG's modulus of the samples E_{ap} was calculated from the following equation;

 $E_{ap} = S \cdot L/A$

^{*} Partly presented at the 22nd Annual Meeting of the Japan Wood Research Society, Tokyo, April 1972.

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where S is the slope of the initial straight part of the curve, L is initial length of the sample and A is cross sectional area determined by measuring the diameter with a traveling microscope under the assumption that the cross section of the sample is round. The values of YOUNG's modulus are given as an average of five tests.

c) Determination of α -cellulose and lignin

Alpha-celluloce in each sample was determined by the chlorite method²⁾, and lignin was determined spectrophotometrically as described previously³⁾.

d) Microphotographs

After tensile tests, hypocotyl samples were excised about 3 mm length and embedded in a epoxy resin, from which cross sectional samples $2 \sim 10 \mu$ thick were cut out with an ultramicrotome. Microphotographs were obtained with a separete interference microscope (Olympus PMB) to determine the cell wall area and with a polarlized microscope (Olympus POM-3) to distinguish crystalline rich cell wall.

e) X-ray diffraction pattern

Equatorial scanning X-ray diffraction patterns of the hypocotyl samples dried in air were obtained by a Roterflex Model RU-3 X-ray diffractometer with a scintillation counter (Rigaku Electric Co.) using Cu-K α radiation at 50 kV and 80 mA.

Results and discussion

The value of apparent YOUNG's modulus of the hypocotyl of pine seedlings increased linearly till 28 days and then the increasing rate decreased as shown in Fig. 1. The modulus of the sample F was about $400 \ kg/cm^2$, while the modulus of mature wood is about $10^5 \ kg/cm^2$.

YOUNG's modulus of cell wall substances E_s was calculated under the assumption that there are little geometric effects of cells, but only the cell wall substances contribute to the modulus



Fig. 1. The changes of apparent Young's modulus of the hypocotyls with growing duration. A, B, C, D, E and F are the plots of the value with the sample excised 2, 7, 14, 21, 28 and 50 days water cultured after germination, respectively.

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with following equation;

$$E_s = E_{ap}/W_a$$

where W_a is the ratio of cross sectional cell wall area to whole cross sectional area of the sample determined by measuring double cell wall thickness on the microphotograph. E_s was plotted against growing days in Fig. 2. The curve fitting in the plots resembled to that of Fig. 1. The modulus E_s seems to approach a constant value, but it is far less than the modulus of cell wall substances of mature wood as in the case of E_{ap} . Above all things the modulus of the cell wall substances increased with growing duration, and it may be attributed to some structural changes in the cell wall.

The amount of crystalline calculated from the areas of (101), (101) and (002) peaks and rest area of X-ray diffraction patterns were plotted against growing duration in Fig. 3. The curve fitting in the plots was sigmoidal and no linear correlation to E_s or E_{ap} was found. And crystallinity of the sample F was less than that of mature wood.

Changes of α -cellulose and lignin contents with growing duration were indicated in Fig. 4. The increasing pattern of both compounds was similar to that of crystallinity as shown in Fig. 3. The lignin content of the sample F was as much as those of mature wood.

While these sigmoidal fitting curves look like to the Verhulst-Pearl's logistic curve which satisfy the following non-linear differential equation⁴⁾,

$$\frac{dN}{dt} = (\varepsilon - \lambda N)N \tag{1}$$

the curves of E_{ap} and E_s seem to satisfy the following linear differential equation,



$$\frac{dN}{dt} = \gamma (N_0 - N) \tag{2}$$

Fig. 2. The changes of Young's modulus of cell wall substances of the hypocotyls with growing duration. A, B, C, D, E and F correspond to the samples as illustrated in Fig. 1.



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Fig. 3. The changes of crystallinity indexes of air-dried hypocotyls determined by X-ray diffraction patterns. A, B, C, D, E and F correspond to the samples as illustrated in Fig. 1.



Fig. 4. The changes of α -cellulose and lignin contents of hypocotyls with growing duration. \oplus and \bigcirc indicate α -cellulose and lignin contents respectively. A, B, C, D, E and F correspond to the samples as illustrated in Fig. 1.

where each N is an amount such as α -cellulose or modulus E_s , t is growing duration, ε and λ are constants called as increasing coefficient and confusing coefficient respectively in the field of population genetics⁵ and γ is a constant. Solutions of these equations were shown in Fig. 5 for each initial condition.

Coefficient ε in the equation (1) increases the rate of N by first order of N, and coefficient λ inhibits the rate by second order of N, if they are positive definites. During cell differentiation and cell wall thickening in the hypocotyl, the amount of constituents and crystallinity are considered to increase in the same manner.

In the equation (2), the rate of amount N decreases when N approaches to a constant N_0 , which is sometimes assumed in chemical reactions or others. Since E_{ap} and E_s are only indexes



Fig. 5. Solution curves of differential equations (1) and (2) under each initial condition, $N=0.0075 \epsilon/\lambda$ and N=0 when t=0 respectively.



Fig. 6. The changes of Young's modulus of crystalline rich cell wall calculated with the equation (3). A, B, C, D, E and F correspond to the samples as illustrated in Fig. 1. The constant moduli Er are 100, 200 and 370 kg/cm² on the plots ①, ⊖ and ○, respectively.

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of mechanical characteristics, there are many difficulties on interpretting the meaning of the equation.

Both in the equation (1) and (2), the amount N approaches to a constant when t increases infinitely. If it is assumed that the constitution and microstructure of the hypocotyl cell walls arrive in a steady state after sufficient growth, the above saturation in E_s or E_{ap} can be attained. But as mentioned above, there are no linear correlations between the modulus and the amount of constituent or crystalline regions. Contributions of these structural parameters to the modulus should be discussed in future in consideration of their interactions during growth.

The modulus E_s is composed of two different moduli; E_c , the modulus of cell walls which are bright under a polarlized microscope and E_r , the modulus of the other cell wall. Assuming that the interaction of these parts is negligible, E_s may be represented as follows;

$$E_s = A \cdot E_c + (1 - A) \cdot E_\tau \tag{3}$$

where A is the ratio of the crystalline rich cell wall area to the whole cell wall area as mentioned above.

Since E_r would be far less than E_c , E_r may be assumed to be invariant with growing days. The modulus E_c determined by the equation (3) substituted with a certain value of E_r was plotted against growing days in Fig. 6. The figure shows that E_c increases with growing days similar to E_s . This indicates that some changes of microstructure of the crystalline rich cell walls occured during cell wall thickening and affected the mechanical characteristics of the wall.

References

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