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Culm Strength of Barley[1]

Correlation Among Maximum Bending Stress, Cell Wall Dimensions, and Cellulose Content

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ABSTRACT

Grass culms are known to differ in breaking strength, but there is little physicochemical data to explain the response. The fourth internode of four brittle and two nonbrittle barley (*Hordeum vulgare L.*) strains were used for physical and chemical studies of culm strength. Inner and outer culm diameters of brittle strains (3.6 ± 0.2 and 5.0 ± 0.1 millimeters) were not significantly different from those of nonbrittle strains (3.9 ± 0.2 and 5.2 ± 0.2 millimeters). Maximum bending stress, at which the culm was broken, was 192 ± 34 g/mm² for brittle and 490 ± 38 g/mm² for nonbrittle strains. Wall thickness and cell dimensions of epidermal, sclerenchyma, and parenchyma cells were measured in culm cross sections. The area of cell wall per unit cell area for each tissue was significantly correlated with the maximum bending stress (r = 0.93 for epidermis, 0.90 for sclerenchyma, and 0.84 for parenchyma). Cell walls of brittle culms had 6 to 64% as much cellulose content as those of nonbrittle culms. Maximum bending stress correlated significantly with cellulose content of the cell walls (r = 0.93), but not with the contents of noncellulosic compounds. The lower cellulose content of the brittle culm was significantly correlated with brittleness.

Brittle (fragile) culms have been investigated mainly from the genetical view-point using maize (3), rice (9, 15, 23), and barley (24, 25). The stiff culm of barley has been studied physiologically (6, 7). The maximum bending stress and hardness of the stiff culm was twice that of normal culms (6), although the Young's modulus of both culms differed by only 16% (7). The chemical nature of the cell walls of stiff and brittle culms has not been studied. Nagao and Takahashi (15) suggested that there was a lower cellulose content in the cell wall of brittle rice culms without providing experimental evidence.

Two hundred and forty barley mutants (OUM[2] 1–240) were produced by treating uzu Akashinriki, a semi-dwarf cultivar, with ethylene methane sulfonate (10). Among them, OUM 5, 77, 97, 105, 125, 131, 133, 136, 148 showed slower coleoptile elongation than Akashinriki (normal), which had isogenic genes of these mutants except for semidwarf gene. Coleoptile of the mutant strains, including uzu Akashinriki, had a lower IAA content than Akashinriki (normal). Close correlation between growth rate and endogenous IAA content ($r = 0.907$) suggested that dwarfism of these strains was caused by reduced IAA content (8).

Three strains (OUM 40, OUM 41, and OUM 42) had slow rates of stem elongation and were found to have brittle culms (11), although hormonal studies on the dwarfism has not been completed. Experiments were performed to study the cause of culm brittleness of these three strains by comparing maximum bending stress, cell wall thickness, and cell wall components of their culms with those of nonbrittle culms.

MATERIALS AND METHODS

Plant Materials

Brittle barleys (*Hordeum vulgare* L.) used were Kamairazu, whose genetic background is not known (25), and OUM 40, OUM 41, and OUM 42 that were developed by treating uzu Akashinriki (semidwarf cultivar) with ethylene methane sulfonate (10). Gene loci for brittleness of OUM 40, OUM 41, and OUM 42 have not been identified (T Konishi, personal communication). Nonbrittle barleys were uzu Akashinriki and Akashinriki (normal), which had isogenic genes of uzu Akashinriki except for the semidwarf gene.

Seeds were sown in the middle of November at the experimental farm of the Research Institute for Resources, Okayama University. Samples were harvested at about 1 week intervals from the times of initiation to cessation of internode elongation during 1985, 1986, 1987, and 1988. Five to 50 culms, with leaf blades and sheaths removed, were immediately immersed in methanol and kept at room temperature until analyses were performed.

Measurement of Cell Wall Thickness and Cell Area

Culms of the fourth internode, harvested on May 8, 1988, were fixed in Nawaschin Claf type III (13) (1% chromic acid:10% acetic acid:35% formalin:water, v/v 3:2:1:4), which avoids the probable shrinkage of cell walls that is often observed with fixing reagents containing alcohol. Cross-sections

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[2] Abbreviations: OUM, Okayama University mutant; chloramine-T, *N*-chloro-4-methylbenzensulfonamide sodium salt; σ_{max}, maximum bending stress; P_{max}, maximum load.

from the middle of the internode of the fixed culms were carefully cut perpendicular to the culm axis with a razor blade. Thickness of the radial and tangential cell walls of epidermal, sclerenchyma, and parenchyma cells were measured under a microscope (Olympus model BHC, Tokyo) using an ocular micrometer with a precision of 0.25 μm. Cross-sections were studied from at least three different culms.

Protoplast and cell dimensions along the radial and tangential axes of each tissue were measured to calculate the ratio of cell wall area to total cell area. In sclerenchyma and parenchyma cells, the cell and protoplast shapes were elliptical. Short (a) and long axes (a′) of the protoplast and short (b) and long axes (b′) of the cell including cell wall were measured. Total cell area for sclerenchyma and parenchyma was calculated by $(b \times b')\pi/4$, and protoplast area by $(a \times a')\pi/4$. The cell wall area was, therefore, calculated by (total cell area − protoplast area). On the other hand, cell and protoplast shapes of epidermal cells are rectangular. The cell wall area was calculated by $(b \times b') - (a \times a')$.

Cell Wall Fractionation

Methanol-fixed fourth internodes were rehydrated for 2 h in 300 mL deionized water at room temperature. Rehydrated culms were blotted dry and homogenized for 30 min in deionized water with an ultrasonic homogenizer (model TP18/10 S2; Ika Werk, Breisgau, FRG) at maximum speed. The homogenate was centrifuged for 10 min at 1000*g*. The residue was washed twice with deionized water, three times with acetone, then with a methanol:chloroform (1:1 v/v) mixture. The washed residue was dried overnight at 40°C and the dried material was then treated for 18 h at 37°C with 200 μg mL^{-1} pronase (Actinase; Kaken Seiyaku Co., Ltd., Tokyo) in 50 mM sodium phosphate buffer (pH 6.5) containing 5% ethanol. The pronase solution was preincubated for 2 h at 37°C to degrade contaminant glycanases. After the material had been washed three times with deionized water, it was treated for 2 h at 37°C with 20 units mL^{-1} porcine pancreatic α-amylase type I-A (Sigma, St. Louis, MO) in 100 mM sodium acetate buffer (pH 6.5).

Preliminary experiment in 1985 and 1986 revealed that the cellulose fraction obtained directly by using a conventional fractionation technique (16, 19) was contaminated with hemicellulosic components (including up to 38% xylose). Therefore, a delignification procedure (5) was used before the fractionation of cell wall components. The enzyme-treated cell wall materials (about 0.2 g fresh weight) were heated at 100°C for 2 h in 75 mL of 60 mM chloramine-T solution after acidification with a few drops of glacial acetic acid. The solution was centrifuged at 1000*g* for 10 min and the precipitate was successively washed three times each with hot ethanol, cold ethanol:ethanolamine (97.3 v/v) and deionized water.

Chloramine-T soluble fraction plus washings were combined and designated as the chloramine-T soluble fraction. Extraction with chloramine-T was repeated until the residue became white. A solution of 4.4 N NaOH was used to extract the white residue, and the collected extracts designated as the alkaline fraction. Final residue, designated as the cellulose fraction, yielded 99.95% glucose assayed by GLC (1) after acid hydrolysis with 15 N H_2SO_4 for 1 h at room temperature and 2 N H_2SO_4 for 10 h at 100°C.

Sugar Analyses

Total sugar contents of each fraction were determined by the phenol-sulfuric acid method (4). Monosaccharide composition of the alkaline fraction was determined by GLC (1). The sugar analysis experiments were repeated three times (1985, 1986, 1987) with data being similar. Data for April 30, 1987, with triplicate samples are presented.

Maximum Bending Stress

Seven-cm sections of the fourth internode fixed in methanol were rehydrated for 2 h in 300 mL of deionized water at room temperature. Rehydrated internodes were measured as described in Figure 1. Maximum bending stress (σ_{max}, g/mm^2) was calculated as follows (26)

$$\sigma_{max} = \frac{P_{max}\, L/4}{\pi(d'^4 - d^4)/32d'}$$

where L is the distance between edges (25 mm), P_{max} is the

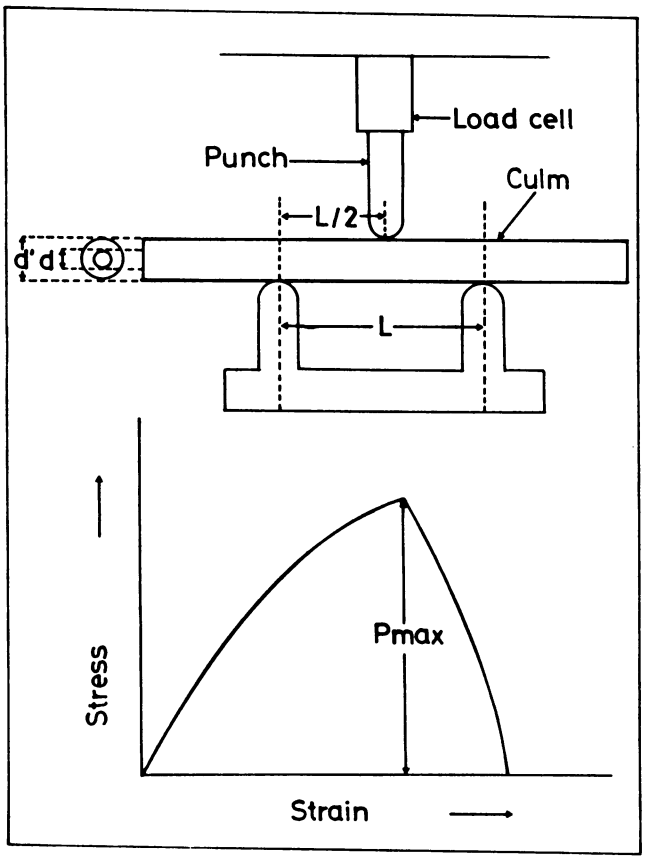

Figure 1. Measurement of maximum bending stress. Above, A 7-cm-long section of culm was placed on the edges 25 mm apart. The center of the culm was pressed by a 2-mm-diameter punch with a constant velocity of 50 mm min^{-1}. Stress and strain were measured with an Auto-GRAPH, DCS-5000 (Shimadzu, Kyoto, Japan). Outer and inner diameter of the culm were measured microscopically. Below, A schematic form of a stress and strain curve.

maximum load (g), and d' and d are the outer and inner diameters of the culm (mm), which were measured microscopically. Bending stress data are for samples taken April 30, 1987 ($n = 5$).

RESULTS

Growth of Culms

Culm lengths of the brittle strains, OUM 40, OUM 41, and OUM 42, were shorter than the nonbrittle strains, normal and uzu, at all growth stages (Fig. 2). Uzu Akashinriki showed less growth than normal Akashinriki. The brittle strain Kamairazu did not show semidwarfness. Similar growth responses were obtained during 1985, 1986, and 1987.

Maximum Bending Stress

On April 30, 1987, inner and outer diameters of the fourth internode of brittle strains were similar to those of nonbrittle strains, indicating that brittleness was not due to geometry of the culm (Table I). Average P_{max} and σ_{max} for culms of brittle strains, however, were significantly lower than those of nonbrittle ones. The results indicate that the brittleness is associated with lower P_{max} and σ_{max} values. Microscopic observation of fractures of brittle culms indicated that the center parts of the cell walls of epidermal, sclerenchyma, and parenchyma cells were broken and the middle lamella between cells was not detached.

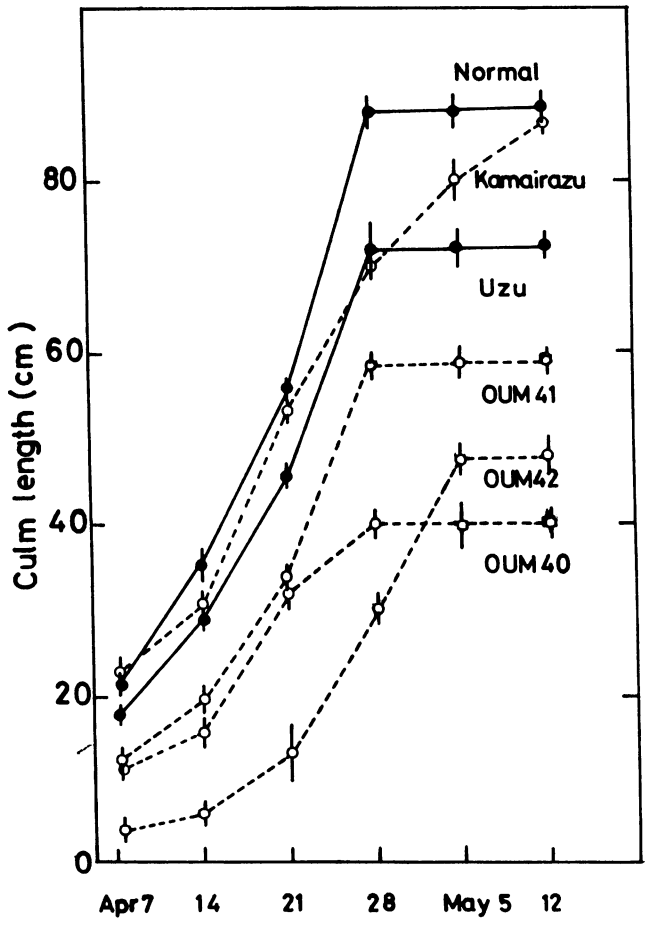

Figure 2. Culm length of brittle (– – –) and nonbrittle (——) strains of barley during 1988. Length from the base to the first internode was measured after removing all leaf blades and sheaths. Means and standard errors are given (n = 11–20).

Cell Wall Thickness and Relative Cell Wall Area

Since culm geometry was not a factor, brittleness expressed by σ_{max} is likely associated with architecture of the cell walls of the culms. Therefore, cell wall thickness of different tissues was measured after fixing the culms in Nawaschin Claf type III.

Thicknesses of cell walls of epidermal, sclerenchyma, and parenchyma tissues is included in Table II. The outer, tangential cell wall of the epidermis is the exterior of the culm. The brittle strains OUM 40 and OUM 42 had significantly thinner cell walls than nonbrittle strains for all tissues, suggesting that culm brittleness was due to reduced cell wall thickness. Thin tangential cell walls of the epidermis were present in Kamairazu, whereas they were present in the epidermis and on both sides of parenchyma cells in strain OUM 41. Cell wall thickness did not fully account for culm brittleness of Kamairazu and OUM 41, but both strains had larger epidermal cells (Table III). Widths of protoplast along the tangential axes of Kamairazu and OUM 41 epidermal cells were significantly larger than those in nonbrittle strains, suggesting that wall areas of Kamairazu and OUM 41 epidermal cell per unit cross-section culm were smaller than those for nonbrittle strains. Cell wall thicknesses and cell dimensions for a given tissue were used to calculate the cell wall area per unit cross-sectional area of that tissue (Table IV). Correlation coefficients between σ_{max} and relative cell wall area of epidermal, sclerenchyma, and parenchyma tissues were 0.93, 0.90, and 0.84, respectively (Fig. 3).

Cell Wall Components

Table V shows the sugar contents of cellulose, alkali-soluble and chloramine-T-soluble fractions of cell walls from brittle and nonbrittle strains. Cellulose content of brittle culms was conspicuously lower than that of nonbrittle culms (6–64% of uzu Akashinriki). Conversely, both alkali-soluble and chloramine-T-soluble fractions for brittle strain were only slightly lower than for nonbrittle strains. Sugar content in the chloramine-T-soluble fractions was extremely small. The monosaccharide composition of the alkali-soluble fraction is shown in Table VI. No major difference occurred between brittle and nonbrittle strains. The major component was xylose for both brittle and nonbrittle strains.

Correlation coefficients between cellulose content and σ_{max} (Fig. 4) were significant ($r = 0.93$), indicating clearly that the brittleness is associated with lower cellulose content in the cell wall.

DISCUSSION

Semidwarf mutants containing low amounts of IAA in the coleoptile have been found in barley (8) and maize (27). Sugar

Table I. *Inner and Outer Diameter, Maximum Load and Maximum Bending Stress of Internode of Brittle and Nonbrittle Strains of Barley*

Data are means and standard errors for five culms sampled on April 30, 1987.

Strain	Diameter		P_{max}	σ_{max}
	Inner	Outer		
	mm		*g*	*g/mm²*
Nonbrittle				
Normal	3.4 ± 0.3	4.7 ± 0.2	324 ± 26	497 ± 62
Uzu	4.3 ± 0.2	5.7 ± 0.2	579 ± 57	484 ± 44
Average	3.9 ± 0.2	5.2 ± 0.2	452 ± 52	490 ± 38
Brittle				
OUM 40	2.8 ± 0.3	4.5 ± 0.3	101 ± 11	98 ± 23
OUM 41	4.1 ± 0.3	5.5 ± 0.3	285 ± 46	297 ± 46
OUM 42	3.5 ± 0.1	4.8 ± 0.1	57 ± 12	69 ± 8
Kamairazu	4.1 ± 0.1	5.3 ± 0.2	252 ± 34	302 ± 65
Average	3.6 ± 0.2	5.0 ± 0.1	174 ± 26	192 ± 34

Table II. *Wall Thickness of Cells in the Fourth Internode for Brittle and Nonbrittle Strains of Barley*

Culms were fixed in Nawaschin Claf type III. Cross-sections were observed under a microscope. Thicknesses of cell walls of epidermal, sclerenchyma, and parenchyma tissues was measured. Data are means and standard errors for culms sampled on May 8, 1988 (n = 20).

Strain	Cell Wall Thickness					
	Epidermis		Sclerenchyma		Parenchyma	
	Radial	Tangential[a]	Radial	Tangential	Radial	Tangential
			μm			
Nonbrittle						
Normal	7.2 ± 0.2	8.8 ± 0.4	5.0 ± 0.3	6.1 ± 0.5	4.7 ± 0.3	2.6 ± 0.1
Uzu	4.9 ± 0.1	5.1 ± 0.1	4.3 ± 0.3	4.6 ± 0.2	5.1 ± 0.3	3.4 ± 0.3
Brittle						
OUM 40	1.8 ± 0.2	2.0 ± 0.1	1.8 ± 0.2	1.4 ± 0.1	1.5 ± 0.3	1.0 ± 0.1
OUM 41	6.4 ± 0.1	3.1 ± 0.2	4.3 ± 0.7	5.3 ± 0.3	1.3 ± 0.1	1.4 ± 0.1
OUM 42	2.4 ± 0.1	1.4 ± 0.1	2.3 ± 0.1	1.9 ± 0.3	1.0 ± 0.1	1.3 ± 0.1
Kamairazu	7.4 ± 0.1	3.6 ± 0.3	5.9 ± 0.6	5.6 ± 0.4	4.8 ± 0.1	3.3 ± 0.2

[a] Outside wall thickness of epidermal cell.

Table III. *Radial and Tangential Protoplast Widths of Epidermal, Sclerenchyma, and Parenchyma Cells of the Fourth Internode of Brittle and Nonbrittle Strains of Barley*

Culms were fixed in Nawaschin Claf type III. Radial and tangential protoplast widths of epidermal, sclerenchyma, and parenchyma cells were measured from cross-sections. Data are means and standard errors for culms sampled on May 8, 1988 (n = 20).

Strain	Protoplast width					
	Epidermis		Sclerenchyma		Parenchyma	
	Radial	Tangential	Radial	Tangential	Radial	Tangential
			μm			
Nonbrittle						
Normal	7.8 ± 0.2	4.1 ± 0.2	7.3 ± 1.0	8.9 ± 1.2	71.6 ± 6.8	53.0 ± 5.2
Uzu	7.7 ± 0.2	9.0 ± 0.6	7.0 ± 1.2	11.7 ± 1.1	56.9 ± 5.4	51.3 ± 3.5
Brittle						
OUM 40	11.7 ± 0.6	7.2 ± 0.7	14.4 ± 1.3	11.1 ± 1.1	60.8 ± 5.9	51.6 ± 5.5
OUM 41	8.1 ± 0.2	13.8 ± 0.7	10.7 ± 1.2	14.6 ± 0.9	87.5 ± 6.5	80.3 ± 6.5
OUM 42	13.6 ± 0.6	14.0 ± 0.7	11.9 ± 1.4	16.2 ± 1.6	51.4 ± 3.3	55.4 ± 5.3
Kamairazu	13.8 ± 0.4	16.8 ± 0.5	9.0 ± 1.2	10.3 ± 1.2	72.6 ± 6.8	72.5 ± 4.6

Table IV. *Ratio of Cross-Sectional Area of Cell Wall to Cross-Sectional Area of Epidermis, Sclerenchyma, and Parenchyma Tissue*

Protoplast area for each tissue was calculated from the data in Table III. Total cell area for each tissue was calculated from data shown in Tables II and III. Cell wall area was calculated by subtracting protoplast area from total cell area, then the ratio of cell wall to total cell area was calculated. See "Materials and Methods" for the calculation method.

Strain	Ratio of Cell Wall Area		
	Epidermis	Sclerenchyma	Parenchyma
Nonbrittle			
Normal	0.829 ± 0.013	0.669 ± 0.031	0.120 ± 0.014
Uzu	0.608 ± 0.011	0.592 ± 0.043	0.159 ± 0.012
Brittle			
OUM 40	0.369 ± 0.012	0.223 ± 0.013	0.046 ± 0.006
OUM 41	0.545 ± 0.005	0.507 ± 0.025	0.036 ± 0.002
OUM 42	0.238 ± 0.012	0.280 ± 0.029	0.043 ± 0.003
Kamairazu	0.467 ± 0.010	0.649 ± 0.021	0.096 ± 0.005

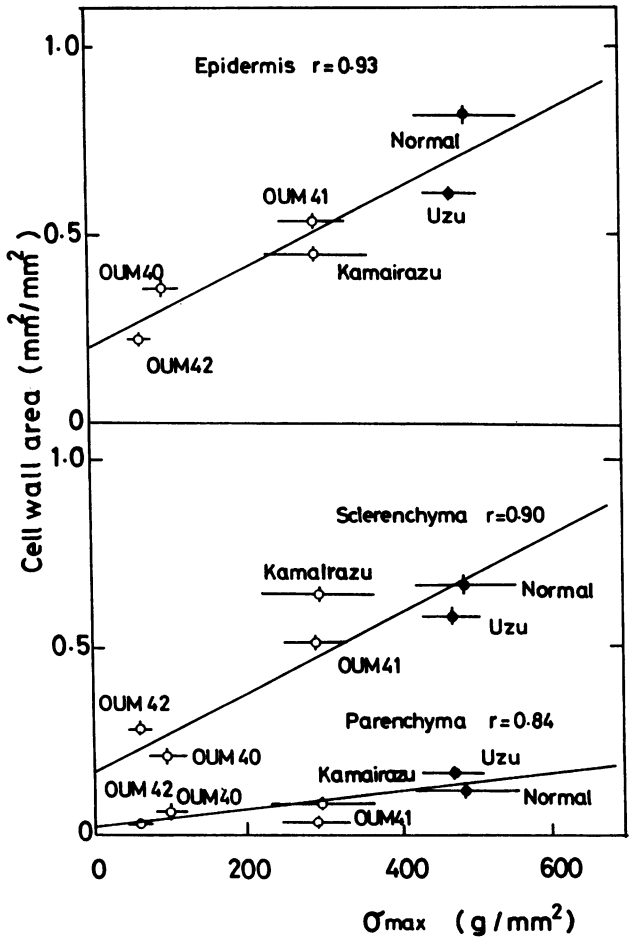

Figure 3. Correlation between maximum bending stress and ratio of cell wall area in epidermis, schlerenchyma, and parenchyma. Data in Tables I and IV were used to calculate the correlation. The correlation coefficient of 0.93 is significant at $P < 0.01$, those of 0.90 and 0.84 at $P < 0.05$.

Table V. *Sugar Content of Cell Walls of the Fourth Internode of Brittle and Nonbrittle Strains on April 30, 1987*

Cell wall material was delignified with 60 mM chroramine-T at 100°C for 2 h. Treated cell walls were divided into chloramine-T soluble (CT-fr), alkali (Alkaline-fr), and cellulose (Cellulose-fr) fractions. Means and standard errors are given ($n = 3$).

Strain	Cellulose fr	Alkaline fr	CT-fr
	mg/unit length of culm (cm)		
Nonbrittle			
Normal	4.84 ± 0.23	2.53 ± 0.04	0.06 ± 0.01
Uzu	3.38 ± 0.16	2.41 ± 0.18	0.05 ± 0.01
Brittle			
OUM 40	0.20 ± 0.01	1.01 ± 0.23	0.04 ± 0.01
OUM 41	1.62 ± 0.04	1.78 ± 0.42	0.13 ± 0.01
OUM 42	0.93 ± 0.07	2.14 ± 0.54	0.04 ± 0.04
Kamairazu	2.15 ± 0.07	2.14 ± 0.25	0.10 ± 0.01

Table VI. *Sugar Composition of the Alkaline Fraction of the Fourth Internode of Brittle and Nonbrittle Strains*

Alkaline fraction was subjected to GLC analysis after hydrolysis with 2 M TFA. Sugar composition are shown by percentage of the fraction.

Strain	Sugar Composition				
	Ara	Xyl	Man	Gal	Glc
	% of alkaline fraction[a]				
Nonbrittle					
Normal	5.0	78.0	0.7	0.3	16.0
Uzu	5.3	76.0	1.1	0.4	17.2
Brittle					
OUM 40	4.8	59.6	9.8		25.8
OUM 41	5.5	84.3	0.7		9.5
OUM 42	7.2	66.1	6.1	1.8	18.9
Kamairazu	5.0	80.0	0.7	0.3	14.1

[a] Detection limit was about 0.01%.

analysis of coleoptile cell walls of IAA-less barley mutants revealed that the low contents of galactose and mannose in noncellulosic polysaccharides were closely correlated with increased duration of the growth period (21). Furthermore, the mechanical properties of the cell walls, such as minimum stress relaxation time and relaxation rate, were significantly correlated ($r = 0.79$ and 0.72, respectively) with the maximum growth rates (22). Collectively, these data suggest that hemicellulosic components in dwarf mutants of barley may be closely related to the stress relaxation times and relaxation rates as reported previously for *Avena* coleoptiles (19, 20) and azuki bean epicotyls (16–18).

Mechanical properties of cell walls of the fourth internode of brittle and nonbrittle strain were measured by a stress relaxation analysis (28). The results, however, failed to show significant differences in the minimum stress relaxation times and relaxation rates between brittle and nonbrittle barley (data not shown). Thus, brittleness is not determined by the stress relaxation parameters although these may be associated with the physicochemical nature of the hemicellulosic polysaccharides.

Culm brittleness of barley mutants could be physically

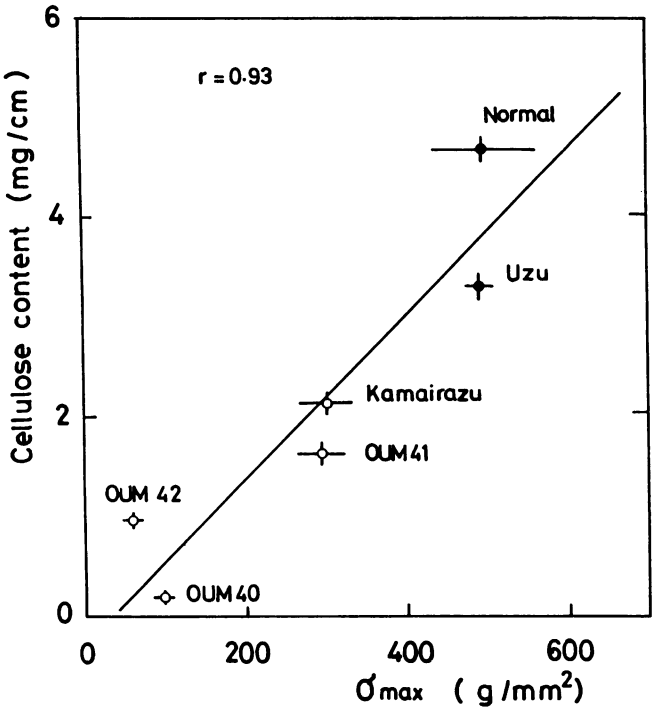

Figure 4. Correlation between cellulose content and maximum bending stress of fourth internodes of barley culms harvested in 1987. Maximum bending stress and cellulose content are shown in Table I and Table V, respectively.

evaluated by P_{max} and σ_{max}. σ_{max} is a more reliable parameter than P_{max}, because P_{max} is affected by the material dimensions. Measurement of σ_{max} showed that strains OUM 40 and OUM 42 were more fragile than Kamairazu and strain OUM 41. This was clearly confirmed by measurement of cell wall thicknesses of the epidermal, sclerenchyma, and parenchyma cells, *i.e.* OUM 40 and OUM 42 had much thinner cell walls than the other brittle strains.

Kamairazu exhibited cell wall thicknesses that tend to be similar to those of nonbrittle strains, but the epidermal cell size was much larger than that of nonbrittle strains. Therefore, the cross-sectional area of epidermal cell wall per unit epidermal area is smaller in Kamairazu than in nonbrittle strains.

σ_{max} is defined as the stress (g/mm^2) applied to the culm when the culm is broken. Therefore, σ_{max} should be correlated with the cross-sectional area of cell wall per unit cell area of tissue, provided the applied load is sustained by cell walls of the culm. Results from this study support that concept.

σ_{max} must reflect the amount of cell wall or the physicochemical nature of the cell wall. The physicochemical nature is based on chemical composition of the constituents and their interactions. Chemical factors involved in stiffness or brittleness are mainly lignin, silica, and cell wall polysaccharides (2). Further, orientation of cellulose fibrils may be involved in the brittleness. The close correlation between σ_{max} and cellulose contents in the culm indicate that a low level of cellulose content, perhaps a smaller number of cellulose fibrils, causes the brittleness of the barley mutants used in these experiments. There is, however, a possibility that disordered orientation of cellulose fibrils or other chemical components like silica or lignin play a role in the expression of brittleness.

The reason why brittle barley strains contain less cellulose content in the cell wall is open to further investigation. It is possible that cellulose synthase (12), integrity of a cellulose forming complex (14), or biosynthesis of a direct substrate for cellulose is impaired in the barley mutants.

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