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ORIGINAL PAPER

Improvement of lodging resistance with QTLs for stem diameter in rice (*Oryza sativa* L.)

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Abstract Varietal differences among ten rice cultivars showed that stem diameter is a key factor in lodging resistance (measured in terms of pushing resistance). Two near-isogenic lines (NILs) were selected from a series of chromosome segment substitution lines developed between cultivars Nipponbar and Kasalath, one containing a single stem diameter QTL (*sdm8*; NIL114), and another with four stem diameter QTLs (*sdm1*, *sdm7*, *sdm8*, *sdm12*; NIL28). Compared with the Nipponbare control, stem diameters were larger in NIL114 and NIL28 by about 7 and 39%, respectively. Pushing resistance in NIL28 was significantly greater than in Nipponbare, but NIL114 was similar to Nipponbare. The two NILs had greater weight of lower stem and culm wall thickness than Nipponbare. NIL28 had higher plant height, which is a negative effect on lodging resistance, than Nipponbare. The non-structural carbohydrate contents of NIL stems were higher than that of Nipponbare, whereas the silicon contents were lower in the NILs, and cellulose contents were lower only in NIL28. The basal internodes of the two NILs were significantly stiffer than those of Nipponbare. These results suggest that increasing stem diameter in rice breeding programs would improve lodging resistance, although the combination of multiple QTLs would be necessary to produce thicker stems with higher pushing resistance, whereas the higher plant height could also result from the combination of multiple QTLs.

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Introduction

Lodging is a major problem in the production of cereal crops, because it causes decreases in yield and quality by reducing photosynthesis in the canopy, damages vascular bundles by bending or breaking stem, and causes problem associated with mechanical harvesting (Weber and Fehr 1966; Kono 1995; Setter et al. 1997). In lowland rice (*Oryza sativa* L.), lodging is characterized by stem bending, stem breakage, and root lodging (Kono 1995). Root lodging occurs in upland rice or with direct sowing, but is infrequently observed in common cultivation of lowland rice (Watanabe 1997). Stem bending type is the main type of lodging in lowland rice. It is caused by the increase in panicle weight during maturation, and by environmental effects (i.e., rain and wind). Stem breaking occurs at lower internodes (below the third internode from the top) in response to bending higher up the stem (Hoshikawa and Wang 1990; Islam et al. 2007).

Several methods have been used to assess lodging resistance (Kono 1995, Watanabe 1997). Pushing resistance has been used primarily as an index of resistance to stem bending or root lodging in several crops (Terashima et al. 1992; Fouéré et al. 1995; Won et al. 1998). Terashima et al. (1992) demonstrated a high positive correlation between pushing resistance and lodging in paddy fields (correlation coefficient was 0.79). Berry et al. (2003) showed that measuring wheat shoot lodging resistance by rotational displacement, which accounted for a little more than half of the stem and root lodging.

Pushing resistance in rice is determined by root morphology, stem bending strength, and other characters. There

is a positive correlation between pushing resistance and culm or root thickness and root weight in deeper soil layers (Terashima et al. 1994; Won et al. 1998). Stem weight and diameter are strongly related to physical strength (Atkins 1938; Zuber et al. 1999). Stem strength is also the product of its chemical and biochemical components. Generally, lignin or cellulose determine physical strength, as lower lignin or cellulose contents cause the culm to be brittle (Kokubo et al. 1989; Taylor et al. 1999; Jones et al. 2001; Ma et al. 2002; Tanaka et al. 2003). In the rice mutant *brittle culm1* (*bc1*), the altered biosynthesis of cellulose, hemicellulose, and lignin in culms reduced secondary cell wall thickness and mechanical strength (Li et al. 2003). Mutations of the *COMT* gene, which is involved in lignin biosynthesis, have a brown midrib3 phenotype in maize (Vignols et al. 1995), and *COMT* gene expression in the developing stem is associated with stem lodging in wheat (Ma et al. 2002). Cellulose also has qualitative characteristics that relate to the strength of cellulose fibers, such as crystallinity (Reddy and Yang 2005). In addition, the accumulated carbohydrate contents of rice stems are related to lodging resistance (Sato 1957; Takaya and Miyasaka 1983; Yang et al. 2001), and a greater accumulation of starch increases the bending strength and stiffness of culms (Takahashi 1960; Matsuzaki et al. 1972; Kashiwagi et al. 2006). Likewise, higher silicon contents are also related to physical strength (Idris et al. 1975; Ma and Yamaji 2006). It has been shown that the locus for pushing resistance in the lower part of the rice plant (*prl5*) increases the weight of the lower stem due to higher carbohydrate contents at maturity, and consequently improved lodging resistance (Kashiwagi and Ishimaru 2004).

Analysis of quantitative trait loci (QTLs) can reveal the genetic basis of relationships among traits, and allows a comprehensive investigation of the genetic relationships among morphological and physiological traits (e.g., Ishimaru et al. 2001b, c, d). In addition, the use of near-isogenic lines (NILs) developed by marker-assisted selection is an effective method for characterizing QTLs in detail (Lin et al. 2000, 2003; Yano 2001; Kashiwagi and Ishimaru 2004), and for clarifying the nature of epistatic interactions between QTLs (Tanksley 1993; Lin et al. 2000). Combining QTL and physiological analyzes with NIL selection can help clarify the function of a particular locus (e.g., Ishimaru 2003). Comparisons between QTLs for lodging and other traits have been made in several cereals. In barley, one QTL has been associated with grain yield and plant height, and reduces lodging severity (Spaner et al. 1999). Seven wheat and spelt lodging resistance QTLs correspond with the QTLs for plant height, culm stiffness, leaf width, leaf-growth, days to ear emergence, and culm thickness (Keller et al. 1999).

Lodging resistance, which is assessed by the physical strength of aerial plant parts and the load they must bear, is clearly determined by various factors, but thus far suitable targets for genetic improvement of lodging resistance are not clear. The aim of this study is to identify targets for genetic improvement of rice lodging resistance. The potential factors for higher resistance can be inferred from some of the varietal differences among ten rice cultivars. Two NILs containing resistance-associated QTLs were selected to determine whether the identified phenotype, in this case stem diameter, could be used to genetically improve lodging resistance.

Materials and methods

Plant materials for determining varietal differences among ten rice cultivars

To analyze the potential factors that determine lodging resistance, ten *Indica* and *Japonica* rice cultivars were divided into three groups according to plant height. The short group, about 100 cm at maturity was Calrose76, Dee-geo-woo-gen, IR8, and Nipponbare. The medium group, about 120 cm was Calrose, Koshihikari, and New plant type (IR65598-112-2). The tall group, 140 cm and over was Ai-jiao-nan-te, Canabongbong, and Kasalath. Seeds were sown in early May 2003 and transplanted into paddies at Tsukuba, Japan (latitude 36°N), in early June with a single plant per hill, spaced at 18 × 30 cm. Six plants of each cultivar were planted per row.

Heading date, morphological traits, and stem characters of the ten cultivars

All of the following measurements were taken from five plants from each cultivar. The heading date was recorded for each cultivar. At the full-ripe stage, plant height, the height of the second leaf from the flag leaf (−2 leaf height), crown width, tiller number, dry weight of the plant body above 40 cm (upper plant weight), and dry weight of panicles per stem (panicle weight) were recorded. −2 leaf height was measured the height from the ground to the base of the second leaf blade. Crown width was measured as the width of stems per plant at 15 cm height. Dry weights were measured after oven-drying at 80°C for 3 days.

Stem diameter, weight, and the starch and silicon contents of the lower stem (below 40 cm) were measured at the full-ripe stage. Plant stems were cut at 40 cm height, and stem diameter was measured with a slide caliper according to the method described previously (Kashiwagi and Ishimaru 2004). The weight of the lower stem was measured after desiccation at 80°C for 3 days.

To measure the contents of starch and silicon, dried lower stem was ground to a powder with a Wonder Blender

(Osaka Chemical Co., Osaka, Japan). Starch was measured enzymatically according to the method of Ishimaru et al. (2001a). Fifty milligrams of the powdered lower stem was reground in liquid nitrogen with a mortar and pestle. The powdered sample was extracted twice with 80% (v/v) ethanol at 80°C and centrifuged at 12,000×*g* for 5 min. The pellet was boiled in distilled water for 2 h and then digested with amyloglucosidase for 15 min at 55°C. The resultant hexoses were determined by the enzymatic method of Bergmeyer and Bernt (1974).

Relative silicon contents were assayed with an energy-dispersive X-ray fluorescence spectrometer (Element Analyzer JSX-3201, Jeol, Tokyo, Japan) according to a method reported previously (Kashiwagi and Ishimaru 2004). Powdered lower stem (200 mg) was formed into a 13-mm diameter tablet with a hydraulic press (Evacuable KBr Die, Shimadzu, Kyoto, Japan). The measurement was performed at 30 kV for 600 s and replicated three times for each sample. Silicon was analyzed at a peak of 1.739 keV, and the relative content was calculated as the counts-per-second ratio using the method of Vázquez et al. (1999).

Measurement of pushing resistance

Whole plant pushing resistance was measured with a prostrate tester (Daiki Rika Kogyou Co., Tokyo, Japan) at the full-ripe stage, according to a method reported previously (Kashiwagi and Ishimaru 2004). The prostrate tester was set perpendicularly to the whole plant at 20 cm height, and pushing resistance was measured when plants inclined to 45°. For measurements of pushing resistance, individual tests were done on each of five plants.

Plant materials for NILs containing QTLs with potential genetic targets

Using marker-assisted selection, chromosome segment substitution lines were produced which were advanced backcross progeny with Nipponbare as a recurrent parent and Kasalath as a donor parent (Yano 2001). Two NILs (NIL28 and NIL114) were selected from these lines based on QTL data for stem diameter previously reported (Kashiwagi and Ishimaru 2004). NIL28 carries Kasalath chromosomal segments with four QTLs for stem diameter on chromosomes 1, 7, 8, and 12 (tentatively named *sdm1*, *sdm7*, *sdm8*, and *sdm12*, respectively), all QTLs have positive effects associated with the kasalath allele (Kashiwagi and Ishimaru 2004). NIL114 carries one QTL on chromosome 8 (*sdm8*) with the second highest LOD score among QTLs for stem diameter in the genetic background of Nipponbare. The selected NILs and Nipponbare as a control were sown in early May 2002 and 2003, and the seedlings were transplanted to paddies in Tsukuba in early June with a single plant per hill, spaced at 18 × 30 cm. Each NIL was planted in three rows, with four plants per row in 2002, and in two rows with eight plants per row in 2003.

Morphological characters of NILs

Stem diameters of nine plants in each line were measured at the full-ripe stage in 2002 and 2003, as described above. The NIL effects on morphological characters other than stem diameter were verified in 2002. Plant height, −2 leaf height, tiller number, crown width, dry weights of panicle per stem and lower stem were measured at the full-ripe stage, as described above. These measurements used nine plants in each line. To verify the effect of the NILs on culm characters, culm diameter and wall thickness were measured at the full-ripe stage in 2003. Culm diameter was measured at the central part of the second and third internodes from the top (internodes II and III) and the basal internode. The wall thickness of internode III was measured at the central part of the internode. Four or more plants in each line were used for the measurement of culm diameter and wall thickness.

Analysis of stem chemical components in NILs

To analyze the chemical components of the lower stem and internode III of the culm, samples were taken at the full-ripe stage in 2002 and 2003, respectively, and dried at 80°C for 3 days. Dried samples were then ground to a powder with a Wonder Blender (Osaka Chemical Co., Osaka, Japan). Carbohydrate contents were measured enzymatically according to the method of Ishimaru et al. (2001a). Powdered lower stem were extracted in the same way as starch described above. The supernatant was collected, dried in a vacuum, and used for the determinations of sucrose and hexoses by the enzymatic method of Bergmeyer and Bernt (1974). Starch content was determined as described above. Nine plants in each line were used to analyze carbohydrates in 2002.

The relative silicon contents of the lower stem were analyzed with an energy-dispersive X-ray fluorescence spectrometer, as described above. Nine plants in each line were used to measure silicon contents in 2002.

Four plants in each line were used to measure the contents of structural carbohydrates in 2003. Lignin content as a percent of total dry weight of internode III was determined using a modified version of Japan TAPPI (Technical Association of the Pulp and Paper Industry) test method no. 61: 2000. Dried internode III was ground to a powder with a Wonder Blender (Osaka Chemical Co.). The powder was weighed, degreased in a solution of 70% benzene, 30% ethanol, and steeped in 72% sulfuric acid for 4 h. The diluted sample solution was boiled for 2 h, and the dried filtrate was weighed to calculate the lignin content.

The cellulose contents of internode III were determined using a modified version of Japan TAPPI test method no. 60: 2000. Before the measurement, internode III was ground to a powder, extracted with ethanol–benzene (1:2, v/v), and then air-dried. The extracted powder was chlorinated with 0.3% chlorine water for 5 min at room temperature. After filtration through a glass filter P100, the residue was washed successively with water, 3% sulfurous acid, and then water. The washed residue was transferred to a beaker with 2% sodium sulfite, and the mixture was boiled for 30 min. The residue was filtered through a glass filter P100 and washed in hot and then cool water. The steps of the treatment process from chlorine water to sodium sulfite were repeated several times until the residue became markedly white. Finally, the white residue was bleached with 0.1% potassium permanganate for 10 min. The residual cellulose was filtered and washed with 3% sulfurous acid, hot water, and ethanol. The cellulose was dried at 105°C for 4 h and weighed.

Measurements of culm cellulose crystallinity

Internodes II, III, and the basal culm internode were sampled at the full-ripe stage in 2003. The culms were bleached in the rod state with 0.5% $NaClO_2$ (buffered at pH 4.7 in acetate buffer) for a week at room temperature. Samples were disintegrated into small fragments, and then approximately 50 mg of each sample was pressed into a 10-mm diameter disk at 9,800 kPa for 1 min. Wide-angle X-ray diffraction (WAXD) patterns were measured using nickel-filtered CuKα radiation produced by a RINT-2500F X-ray generator (Rigaku, Tokyo, Japan) with a 1-mm diameter pinhole collimator. Each WAXD image of a sample was recorded on a flat imaging plate (BAS-SR 127 mm × 127 mm; FUJIFILM Co., Tokyo, Japan) at 40 kV and 50 mA for 60 min at a distance of 60 mm; images were analyzed using R-AXIS-DS3 system (Rigaku). WAXD intensity curves were drawn using a transmission method with a scintillation counter at 40 kV and 200 mA, $2\theta = 5°–35°$, scan speed = 0.5° min^{-1}, and scan step = 0.02°. Percent cellulose crystallinity was estimated from the ratio of the areas of the crystalline and noncrystalline regions in the WAXD intensity curve of the disk sample (Togawa and Kondo 1999) from a bulk sample of five plants in each line.

Measurement of lodging resistance in NILs

Pushing resistances and culm stiffness were analyzed at the full-ripe stage as lodging resistance. Pushing resistance of the whole plant was measured in 2002, as described above. Likewise, pushing resistance of the lower part of the plant was measured in 2002 after the stem was cut at 40 cm height and the upper parts were removed. Six or more plants in each line were used to measure pushing resistance. Culm stiffness was measured by a compression test with a Tensilon UTM-II-20 (Toyo Baldwin Co., Ltd., Tokyo, Japan) in 2003, according to a method reported previously (Kashiwagi et al. 2006). The central part of a fresh culm was compressed with a 50-mm diameter compression jig at a constant velocity of 2 mm min^{-1}. Culm stiffness was recorded at the early stage of the compression. The measurements of culm stiffness used 16 plants in each line.

Statistical analysis

Data of morphological traits, chemical components, and physical strengths were examined using four or more individual plants for replication. Only cellulose crystallinity was measured using a bulk sample of five plants. Accordingly, data of cellulose crystallinity did not have a standard error and statistical difference. Statistical analyzes were performed using Microsoft Excel 2004 (ver. 11.3.7), for correlation and significant difference. Significant differences between means were analyzed by Students t test at the level of $^{***}P_{0.05}$.

Results

Correlation between pushing resistance and other traits

Among the ten cultivars examined, stem diameter ($r = 0.805$, $P < 0.01$) and lower stem weight ($r = 0.718$, $P < 0.05$) were significantly correlated with pushing resistance of the whole plant (Table 1). None of other general characteristics measured (heading date, plant height, −2 leaf height, crown width, tiller number, upper plant weight,

Table 1 Correlation (r) between pushing resistance of the whole plant and morphological traits and stem characters of ten cultivars

Traits	Pushing resistance of the whole plant
Heading date	0.570
Morphological traits	
Plant height	0.562
−2 leaf height	0.333
Crown width	0.272
Tiller number	0.102
Upper plant weight	0.597
Panicle weight	0.344
Stem characters	
Stem diameter	0.805**
Weight of lower stem	0.718*
Starch content in lower stem	−0.191
Silicon content in lower stem	−0.513

**, * Significant levels of $P < 0.01$ and $P < 0.05$, respectively

and number of panicles) were correlated with pushing resistance of the whole plant. Starch and silicon contents in the lower stem were not significantly correlated with pushing resistance ($P > 0.05$).

Stem and culm diameters in NILs

In 2002, stem diameter at 40 cm in Nipponbare was 3.79 ± 0.10 mm, and stem diameters in NIL114 (one QTL) and NIL28 (four QTLs) were 1.06 and 1.35 times as high as Nipponbare, respectively (Fig. 1). In 2003, stem diameter in Nipponbare was 3.39 ± 0.07 mm, and stem diameters in NIL117 and NIL28 were 1.08 and 1.42 times as high as Nipponbare, respectively. In each year, stem diameter was significantly different between Nipponbare and the NILs ($P < 0.05$). The diameters of internode II, internode III, and the basal internode in Nipponbare were 2.58 ± 0.09, 3.16 ± 0.11, and 3.82 ± 0.11 mm, respectively, in 2003 (Fig. 1). The diameters of internodes II and III in NIL114 were significantly larger than in Nipponbare ($P < 0.01$), but there was no significant difference between their basal internode diameters ($P > 0.05$). Nipponbare and NIL28 had significant differences for all internodes ($P < 0.01$). Compared between NILs, the diameters of all internodes in NIL28 were significantly larger than in NIL114.

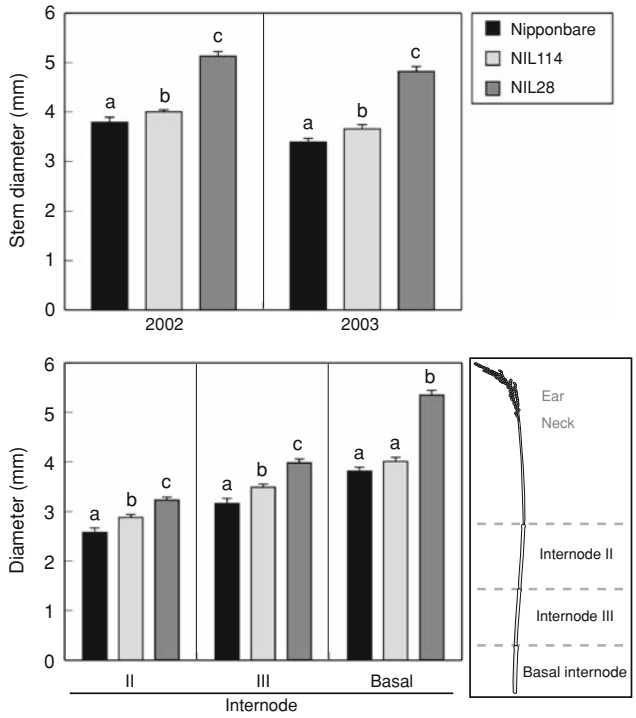

Fig. 1 Diameters of stem and culm internodes in Nipponbare (control), NIL114 and NIL28. Stems were measured in 2002 and 2003, and culm internodes were measured in 2003. The data represent the mean of nine or more independent plants in each line; *vertical bars* indicate standard errors. *Different letters* above columns indicate statistically significant differences between means ($P < 0.05$, Students *t* test)

Morphological characters of NILs

To examine the effects of stem diameter QTLs on other morphological characters, the selected NILs were compared with Nipponbare as the control (Table 2). NIL114 had a significantly greater crown width, panicle weight per stem, lower stem weight, and culm wall thickness compared with Nipponbare. NIL28 had significantly greater plant height, −2 leaf height, crown width, panicle weight per stem, lower stem weight, and culm wall thickness, and a significantly smaller tiller number compared with Nipponbare. NIL28 had significantly greater lower stem weights and thinner culm walls than NIL114.

NILs stem chemical components

The starch contents in NIL114 and NIL28 were 584.4 and 1,038.0 μmol hexose g^{-1} DW higher than in Nipponbare ($P < 0.001$) (Table 2). The contents of sucrose and hexoses in NIL114 were 156.3 and 24.9 μmol hexose g^{-1} DW higher than in Nipponbare ($P < 0.05$), and in NIL28 were 339.3 and 54.5 μmol hexose g^{-1} DW higher than in Nipponbare ($P < 0.01$). NIL28 stem had higher nonstructural carbohydrate contents in 2003 than Nipponbare and NIL114 stems (data not shown). The relative contents of silicon in NIL114 and NIL28 were 11.5 and 22.3 counts s^{-1} lower than in Nipponbare, respectively ($P < 0.001$). There were no significant differences in lignin content between Nipponbare and the selected NILs. The cellulose content in NIL28 was significantly lower than that in Nipponbare (decrease of 7% DW).

Degree of crystallinity of culm cellulose

In Nipponbare, NIL114, and NIL28, the basal internodes had the highest degree of cellulose crystallinity (42.0, 41.4, and 35.6%, respectively) and internode III had the lowest (29.9, 29.3, and 28.0%, respectively; Table 3). Among the three lines, the cellulose crystallinity of NIL28 was the lowest in each internode.

Contribution of QTLs for stem diameter to lodging resistance

Lodging resistance was tested by measuring the pushing resistance of the whole plant, the pushing resistance of the lower part of the stem, and culm stiffness (Fig. 2). The pushing resistances of the whole plant and the lower part in Nipponbare were 0.34 ± 0.02 and 0.61 ± 0.05 N cm^{-2}, respectively. The pushing resistance of the whole plant and the lower part of NIL28 were 0.24 and 0.45 N cm^{-2} higher than in Nipponbare ($P < 0.01$). There was no significant difference in the pushing resistance of the whole plant or

Table 2 Morphological characters and components in stems of Nipponbare, NIL114 and NIL28

	Nipponbare	NIL114	NIL28
QTL for stem diameter		*sdm8*	*sdm1*, *sdm7*, *sdm8*, *sdm12*
Morphological traits			
Plant height (cm)	104.9 ± 1.2a	107.2 ± 1.3a	134.9 ± 1.3b
−2 leaf height (cm)	36.6 ± 0.7a	36.5 ± 0.8a	56.1 ± 1.2b
Tiller number	18.2 ± 0.7a	18.2 ± 1.0a	13.4 ± 0.7b
Crown width (mm)	63.3 ± 2.7a	72.5 ± 3.4b	75.6 ± 3.2b
Panicle weight per stem (g)	2.0 ± 0.1a	2.4 ± 0.0b	2.3 ± 0.1b
Weight of lower stem (g)	0.33 ± 0.02a	0.57 ± 0.03b	0.91 ± 0.06c
Culm wall thickness (mm)	0.54 ± 0.05a	0.76 ± 0.02b	0.71 ± 0.01c
Stem chemical components			
Starch (μmol hexose g^{-1} DW)	208.0 ± 59.7a	792.4 ± 143.2b	1,246.0 ± 126.6c
Sucrose (μmol hexose g^{-1} DW)	332.1 ± 59.7a	488.4 ± 65.7b	671.4 ± 41.5c
Hexoses (μmol hexose g^{-1} DW)	83.9 ± 11.2a	108.8 ± 5.8b	138.4 ± 9.7c
Silicon (counts s^{-1})	46.7 ± 2.4a	35.2 ± 1.5b	24.4 ± 1.3c
Lignin (DW%)[a]	11.7 ± 0.2a	13.2 ± 0.8a	10.8 ± 0.7a
Cellulose (DW%)[a]	39.9 ± 1.3a	38.1 ± 0.8a	32.9 ± 0.5b

The data are expressed as mean ± SE of four or more plants, different letters following datum indicate statistically significant differences at $P < 0.05$ between mean (Students *t* test), superscript "a" indicates the traits measured in 2003

Table 3 Crystallinity of culm cellulose in Nipponbare, NIL114 and NIL28

	Nipponbare	NIL114	NIL28
QTL for stem diameter		*sdm8*	*sdm1*, *sdm7*, *sdm8*, *sdm12*
Cellulose crystallinity (%)			
Internode II	33.7	35.8	28.7
Internode III	29.9	29.3	28.0
Basal internode	42.0	41.4	35.6
Mean	35.2 ± 3.6	35.5 ± 3.5	30.8 ± 2.4

The values were determined with a bulk sample of five plants, mean indicates the mean value ± SE across three internodes

the lower part of the stem between NIL114 and Nipponbare ($P > 0.05$). NIL28 had 0.23 and 0.32 N cm^{-2} higher pushing resistances for the whole plant and the lower part, respectively, than NIL114 ($P < 0.05$).

The stiffnesses of internode II, internode III, and the basal internode were 0.45 ± 0.04, 0.68 ± 0.04, and 0.63 ± 0.05 kg mm^{-1}, respectively in Nipponbare, those in NIL114 were 0.12, 0.18, and 0.25 kg mm^{-1} higher than in Nipponbare ($P < 0.05$), and those in NIL28 were 0.18, 0.15, and 0.38 kg mm^{-1} higher ($P < 0.05$). There were no significant differences in culm stiffness between NIL114 and NIL28.

Discussion

To clarify potential genetic targets for improvement in lodging resistance, we analyzed various morphological and stem characters of ten rice cultivars. Only stem diameter was positively correlated with pushing resistance (Table 1). Field studies of wheat and soybean have shown that lodging degree (high score means lodged) was negatively correlated with stem diameter (Mancuso and Caviness 1991;

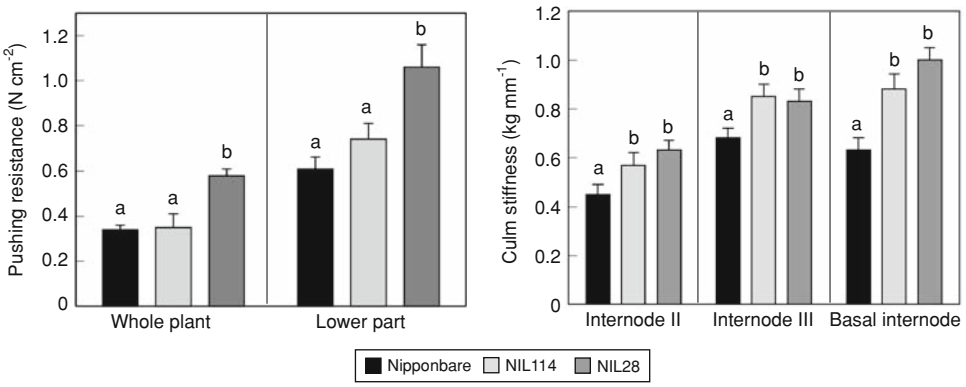

Fig. 2 Pushing resistance and culm stiffness of Nipponbare (control), NIL114 and NIL28. The data represent the mean of six or more independent plants in each line; *vertical bars* indicate standard errors. *Different letters* above columns indicate statistically significant differences between means ($P < 0.05$, Students *t* test)

Zuber et al. 1999; Tripathi et al. 2003). Keller et al. (1999) reported that two QTLs for lodging resistance corresponded with QTLs for culm thickness in a population between wheat and spelt. These findings suggest that stem diameter is an important trait that underlies in lodging resistance, as verified by pushing resistance.

Plant height has been proposed as the critical factor for lodging resistance. However, there was no correlation between plant height and pushing resistance among the ten cultivars tested (Table 1), indicating that, at least in these ten varieties, pushing resistance was not related with plant height. In this study, pushing resistance was measured at a fixed 20 cm height from the ground. This method would mainly assess the physical strength of basal parts. Berry et al. (2003) suggested that a modified test for lodging resistance in which pushing height was adjusted to 50% of crop height appeared to account for the effects of both stem strength and height on lodging resistance appeared the most useful for providing reasonable reliable data in a short time. Higher plant height makes the center of gravity higher (Watanabe 1997) and the leverage force greater. Pushing resistance indicates the cumulative physical strength of basal parts rather than the height of the center of gravity or the leverage force by the upper part of the plant. Improved lodging resistance thus would seem to have two seemingly unlinked targets: reduced plant height, and pushing resistance.

The NIL QTLs clearly affect the internode diameters (Fig. 1). NIL28, which contains four QTLs, had greater diameter at all internodes compared with NIL114 with one QTL (*sdm8*). Based on the results of NIL114, it appears that *sdm8* controls the diameters of internodes II and III, but not the basal internode. Interaction among plural loci or genes can make a greater effect on the trait than the effect of a single, e.g., the interaction of two QTLs delaying heading under short day-length (Lin et al. 2000) and of two dwarf genes (Mackill and Rutger 1979). Multiple loci may thus result in a greater stem diameter. If so, then a single locus may not be expected to result in a striking increase in stem diameter.

Culm diameter in wheat and rice is correlated with breaking strength (Atkins 1938; Ohe et al. 1996). Pushing resistances of the whole plant and of the lower part in NIL28 were significantly higher than in Nipponbare and NIL114 (Fig. 2). Therefore, a combination of multiple QTLs for stem diameter is likely to be required to improve lodging resistance. In addition, our findings suggest that an increase in basal internode diameter could contribute to greater pushing resistance.

NIL28 also had a greater lower stem weight (Table 2). The initial experiment in this study indicated that the weight of lower stems positively correlates with pushing resistance of the whole plant (Table 1). In agreement with a previous studies, stem weight is generally positively correlated with stem diameter (Atkins 1938; Tripathi et al. 2003), Zuber et al. (1999) reported that stem diameter and stem weight (mg cm^{-1}) were indicative of lodging resistance in wheat. Thus, the thicker and heavier stem of NIL28 is probably responsible for its greater pushing resistance.

Culm wall thickness was highly negatively correlated with lodging score (high score means lodged) in field studies of wheat (Tripathi et al. 2003). Both of the NILs examined in this study had thicker culm walls than Nipponbare, and NIL114 had thicker walls than NIL28 (Table 2, Fig. 2). This indicates that QTL (*sdm8*) may contain, or be located close to, loci for culm wall thickness. *sdm8* would appear to be a good target for further genetic analysis for improving culm stiffness. There was a significant difference of culm wall thickness between two NILs with *sdm8*. Because culm wall thickness is determined by internode diameter and lumen, the thinner culm wall of NIL28, as compared with NIL114, might be due to a change in the diameters of internode and lumen by QTLs other than *sdm8*.

The heavier stem of NIL 28 is due to the nonstructural carbohydrates, especially starch, which are higher than in NIL114 (Table 2). This trait was stable in 2 years (data not shown). Starch contents of the culm can also be partly responsible for lodging resistance, because rice tends to lodge when the starch contents of culm parenchymatous cells is very low (Sato 1957), and the ability of the basal culm to re-accumulate starch during the later stages of maturity contribute to lodging resistance of breaking type rice (Yagi 1983). In fact, wheat cultivars with strong lodging resistance remobilize a smaller proportion of prestored assimilates into the grain (Yang et al. 2000), and the NIL with a QTL for greater pushing resistance (*prl5*) had the ability to re-accumulate high levels of carbohydrates in the culm (Kashiwagi et al. 2006). It is also possible that higher levels of nonstructural carbohydrates would increase culm turgor pressure, and that culms containing these nonstructural carbohydrates are wrapped in the leaf sheaths with delayed senescence, thus increasing stem strength (Takaya and Miyasaka 1983). The contribution of nonstructural carbohydrates to lodging resistance would thus be via the physical strength of cell tissue in culms or leaf sheaths. However, at present the function of nonstructural carbohydrates on stem physical strength is not clear. Further investigation of the anatomical contribution of nonstructural carbohydrates is needed for clarification of this point.

The structural carbohydrates (cellulose and lignin) and silicon are largely responsible for the material strength of culms. Cellulose and lignin contents correlate with bending and breaking resistance in barley and rice culms (Kokubo et al. 1989; Ookawa and Ishihara 1993; Li et al. 2003). In addition, cellulose crystallinity correlates with increased

fiber strength (Reddy and Yang 2005). Silicon is deposited in silicified cells in the epidermis and vascular tissues of leaf blades, leaf sheaths, and stems, and enhances the strength and rigidity of cell walls, thus increasing lodging resistance (Ma and Yamaji 2006). Despite their greater physical strength, the NILs did not have higher amounts of culm structural carbohydrates or silicon, nor did they have higher cellulose crystallinity (Tables 2, 3). The greater culm stiffness of the NILs is thus not a product of the quantitative or qualitative differences in their cellulose, lignin, or silicon.

The NILs used in this study had significantly greater stem diameters and physical strength. These NILs had several other altered morphological traits and stem components. It is uncertain whether these effects are pleiotropic or are caused by other QTLs, which could be separated by further backcrossing. The results of this study suggest the possibility that QTLs for stem diameter concomitantly increase stem weight, culm wall thickness, stem nonstructural carbohydrates, and thus cumulatively result in greater physical strength. In addition, these same QTLs could also make higher plant height, lower tiller number, and greater crown width. Tall plant and poor tillering have negative effects on lodging resistance and grain yield, thus stem diameter QTLs may not be the best target for rice breeding. However, there was no overlap between QTLs for increased stem diameter and QTLs for plant height, tiller number, and crown width (Kashiwagi and Ishimaru 2004). The NILs had long overlapping chromosomal segments with QTLs for stem diameter, and apparently superfluous regions. Further definition of the relationship between QTLs for stem diameter and other traits, especially plant height and tiller number, would require a series of NILs containing only the chromosomal segment or segments responsible for stem diameter, and each of the other QTLs in various combinations.

In conclusion, stem diameter is currently the potential target in breeding for better lodging resistance. Multiple QTLs for stem diameter from cv. Kasalath was required for the best pushing resistance. For more efficient breeding, further studies are required to analyze QTLs for stem diameter using NILs with less DNA flanking the diameter QTL and cultivars with thicker stems, e.g., New plant type lines developed by the International Rice Research Institute (Peng et al. 1999), and to verify the contribution of single QTL for thicker stem towards lodging resistance.

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