Identification and Functional Analysis of a Locus for Improvement of Lodging Resistance in Rice[1][w]

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We identified a new locus responsible for increased pushing resistance of the lower part of rice (*Oryza sativa*) and analyzed its physiological function to understand how to improve lodging resistance in rice. Quantitative trait loci (QTLs) controlling pushing resistance of the lower part were analyzed in a population of backcross inbred lines of *japonica* Nipponbare × *indica* Kasalath plants cut out at 40 cm to exclude the effect of the weight of the upper parts. Five QTLs for pushing resistance were detected; only one QTL from Kasalath on chromosome 5 (*prl5*) had a positive effect. The likelihood odds ratio curve of *prl5* echoed that for lodging resistance by typhoon. We selected three near-isogenic lines (NILs) in which the chromosomal region of *prl5* was substituted with that of Kasalath in the Nipponbare background. The dry weights and densities and the contents of accumulated carbohydrate in stems below 40 cm (lower stems) in each NIL were significantly higher than those of Nipponbare. There was no difference between Nipponbare and the NILs in yield, root characteristics, or the weights of the upper parts. Pushing resistance of the lower part and lodging resistance in the NILs were up to twice as high as in Nipponbare. These results suggest that *prl5* might affect the characteristics of the lower stems of the NILs, thus increasing lodging resistance.

Lodging is a common problem in most cereals and various other crops, including wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), oats (*Avena sativa*; Mulder, 1954; Pinthus, 1973), corn (*Zea mays*; Carter and Hudelson, 1988; Minami and Ujihara, 1991), grain sorghum (*Sorghum bicolor*; Larson and Maranville, 1977), soybean (*Glycine max*; Noor and Caviness, 1980; Cooper, 1981), tomato (*Lycopersicon esculentum*; Adelana, 1980), tobacco (*Nicotiana tabacum*; McKee and Aycock, 1976; Walton and Casada, 1979), and rice (*Oryza sativa*; Setter et al., 1997). It can reduce yield, quality of production, and mechanical harvesting efficiency (Weber and Fehr, 1966; Kono, 1995). Shading of leaves and constriction of conducting tissues decreases photosynthesis and photoassimilation, causing poor grain filling and lower yield. Seed set and grain quality are reduced. It was estimated that lodging caused a loss of 26 kg ha^{-1} in rice production in southern India (Duwayri et al., 2000) and a loss of as much as 22% of yield in soybean (Noor and Caviness, 1980).

Plant height has been the main target for improvement of lodging resistance. The detection and introduction of semidwarf lines was one of the main factors responsible for the higher yields of rice and wheat in the "green revolution" (Keller et al., 1999; Khush, 1999). Most dwarfing genes (e.g. *sd-1*) are inherited in an autosomal recessive pattern as recessive alleles (Aquino and Jennings, 1966; Foster and Rutger, 1978; Mackill and Rutger, 1979). This makes it difficult to introduce them into modern cultivars. Recently, there have been several reports of the isolation of genes related to plant height. *Heterotrimeric G protein* (*RGA1*), a gene for "daikoku" dwarf that functions in the transduction of GA signals, was cloned in rice (Ashikari et al., 1999; Fujisawa et al., 1999). A rice homeobox gene, *OSH15*, has a role in shoot apical meristem and final plant height (Sato et al., 1999). Concurrently, three groups isolated and cloned *sd-1* (Monna et al., 2002; Sasaki et al., 2002; Spielmeyer et al., 2002). However, these genes cannot be used yet in practical agriculture or breeding because their effects on yield and other important traits is unclear. For example, seeds of transgenic rice plants with a cosmid vector containing *RGA1* were much shorter and smaller than those of control plants (Ashikari et al., 1999), and a homozygous mutation of *OSH15* by retrotransposon reduced panicle length (Sato et al., 1999).

The optimum plant height for maximum photosynthetic capacity in a canopy is between 70 and 100 cm in wheat (Flintham et al., 1997). New rice cultivars called "New Plant Type" developed for their higher photosynthetic capacity in the canopy are about 100 cm in height (Kumar et al., 1999). Because a reduction in plant height to improve lodging resistance may reduce the photosynthetic capacity of a canopy, another target is needed for further improvement in lodging resistance.

Plant height is not necessarily the most important factor determining lodging resistance in rice (Ookawa and Ishihara, 1992; Easson et al., 1993). The

susceptibility to lodging differed among cultivars with similar plant height (Ookawa and Ishihara, 1992; Easson et al., 1993). Terashima et al. (1992) reported similar results in modern rice cultivars tested for lodging resistance. Therefore, there may be other factors determining lodging resistance than plant height, at least in rice.

The development of a molecular genetic map and DNA markers has made it easy to analyze quantitative trait loci (QTLs) for complex traits (Tanksley, 1993). With QTL analysis, it is possible to analyze the genetic basis of the relationship among traits (e.g. Ishimaru et al., 2001b, 2001c, 2001d). In addition, the use of near-isogenic lines (NILs) is an effective method for characterizing QTLs in detail (Lin et al., 2000, 2003; Yano, 2001). Combining physiology and quantitative genetics is valuable not only in breeding but also in physiology and functional genomics (Cong et al., 2002; Ishimaru, 2003). Cong et al. (2002) clarified the mechanism determining fruit weight in tomato by physiological analyses with NILs for alleles at the locus for *fw2.2*, a QTL that accounts for as much as 30% of the difference in fruit size between wild and cultivated tomatoes.

From the point of view of dynamics, lodging resistance in a plant is determined by two main factors, the weight of the upper part (ears and upper leaves and stems) and the pushing resistance of the lower part (Mulder, 1954). Pushing resistance was used as the index of lodging resistance in maize and rice (Idris et al., 1975; Terashima et al., 1992); however, in its measurement, the whole plant was used, and pushing resistance of the lower part was not measured without the effect of the upper part. The relationship between pushing resistance of the lower part and lodging resistance has not been clarified.

We hypothesized that improving pushing resistance of the lower part might improve lodging resistance. To test this hypothesis, we looked for QTLs for pushing resistance in rice backcross inbred lines (BILs) that had their upper parts cut off to exclude their effects. Then, we attempted to identify one QTL and to elucidate its physiological function and its effect on lodging resistance in selected NILs in which its chromosomal region was substituted with that of another line.

RESULTS

Variation in Pushing Resistance in the Lower Part and Correlation between Pushing Resistance and Other Traits in BILs

Ninety-eight BILs and two parental lines (Nipponbare and Kasalath) were cut off at 40 cm from the ground at the full-ripe stage, and pushing resistance of the lower part was measured when plants were pushed in the middle (20 cm) to 45° from the vertical (Fig. 1A). BILs showed continuous variation in pushing resistance of the lower part, between 0.11 and
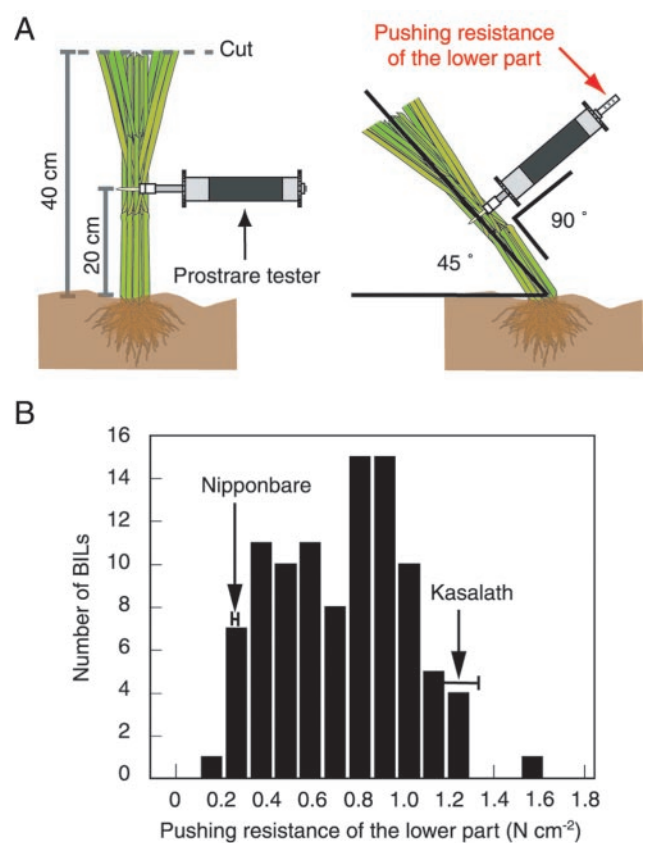

Figure 1. Measurement of pushing resistance of the lower part and frequency distributions of pushing resistance in BILs of Nipponbare × Kasalath. Pushing resistance of the lower part was measured when plants were bent to 45° (A). The values in the histogram are the means of five independent plants in each line (B). Phenotypes of parental lines (Nipponbare and Kasalath) are shown by arrows; crossbars = SEs.

1.60 N cm^{-2} (Fig. 1B). There were two peaks (at 0.32–0.64 and 0.74–0.95 N cm^{-2}). Pushing resistance in Kasalath (1.26 ± 0.17 N cm^{-2}) was 4.5 times the value in Nipponbare (0.28 ± 0.03 N cm^{-2}). A low positive correlation was found between pushing resistance and crown width ($r = 0.201$) in BILs, but there was no significant correlation with stem diameter, stem number, or plant height (data not shown).

QTL Detection

QTLs controlling pushing resistance of the lower part, traits related to plant type (stem diameter, stem no., plant height, and crown width), and lodging resistance in a typhoon in 2002 were detected (Fig. 2). Five QTLs for pushing resistance of the lower part were detected on chromosomes 4 to 6, 11, and 12 and accounted for 0.636 (r^2) of the total phenotypic variation (Fig. 2; Table I). Those on chromosomes 6 and 12 had the highest LOD scores and overlapped with QTLs for plant height. Kasalath had a positive allele only at the QTL on chromosome 5 (tentatively named *prl5*). Six QTLs for stem diameter were located on

Figure 2. QTLs for pushing resistance of the lower part, stem diameter, stem number, plant height, crown width, and lodging resistance by typhoon. N and K in parentheses indicate that Nipponbare and Kasalath alleles had a positive effect. QTL for lodging resistance by typhoon was analyzed with 98 BILs in 2002. QTLs for the other traits were analyzed with 98 BILs in 2001.
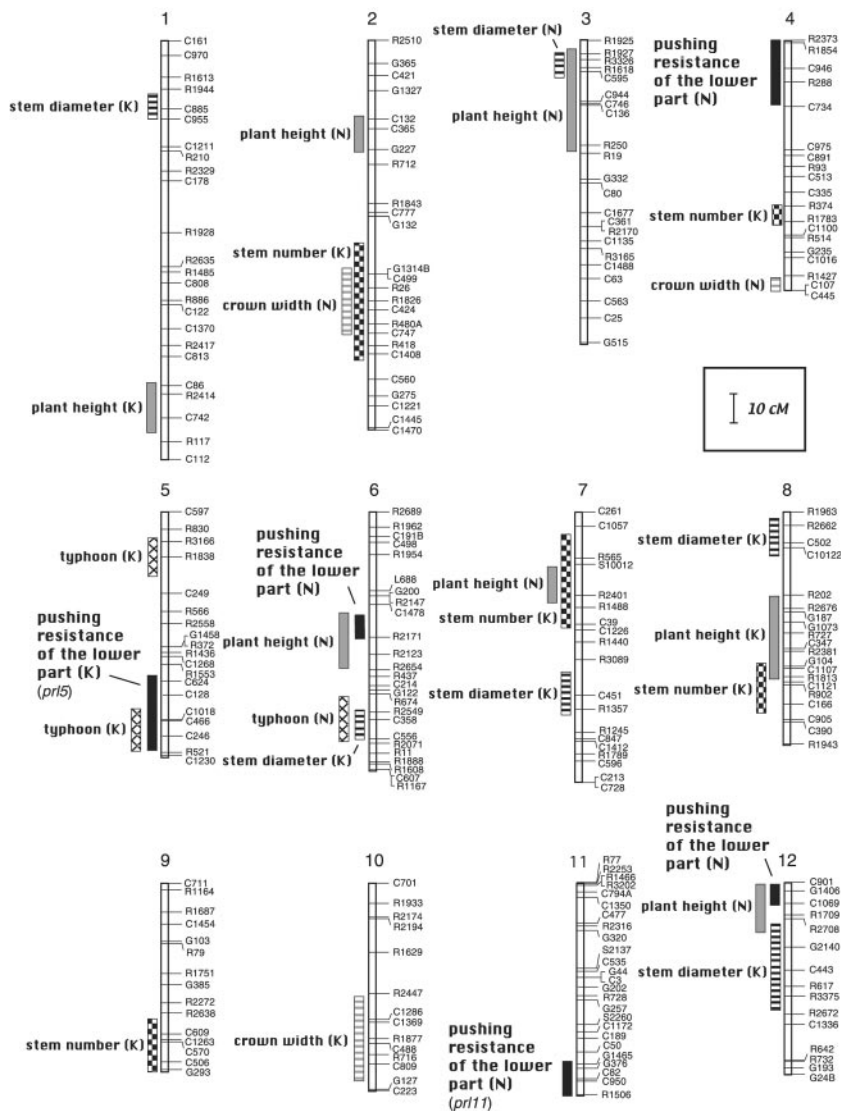
chromosomes 1, 3, 6 to 8, and 12 and explained 84.7% of total phenotypic variation (Table I). The QTLs for pushing resistance did not overlap with those controlling stem diameter or crown width. Two QTLs for lodging resistance by typhoon were detected on chromosome 5, having positive alleles from Kasalath, and one was detected on chromosome 6, having a positive allele from Nipponbare. On chromosome 5, QTL for lodging resistance by typhoon near marker C246 overlapped with the QTL for pushing resistance of the lower part.

LOD Curve of *prl5* and Selection of NILs

Figure 3 shows LOD curves of *prl5* and of the QTL for lodging resistance by typhoon on chromosome 5. *prl5* shows three peaks, the highest of which was detected near C1018. The LOD curve of *prl5* echoed that for lodging resistance by typhoon. Among a series of rice NILs developed by Yano's group (Yano,

2001), by marker-assisted selection we selected three (nos. 43, 60, and 63) that carry a Kasalath chromosomal segment containing *prl5* in a Nipponbare genetic background (Fig. 3; Supplemental Fig. 1). In addition, NIL43 carries a Kasalath segment containing the QTL for pushing resistance on chromosome 11 with a positive effect by the Nipponbare allele (*prl11*). These NILs were used for further biochemical and physiological analyses to identify one of the QTLs for pushing resistance.

Pushing Resistance of the Lower Part and Lodging Resistance

The pushing resistances of the lower part in NIL43, NIL60, and NIL63 were 1.8, 1.4, and 2.4 times, respectively, as high as that in Nipponbare; all differences were significant (Fig. 4). Lodging resistance was measured as pushing resistance with whole plants. The lodging resistances in NIL43, NIL60, and NIL63 were

Table I. *QTLs for pushing resistance of the lower part, stem diameter, and lodging resistance by typhoon*

Traits	No. of QTLs Detected	Chromosome No. (QTL name)	Nearest Marker Locus to Putative QTL	Likelihood Odds Ratio (LOD)	DPE[a]	r^{2} [b]
Pushing resistance of the lower part	5	4	C946	2.02	N	0.089
		5 (*prl5*)	C1081	2.08	K	0.113
		6	R2171	3.98	N	0.174
		11 (*prl11*)	C82	1.78	N	0.130
		12	G1406	2.68	N	0.130
Stem diameter	6	1	C885	4.48	K	0.190
		3	C595	2.62	N	0.148
		6	C358	2.36	K	0.099
		7	C451	2.78	K	0.122
		8	C10122	3.42	K	0.169
		12	R3375	2.67	K	0.119
Lodging resistance by typhoon[c]	3	5	R1838	3.03	K	0.133
		5	C246	1.66	K	0.075
		6	R2549	3.52	N	0.152

[a] DPE, Detection of phenotypic effect. N and K, Nipponbare and Kasalath alleles increased that value, respectively. [b] r^{2}, Phenotypic variation explained by each QTL. [c] Lodging resistance by typhoon was observed on October 2, 2002, the day after the season's 21st typhoon had hit.

2.4, 1.9, and 2.3 times, respectively, as high as that in Nipponbare; all differences were significant. The pushing resistance in NIL63 was significantly higher than those in NIL43 and NIL60 (1.3 and 1.7 times, $P[f] < 0.01$).

Morphological Traits

Plant height in NIL60 and NIL63 was significantly greater than that in Nipponbare, whereas that in NIL43 was significantly less than those in the other NILs and Nipponbare (Table II). The heights of the

second leaf below the flag leaf (-2 leaf height) from the ground and the stem numbers were similar among plants. The stem diameter of NIL63 was significantly larger than that of Nipponbare ($P[f] < 0.01$). Crown widths in all NILs were significantly larger than in Nipponbare. There was no difference
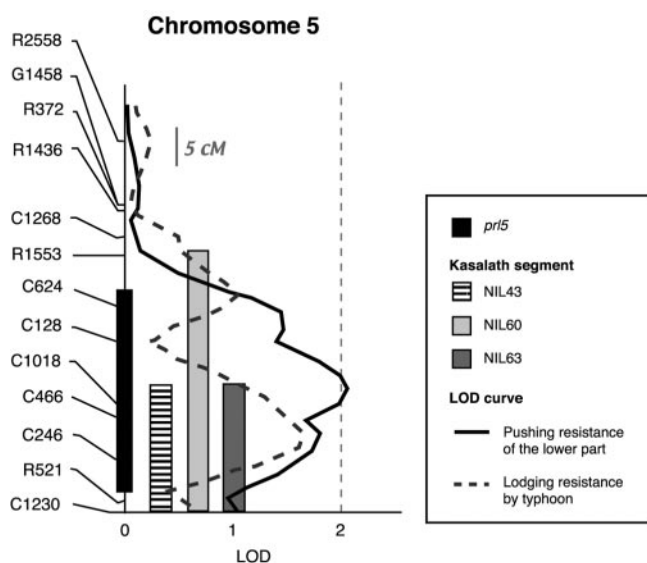

Figure 3. LOD scores of QTLs controlling pushing resistance of the lower part (black line) and lodging resistance by typhoon (dashed line) on chromosome 5. The bars show the Kasalath segment in the NILs. The chromosome region of *prl5* is shown as a black bar. QTLs for pushing resistance of the lower stem and lodging resistance by typhoon were analyzed with 98 BILs in 2001 and 2002, respectively.
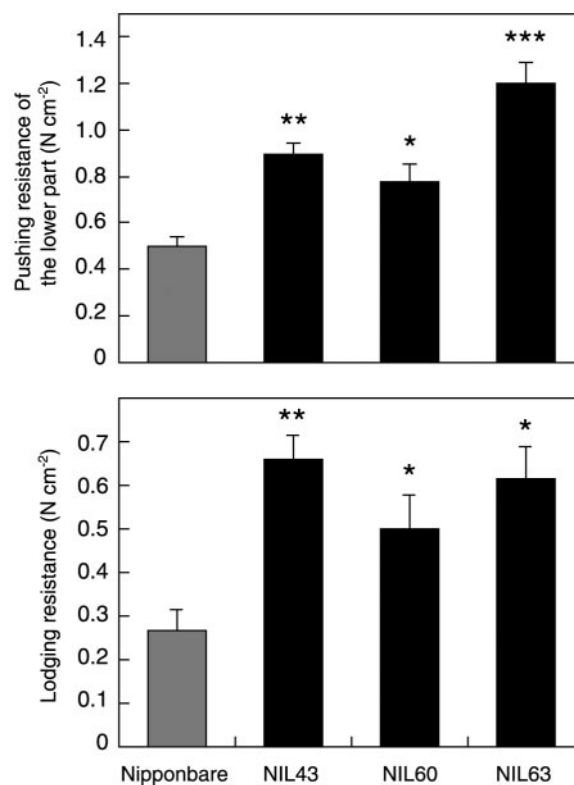

Figure 4. Pushing resistance of the lower part and lodging resistance in NILs and Nipponbare (control). The values are the means of six independent plants in each line; vertical bars = SE. *, **, ***; Significant at $P = 0.05$, 0.01, and 0.001, respectively.

Table II. *Morphological traits and characters and components in lower stems of Nipponbare and NILs*

Data are expressed as means ± SE of nine or more plants.

Traits	Nipponbare	NIL43	NIL60	NIL63
Traits related to plant type and yield				
Plant height (cm)	106.0 ± 1.5	101.6 ± 1.4**[a]	112.0 ± 1.0**	115.8 ± 0.8***
−2 leaf height (cm)	37.0 ± 0.8	35.7 ± 1.0	35.3 ± 1.4	39.1 ± 1.2
Stem diameter (mm)	3.8 ± 0.1	4.0 ± 0.1	4.0 ± 0.1	4.3 ± 0.1**
Stem no.	17.4 ± 1.1	17.8 ± 1.1	16.9 ± 1.3	18.2 ± 1.2
Crown width (mm)	58.1 ± 1.7 (100%)[c]	77.5 ± 3.4*** (133%)	67.8 ± 3.6* (117%)	67.3 ± 2.6** (116%)
Yield (g plant^{-1})	28.2 ± 1.6	28.3 ± 1.9	36.1 ± 3.6	31.1 ± 2.6
Upper parts wt (g)	16.8 ± 1.0	18.3 ± 1.0	16.7 ± 1.2	24.1 ± 1.9***
Characters of lower stems				
Wt (g)	1.0 ± 0.1 (100%)	1.5 ± 0.1*** (150%)	1.6 ± 0.1*** (160%)	2.1 ± 0.1*** (210%)
Density[b] (g cm^{-3})	0.08 ± 0.00 (100%)	0.10 ± 0.01** (125%)	0.11 ± 0.01** (138%)	0.12 ± 0.01*** (150%)
Components in lower stems				
Starch (μmol hexose g^{-1} dry wt)	131.4 ± 51.5 (100%)	457.8 ± 122.2** (348%)	659.2 ± 136.8** (502%)	1,334.1 ± 178.9*** (1,015%)
Suc (μmol hexose g^{-1} dry wt)	320.5 ± 68.0 (100%)	465.5 ± 44.3* (145%)	505.3 ± 21.6* (158%)	641.0 ± 25.5** (200%)
Hexoses (μmol hexose g^{-1} dry wt)	66.3 ± 10.8 (100%)	71.6 ± 4.8 (108%)	89.8 ± 10.8 (135%)	124.4 ± 10.1** (188%)
Silicon (counts s^{-1})	41.0 ± 1.6 (100%)	30.8 ± 2.0*** (75%)	34.4 ± 1.2* (84%)	23.6 ± 2.1*** (58%)

[a] ***,**,*, Significant at 0.001, 0.01, and 0.05 at probability level, respectively. [b] Density of lower stem = lower stem wt/[π × (stem diameter/2)^2 × stem length]. [c] Percentage relative to Nipponbare.

in yield among lines. The root dry weight to 10-cm depth in Nipponbare was 3.35 ± 0.34 g (data not shown), that in NIL43 was similar, and that in NIL60 was 20% less than that in Nipponbare ($P[f] < 0.01$), whereas that in NIL63 was 30% greater ($P[f] < 0.05$). There were no significant differences in root dry weight below 10 cm (data not shown). The dry weights of the upper parts (leaves and stems above 40 cm) in the NILs were not lower than that in Nipponbare, and the dry weight of the upper parts in NIL63 was higher than that in Nipponbare. Compared with Nipponbare, heading date was 12 d earlier in NIL60 and 2 d later in NIL43 and NIL63 (data not shown).

Characters and Components in Lower Stems

The dry weights of stems below 40 cm (lower stem) were significantly greater in all NILs than that in Nipponbare by 50% to 110% (Table II). The densities of the lower stems were significantly greater in all NILs than in Nipponbare by 25% to 50%. The contents of accumulated carbohydrates in lower stems were significantly higher in the NILs than in Nipponbare: The contents of starch in NIL43, NIL60, and NIL63 were 3.5, 5.0, and 10.2 times and those of Suc were 1.5, 1.6, and 2.0 times as high as those in Nipponbare. The relative contents of silicon in the lower stems were estimated from energy-dispersive x-ray fluorescence spectra. The silicon peaks of Nipponbare were all higher than those of the NILs; NIL63 showed the lowest peak (see Supplemental Fig. 2). The relative contents (in counts per second) of silicon in Nipponbare also were significantly higher than those in NIL43, NIL60, and NIL63; again, NIL63 had the lowest content (58% of that in Nipponbare; Table II).

DISCUSSION

Pushing resistance of the lower part was measured in rice BILs that had its upper part cut off to exclude its effect. We detected five QTLs for pushing resistance of the lower part (Fig. 2; Table I). Among them, only *prl5* had a positive allele from Kasalath and did not overlap with other QTLs related to plant type. The LOD curve of *prl5* echoed that for lodging resistance by typhoon, and these QTLs had the same positive allele (Fig. 3). These results indicate that *prl5* should be the main target for identification. NIL43, NIL60, and NIL63 contain the chromosomal region of *prl5* from Kasalath in a Nipponbare background, and NIL43 also carries *prl11* (Fig. 3; Supplemental Fig. 1). Heading date and plant height differed among NILs (data not shown; Table II): In addition to *prl5*, each NIL carries different Kasalath segments (see Supplemental Fig. 1), which caused these differences.

Pushing resistance of the lower part and lodging resistance in each NIL were significantly higher than in Nipponbare (Fig. 4). These results support the existence of *prl5*. Lodging resistance in a plant is determined by the weight of the upper parts and the pushing resistance (Mulder, 1954). Plant height depended on the line, and there was no difference in yield between the NILs and Nipponbare (Table II). The dry weights of the upper parts were not lower in the NILs (Table II). These results show that *prl5* improved lodging resistance not by decreasing the weight of the upper parts but by increasing the pushing resistance of the lower part.

By comparing the characters of NILs that carry different segments from the donor parent, we can elucidate epistatic interactions among QTLs (Lin et al., 2000, 2003; Yano, 2001). In addition to *prl5*, NIL43 carries a Kasalath segment containing *prl11*, which

has a negative effect on pushing resistance (Fig. 2; Supplemental Fig. 1). Pushing resistance in NIL43 was significantly higher than in Nipponbare but significantly lower than in NIL63, which carries the same Kasalath segment containing *prl5* (Fig. 3). These results suggest that an epistatic interaction might occur between *prl5* and *prl11* and that *prl11* might be antagonistic to *prl5*.

Terashima et al. (1994) found a positive correlation between lodging resistance and deep root weight in several rice cultivars. However, we found no difference in root morphological traits between the NILs and Nipponbare (data not shown). The crown widths in NILs were significantly larger than in Nipponbare, but there was no difference in stem diameter or stem number among lines (Table II). Therefore, the distance between stems in NILs was wider. The chromosome segments from Kasalath in the NILs might be responsible for the greater crown widths. In NIL63, which had the greatest pushing resistance, the crown width was 1.16 times that in Nipponbare. Comparing between NILs and Nipponbare, the relation was not observed between crown width and the pushing resistance. QTLs for pushing resistance did not overlap with those for crown width (Fig. 2). These results suggest that traits related to plant type and root morphology might not be the main factors determining the higher pushing resistance of the NILs.

Higher contents of accumulated carbohydrates in rice stems contribute greatly to lodging resistance (Sato, 1957; Takaya and Miyasaka, 1983; Yang et al., 2001). The content of cellulose or lignin is related to the stem rigidity (Taylor et al., 1999; Jones et al., 2001). However, the relationship between contents and lodging resistance has not been clear. The application of abundant nitrogen at the ripening stage increases the accumulation of carbohydrates in stems and improves lodging resistance but reduces cellulose content (Matsuzaki et al., 1972). At harvest, the contents of accumulated carbohydrates in the NILs were much higher than those in Nipponbare, particularly those of starch (Table II). This higher accumulation of carbohydrates might cause the heavier dry weights and the greater densities in the lower stems of the NILs. In stems, the higher accumulation of starch contributes to the higher bending strength (Takahashi, 1960; Matsuzaki et al., 1972). These results suggest that the higher contents of accumulated carbohydrates in the lower stems might enhance the bending strength of the basal stem and, as a consequence, cause the higher lodging resistance in the NILs. Silicon in the cell wall was thought to contribute to mechanical strength in rice stems (Takahashi, 1995). However, the relative silicon contents in the lower stems of NILs were significantly lower than that in Nipponbare (Table II). Lodging resistance in NIL63, with a much lower silicon content, was higher

than in other plants. This result suggests that silicon in the lower stems of NILs might not contribute the higher lodging resistance and that a higher carbohydrate content might decrease the relative silicon content because the densities of the lower stem increased significantly in NILs. Together, these results suggest that *prl5* might affect the content of carbohydrates in the lower stems and, as a consequence, increase pushing resistance in NILs.

In rice, yield is determined by the amount of accumulated carbohydrates in stems before heading and the photosynthates produced during the reproductive stages (Yoshida, 1972). The former contributes about 30% of the final yield (Cock and Yoshida, 1972). In a recent study, our group found that an increase in accumulated carbohydrates before heading could improve yield in Nipponbare (Ishimaru, 2003). At harvest, the higher content of carbohydrates in the lower stems caused by *prl5* did not reduce yield in the NILs relative to Nipponbare (Table II). Retardation of senescence in leaves causes unused carbohydrates to remain in the stems (Cao et al., 1992; Zhu et al., 1997). Senescence was delayed in leaves of NILs relative to Nipponbare (T. Kashiwagi, unpublished data). These results suggest that *prl5* might not delay the release of stored carbohydrates in stems but delay senescence in leaves. Further study is needed of carbon metabolism before and after heading and in relation to senescence of leaves to clarify the effect of *prl5* on the accumulation of carbohydrates in NILs.

In conclusion, we have identified a new locus responsible for pushing resistance of the lower part and, thus, lodging resistance. Physiological analyses of NILs containing Kasalath segments including *prl5* suggested that the higher contents of carbohydrates in the lower stems because of *prl5* might determine the higher pushing resistance. The results of this study strongly suggest that pushing resistance of the lower part could be a new target for improving lodging resistance in rice. We have shown that *prl5* can improve lodging resistance without negative effects on other traits (e.g. yield and plant height); therefore, it might be suitable for introduction into modern rice cultivars.

MATERIALS AND METHODS

Plant Materials

Rice (*Oryza sativa* subsp. *japonica*) cv Nipponbare, Japan's high-yielding cultivar (Saitoh et al., 1993), was crossed with *indica* cv Kasalath, and a resultant F_1 plant was crossed with Nipponbare to produce seeds of BILs. Ninety-eight BILs (F_7) were developed from the resultant BILF$_1$ plants by the single-seed descent method (Lin et al., 1998). The 98 BILs and their parental lines were sown on May 14, 2001 and 2002, and their seedlings were transplanted in early June and grown under natural conditions in Tsukuba, Japan (latitude 38°N) in random design to reduce the effects of environmental factors.

Measurement of Pushing Resistance of the Lower Part and Other Traits in BILs

At the full-ripe stage (45 d after heading), plants were cut off at 40-cm height, the prostrate tester (Daiki Rika Kogyou Co., Tokyo) was set perpendicularly at the middle of the plant (20 cm), and pushing resistance of the lower part was measured when plants were pushed to an angle of 45° from the vertical (Fig. 1A). Five plants in each line were used. The stem diameter was measured at 40 cm. Lodging resistance by typhoon was observed on October 2, 2002, the day after the season's 21st typhoon had hit. That resistance rating was recorded on a scale of 0 (prostrating) to 1 (no or little prostrating). This typhoon had a maximum momentary wind velocity of 31.6 m s^{-1} and a maximum rainfall rate of 21 mm h^{-1} (http://www.tokyo-jma.go.jp/home/mito/).

QTL Analyses

Chromosome locations of putative QTLs were determined by single-point analysis with the General Linear model procedure of QGENE version 3.06 (Nelson, 1997) according to the method of Ishimaru et al. (2001d). P of 0.01 was used as the threshold to detect significant differences in mean values of two genotypic classes, homozygous for Nipponbare and homozygous for Kasalath alleles (Ishimaru et al., 2001d). To represent a QTL on the map, we selected the chromosome region corresponding to log(LOD) > LOD$_{max}$ − 1 with an LOD − 1 interval method (Hirel et al., 2001; Ishimaru et al., 2001b). QTLs for plant height and crown width were reported (by Ishimaru et al. (2001d), and QTL for stem number was unpublished data (K. Ishimaru).

Selection and Growth Conditions of NILs

By marker-assisted selection, we selected NIL43, NIL60, and NIL63 from advanced backcross progeny of Nipponbare as the recurrent parent and Kasalath as the donor parent bred by Yano's group at the National Institute of Agrobiological Sciences (Yano, 2001). These NILs carry a chromosomal segment from Kasalath including *prl5* in the genetic background of Nipponbare (Fig. 3; Supplemental Fig. 1). NIL43 also carries a chromosomal segment from Kasalath including QTLs for crown width on chromosome 10 with a positive effect by the Kasalath allele and for pushing resistance on chromosome 11 (*prl11*) with a negative effect by the Kasalath allele. NIL60 and NIL63 did not have any other chromosomal segments from Kasalath that include QTLs that we have already reported (Ishimaru et al., 2001b, 2001c, 2001d). The NILs and Nipponbare were sown on May 15, 2002, and the seedlings were transplanted in a randomized block design with three replicates in Tsukuba in early June.

Lodging Resistance and Morphological and Physiological Traits in NILs

Pushing resistance in NILs and Nipponbare was measured as above. Lodging resistance was measured as for pushing resistance but on whole plants. Six plants in each line were used for measurement. Next, plant height, −2 leaf height, stem diameter, crown width, and the weight and number of ears were measured. Then, plants were divided into two parts at 40 cm; the lower stem and the upper part were sampled, oven dried at 80°C for 3 d, and weighed. The density of a lower stem was calculated as dry weight/[$\pi \times$ (stem diameter/2)$^2 \times$ stem length]. Roots were divided into three parts (<10 cm from the ground surface, 10–20 and 20–30 cm) in a modification of the method of Terashima et al. (1994), and sampled root in each layer was oven dried at 80°C for 3 d and weighed. Yield per plant was calculated according to the method of Xing et al. (2002).

Measurements of Components in Lower Stems of NILs

Dried samples of lower stems were powdered at 15,000 rpm for 90 s in a Wonder Blender (Osaka Chemical Co., Osaka). Carbohydrate contents were measured enzymatically according to the method of Ishimaru et al. (2001a). Samples of approximately 50 mg were powdered in liquid nitrogen in a mortar with a pestle and extracted twice with 80% (v/v) ethanol at 80°C. After centrifugation at 12,000g for 5 min, the supernatants were collected,

dried in a vacuum, and used for the determination of Suc by an enzymatic method (Bergmeyer and Bernt, 1974). To determine starch content, the pellets were boiled in distilled water for 2 h and then digested with amyloglucosidase for 15 min at 55°C. The resultant hexoses were determined enzymatically as described above. Samples of lower stems (200 mg dry weight) were pressed at 15 kN cm^{-2} in a hydraulic press (Evacuable KBr Die, Shimadzu, Kyoto) to form a 13-mm-diameter tablet. The relative content of silicon in the pellet was analyzed with an energy-dispersive x-ray fluorescence spectrometer with an element analyzer (JSX-3201, JEOL, Tokyo). The measurement was carried out at 30 kV for 600 s and replicated three times for each sample. Silicon was analyzed at a peak of 1.739 keV; the relative content was calculated as the counts-per-second ratio by the method of Vázquez et al. (1999).

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