Lodging tolerance (LT) is an important trait for high yield and combine-harvesting efficiency in soybean 
*Glycine max* (L.) Merr. Many previous studies have investigated quantitative trait loci (QTLs) for lodging
score (LS) in soybean. Most of the investigated QTLs were located in the proximal region of maturity or
growth habit loci. The aim of this study was to identify genetic factors for LT not associated with maturity or
growth habit. QTL analysis was performed using a recombinant inbred line (RIL) population derived from a
cross between ‘Toyoharuka’ (TH), a lodging-tolerant cultivar, and ‘Toyomusume’ (TM). The genotypes of TH
and TM were estimated as both *e1e2E3E4* and *dt1*. The average LS over 4 years was used for QTL analysis,
identifying a major and stable QTL, *qLS19-1*, on chromosome 19. The LS of the near-isogenic line (NIL) with
the TH allele at Sat_099, the nearest marker to *qLS19-1*, was significantly lower than the NIL with the TM
allele at that position. The TH allele at Sat_099 rarely had a negative influence on seed yield or other agronom-
ict traits in both NILs and the TM-backcrossed lines. Our results suggest that marker-assisted selection for
*qLS19-1* is effective for improving LT in breeding programs.

**Key Words:** soybean, lodging, quantitative trait loci, marker-assisted selection.

**Introduction**

Lodging tolerance (LT) is an important trait for high yield and combine-harvesting efficiency in soybean 
*Glycine max* (L.) Merr. Numerous studies have investigated the effect of lodging on yield (Noor and Caviness 1980, Saito et al. 2012, Weber and Fehr 1966, Woods and Swearingin 1977). Complete lodging at the seed maturation stage decreases yield by more than 30% (Saito et al. 2012). Likewise, many studies have investigated the effect of lodging on combine-
harvesting efficiency (Ono et al. 1990, Uchikawa et al. 2006, Weber and Fehr 1966). Combine-harvesting loss
through lodging of soybeans is estimated to be about 20% (Uchikawa et al. 2006).

Genetic analysis of LT is important in the breeding of lodging-tolerant cultivars. Many studies have investigated quantitative trait loci (QTLs) for lodging score (LS) in soybean (Lee et al. 1996, Mansur et al. 1993, Orf et al. 1999, Specht et al. 2001). However, in these studies, the maturity or growth habit was segregated in the population used for

QTL analysis. Determinate and indeterminate growth habits are controlled by alleles at the *Dt1* locus (Bernard 1972). The stem growth habit influences other agronomic traits: for example, determinate phenotypes generally reach shorter
heights and have increased LT than indeterminate phenotypes of similar maturity (Cober and Morrison 2010, Foley et al. 1986). In soybean, several maturity loci are reported to control the time to flowering and maturity. These are desig-
nated as *E* loci (Cober et al. 1996). Recently, the candidate genes *E3* and *Dt1* were reported to be linked (Liu et al. 2010, Watanabe et al. 2009). Previous studies reported that most QTLs for LS were located in the proximal region of
*E3* or *Dt1* loci (Lee et al. 1996, Mansur et al. 1993, Orf et al. 1999, Specht et al. 2001). Therefore, it is not clear whether genes responsible for these QTLs are closely linked to *E3* and *Dt1* or are pleiotropic.

In this study, we selected a population in which parents reached similar maturity and were determinate for QTL
analysis. We performed QTL analysis using recombinant inbred lines (RILs) derived from a cross between the
lodging-tolerant cultivar ‘Toyoharuka’ (TH) and the high lodging cultivar ‘Toyomusume’ (TM) (Sasaki et al. 1988, Tanaka et al. 2009). Moreover, we developed near-isogenic lines (NILs) from the RIL, and backcrossed (BC) lines using
the nearest marker to a major QTL to investigate the effect on LT, seed yield, and other agronomic traits. We also investigated the effect of a major QTL in the breeding line Toiku 248 (T248) background by marker-assisted selection (MAS).

Materials and Methods

Plant materials and field tests

All cultivars and breeding lines were developed at the Tokachi Agricultural Experiment Station (TAES), Memuro, Hokkaido, Japan. A RIL population (192 lines) was developed by a single seed decent method from a TH × TM cross (Ikeda et al. 2009, Ohnishi et al. 2011). Both parental cultivars are determinate and reach maturity at similar times (Tanaka et al. 2009). The generation of the RIL population was F₆:7 in 2008, F₆:8 in 2009, F₆:9 in 2011, and F₆:10 in 2012.

TOIU 248, a modern breeding line with high lodging resistance (Ikeda et al. 2009, Ohnishi et al. 2011). Both parental cultivars are determinate and reach maturity at similar times (Tanaka et al. 2009). The generation of the RIL population was F₆:7 in 2008, F₆:8 in 2009, F₆:9 in 2011, and F₆:10 in 2012.

Toiku 248, a modern breeding line with high lodging resistance (Ikeda et al. 2009, Ohnishi et al. 2011). Both parental cultivars are determinate and reach maturity at similar times (Tanaka et al. 2009). The generation of the RIL population was F₆:7 in 2008, F₆:8 in 2009, F₆:9 in 2011, and F₆:10 in 2012.

Calculation of broad-sense heritability for LS

The broad-sense heritability for LS was calculated using data from 2008. The environmental variance was calculated according to the LSs of the parents (three replicates). The phenotypic variance was calculated according to the LSs of the 192 RILs. The genetic variance and broad-sense heritability were calculated as follows: (genetic variance) = (phenotypic variance) – (environmental variance); (broad-sense heritability) = (genetic variance)/(phenotypic variance).

Molecular marker analysis and linkage mapping

Polymorphic SSR markers were added to the linkage map previously developed by Ikeda et al. (2009) to reconstruct a higher density linkage map. The F₉ RIL plants were used for genotyping of the SSR markers. DNA extraction and PCR for the markers were as described previously (Hwang et al. 2009, Sayama et al. 2010). We analyzed the 243 molecular markers using the SSR genotyping panel system (Sayama et al. 2011). In addition to the markers in the panel, six polymorphic SSR markers: BARCSOYSSR_19_1200, BARCSOYSSR_19_1212, BARCSOYSSR_19_1255, BARCSOYSSR_19_1271, BARCSOYSSR_19_1286, and BARCSOYSSR_19_1321 (Song et al. 2010) were also genotyped. These markers are located in the proximal region of a major QTL, qLS19-1. MAPMAKER/EXP 3.0b (Lincoln et al. 1993) was used to determine molecular linkage groups (MLGs) and marker positions. The design of molecular markers for E1, E2, E3, E4 and D1l loci was based on previous studies (Liu et al. 2008, 2010, Watanabe et al. 2009, 2011, Xia et al. 2012, Yamanaka et al. 2001, 2005). The genotypes at the E1 to E4 loci of the parental cultivars were estimated as described by Sayama et al. (2010).

QTL analysis

QTL analysis was performed using QTL Cartographer version 2.5 (Wang et al. 2007). Composite interval mapping (Zeng 1994) was implemented with a threshold logarithm of odds (LOD) score calculated by a permutation test to identify QTLs. The LOD threshold value at the 5% probability level was calculated using a thousand-replicate permutation test.
The NILs were planted on 18th May 2011 and 22nd May 2012. Each plot consisted of four rows, with lengths of 1.5 m (2010) or 3.5 m (2011), spaced at 60 cm with a plant interval of 6.7 cm; giving a plant population density of 25.0 plants m\(^{-2}\). A randomized complete block design with three replicates was used for these experiments. Flowering time was defined as the time at which more than 50% of plants in the plot were flowering. Maturing time was defined as the time when more than 80% of plants defoliated and turned yellow, with pods rattling when shaken. At the time of maturing, LS was recorded for each plant. The average LS for each plot was used for statistical analysis. Before harvesting, ten central consecutive plants were selected from each plot for morphological measurement. Main stem length, number of main stem nodes, and the number of branches were recorded for phenotypic evaluation. Mature plants were harvested by hand in each plot. The Tukey–Kramer multiple comparison test was used for testing significant differences in the agronomic traits among the cultivars and NILs. ‘Cultivar’ and ‘year’ were considered the two factors.

**Evaluation of LT in backcrossed lines**

The BC lines were developed as follows: F\(_1\) plants from the cross between TH and TM were obtained, and backcrossed with TM. The BC\(_1\)F\(_1\) plants with heterozygous genotypes at Sat\(_{099}\) were backcrossed with TM. BC\(_2\)F\(_1\) plants with heterozygous genotypes at Sat\(_{099}\) were then selected. The BC\(_2\)F\(_2\) plants were genotyped at Sat\(_{099}\) and sorted into TH, TM, and heterozygous genotypes. The two BC\(_2\)F\(_3\) lines with TH genotypes at Sat\(_{099}\) were selected from the individual BC\(_2\)F\(_1\) plants. These lines were named as TMBC2-1 and TMBC2-2. The BC\(_2\) generation was F\(_4\) in 2012 and F\(_3\) in 2013. The BC\(_2\) lines were planted on 5th June 2012 and 21st May 2013. Each plot consisted of two (2012) or four rows (2013) with lengths of 3 m, these were spaced 60 cm apart with a 20 cm inter-hill with two plants per hill; giving a plant population density of 16.7 plants m\(^{-2}\). A randomized complete block design with two (2012) or three replicates (2013) was used for these experiments. The measurement methods were as described above in the section ‘Evaluation of lodging tolerance in NILs’.

**MAS for qLS19-1 in a T248 × TH background**

F\(_1\) plants were obtained from a cross between T248 and TH, and F\(_2\) plants genotyped at Sat\(_{099}\) were selected randomly. The eight breeding lines and parental lines were planted on 21st May 2012. Each plot consisted of two rows with lengths of 3.5 m spaced at 60 cm, with a 20 cm inter-hill with two plants per hill; giving a plant population density of 16.7 plants m\(^{-2}\). A randomized complete block design with two replicates was used. LS was recorded in each plot at the time of maturing. Dunnett’s test was performed in each agronomic trait using T248 as the reference.

**Results**

**Evaluation of LT in the parents**

‘Toyoharuka’ was lodging tolerant while TM displayed high lodging at the flowering to maturing stage (Fig. 1A, 1B). The LS of TH was significantly lower than that of TM, even though the main stem length and the number of main stem nodes were similar (Table 1). The number of branches in TH was significantly less than that of TM (Table 1). The genotypes at the E1 to E4 loci of TH and TM were estimated to be the same, el\(_{e2}E3\)e\(_4\) (Table 1). The determinate genotypes of both TH and TM were dt\(_1\) (Table 1). These results suggested that LT in the RIL population could be evaluated without the effects of the E1, E2, E3, E4 and Dt\(_1\) loci.

**Broad-sense heritability for LS**

The calculated genetic variance and environmental variance for LS were 1.48 and 0.46, respectively. The broad-sense heritability for LS was calculated as 0.76. These results suggested that LS showed relatively high heritability.

**SSR analysis and linkage mapping**

A higher density linkage map was constructed based on available SSR marker locations and their polymorphisms in the parental cultivars (Hwang et al. 2009, Sayama et al. 2011). In all, 177 markers were polymorphic between parents. The resultant genetic linkage map comprised 20 molecular linkage groups (MLGs) and covered 2512 cM. The entire genome size was larger than that previously reported by Ikeda et al. (2009).

**QTL analysis for LS in RILs**

The LSs of the RILs were evaluated over 4 years because there were no replicates in each year. There were positive correlations between the LSs of the RILs in each pair of years (Table 2). Two-way analysis of variance (ANOVA) was used to test differences among the RILs in LS, with ‘RIL’ and ‘year’ as the two factors. The ANOVA revealed that there were significant differences among the RILs (\(P < 0.001\)). Therefore, we considered years as replicates, and the average LS over 4 years was used for the QTL analysis.

The average LSs over 4 years for TH and TM were 0.8 and 2.7, respectively (Fig. 2). In the RIL population, average LSs varied from 0.0 to 3.7, and normally distributed (Fig. 2). QTL analysis using the average LS over 4 years was then performed using the 192 RILs. The LOD threshold value at the 5% probability level was 3.5. Two QTLs,
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...were detected on chromosome-19 (Chr-19) (MLG-L) and Chr-13 (MLG-F), respectively (Table 3). The \textit{qLS19-1} and \textit{qLS13-1} loci had LOD scores of 11.4 and 3.7, respectively (Table 3). The TH allele at \textit{qLS19-1} promoted a stronger LT (Table 3). The LOD score peak of \textit{qLS19-1} was located at Sat_099 (Fig. 3A). The LOD score peak of \textit{qLS13-1} was located in the region...

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**Table 1.** Agronomic traits of the parents (25.0 plants m\(^{-2}\); average in 2008 to 2011)

| Cultivar | Lodging score\(^a\) | Main stem length (cm) | No. of main stem nodes | No. of branches (m\(^{-2}\)) | Genotype | 
|----------|----------------------|------------------------|-------------------------|-----------------------------|----------|
| TH       | 0.7                  | 67                     | 9.9                     | 18.8                        | \textit{ele2E3E4 dt1} |
| TM       | 2.2 **               | ns                     | ns                      | 53.6                        | \textit{ele2E3E4 dt1} |

\(^a\) Lodging score: 0 (no lodging)–4 (completely lodged).

\(^b\) ns, non-significant at \(P < 0.05\).

**Table 2.** Correlation coefficients between lodging scores of RILs in each pair of years

|       | 2008     | 2009     | 2011     | 2012     |
|-------|----------|----------|----------|----------|
| 2008  |          | 0.461*** | 0.340*** | 0.424*** |
| 2009  | 0.461*** |          | 0.440*** | 0.268*** |
| 2011  | 0.340*** | 0.440*** |          | 0.390*** |
| 2012  | 0.424*** | 0.268*** | 0.390*** |          |

*** Significant at \(P < 0.001\).

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\textit{qLS19-1} and \textit{qLS13-1}, were detected on chromosome-19 (Chr-19) (MLG-L) and Chr-13 (MLG-F), respectively (Table 3). The \textit{qLS19-1} and \textit{qLS13-1} loci had LOD scores of 11.4 and 3.7, respectively (Table 3). The TH allele at \textit{qLS19-1} promoted a stronger LT (Table 3). The LOD score peak of \textit{qLS19-1} was located at Sat_099 (Fig. 3A). The LOD score peak of \textit{qLS13-1} was located in the region...
between Satt334 and Sat_313 (Fig. 3B). The nearest marker to qLS13-1 was Sat_313 (Table 3).

The LSs of the RILs with TH alleles at Sat_099 were significantly lower than the TM alleles in each year (P < 0.01, Table 4). The main stem lengths of the RILs with TH alleles at Sat_099 were similar to those of the TM alleles in 2008 and 2012 (Table 4). The seed yields of the RILs with TH alleles at Sat_099 were similar to those of the TM alleles in each year (Table 4). These results indicated that qLS19-1 was a stable QTL, and rarely had a negative influence on seed yield.

In contrast, the TM allele at Sat_313 contributed stronger tolerance (Table 3). The LSs of the RILs with TM alleles at Sat_313 were lower than those with TH alleles in 2012 (P < 0.05, Table 5). The main stem lengths of the RILs with TM alleles at Sat_313 were shorter than those with TH alleles in each year (P < 0.01, Table 5). The seed yields of the RILs with TM alleles at Sat_313 were lower than those with TH alleles in 2009 and 2011 (P < 0.05, Table 5). These results indicated that qLS13-1 was not a stable QTL, and frequently had a negative influence on seed yield.

QTL analysis for LS was also performed each year. However, no significant QTLs were detected (data not shown).

**Evaluation of LT in NILs**

As it was a major and stable QTL, further study focused on the qLS19-1 locus (Tables 3, 4). NILs were developed from RILs heterozygous at Sat_099, the nearest marker to qLS19-1. The LS of NIL-TH was lower than that of NIL-TM (P < 0.05; Table 6). The 100-seed weight of NIL-TH was heavier than that of NIL-TM (P < 0.05; Table 6). The other agronomic traits: flowering date, maturing date, main stem lengths, number of main stem nodes, number of branches, and seed yield were similar in NIL-TH and NIL-TM (Table 6). These results suggested that the TH allele at the Sat_099 locus promoted a stronger LT, and rarely had a negative influence on seed yield in the NILs.

**Evaluation of LT in backcrossed lines**

The BC2 lines were developed by MAS for qLS19-1. TMBC2-2 was lodging tolerant while TM, a backcrossed parent, had high lodging at the young pod to maturing stage (Fig. 1C, 1D). The LSs of TMBC2-1 and TMBC2-2 were lower than in TM (P < 0.05; Table 7). The maturing times of TMBC2-1 and TMBC2-2 were 2–4 days shorter than observed in TM (Table 7). This may be because TM had severe lodging (Table 7) and a later maturing date. The main

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**Table 3. QTL analysis of lodging score (four year average)**

| Chr (LG) | Position (cM) | Nearest marker | LODa | R2 (%) | Additive effectd | QTL name |
|----------|---------------|----------------|------|--------|-----------------|----------|
| 19 (L)   | 110.9         | Sat_099        | 11.4 | 19.8   | -0.40           | qLS19-1  |
| 13 (F)   | 123.3         | Sat_313        | 3.7  | 10.9   | 0.29            | qLS13-1  |

*Chr, chromosome; LG, linkage group.

*LOD, logarithm of odds determined by composite interval mapping; The threshold LOD value at 5% probability level was calculated by a thousand-replicate permutation test. The value was 3.5.

*Percentage phenotypic variance explained by the QTL.

*The effect of the TH allele on the QTL. Lodging score: 0 (no lodging)–4 (completely lodged).

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**Fig. 3.** LOD score plot of QTLs associated with lodging score in the RILs. (A) qLS19-1 located on Chr-19. (B) qLS13-1 located on Chr-13. The LOD threshold value at the 5% probability level was 3.5. Arrows show the location of the E3 and Dt1 loci.
Table 4. Relationship between the marker genotype at qLS19-1 and agronomic traits in the RILs

| Year | Generation of RILs | Planting density (plants m\(^{-2}\)) | qLS19-1 genotype (Sat_099) | Lodging score\(^e\) | Main stem length (cm) | Seed yield (kg 10\(a\)^{1}\) |
|------|-------------------|----------------------------------|---------------------------|---------------------|----------------------|-------------------------|
| 2008 | \(F_{6:7}\)       | 25.0                             | TH                        | 1.6                 | 74                   | ND                      |
|      |                   |                                  | TM                        | 2.6                 | 75                   | ND                      |
|      |                   |                                  |                           | **                  | ns\(^b\)              |                         |
| 2009 | \(F_{6:8}\)       | 25.0                             | TH                        | 0.3                 | 55                   | 396                     |
|      |                   |                                  | TM                        | 0.6                 | 60                   | 400                     |
|      |                   |                                  |                           | **                  | **                   | ns                      |
| 2011 | \(F_{6:9}\)       | 16.7                             | TH                        | 1.3                 | ND                   | 401                     |
|      |                   |                                  | TM                        | 2.2                 | ND                   | 419                     |
|      |                   |                                  |                           | **                  | **                   | ns                      |
| 2012 | \(F_{6:10}\)      | 16.7                             | TH                        | 1.3                 | 73                   | 514                     |
|      |                   |                                  | TM                        | 2.0                 | 73                   | 492                     |

**Significant at \(P < 0.01\).
\(^a\) Lodging score: 0 (no lodging)–4 (completely lodged).
\(^b\) ns, non-significant at \(P < 0.05\).
\(^c\) ND, no data.

Table 5. Relationship between the marker genotype at qLS13-1 and agronomic traits in the RILs

| Year | Generation of RILs | Planting density (plants m\(^{-2}\)) | qLS13-1 genotype (Sat_313) | Lodging score\(^e\) | Main stem length (cm) | Seed yield (kg 10\(a\)^{1}\) |
|------|-------------------|----------------------------------|---------------------------|---------------------|----------------------|-------------------------|
| 2008 | \(F_{6:7}\)       | 25.0                             | TH                        | 2.4                 | 77                   | ND                      |
|      |                   |                                  | TM                        | 2.0                 | 73                   | ND                      |
|      |                   |                                  |                           | ns\(^b\)             | **                   |                         |
| 2009 | \(F_{6:8}\)       | 25.0                             | TH                        | 0.5                 | 60                   | 418                     |
|      |                   |                                  | TM                        | 0.4                 | 56                   | 390                     |
|      |                   |                                  |                           | ns                  | **                   | *                       |
| 2011 | \(F_{6:9}\)       | 16.7                             | TH                        | 1.8                 | ND                   | 424                     |
|      |                   |                                  | TM                        | 1.6                 | ND                   | 401                     |
|      |                   |                                  |                           | ns                  | **                   | *                       |
| 2012 | \(F_{6:10}\)      | 16.7                             | TH                        | 1.8                 | 76                   | 493                     |
|      |                   |                                  | TM                        | 1.4                 | 71                   | 521                     |

**,** Significant at \(P < 0.05\) and \(P < 0.01\), respectively.
\(^a\) Lodging score: 0 (no lodging)–4 (completely lodged).
\(^b\) ns, non-significant at \(P < 0.05\).
\(^c\) ND, no data.

Table 6. Relationship between the marker genotype at qLS19-1 and agronomic traits in the NILs (25.0 plants m\(^{-2}\); average in 2010 and 2011)

| Cultivar or line | qLS19-1 genotype (Sat_099) | Lodging score\(^e\) | Flowering time (days) | Maturing time (days) | Main stem length (cm) | No. of main stem nodes | No. of branches (plant\(^{-1}\)) | Seed yield (kg 10\(a\)^{1}\)) | 100-seed weight (g) |
|-----------------|---------------------------|---------------------|----------------------|---------------------|----------------------|------------------------|-----------------------------|-----------------------------|-----------------|
| TH              | TH                        | 0.7 \(d^b\)        | 57 a                 | 133 a               | 68 a                 | 10.1 a                 | 0.9 c                      | 393 b                       | 38.2 c          |
| TM              | TM                        | 2.6 a               | 57 a                 | 136 a               | 71 a                 | 10.1 a                 | 2.0 a                      | 431 ab                      | 38.6 c          |
| NIL-TH          | TH                        | 1.3 c               | 56 a                 | 131 a               | 68 a                 | 9.9 a                  | 1.5 b                      | 427 ab                      | 43.0 a          |
| NIL-TM          | TM                        | 1.9 b               | 55 a                 | 131 a               | 66 a                 | 9.9 a                  | 1.9 ab                     | 443 a                       | 40.7 b          |

\(^a\) Lodging score: 0 (no lodging)–4 (completely lodged).
\(^b\) Values within a trait with the same letters were not significantly different at \(P < 0.05\) (Tukey–Kramer multiple comparison test).

The stem length of TMBC2-1 was shorter than seen in TM (Table 7). The 100-seed weight of TMBC2-1 was heavier than that of TM (\(P < 0.05\); Table 7). Other agronomic traits, including flowering date, number of branches, and seed yield were similar in TMBC2-1, TMBC2-2, and TM (Table 7). These results suggested that the TH allele at Sat_099 promoted stronger LT, and rarely had a negative influence on seed yield in the BC lines.

**MAS for qLS19-1 in the T248 × TH background**

We developed breeding lines by MAS for qLS19-1 from a T248 × TH cross to investigate the effects of qLS19-1 in a different background. According to the marker genotypes, the genotype at the E1 to E4 loci of T248 was estimated as e1e2E3e4. T248 was determinate (\(dt1\) genotype). The frequency distribution of LS in the F\(_3\) lines is shown in Fig. 4. The average LSs of lines with the TH allele were lower than...
those with the T248 allele \((P < 0.01)\). In 2012, eight breeding lines with the TH allele at Sat_099 were tested. The LSs of the six lines were significantly lower than in T248 (Table 8). We obtained three breeding lines, 2129-2, 5, and 7; in these the LSs were significantly lower, and the yield significantly greater, than that of T248 (Table 8). The maturing times of 2129-2, 5, and 7 were 5 days shorter than observed in T248 (Table 8). This may be because T248 had severe lodging (Table 8) and a later maturing date.

**Discussion**

In previous studies, QTLs for LS frequently influenced other agronomic traits, such as flowering date, plant height, and determinate habit (Lee *et al*. 1996, Mansur *et al*. 1993, Orf *et al*. 1999, Specht *et al*. 2001). In this study, the qLS13-1 was not stable, and frequently had a negative influence on seed yield (Table 5). Matsukawa and Banba (1986) reported a positive correlation between main stem length and LS. The main stem lengths of RILs with TM alleles at Sat_313 were shorter than those with TH alleles in each year (Table 5). In fact, a QTL for main stem length was detected in the proximal region of qLS13-1 (data not shown). Therefore, we speculate that qLS13-1 may be a QTL for main stem length. In contrast, qLS19-1 was identified as a stable QTL, and rarely had a negative influence on seed yield or other agronomic traits (Tables 3, 4, 6, 7). No QTLs for main stem length were detected in the proximal region of qLS19-1 (data not shown). The MAS of qLS19-1 was also effective in the T248 × TH background (Fig. 4, Table 8). These results suggest that MAS for qLS19-1 will be of great use for improving LT in breeding programs.

Combine-harvesting loss through lodging of soybeans is estimated to be about 20% (Uchikawa *et al*. 2006). In this study, seed yield was determined by hand-harvesting. The

### Table 8. Agronomic traits of the F4 lines derived from the Toiku 248 (T248) × Toyoharuka (TH) cross (16.7 plants m\(^{-2}\); 2012)

| Cultivar or line | qLS19-1 genotype (Sat_099) | Lodging score\(^a\) | Flowering time (days) | Maturing time (days) | Main stem length (cm) | No. of main stem nodes | No. of branches (plant\(^{-1}\)) | Seed yield (kg 10a\(^{-1}\)) | 100-seed weight (g) |
|-----------------|-----------------------------|----------------------|-----------------------|----------------------|----------------------|------------------------|-----------------------------|------------------------|------------------|
| T248            | T248                        | 4.0                  | 65                    | 140                  | 82                   | 11.5                   | 1.6                         | 407                    | 43.0             |
| TH              | TH                          | 1.8*                 | 62                       | 137                 | 77                   | 136*                   | 1.3                         | 338                    | 43.1             |
| 2129-2          | TH                          | 2.8                  | 63*                   | 136                  | 85                   | 135*                   | 2.8                         | 489*                   | 47.5             |
| 2129-3          | TH                          | 1.0**               | 62**                  | 136                | 76                   | 135*                   | 2.3                         | 380                    | 42.7             |
| 2129-4          | TH                          | 1.5*                 | 61**                  | 136                  | 76                   | 135*                   | 2.3                         | 407                    | 43.0             |
| 2129-5          | TH                          | 2.8                  | 64                    | 138                 | 88                   | 135*                   | 2.8                         | 485*                   | 47.5             |
| 2129-7          | TH                          | 2.0*                 | 65                    | 135*                 | 91**                  | 135*                   | 2.3                         | 407*                   | 43.0             |
| 2129-8          | TH                          | 1.8*                 | 64                    | 134**              | 79                    | 135*                   | 2.3                         | 485*                   | 47.5             |

\(^a\) Lodging score: 0 (no lodging)–4 (completely lodged).

\(^*\) Significant at \(P < 0.05\) and \(P < 0.01\), respectively. Dunnett’s test was performed for each agronomic trait using T248 as the reference.
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Seed yields were similar in TM and TMBC lines (Table 7). We speculate that the combine-harvesting loss of TM might be greater than that of TMBC lines because TM had severe lodging (Table 7, Fig. 1D). In the future, combine-harvesting tests will be required to clarify whether TMBC lines yield more than TM or not.

The TH allele at Sat_099 promoted a heavier seed. The 100-seed weights of NIL-TH and TMBC2-1 were heavier than those of NIL-TM and TM, respectively (Tables 6, 7). We could not confirm whether a difference in the 100-seed weight was caused by the other gene linked to qLS19-1 or by pleiotropism of qLS19-1. In either case, heavier seeds are preferred for boiled-bean processing in Japan (Kato et al. 2014, Tanaka 2011). Therefore, we feel that the effect of the TH allele at Sat_099 on the 100-seed weight is not disadvantageous for breeding programs in Japan.

The number and distribution of branches in soybean affects LT (Sayama et al. 2010). The number of branches in TH was significantly less than in TM (Tables 3, 6, 7). However, the number of branches in the NIL-TH and BC lines was similar to that of NIL-TM and TM, respectively (Tables 6, 7). The number of branches in the BC lines was significantly greater than in TH although LT in the BC lines was comparable to that in TH (Table 7). Therefore, the effect of qLS19-1 could not be explained by the number of branches alone. Saito et al. (2012) reported that the number of branches was higher, and that branches compensated seed yield when plants lodged at the flowering stage. In this study, the number of branches was only measured at the maturing stage. Therefore, it will be important to investigate the number of branches after the flowering stage to clarify the relation between LT in TH and the number of branches.

The agronomic traits of TMBC2-1 and TMBC2-2 were slightly different (Table 7). It is possible that another genomic region might influence main stem length or number of main stem nodes in the BC lines. To confirm the effect of qLS19-1 more accurately, it will be important to develop NILs from TMBC lines with more BCs to TM.

The candidate genes of E3 and Dt1 have been reported and are considered to be linked (Liu et al. 2010, Watanabe et al. 2009). Previous studies reported that QTLs for LS were located in the E3 and Dt1 locus on Chr-19 (Lee et al. 1996, Mansur et al. 1993, Orf et al. 1999, Specht et al. 2001). These QTLs are recorded as Ldge 1-1, 4-2, 4-3, 8-4, and 9-5 in SoyBase (www.soybase.org). Moreover, the QTLs for traits associated with lodging, branch number, or max internode length also located to the proximal region of the E3 and Dt1 loci (Liu et al. 2007, Sayama et al. 2010). However, it was not determined whether genes responsible for these QTLs are closely linked to E3 and Dt1 or the pleiotropism of them. In this study, qLS19-1 was located in the proximal region of E3 and Dt1 (Table 3, Fig. 3A). However, the LOD score peak of qLS19-1 was located in the region upstream of E3 and Dt1 on Chr-19 (Fig. 3A), and the genotypes of the cultivars and breeding lines used in this study were E3 and dt1 (Table 1). Therefore, the gene responsible for qLS19-1 is unlikely to be either E3 or Dt1.

There have been numerous reports on QTLs associated with LT. Kashiwagi and Ishimaru (2004) identified a QTL for pushing resistance of the lower part in rice. Ootakawa et al. (2010) identified an effective QTL, and isolated the candidate gene for culm strength in rice. In soybean, Chen et al. (2011) reported QTLs associated with stem strength, and Sayama et al. (2010) identified QTLs for branch number. In this study, other QTLs may also be involved, as the distribution of LSs could not be explained by qLS19-1 alone (Fig. 2). To detect these other QTLs, it might prove effective to perform QTL analysis using the traits associated with LT.

In summary, we identified a stable QTL for LT. The TH allele at Sat_099 rarely had a negative influence on seed yield or other agronomic traits in both NILs and BC lines. Moreover, the TH allele at Sat_099 promoted a stronger LT in the T248 × TH background. Our results suggest that MAS for qLS19-1 is effective for improving LT in breeding programs.

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