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Quantitative trait loci associated with lodging tolerance in soybean cultivar 'Toyoharuka'

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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 agronomic 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 designated 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

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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 $F_{6:7}$ in 2008, $F_{6:8}$ in 2009, $F_{6:9}$ in 2011, and $F_{6:10}$ in 2012. Toiku 248, a modern breeding line with high lodging derived from the Toiku 239 × Toiku 238 cross, was used for MAS. Toiku 239 and Toiku 238 have the same origin, TM, in their pedigrees. All field tests were performed in the experimental field of TAES, located at the latitude 42°89′N. Fertilizer was applied according to Hokkaido fertilization standards (0.2 N–1.8 P_2O_5–0.9 K_2O–0.4 MgO kg a^{-1}).

Evaluation of lodging tolerance in the parents

TH and TM were planted on 22nd May 2008, 18th May 2009, 19th May 2010, and 19th May 2011. Each plot consisted of one (2008), two (2009), or four rows (2010 and 2011), with a length of 1.5 m (2008 to 2010) or 3.5 m (2011), spacing of 60 cm, and 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 the experiments. At the time of maturing, LS was recorded in each plot for LT as: 0 (no lodging) to 4 (completely lodged) (Matsukawa and Banba 1986, Saito *et al.* 2012). Before harvesting, ten central consecutive plants were selected from each plot for morphological measurement. Main stem length (distance from cotyledonary node to terminal node), number of main stem nodes, and the number of branches (branches with more than two nodes) were recorded for phenotypic evaluation. The Tukey–Kramer multiple comparison test was used to detect significant differences in agronomic traits among the cultivars. 'Cultivar' and 'year' were considered the two factors.

Evaluation of LT in RILs

The 192 RILs were planted on 22nd May 2008, 18th May 2009, 18th May 2011, and 22nd May 2012. Each RIL was planted in a plot consisting of 1.5 m row spaced 60 cm apart, with a plant interval of 6.7 or 10 cm; giving a plant population density of 25.0 (2008 and 2009) or 16.7 (2011 and 2012) plants m^{-2}. The order of the RILs was randomized in each year to eliminate confounding effects from neighboring RILs. At the time of maturity, LS was recorded in each plot. The average LS over the 4 years was used for QTL analysis. Before harvesting, ten central consecutive plants were selected from each plot for measurement of main stem length. A student's t-test was used to determine significant differences between genotypes.

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 $F_{6:9}$ 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 *Dt1* 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.

Evaluation of LT in NILs

Near-isogenic lines were developed from a RIL in which the genomic region of interest is segregated, with the other regions being fixed (Ikeda *et al.* 2009, Tuinstra *et al.* 1997, Yamanaka *et al.* 2005). In this study, NILs were developed from the RIL heterozygous at Sat_099, the nearest marker to a major QTL. DNA was extracted from the F_9 seeds. The seeds were genotyped at Sat_099, and sorted into TH, TM, and heterozygous genotypes. The F_9 progeny of TH and TM genotypes were named as NIL-TH and NIL-TM, respectively. The NILs generations were F_{10} in 2010, and F_{11} in 2011.

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_1F_1 plants with heterozygous genotypes at Sat_099 were backcrossed with TM. BC_2F_1 plants with heterozygous genotypes at Sat_099 were then selected. The BC_2F_2 plants were genotyped at Sat_099 and sorted into TH, TM, and heterozygous genotypes. The two BC_2F_3 lines with TH genotypes at Sat_099 were selected from the individual BC_2F_1 plants. These lines were named as TMBC2-1 and TMBC2-2. The BC_2 generation was F_4 in 2012 and F_5 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 sorted into T248, TH, and heterozygous genotypes in 2010. All F_2 plants with TH (18 plants) or T248 (22 plants) alleles were harvested. Therefore, the F_3 lines were developed by MAS. All 40 F_3 lines were planted on 19th May 2011. Each plot consisted of a 3 m row spaced at 60 cm, with a plant interval of 6.7 cm: giving a plant population density of 25.0 plants m^{-2}. LS was recorded in each plot at the time of maturing. A student's t-test was used to determine significant differences between the genotypes. In 2012, only the F_4 lines with TH allele were tested. Eight lines 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, *e1e2E3E4* (Table 1). The determinate genotypes of both TH and TM were *dt1* (Table 1). These results suggested that LT in the RIL population could be evaluated without the effects of the *E1*, *E2*, *E3*, *E4* and *Dt1* 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,

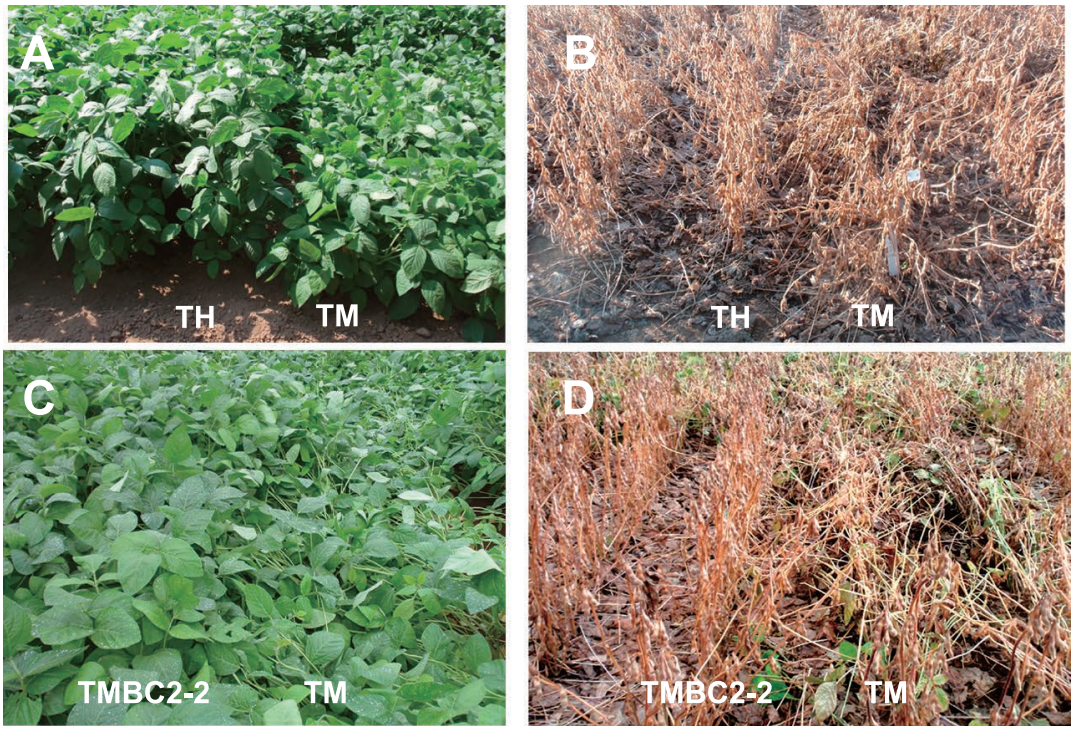

Fig. 1. Representative plants in the field. (A) Shape of the Toyoharuka (TH) and Toyomusume (TM) plants used to create RILs. The photograph was taken on 28th July 2011. (B) TH and TM at harvesting time. The photograph was taken on 5th October 2011. (C) TMBC2-2, a backcrossed line containing the TH genotype at Sat_099, and TM. The photograph was taken on 7th August 2012. (D) TMBC2-2 and TM at harvesting time. The photograph was taken on 12th October 2012.

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	
					E loci	Determinate
TH	0.7	67	9.9	18.8	*e1e2E3E4*	*dt1*
TM	2.2	68	9.9	53.6	*e1e2E3E4*	*dt1*
	**	ns[b]	ns	**		

** Significant at $P < 0.01$.

[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 $P < 0.001$.

qLS19-1 and *qLS13-1*, were detected on chromosome-19 (Chr-19) (MLG-L) and Chr-13 (MLG-F), respectively (Table 3). The *qLS19-1* and *qLS13-1* loci had LOD scores of 11.4 and 3.7, respectively (Table 3). The TH allele at *qLS19-1* promoted a stronger LT (Table 3). The LOD score peak of *qLS19-1* was located at Sat_099 (Fig. 3A). The LOD score peak of *qLS13-1* was located in the region

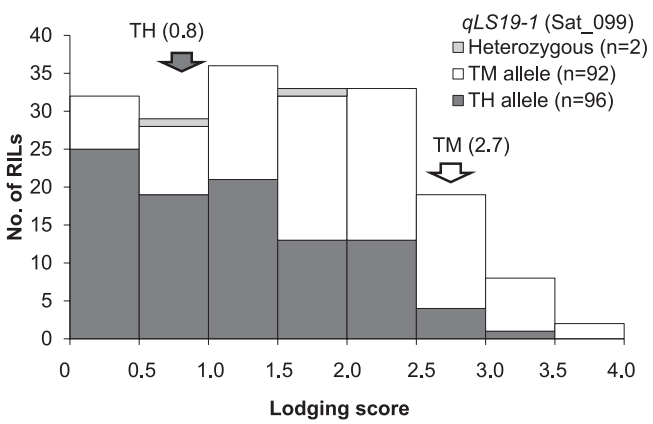

Fig. 2. Frequency distribution of the average lodging score over four years in the RILs. The lodging scores (LSs) of Toyoharuka (TH) and Toyomusume (TM) are shown in parentheses using a LS scale of 0 (no lodging)–4 (completely lodged).

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.

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

Chr (LG)[a]	Position (cM)	Nearest marker	LOD[b]	R^2 (%)[c]	Additive effect[d]	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*

[a] Chr, chromosome; LG, linkage group.

[b] 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.

[c] Percentage phenotypic variance explained by the QTL.

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

Evaluation of LT in backcrossed lines

The BC_2 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

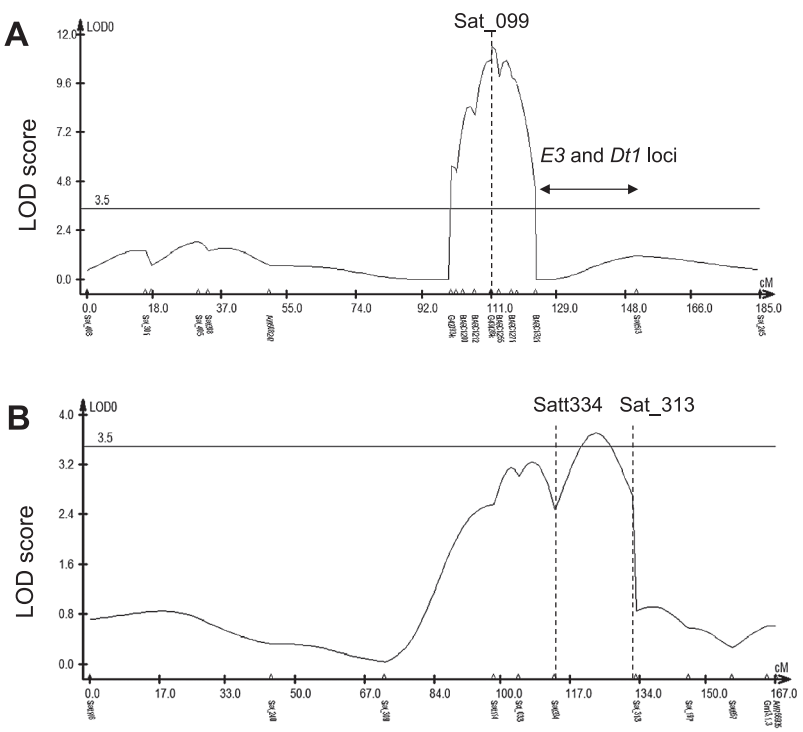

Fig. 3. LOD score plot of QTLs associated with lodging score in the RILs. (A) *qLS19-1* located on Chr-19. (B) *qLS13-1* located on Chr-13. The LOD threshold value at the 5% probability level was 3.5. Arrows show the location of the *E3* and *Dt1* loci.

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[a]	Main stem length (cm)	Seed yield (kg $10a^{-1}$)
2008	$F_{6:7}$	25.0	TH	1.6	74	ND[c]
			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
				**	ns	ns

**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[a]	Main stem length (cm)	Seed yield (kg $10a^{-1}$)
2008	$F_{6:7}$	25.0	TH	2.4	77	ND[c]
			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
				*	**	ns

*,** 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[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)
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).

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

Table 7. Relationship between the marker genotype at *qLS19-1* and agronomic traits in the backcrossed lines (16.7 plants m^{-2}; average in 2012 and 2013)

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)
TH	TH	2.0 b[b]	55 a	130 b	82 a	11.5 a	1.6 b	407 a	43.0 b
TM	TM	3.6 a	54 a	135 a	78 ab	10.6 b	2.8 a	338 b	43.1 b
TMBC2-1	TH	1.7 b	54 a	133 ab	69 c	9.8 c	2.3 a	349 b	47.5 a
TMBC2-2	TH	1.3 b	54 a	131 b	71 bc	9.7 c	2.3 a	380 ab	42.7 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).

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.

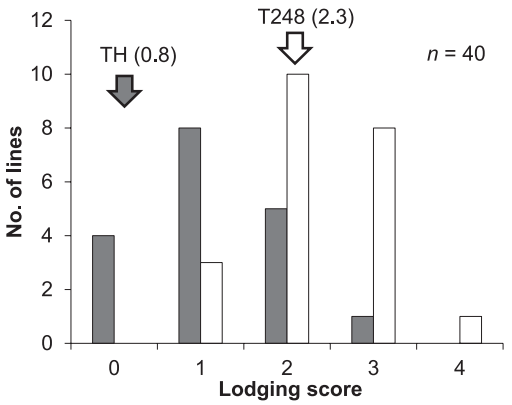

Fig. 4. The effect of *qLS19-1* on lodging tolerance in the Toiku 248 background. Frequency distribution of the lodging score (LS) in F_3 lines derived from a Toiku 248 (T248) × Toyoharuka (TH) cross in 2011. LSs of the parental lines are shown in parentheses. LS scale: 0 (no lodging)–4 (completely lodged). The average LS of lines with either TH or T248 alleles was 1.3 or 2.3, respectively ($P < 0.01$). Shaded bars: lines with the TH allele at Sat_099 ($n = 18$). White bars: lines with the T248 allele at Sat_099 ($n = 22$). The plant population density was 25.0 plants m^{-2}.

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 F_4 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)	Seed yield (kg $10a^{-1}$)
T248	T248	4.0	65	140	82	348
TH	TH	1.8*	62**	137	77	473*
2129-1	TH	1.8*	62**	136*	78	451
2129-2	TH	1.8*	60**	135*	90*	465*
2129-3	TH	2.8	63*	136	85	489*
2129-4	TH	1.0**	62**	136	76	444
2129-5	TH	1.5*	61**	135*	72**	485*
2129-6	TH	2.8	64	138	88	450
2129-7	TH	2.0*	65	135*	91**	476*
2129-8	TH	1.8*	64	134**	79	435

[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.

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 yields 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 effects 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. Ookawa *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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