Note

Identification and validation of quantitative trait loci associated with seed yield in soybean

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In soybean [*Glycine max* (L.) Merrill], the genetic analysis of seed yield is important to aid in the breeding of high-yielding cultivars. Seed yield is a complex trait, and the number of quantitative trait loci (QTL) involved in seed yield is high. The aims of this study were to identify QTL associated with seed yield and validate their effects on seed yield using near-isogenic lines. The QTL analysis was conducted using a recombinant inbred line population derived from a cross between Japanese cultivars 'Toyoharuka' and 'Toyomusume', and eight seed yield-associated QTL were identified. There were significant positive correlations between seed yield and the number of favorable alleles at QTL associated with seed yield in the recombinant inbred lines for three years. The effects of *qSY8-1*, a QTL promoting greater seed yield, was validated in the Toyoharuka background. In a two-year yield trial, the 100-seed weight and seed yield of Toyoharuka-NIL, the near-isogenic line having the Toyomusume allele at *qSY8-1*, were significantly greater than those of Toyoharuka (106% and 107%, respectively) without any change for days to flowering and maturity. Our results suggest that *qSY8-1* was not associated with maturity genes, and contributed to the 100-seed weight.

Key Words: soybean, seed yield, 100-seed weight, quantitative trait locus.

Introduction

Soybean [*Glycine max* (L.) Merrill] is an important protein and oil crop. The genetic analysis of seed yield is important to aid in the breeding of high-yielding cultivars. Seed yield is a complex trait. The number of bi-parental quantitative trait loci (QTL) for seed yield in the soybean database SoyBase (Grant *et al.* 2010, https://www.soybase.org/) was 165 in June 2020. Many QTL have been detected only in a single population, and the effects of each QTL are small in many cases. In soybean, seed yield consists of three main components: pod number, seed number per pod, and 100-seed weight. The genetic factors that affect these yield components in soybean have been reported (Fujii *et al.* 2018, Hoeck *et al.* 2003, Hyten *et al.* 2004, Kato *et al.* 2014, Yan *et al.* 2014). In particular, numerous QTL associ-

ated with 100-seed weight have been reported, and over 250 QTL are registered in the SoyBase.

Lodging tolerance is an important trait that affects yield in soybean (Cooper 1971, Weber and Fehr 1966, Woods and Swearingin 1977). In our previous study, a major QTL for lodging score on chromosome (Chr) 19, *qLS19-1*, was identified using recombinant inbred lines (RILs) from a cross between 'Toyoharuka' and 'Toyomusume' (Yamaguchi *et al.* 2014). The Toyoharuka allele at *qLS19-1* had positive effects on lodging tolerance (Yamaguchi *et al.* 2014). Using the same RIL population, Ohnishi *et al.* (2011) detected a QTL for tolerance to seed coat discoloration induced by chilling temperature, and Ikeda *et al.* (2009) detected a QTL for chilling tolerance at the flowering stage. Both QTL are located in the proximal region of the *I* locus, and the Toyoharuka allele, which was designate *Ic* allele, had positive effects on chilling tolerance. These two loci would be useful to improve seed quality and seed yield with reducing lodging. The aims of this study were to identify QTL associated with seed yield using a RIL population derived from a cross between Toyoharuka and Toyomusume,

and to validate their effects on seed yield using near-isogenic lines. We also discuss the additional use of *qLS19-1* and *Ic* alleles with seed yield QTL detected in this study for further soybean breeding program.

Materials and Methods

Plant materials

An RIL population (192 lines) was bred using the single-seed descent method from a Toyoharuka × Toyomusume cross (Ikeda *et al.* 2009, Ohnishi *et al.* 2011). The parent cultivars and RILs were bred at the Tokachi Agricultural Experiment Station, Memuro, Hokkaido, Japan. They all have the determinate growth habit phenotype. The generations of the RIL population were F_8 in 2009, F_9 in 2011, and F_{10} in 2012. Toyoharuka-NIL, the near-isogenic line for *qSY8-1* (**Table 1**) derived from Toyoharuka (recurrent parent) × Toyomusume (donor parent of *qSY8-1*), was bred by the backcrossing method using the Ic marker, the marker closest to this QTL, as described previously (Yamaguchi *et al.* 2019). The generations of Toyoharuka-NIL were BC_4F_4 and BC_4F_5 in 2010 and 2011, respectively.

Evaluation of RIL seed yields

The field tests in 2009 and 2011 were performed at Tokachi Agricultural Experiment Station, located at latitude 42°89′ N and longitude 140°07′ E. The field test in 2012 was performed at the Central Agricultural Experiment Station, located at latitude 43°05′ N and longitude 141°75′ E. The 192 RILs were planted on 18 May in 2009, 18 May in 2011, and 22 May in 2012. Each plot consisted of one (in 2011 and 2012) or two rows (in 2009). Each RIL was planted in a plot consisting of 1.5-m rows spaced 60-cm apart, with a plant interval of 6.7 (in 2009) and 10 cm (in 2011 and 2012), resulting in plant population densities of 25.0 (in 2009) and 16.7 (in 2011 and 2012) plants m^{-2}. The order of the RILs was randomized in each year to eliminate confounding effects from neighboring RILs. The 24 (in 2009) or 12 (in 2011 and 2012) plants in each plot were harvested by hand. Pearson's correlation coefficient was determined between seed yields or 100-seed weights for each pair of years.

The broad-sense heritability values for seed yield and 100-seed weight were calculated using data from 2009. The environmental variance was calculated on the basis of the parental traits (four replicates). The phenotypic variance was calculated on the basis of the 170 RILs' traits. The broad-sense heritability was calculated as follows:

Broad-sense heritability = 1 – (Environmental variance)/ (Phenotypic variance).

Molecular marker analysis and linkage mapping

DNA extraction and PCR analyses of the markers were performed as described previously (Hwang *et al.* 2009, Sayama *et al.* 2010). The F_6 RIL plants were used for geno-typing with simple sequence repeat (SSR) markers. Polymorphisms in SSR markers were analyzed using the SSR genotyping panel system (Sayama *et al.* 2011). The previously reported linkage map (Yamaguchi *et al.* 2014) was reconstructed using 149 co-dominant markers. The computer program AntMap Ver. 1.2 (Iwata and Ninomiya 2006) was used to determine molecular linkage groups and the positions of the polymorphic markers. AntMap seeks the linear order of loci that minimizes the sum of adjacent recombination fractions or that maximizes the log likelihood of locus order.

QTL analysis for seed yield and 100-seed weight

A QTL analysis was performed using the composite interval mapping method (Zeng 1994) in QTL Cartographer version 2.5 (Wang *et al.* 2007). The logarithm of the odds (LOD) threshold value at the 5% probability level was calculated using 500 permutations. We defined QTL detected in different years at a similar position and having a genetic distance between QTL peaks of less than 10 cM on a Chr, as the same QTL.

Pearson's correlation coefficient was determined between seed yield and the number of favorable alleles at each QTL associated with seed yield in the RILs in each year. In the high-yielding RILs, the percentage of favorable alleles was calculated as follows:

Rate of the favorable alleles (%) = (Number of the favorable alleles)/(Number of the favorable and unfavorable alleles) × 100.

The percentage of genomic regions originating from Toyomusume was calculated using the 149 polymorphic

Table 1. Correlation coefficients between seed yields or 100-seed weights of the recombinant inbred soybean lines for each pair of trial years

Trait	Year	Seed yield			100-seed weight		
		2009	2011	2012	2009	2011	2012
Seed yield	2009	–			0.188*	–	–
	2011	0.490***	–		–	0.120	–
	2012	0.409***	0.526***	–	–	–	0.164*
100-seed weight	2009	0.188*	–	–	–		
	2011	–	0.120	–	0.642***	–	
	2012	–	–	0.164*	0.520***	0.636***	–

* Significant at $P < 0.05$.
*** Significant at $P < 0.001$.

markers as follows:

Genomic regions originating from Toyomusume (%) = (Number of Toyomusume alleles)/(number of Toyoharuka and Toyomusume alleles) × 100.

Yield trials of Toyoharuka-NIL

The yield trials of Toyoharuka-NIL were conducted at the Tokachi Agricultural Experiment Station in 2010 and 2011. Parental cultivar Toyoharuka was used as the control. Seeds were planted on 18 May in 2010 and 17 May in 2011. Each plot consisted of three rows with lengths of 3.5 m. The plot area was $6.3\,m^2$. These were spaced 60-cm apart with a 20-cm inter-hill having two plants per hill. This resulted in a plant population density of 16.7 plants m^{-2}. A randomized complete block design with three replicates was used. The time of flowering was defined as the time at which more than 50% of the plants in the plot were flowering. The time of maturity was defined as the time when more than 80% of the plants defoliated and turned yellow, with pods rattling when shaken. At the time of maturity, a lodging score from 0, indicating no lodging, to 4, indicating completely lodged, was recorded for each plot. The 72 plants in each plot were harvested by hand, and total above-ground yield (including pod, stem, branch, and seed) and seed yield were recorded for each plot. Seed yield was adjusted to 15% moisture.

A combined analysis of variance was performed using the mixed model procedure of the JMP 10 statistical package (SAS Institute 2012). Statistical significance was evalu-ated at $P \leq 0.05$, unless otherwise stated. Years and replications within year were considered random effects, whereas cultivars were considered fixed effects.

Results

Evaluation of seed yield and 100-seed weight in the RIL population

There were significant positive correlations between seed yield of the RILs in each pair of years (**Table 1**). The seed yields were normally distributed, and transgressive segre-gation occurred in each year (**Fig. 1**). This result indicated that it is possible to select lines producing yield greater than the parental cultivars from this population. The 100-seed weights, one yield component, were also evaluated in the RILs. There were significant positive correlations between 100-seed weights of the RILs in each pair of years (**Table 1**). However, there were only weak positive correlations between the seed yield and 100-seed weight in each year (**Table 1**). The broad-sense heritability values for seed yield and 100-seed weight were calculated as 0.91 and 0.85, respectively, indicating their high heritability levels.

QTL analysis of seed yield and 100-seed weight

The resulting genetic linkage map comprised 23 molecu-lar linkage groups and covered 1,335 cM. Chr-8, -12 and -19 were divided into two linkage groups each, because Toyoharuka was very closely related to Toyomusume based
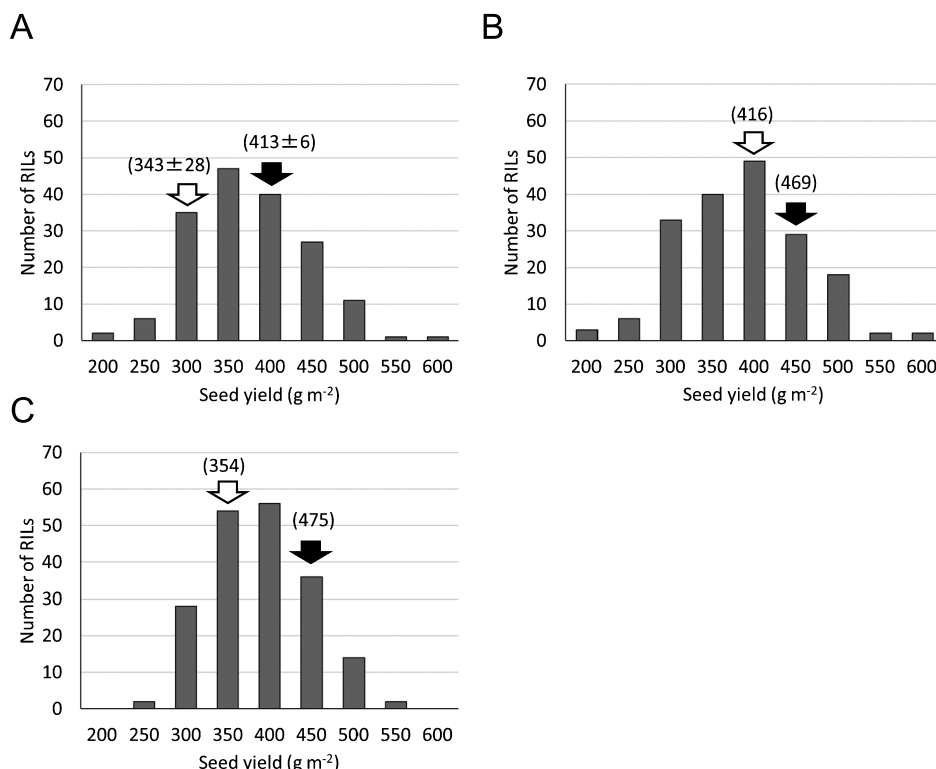
Fig. 1. Frequency distribution of seed yield in each year. (A) 2009. (B) 2011. (C) 2012. Black and white arrows indicate the seed yields of Toyomusume and Toyoharuka, respectively. The seed yields of the parental cultivars are shown in parentheses (average ± standard deviation).

on a clustering analysis (Hwang *et al.* 2008), and no polymorphic markers were found in some regions. The average number of marker loci in each molecular linkage group was 6.5, and the average distance between markers was 9.0 cM.

The results of the QTL analysis of seed yield are shown in **Table 2** and **Supplemental Fig. 1**. In total, eight QTL were detected. The relationships between the closest marker to each QTL and seed yield in the RILs are shown in **Supplemental Table 1**. A QTL analysis of 100-seed weight was also performed, and 13 QTL were detected (**Table 3**). Only three QTL associated with 100-seed weight, *qSW5-1*, *qSW8-1*, and *qSW11-1*, were identified

near QTL for seed yield (**Tables 2**, **3**). This result was corroborated by a weak correlation between seed yield and 100-seed weight (**Table 1**). The Toyomusume alleles at *qSW5-1* and *qSW8-1* promoted heavier seeds, resulting in greater seed yields (**Tables 2**, **3**). In contrast, the Toyoharuka allele at *qSW11-1* promoted heavier seeds, but resulted in a low yield (**Tables 2**, **3**).

There were significant positive correlations between seed yield and the number of favorable alleles at QTL associated with seed yield in the RILs for three years (**Fig. 2**). The 12 RILs produced higher yields than their parental cultivars in each year (**Table 4**), and they were considered as stable

Table 2. Quantitative trait locus (QTL) analysis of seed yield in the recombinant inbred soybean lines

Chr[a]	Year	Location	Position (cM)	Nearest marker	LOD[b]	$R^{2\,c}$ (%)	Favorable allele	Additive effect[d] (g m^{-2})	QTL name
5	2011	Tokachi	3.0	Sat_267	6.9	14.7	Toyomusume	27.9	*qSY5-1*
	2012	Central	19.5	Sat_271	3.3	6.4	Toyomusume	15.2	*qSY5-2*
8-1	2009	Tokachi	44.2	Ic	9.0	17.7	Toyomusume	26.9	*qSY8-1*
	2011	Tokachi	40.2	Ic	4.3	9.0	Toyomusume	22.9	*qSY8-1*
11	2012	Central	26.9	Satt519	7.0	15.2	Toyomusume	23.3	*qSY11-1*
13	2009	Tokachi	43.8	Sct_033	3.5	7.5	Toyoharuka	19.4	*qSY13-1*
	2011	Tokachi	76.8	Sat_197	6.9	15.7	Toyoharuka	28.9	*qSY13-2*
14	2009	Tokachi	23.4	BARCSOYSSR_14_0370	4.0	7.3	Toyomusume	18.9	*qSY14-1*
	2012	Central	23.4	BARCSOYSSR_14_0370	3.4	6.4	Toyomusume	15.2	*qSY14-1*
18	2009	Tokachi	6.7	Satt594	3.6	6.7	Toyomusume	18.2	*qSY18-1*

[a] Chr, chromosome. Chr-8 was divided into two linkage groups.
[b] LOD, logarithm of odds determined by composite interval mapping. The LOD threshold value at the 5% probability level was 3.1 in 2009, and 3.2 in 2011 and 2012. The LOD threshold value at the 1% probability level was 4.4 in 2009, 4.1 in 2011, and 3.9 in 2012.
[c] Percentage phenotypic variance explained by the QTL.
[d] Effect of the favorable allele promoting a greater seed yield on the QTL (g m^{-2}).

Table 3. Quantitative trait locus (QTL) analysis of 100-seed weight in the recombinant inbred soybean lines

Chr[a]	Year	Location	Position (cM)	Nearest marker	LOD[b]	$R^{2\,c}$ (%)	Favorable allele	Additive effect[d] (g)	QTL name
2	2011	Tokachi	114.7	Sat_237	3.3	1.0	Toyomusume	0.3	*qSW2-1*
5	2011	Tokachi	12.5	Satt200	3.6	3.0	Toyomusume	0.5	*qSW5-1*
8-1	2009	Tokachi	45.2	Ic	8.2	4.5	Toyomusume	0.7	*qSW8-1*
9	2009	Tokachi	1.0	GMES5734	8.1	4.4	Toyoharuka	0.5	*qSW9-1*
9	2009	Tokachi	23.2	Satt264	8.0	3.4	Toyomusume	0.4	*qSW9-2*
10	2009	Tokachi	7.0	Gm10.1.1	8.8	9.0	Toyomusume	0.8	*qSW10-1*
	2012	Central	7.9	Gm10.1.1	4.0	7.3	Toyomusume	0.7	*qSW10-1*
11	2011	Tokachi	17.9	GMES0027	4.3	9.7	Toyoharuka	0.9	*qSW11-1*
	2009	Tokachi	21.9	GMES0027	9.8	8.6	Toyoharuka	0.7	*qSW11-1*
	2012	Central	29.9	Satt519	9.5	22.4	Toyoharuka	1.4	*qSW11-1*
12-2	2009	Tokachi	1.0	Sat_206	3.5	2.3	Toyomusume	0.4	*qSW12-1*
13	2009	Tokachi	57.8	Sat_313	7.2	3.6	Toyoharuka	0.6	*qSW13-1*
14	2012	Central	69.2	Satt556	3.7	7.5	Toyoharuka	0.8	*qSW14-1*
	2011	Tokachi	92.0	Sat_424, Satt726	4.5	4.7	Toyoharuka	0.6	*qSW14-2*
19-1	2011	Tokachi	19.5	Sat_301	3.3	2.2	Toyoharuka	0.5	*qSW19-1*
19-2	2011	Tokachi	20.8	BARCSOYSSR_19_1286	4.2	5.3	Toyoharuka	0.6	*qSW19-2*

[a] Chr, chromosome. Chr-8, 12, and -19 were divided into two linkage groups for each.
[b] LOD, logarithm of odds determined by composite interval mapping. The LOD threshold value at the 5% probability level was 7.1 in 2009, 3.3 in 2011, and 3.2 in 2012. The LOD threshold value at the 1% probability level was 8.3 in 2009, and 3.9 in 2011 and 2012.
[c] Percentage phenotypic variance explained by the QTL.
[d] Effect of the favorable allele promoting a heavier seed on the QTL (g).
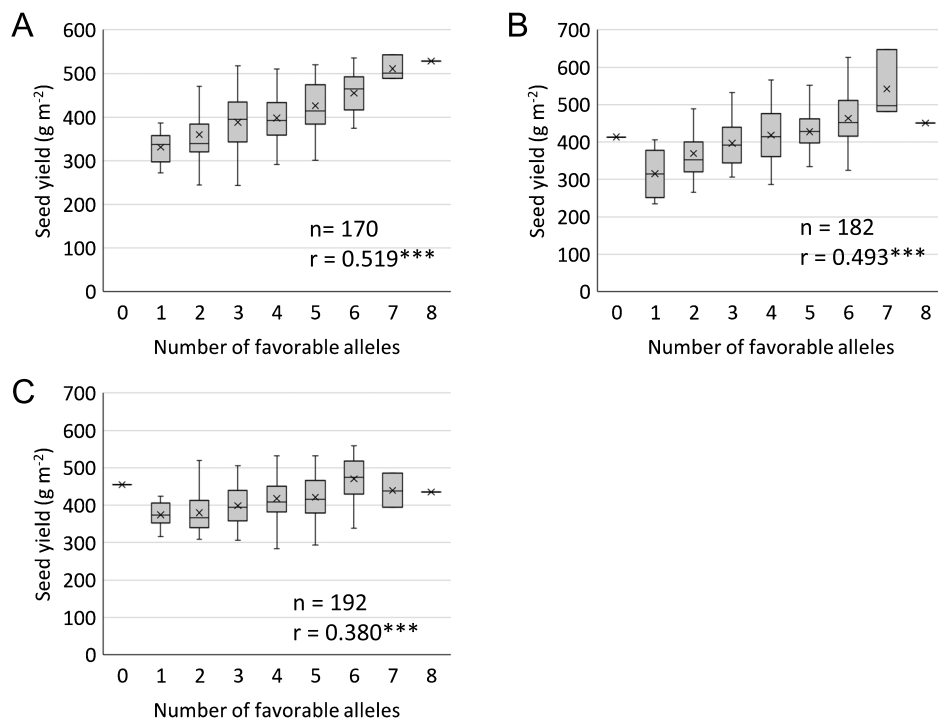
Fig. 2. Box plot of seed yield in the recombinant inbred lines for each trial year. Crosses (×) indicate the average values. (A) 2009. (B) 2011. (C) 2012. Pearson's correlation coefficient was determined between seed yield and the number of favorable alleles at each QTL associated with seed yield in each year. *** Significant at $P < 0.001$.

Table 4. The 12 recombinant inbred lines (RILs) producing higher yields than their parental cultivars in each year

| Cultivar/line | Alleles at QTL associated with seed yield[a] | | | | | | | | Number of favorable alleles | qLS19-1 allele[b] | Genomic regions originating from Toyomusume (%)[c] | Seed yield (g m⁻²) | | | |
	qSY5-1	qSY5-2	qSY8-1	qSY11-1	qSY13-1	qSY13-2	qSY14-1	qSY18-1				2009	2011	2012	Average
Toyoharuka	U	U	U	U	F	F	U	U	2	F	0	354	416	343	371
Toyomusume	F	F	F	F	U	U	F	F	6	U	100	475	469	413	452
RIL-109	F	U	F	F	F	F	F	F	7	U	60	486	647	543	559
RIL-57	F	F	F	F	U	F	U	F	6	U	65	559	626	485	557
RIL-100	F	F	U	F	F	F	F	U	6	U	57	518	503	535	519
RIL-112	F	F	F	F	F	U	U	F	6	U	63	525	540	477	514
RIL-167	F	F	F	U	F	U	F	F	6	U	61	550	475	477	501
RIL-42	F	F	F	F	U	U	F	F	6	U	71	517	509	462	496
RIL-181	F	F	U	F	F	F	U	F	6	F	43	489	548	416	484
RIL-96	S	F	F	F	F	U	U	F	5	U	57	480	552	520	517
RIL-29	F	F	U	F	F	F	S	U	5	F	33	496	478	414	463
RIL-34	F	S	S	F	U	F	U	F	4	F	42	500	516	495	504
RIL-86	F	U	U	F	F	F	U	U	4	F	47	527	565	421	504
RIL-18	U	U	F	F	U	F	U	U	3	F	42	482	532	518	511
Rate of the favorable alleles (%)	91	73	64	92	67	67	36	67		42					

[a] The closest markers to the peak positions of each QTL were used. F, the favorable allele. U, the unfavorable allele. S, segregation. Rate of the favorable alleles (%) = (Number of the favorable alleles)/(Number of the favorable and unfavorable alleles) × 100.
[b] The Toyoharuka allele at qLS19-1 has positive effects on lodging tolerance (Yamaguchi et al. 2014).
[c] Genomic regions originating from Toyomusume (%) = (Number of Toyomusume alleles)/(Number of Toyoharuka and Toyomusume alleles) × 100. The 149 polymorphic markers were used for calculation.

high-yielding RILs. In the high-yielding RILs, the rate of the favorable alleles at *qSY14-1* (36%) was low compared to the other QTL (64–92%) (**Table 4**). This result indicates that the effect of *qSY14-1* may be low or unstable in the

high-yielding RILs. In contrast, the high rates of the favorable alleles at *qSY5-1* and *qSY11-1* suggest that these QTL may be useful for high-yielding breeding.

Table 5. Yield trials of the near-isogenic line for *qSY8-1* (means in 2010 and 2011)

Cultivar/line	*qSY8-1* allele	Days to flowering (days)	Days to maturing (days)	Main stem length (cm)	Lodging score[a]	100-seed weight (g)	Total above-ground yield (t ha⁻¹)	Seed yield (t ha⁻¹)
Toyoharuka	Toyoharuka	58	132	64	0.3	38.2	6.61	3.68
Toyoharuka-NIL	Toyomusume	58	133	72	1.0	40.6	7.16	3.94
		ns[b]	ns	*	ns	**	*	*

[a] Lodging score: 0 (no lodging)–4 (completely lodged).

[b] ns, not significant.

  * Significant at $P < 0.05$.

** Significant at $P < 0.01$.

Validation of the effect of qSY8-1 on seed yield and 100-seed weight

The *qSW8-1* was detected in the proximal region of *qSY8-1* (**Tables 2**, **3**). To confirm the effects of *qSY8-1*, Toyoharuka-NIL was developed. In a two-year yield trial, main stem length, total above-ground yield, seed yield, and 100-seed weight were significantly different between Toyoharuka and Toyoharuka-NIL (**Table 5**). The 100-seed weight and seed yield of Toyoharuka-NIL were significantly greater than those of Toyoharuka (106% and 107%, respectively) (**Table 5**). Main stem length of Toyoharuka-NIL was significantly greater than that of Toyoharuka (**Table 5**). Days to flowering, days to maturity, and lodging score were not significantly different between Toyoharuka and Toyoharuka-NIL (**Table 5**). Thus, the effects of *qSY8-1* were validated in the Toyoharuka background, and *qSY8-1* contributes to the 100-seed weight.

Discussion

Seven QTL associated with seed yield, except *qSY8-1*, detected in this study appear to be located in the proximal regions of previously reported loci (**Supplemental Table 2**). Six QTL associated with 100-seed weight located near *qSY8-1* have been reported (Han *et al.* 2012, Hoeck *et al.* 2003, Kim *et al.* 2010, Kulkarni *et al.* 2017, Orf *et al.* 1999, Yang *et al.* 2011). However, their effects on seed yield have been not validated in yield trials. The Toyomusume allele at *qSY8-1* had positive effects on seed yield and 100-seed weight compared with the Toyoharuka allele in the Toyoharuka background (**Table 5**). Main stem length and total above-ground yield of Toyoharuka-NIL were significantly greater than those of Toyoharuka although there were no differences in days to flowering and maturity between them (**Table 5**). These results indicate that the vegetative growth of Toyoharuka-NIL may be more vigorous than that of Toyoharuka. Therefore, we hypothesize that the vigorous plant growth in Toyoharuka-NIL may improve the source ability for bigger seed size. In our previous study, the near-isogenic line for *qSY8-1* was bred from Toyoharuka (having unfavorable allele at *qSY8-1*) × Toyomizuki (recurrent parental cultivar) by the backcrossing method (Yamaguchi *et al.* 2019). In a three-year yield trial,

the Toyoharuka allele at *qSY8-1* did not have negative effects on seed yield and 100-seed weight compared with the Toyomizuki allele (Yamaguchi *et al.* 2019). Evaluations of *qSY8-1* in different backgrounds and more environments are needed to characterize the QTL's stability.

The *qSY8-1* was detected in the proximal region of the *I* locus, which controls seed coat pigmentation in soybean (**Table 2**). In our previous study, the Toyoharuka allele at the *I* locus had positive effects on tolerance to seed coat discoloration induced by chilling temperature compared to the Toyomusume allele (Ohnishi *et al.* 2011, Yamaguchi *et al.* 2019). Thus, marker-assisted selection for the Toyomusume allele at *qSY8-1* may be advantageous for high-yielding but disadvantageous for tolerance to seed coat discoloration in breeding programs. We could not confirm whether *qSY8-1* was closely linked to the *I* locus or pleiotropism of the *I* locus. Fine mapping and gene isolation for *qSY8-1* may clarify the relationship between them in the future.

Days to flowering and maturity, as well as the seed filling period, affect the seed yield of soybean (Cober and Morrison 2010, Yamada *et al.* 2012). In total, 11 maturity genes, *E1–E11*, controlling flowering time and maturity, have been identified in soybean (Cober *et al.* 2010, Grant *et al.* 2010, Kong *et al.* 2014, Samanfar *et al.* 2017, Wang *et al.* 2019). The QTL controlling maturity or the reproductive stage have been identified in the proximal regions of *qSY11-1* and *qSY18-1* (Komatsu *et al.* 2012, Wang *et al.* 2015, Yao *et al.* 2015). Ikeda *et al.* (2009) also detected a QTL for maturity in the proximal region of *qSY11-1* in the RIL population from a Toyoharuka × Toyomusume cross. Therefore, *qSY11-1* may be pleiotropic. Both *qSY8-1* and maturity gene *E10* (Samanfar *et al.* 2017) were identified on Chr-8, but the physical distance between *qSY8-1* and *E10* was estimated to be over 38 megabases (Mb) (*qSY8-1*, 5.7 to 8.2 Mb; *E10*, 46.6 Mb). In fact, days to flowering and maturity for Toyoharuka-NIL having the Toyomusume allele at *qSY8-1* were similar to those for Toyoharuka (**Table 5**). Thus, *qSY8-1* was not associated with maturity genes.

In the high-yielding RILs, the percentage of genomic regions originating from Toyomusume varied from 33% to 71% (**Table 4**). A few high-yielding RILs had only two or

three favorable alleles at QTL associated with seed yield (**Table 4**). These results indicated that more QTL associated with seed yield exist in Toyoharuka and Toyomusume. To detect more of these QTL, it is necessary to increase the recombination events in the population with large size population, and to develop a new experimental population segregated undetected QTL.

There were significant positive correlations between seed yield and the number of favorable alleles at QTL associated with seed yield (**Fig. 2**). In the high-yielding RILs, 7 out of 12 RILs had 6 or 7 favorable alleles at QTL associated with seed yield (**Table 4**). Thus, pyramiding the six favorable alleles or more may be effective for producing high-yielding soybean lines. Soybean plants with higher above-ground weights are easily lodged (Shimada *et al.* 2002). The effect of *qSY8-1* originated from Toyomusume indicated that the higher above-ground weights would be useful to enhance yield. The chromosomal location of *qLS19-1*, the QTL for lodging tolerance (Yamaguchi *et al.* 2014), is different from seed yield QTL detected in this study. Therefore, to confirm the pyramiding effects on seed yield, it is necessary to develop and evaluate the pyramiding lines having favorable alleles at QTL associated with seed yield and *qLS19-1* in the future.

Author Contribution Statement

NY designed research. NY and FK conducted the field experiments. FT, YS, MS, and MI conducted the DNA marker experiments. NY, FT, YS, and FK analyzed the data. NY wrote the manuscript. All authors read and approved the manuscript.

Acknowledgments

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