Introduction

Single seed weight (SSW), or seed size, is a seed yield component (SYC) in soybean, and it is suggested that the genetic factors regulating SSW are involved in the control of other SYCs. The quantitative trait loci (QTLs) for SSW and their effects on the other SYCs were investigated using a recombinant inbred line population derived from typical small- and large-seeded cultivars that were cultivated in two different environments. QTL analysis detected four environmentally stable QTLs for SSW, two of which coincided with the defined loci, \( q_{Sw17-1} \) and \( Ln \). The effects of the other loci, \( q_{Sw12-1} \) and \( q_{Sw13-1} \), were confirmed by analyzing residual heterozygous line progenies derived from the recombinant population. These four QTL regions were also involved in the control of an additional SYC, namely the large-seeded allele at each locus that reduced either the number of pods per plant or the number of ovules per pod. These results suggest the presence of at least two different regulatory mechanisms for SSW. Isolation of genes responsible for these QTLs provides an important tool in the understanding and utilization of SSW diversity for soybean breeding.

Key Words: quantitative trait locus, recombinant inbred line, seed weight, seed yield component, soybean, trade-off.

Identification and dissection of single seed weight QTLs by analysis of seed yield components in soybean

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Single seed weight (SSW), or seed size, is a seed yield components (SSW) in soybean, and it is suggested that the genetic factors regulating SSW are involved in the control of other SYCs. The quantitative trait loci (QTLs) for SSW and their effects on the other SYCs were investigated using a recombinant inbred line population derived from typical small- and large-seeded cultivars that were cultivated in two different environments. QTL analysis detected four environmentally stable QTLs for SSW, two of which coincided with the defined loci, \( q_{Sw17-1} \) and \( Ln \). The effects of the other loci, \( q_{Sw12-1} \) and \( q_{Sw13-1} \), were confirmed by analyzing residual heterozygous line progenies derived from the recombinant population. These four QTL regions were also involved in the control of an additional SYC, namely the large-seeded allele at each locus that reduced either the number of pods per plant or the number of ovules per pod. These results suggest the presence of at least two different regulatory mechanisms for SSW. Isolation of genes responsible for these QTLs provides an important tool in the understanding and utilization of SSW diversity for soybean breeding.

Key Words: quantitative trait locus, recombinant inbred line, seed weight, seed yield component, soybean, trade-off.
could be compensated for by the change in NS through other seed yield components (SYCs). The narrow leaflet phenotype, controlled by the Ln locus (Bernard and Weiss 1973, Jeong et al. 2012), is associated with both decreased SSW and increased NOP (Dinkins et al. 2002, Jeong et al. 2011). The induced mutant line for the Gm-JAG1 (Glyma20g25000) gene clearly demonstrated the increase in NSP compensated reductions of SSW (Sayama et al. 2011). The induced mutant line for the Gm-JAG1 gene clearly demonstrated the increase in NSP compensated reductions of SSW (Sayama et al. 2011). The induced mutant line for the Gm-JAG1 gene clearly demonstrated the increase in NSP compensated reductions of SSW (Sayama et al. 2011). The induced mutant line for the Gm-JAG1 gene clearly demonstrated the increase in NSP compensated reductions of SSW (Sayama et al. 2011). The induced mutant line for the Gm-JAG1 gene clearly demonstrated the increase in NSP compensated reductions of SSW (Sayama et al. 2011). The induced mutant line for the Gm-JAG1 gene clearly demonstrated the increase in NSP compensated reductions of SSW (Sayama et al. 2011). The induced mutant line for the Gm-JAG1 gene clearly demonstrated the increase in NSP compensated reductions of SSW (Sayama et al. 2011).

In the present study, we detected four significant and environmentally stable quantitative trait loci (QTLs) for SSW in a recombinant inbred line (RIL) population derived from a cross between the typical Japanese small- and large-seeded cultivars ‘Natto-shoryu’ and ‘Tachinagaha’ (Supplemental Fig. 1), and examined these QTLs using their effects on other SYCs. Subsequently, the effects of two notable QTLs were verified in the progeny of the residual heterozygous lines derived from the RIL population. Our results suggest the presence of at least two different regulatory mechanisms for SSW through trade-offs among other SYCs, such as between SSW and NP, and between SSW and NOP.

**Materials and Methods**

**Plant materials**

An RIL population consisting of 181 lines was developed by single seed descent from a cross between ‘Natto-shoryu’ (NARO Genebank JP29161) and ‘Tachinagaha’ (NARO Genebank JP67666), and designated as NsT-RILs. DNA samples were taken from leaflets of single F2 seedlings from each line for linkage map construction and genotyping for the E3 (Buzzell 1971), a major flowering locus segregating in the NsT-RILs (see below), and the F3 population was used for trait evaluation for QTL analysis. The residual heterozygous lines (RHLs; Yamanaka et al. 2005) derived from the NsT-RILs were used to verify the effects of environmentally stable and notable QTLs for SSW. The RHLs for the QTLs were selected based on the genotypes of the F2 RIL population, and genotypes for seeds derived from heterozygous F2 plants were determined using DNA markers and segregating progenies for each QTL.

**Field experiments**

The NsT-RILs and their parental cultivars were evaluated by field experiments at two locations, Mito (Plant Biotechnology Institute, Ibaraki Agricultural Center, located at 36°26′N, 140°26′E) and Tsukuba (National Institute of Agrobiological Sciences, located at 36°01′N, 140°06′E) in 2012. A starter fertilizer was applied at 3, 10, and 10 g m⁻² of N, P₂O₅, and K₂O, respectively. Experimental plots for the NsT-RILs and parental cultivars were arranged according to the E3 genotypes, because flowering time (maturity) loci affect plant size and E3 genotypes differ between the two parental cultivars (Tsubokura et al. 2014). Therefore, the E3 (n = 84 including ‘Natto-shoryu’) and e3 (n = 89 including ‘Tachinagaha’) genotype groups were planted in separate blocks, and the heterozygous genotypes (n = 10) were placed between the two homozygous genotype groups. Within the groups, each line or cultivar was planted in single row plots following a randomized block design with one and two replicates at Mito and Tsukuba, respectively. Each row was placed 0.6 and 0.8 m apart, and each plot was composed of five plants that were spaced 0.15 and 0.2 m apart for the Mito and Tsukuba experimental conditions, respectively. Seeds were sown on June 20, 2012, at Mito and July 2, 2012, at Tsukuba. Three plants in each plot, excluding the plants at both plot ends, were individually harvested.

The RHL progeny and the parental cultivars were grown at Tsukuba in 2014. Seeds were sown in nursery beds on June 19, and robust seedlings were transplanted into the field on June 30. Starter fertilizer with the same composition used in the RIL evaluation was applied before transplantation. The plot was composed of seven plants placed 0.2 m apart, in rows spaced 0.8 m apart, arranged according to a completely randomized block design with four replicates. Three plants, excluding the two plants at both ends of the plots, were individually harvested for further analysis.

**Phenotypic measurements**

Trait evaluation for SYCs was conducted on the basis of individual plants. SSW, SY, NP, NOP, and RSS were measured in individual plants from the RIL F₆ population, the RHL progenies, and the parental cultivars. Pods were cut off from the stem and classified based on ovule number per pod by visual inspection as previously reported (Jeong et al. 2011). After threshing the pods, seeds were counted and weighed. Immediately after weighing, seed water content was measured using a grain moisture tester (TB-2, OGA Electric, Tochigi, Japan). Pest-damaged seeds were removed from SSW evaluation in order to decrease experimental error, and SY was also corrected by multiplying the SSW and the total number of seeds per plant. The SSW and SY values were displayed to reflect 15% moisture content. NOP was calculated by dividing the number of ovules per plant by NP, and RSS by dividing the number of seeds per plant by the number of ovules per plant.

**Linkage map construction**

A whole-genome simple sequence repeat (SSR) marker panel (WGSP) developed by Sayama et al. (2011) was modified, and applied to the construction of our linkage map. WGSP was originally developed to construct linkage maps by a simple procedure and at a low cost, using a selection of publicly available SSR markers according to their positions on soybean linkage maps and polymorphism information content (PIC) values (Cregan et al. 1999, Hisano et al. 2007, Hwang et al. 2009, Song et al. 2004, Xia et al. 2007). This original WGSP is comprised of 304 SSR markers and...
Genetic control of single seed weight in soybean

The cycling program consisted of 15 min at 95°C for activation, followed by 35 cycles: 30 s at 95°C for denaturation, 1.5 min at 50°C for primer annealing, and 1.5 min at 60°C for extension. At the end of the cycles, the reaction mixtures were held at 60°C for 30 min, and then cooled to 4°C. The lengths of PCR products were determined using a 3730 DNA Analyzer equipped with POP-7 polymer separation matrix and 36-cm capillaries (Thermo Fisher Scientific), and allele calling and binning was performed with GeneMapper software v5.0 (Thermo Fisher Scientific). Linkage analysis was performed with MAPMAKER/EXP 3.0b software (Lander et al. 1987), and genetic distance was calculated with Kosambi’s map function (Kosambi 1943).

Data analysis

Statistical analyses for SY and its components, SSW, NP, NOP, and RSS, were performed using R statistical software ver. 3.1.3 (R Core Team 2015). The effects of experimental factors (genotype/allele, experimental condition, or interaction between genotype/allele and experimental condition) influencing the trait values were assessed by analysis of variance (ANOVA) using type-I sum of square (SS); then the effect size (η²) of a given factor was presented as the calculated proportion of SS for the factor to the total SS. Hence, the η² of the genotype factor of each trait in the RIL population, reflected in a broad sense heritability for that trait. The correlation coefficient is a proportion of the genotypic covariance between two traits to the square root of the product of genotypic variances of the two traits. Trait values between two genotypes were compared by Welch’s t-test.

QTL analysis was performed using the Windows QTL Cartographer ver. 2.5 (Wang et al. 2013). The composite interval mapping (CIM) method was applied to detect the position and effects of QTLs. The genome was scanned at 1-cM intervals and additive effects and logarithm of odds (LOD) were estimated. A permutation test was conducted 1,000 times for each trait and experiment, and the threshold value of the LOD peak was set at P = 0.05. The QTL position with the 1- and 2-LOD significant intervals (van Ooijen 1992) on the linkage map was depicted using MapChart software (Voorrips 2002).

Results

Phenotypic variation of SYCs in the NsT-RILs

The SSW and other SYCs were evaluated in the NsT-RIL population and their parental cultivars for two different experimental conditions (Fig. 1). Other measures of SYCs, including NS and NSP, and additional statistic data are also shown in Supplemental Table 3.

SSW values in ‘Natto-shoryu’ were 128 and 148 mg, and 365 and 400 mg in ‘Tachinagaha’ at Mito and Tsukuba experimental conditions, respectively. These values indicated significant differences in SSW between the two parental cultivars for each experimental condition. SSW values in the NsT-RILs ranged from 145 to 339 mg and 161 to
Construction of the genetic linkage map of the NsT-RILs by WGSP ver. 2

WGSP ver. 2 was used to construct a linkage map of the NsT-RIL population with a total of 352 SSR markers. Fragment analysis identified polymorphisms between the parental cultivars of the RIL population at 221 loci; 210 of these loci were co-dominantly segregated. Of the 72 new markers that were added in the updated WGSP, 45 were segregated between the parental cultivars and 41 were co-dominant (Supplemental Table 1). These co-dominant SSR markers, as well as the two additional genic markers for the Ln and E3 loci, were used to genotype the NsT-RILs and calculate the genetic distance with Kosambi’s map function (Kosambi 1943) (Supplemental Table 1). The genetic linkage map covered a total of 2,824.3 cM over the 20 soybean chromosomes with an average marker interval of 14.7 cM. The marker order and distance between loci for the constructed linkage maps, including Ln and the E3, accorded well with...
those of the previous linkage maps (Hwang et al. 2009, Jeong et al. 2012, Sayama et al. 2011, Watanabe et al. 2009). The Ln and E3 genotypes of each line agreed with phenotypic data from the preliminary evaluation in the F₅ generation (data not shown).

**QTL mapping of SSW and other SYCs**

Our QTL analysis detected a total of five QTLs that control SSW (Fig. 2, Supplemental Table 5). Of these, the effects of the QTLs on Chr. 12, 13, 17, and 20 were stable across the two experimental conditions, and the four QTLs explained a total of 34.6% and 43.5% of the phenotypic variations at Mito and Tsukuba, respectively. The small-seeded alleles at the QTLs on Chr. 12, 13, and 17 were derived from ‘Natto-shoryu’, while the small-seeded allele on Chr. 20 was derived from ‘Tachinagaha’. The QTLs on Chr. 13, 17, and 20 mapped to the corresponding regions of qSw13-1, qSw17-1, and qSw20-1, which were identified from an RIL population that also had ‘Tachinagaha’ as a parent (Kato et al. 2014). Thus, we also designated these environmentally stable QTLs as qSw12-1, qSw13-1, qSw17-1, and qSw20-1 based on the previous nomenclature system; the designations represent the initials of trait names with chromosome number and the rank of the additive effect among the QTLs on the same chromosome. The proximal markers of these QTLs were Sat469 or Sat_206 for qSw12-1, CSSR535 for qSw13-1, CSSR172 for qSw17-1, and Ln for qSw20-1 (Fig. 2).

QTL analyses of NP, NOP, RSS, and SY were also conducted and the detailed results are listed in Supplemental Table 5. Two QTLs for NP were located at the corresponding regions for qSw12-1 and E3, and were stable at both of the experimental conditions. Both the alleles that increased NP were derived from ‘Natto-shoryu’. Two QTLs that controlled NOP were mapped to the same chromosomal regions as the qSw13-1 and qSw20-1. These two QTLs had significant effects on NOP and explained nearly 50% of the phenotypic variation at both experimental conditions. The small-seeded allele at these QTLs was associated with a positive effect on NOP. The QTLs for RSS were closely mapped to the qSw12-1 and E3 loci. The QTL close to the E3 locus was consistently detected in the RIL population grown at the experimental conditions, but the effect on RSS was entirely different between these conditions. For example, the allele from ‘Natto-shoryu’ increased RSS at Mito, but decreased RSS at Tsukuba. The QTLs for SY were mapped to Chr. 12 and 19, and located near qSw12-1 and E3, respectively. The QTL on Chr. 19 was stable across the experimental conditions. The late flowering allele at E3 from ‘Natto-shoryu’ increased SY and NP at both conditions. The QTLs for NS and NSP are also shown in Supplemental Table 5.

**Effect of the SSW QTL on other SYCs**

The effects of the chromosomal regions regulating SSW on the other SYCs were investigated by ANOVA in the NsT-RILs (Table 2). The genotype of each line was estimated based on the allele genotypes of the flanking markers of the QTLs. Although the flanking markers for qSw12-1 were different between the experimental conditions, Sat_206 was...
Effects of each QTL allele detected for single seed weight (SSW) on other seed yield components in the NsT-RILs grown at Mito and Tsukuba experimental conditions

| QTL   | Allele                  | Experimental condition | SSW (mg) | NP    | NOP   | RSS (%) | SY (g) |
|-------|-------------------------|------------------------|---------|-------|-------|---------|--------|
| qSw12-1 (Sat_206) | 'Natto-shoryu' (n = 90) | Mito                   | 216 ± 36 | 155 ± 39 | 2.27 ± 0.17 | 71.6 ± 8.0 | 53.6 ± 15.8 |
|       |                         | Tsukuba                | 220 ± 30 | 165 ± 32 | 2.31 ± 0.19 | 85.2 ± 4.7 | 70.2 ± 12.0 |
|       | 'Tachinagaha' (n = 74)  | Mito                   | 244 ± 31 | 130 ± 28 | 2.29 ± 0.19 | 67.8 ± 10.1 | 48.7 ± 12.2 |
|       |                         | Tsukuba                | 242 ± 29 | 139 ± 28 | 2.32 ± 0.20 | 83.5 ± 5.3 | 63.7 ± 11.3 |
| ANOVA | Allele                  |                        | NS (0.000) | NS (0.000) | NS (0.000) | NS (0.000) | NS (0.000) |
|       | Experimental condition  |                        | NS (0.002) | NS (0.000) | NS (0.000) | NS (0.000) | NS (0.001) |

| qSw13-1 (CSSR535) | 'Natto-shoryu' (n = 89) | Mito                   | 219 ± 39 | 143 ± 37 | 2.38 ± 0.14 | 68.7 ± 9.4 | 50.3 ± 14.1 |
|                   |                         | Tsukuba                | 218 ± 31 | 151 ± 35 | 2.42 ± 0.17 | 85.1 ± 5.2 | 65.9 ± 12.9 |
|                   | 'Tachinagaha' (n = 78)  | Mito                   | 238 ± 32 | 144 ± 36 | 2.17 ± 0.14 | 70.2 ± 9.7 | 51.7 ± 15.4 |
|                   |                         | Tsukuba                | 241 ± 28 | 157 ± 31 | 2.21 ± 0.15 | 84.3 ± 5.1 | 68.9 ± 11.5 |
| ANOVA | Allele                  |                        | NS (0.000) | NS (0.000) | *** (0.321) | NS (0.000) | NS (0.005) |
|       | Experimental condition  |                        | NS (0.000) | NS (0.000) | NS (0.000) | NS (0.003) | NS (0.001) |

| qSw17-1 (CSSR172) | 'Natto-shoryu' (n = 96) | Mito                   | 224 ± 36 | 146 ± 38 | 2.26 ± 0.18 | 69.7 ± 9.9 | 50.1 ± 14.2 |
|                   |                         | Tsukuba                | 223 ± 30 | 156 ± 34 | 2.29 ± 0.18 | 85.3 ± 4.7 | 66.5 ± 12.5 |
|                   | 'Tachinagaha' (n = 70)  | Mito                   | 240 ± 35 | 132 ± 29 | 2.27 ± 0.17 | 68.5 ± 9.0 | 48.6 ± 12.1 |
|                   |                         | Tsukuba                | 239 ± 32 | 148 ± 29 | 2.31 ± 0.20 | 84.1 ± 5.4 | 67.1 ± 11.1 |
| ANOVA | Allele                  |                        | NS (0.000) | NS (0.000) | NS (0.001) | NS (0.000) | NS (0.000) |
|       | Experimental condition  |                        | NS (0.000) | NS (0.000) | NS (0.000) | NS (0.000) | NS (0.000) |

| qSw20-1 (Ln)     | 'Natto-shoryu' (n = 79) | Mito                   | 240 ± 34 | 139 ± 33 | 2.16 ± 0.13 | 68.9 ± 9.6 | 49.0 ± 13.2 |
|                  |                         | Tsukuba                | 241 ± 28 | 159 ± 33 | 2.17 ± 0.12 | 85.1 ± 5.0 | 69.5 ± 12.1 |
|                  | 'Tachinagaha' (n = 91) | Mito                   | 218 ± 35 | 147 ± 39 | 2.39 ± 0.14 | 69.9 ± 9.2 | 52.8 ± 15.7 |
|                  |                         | Tsukuba                | 218 ± 31 | 147 ± 32 | 2.45 ± 0.15 | 84.5 ± 4.9 | 65.0 ± 11.8 |
| ANOVA | Allele                  |                        | NS (0.000) | NS (0.001) | *** (0.465) | NS (0.000) | NS (0.000) |
|       | Experimental condition  |                        | NS (0.000) | NS (0.000) | NS (0.000) | NS (0.004) | NS (0.000) |

The genotype at each environmentally stable QTL for SSW was estimated by the alleles of the flanking markers; Sat_206 for qSw12-1, CSSR535 for qSw13-1, CSSR172 for qSw17-1, and Ln for qSw20-1.

SSW, single seed weight; NP, number of pods per plant; NOP, number of ovules per pod; RSS, rate of seed-set; SY, seed yield per plant.

Trait value of each genotype group is shown as mean ± standard deviation.

*, **, and *** represent statistical significance at P < 0.05, 0.01, and 0.001 by ANOVA, respectively; NS = not significant.

Values in parentheses indicate effect size (η²) of each factor calculated as a proportion of the sum of squares of the factor to the total sum of squares.

Confirmation of the effects of qSw12-1 and qSw13-1 on SYCs using RHL progenies

The effects of the chromosomal regions containing qSw12-1 and qSw13-1 on SYCs were verified in the RHL progenies, which were selected from the NsT-RIL population as being heterozygous around each locus based on the genotype of the flanking markers. NsT-RILs 086 and 103 had heterozygous genotypes around the qSw12-1 and qSw20-1 contained QTLs for NOP, and the small-seeded alleles were associated with increased NOP. The effect size of these two QTLs was as large as 0.786 in total for NOP in the NsT-RILs. In addition, the chromosomal region around qSw12-1 also related to RSS and SY; at this locus, the allele derived from 'Natto-shoryu' increased both traits. This analysis indicated the genotype-experimental condition interactions of the qSw20-1 region on NP and SY, but the effect sizes of these interactions were as small as 0.002 and 0.017, respectively.
while NsT-RILs 018 and 111 were selected as RHLs for $qSw13-1$. Those RILs did not segregate at the other QTL regions that affected SYCs in the F$_5$ generation. The ‘Natto-shoryu’ and ‘Tachinagaha’ genotypes in the $qSw12-1$ or $qSw13-1$ regions were developed from the corresponding RHLs, and then applied for the evaluation of each trait.

For $qSw12-1$, ‘Tachinagaha’ genotypes had significantly larger SSW than the ‘Natto-shoryu’ genotypes in the RHL-086 and RHL-103 genetic backgrounds (Table 3). NP in the ‘Natto-shoryu’ genotype was higher than in the ‘Tachinagaha’ genotype in RHL-103. Similarly, the ‘Natto-shoryu’ genotype had higher NP than the ‘Tachinagaha’ genotype in RHL-086, although this difference was not significant at $P < 0.05$. The genotypes in the $qSw12-1$ region did not affect NOP and SY in either background. The effects of the chromosomal regions that contain $qSw12-1$ on SSW and NP were consistent with the results from the QTL analysis.

SSW and NOP differed among the genotypes in the $qSw13-1$ region in both RHL-018 and RHL-111 backgrounds (Table 4). The ‘Natto-shoryu’ genotype decreased SSW and increased NOP. RSS was significantly different between the ‘Natto-shoryu’ and ‘Tachinagaha’ genotypes in RHL-018, but the difference was not observed in RHL-111 backgrounds. The effects of the $qSw13-1$ region on SSW and NOP were uniquely identical to the results of the QTL analysis.

### Discussion

The ‘Natto-shoryu’ and ‘Tachinagaha’ cultivars differed substantially in SSW and the RIL population derived from these cultivars, NsT-RILs, exhibited a wide variation in SSW, which ranged from 145 to 339 mg (Fig. 1, Supplemental Table 3). As no obvious correlation between SSW and SY was observed (Table 1), the impact of the difference in SSW on SY would be compensated for by the change in NS (Supplemental Table 4). All the components of NS, namely, NP, NOP, and RSS, negatively correlated with SSW (Table 1). Therefore, the effects of the genetic factors regulating SSW on other SYCs could be dissected using this RIL population.

Composite interval mapping detected four significant and environmentally stable QTLs for SSW from the NsT-RILs that mapped toChr. 12, 13, 17, and 20; designed as $qSw12-1$, $qSw13-1$, $qSw17-1$, and $qSw20-1$, respectively (Fig. 2, Supplemental Table 5). The four environmentally stable SSW QTLs explained approximately 40% of the phenotypic variance in SSW, and the other part of the phenotypic variance is probably controlled by many other undefined QTLs with smaller effects on SSW. Although the large-seeded allele at $qSw20-1$ was derived from the small-seeded parent, the NsT-RILs did not exhibit transgressive segregation in SSW. This suggested that the small- or large-seeded parents accumulated negative or positive effect alleles at most of the SSW QTLs, other than $qSw20-1$; it was extremely difficult to accumulate an adequate number of positive or negative effect alleles in order to achieve the transgressive segregation because approximately half of the chromosomal segments of each recombinant line was derived from one parental cultivar. The difficulty in achieving extreme allele combination has also been discussed in other reports (Alt et al. 2002, Cober and Voldeng 2008, Johnson et al. 2001).

The stable QTL on Chr. 17, $qSw17-1$, was mapped to almost the same position as that previously reported by Kato et al. (2014), which was identified using two different RIL populations derived from crosses between Japanese large-seeded and US conventional soybean cultivars. The $qSw20-1$ is closely linked to the $Ln$ locus; whose recessive allele governs narrow leaflet morphology. Previous studies indicated a relationship between small seed and narrow leaflet phenotypes controlled by the $Ln$ locus (Dinkins et al. 2002, Jeong et al. 2011, Sayama et al. 2017). ‘Tachinagaha’ is a narrow leaflet cultivar and the small-seeded $ln$ allele at $qSw20-1$ was certainly derived from this narrow leaflet parent in the NsT-RIL population (Supplemental Table 5). Previously mapped QTLs for SSW were located around the chromosomal regions where $qSw12-1$ and the $qSw13-1$ were detected (Hoeck et al. 2003, Hyten et al. 2004, Kato et al. 2014, Mian et al. 1996, Specht et al. 2001), but the presence of these QTLs has not been corroborated to date.

Confirmation of the effect of QTLs in different environmental conditions or genetic backgrounds is an important process because there are several cases of QTLs that are ineffective in subsequent practices, such as marker-assisted selection (Diers et al. 1992, Fasoula et al. 2004). This is caused by confounding factors such as the genetic structure of tested populations, environmental effects, and differences in marker sets used for genotyping (Beavis 1994). Fasoula et al. (2004) verified QTLs for seed protein, seed oil, and SSW in soybean using independent sets of RIL populations derived from the same parental cultivars. As a result, only one third of the QTLs for those traits were verified and the effects of other QTLs were not observed. The effects of the $qSw17-1$ and the $qSw20-1$ on SSW were confirmed in several genetic studies (Dinkins et al. 2002, Hirata et al. 2014, Jeong et al. 2011, Zhou et al. 2015); however, $qSw12-1$ and $qSw13-1$ have not been corroborated. We have successfully verified the effects of these QTL regions on SSW using segregants of residual heterozygous lines (RHLs; Yamanaka et al. 2005) derived from the NsT-RIL population (Tables 3, 4). For the $qSw12-1$ region, the ‘Natto-shoryu’ allele decreased SSW, and the effects of the $qSw13-1$ allele were in agreement with the QTL mapping results. Therefore, the responsible gene(s) for these traits was expected to be located in the chromosomal region that commonly segregated in the two sets of RHLs.

In the NsT-RILs, SSW was negatively correlated with NP, NOP, and RSS with respect to their genetic variation, indicating that the impact of increased SSW might be largely compensated for by decreases in the other SYCs, which are components of NS (Table 1, Supplemental Table 4).
### Table 3. Effect of the qSw12-1 alleles on single seed weight (SSW) and other seed yield components in segregated progeny derived from residual heterozygous lines (RHLs)

| RHL     | Estimated allele at qSw12-1 | Marker allele on Chr. 12 | SSW (mg) | NP | NOP | RSS (%) | SY (g) |
|---------|----------------------------|--------------------------|----------|----|-----|---------|--------|
|         | Sat192                     | GMES4012                 | Satt469  | Sat206 | Satt302 | Satt142 |
| RHL-086 'Natto-shoryu' | N | N | N | N | N | N | 245 ± 14 *** | 138 ± 26 NS | 2.21 ± 0.04 NS | 88.7 ± 2.7 NS | 66.5 ± 13.8 NS |
| RHL-086 'Tachinagaha' | N | N | T | T | T | T | 278 ± 13 | 118 ± 24 | 2.21 ± 0.04 | 89.4 ± 2.6 | 64.7 ± 12.0 |
| RHL-103 'Natto-shoryu' | T | N | N | N | T | T | 186 ± 8 *** | 239 ± 37 * | 2.03 ± 0.02 | 91.4 ± 1.6 | 82.0 ± 12.2 NS |
| RHL-103 'Tachinagaha' | T | T | T | T | T | T | 219 ± 14 | 207 ± 39 | 2.03 ± 0.02 | 90.2 ± 2.1 | 83.0 ± 11.1 |

SSW, single seed weight; NP, number of pods per plant; NOP, number of ovules per pod; RSS, rate of seed-set; SY, seed yield per plant.

Data were obtained from 12 plants (3 plants with 4 replications) for each genotype. The flanking markers of qSw12-1 are indicated in boldface type.

N and T indicate the ‘Natto-shoryu’ and ‘Tachinagaha’ alleles at each marker locus, respectively.

Trait value of each genotype group is shown as mean ± standard deviation.

* and *** represent statistical significance at \( P < 0.05 \) and \( 0.001 \) by Welch’s t-test, respectively; NS = not significant.

### Table 4. Effect of the qSw13-1 alleles on single seed weight (SSW) and other seed yield components in segregated progeny derived from residual heterozygous lines (RHLs)

| Line | Estimated allele at qSw13-1 | Marker allele on Chr. 13 | SSW (mg) | NP | NOP | RSS (%) | SY (g) |
|------|----------------------------|--------------------------|----------|----|-----|---------|--------|
|      | Sat_039                    | CSSR535                  | Satt663  | Satt114 |
| RHL-018 'Natto-shoryu' | N | N | N | N | N | 235 ± 23 ** | 104 ± 24 NS | 2.18 ± 0.05 *** | 91.6 ± 1.7 ** | 49.3 ± 14.8 NS |
| RHL-018 'Tachinagaha' | N | T | T | T | N | 258 ± 25 | 91 ± 25 | 2.04 ± 0.08 | 89.3 ± 2.0 | 42.6 ± 15.2 |
| RHL-111 'Natto-shoryu' | T | N | N | T | N | 232 ± 15 ** | 113 ± 18 NS | 2.46 ± 0.06 *** | 87.1 ± 2.5 NS | 56.3 ± 11.8 NS |
| RHL-111 'Tachinagaha' | T | T | T | T | T | 253 ± 15 | 128 ± 24 | 2.24 ± 0.12 | 88.9 ± 2.6 | 64.5 ± 13.6 |

SSW, single seed weight; NP, number of pods per plant; NOP, number of ovules per pod; RSS, rate of seed-set; SY, seed yield per plant.

Data were obtained from 12 plants (3 plants with 4 replications) for each genotype. The flanking marker of qSw13-1 is indicated in boldface type.

N and T indicate the ‘Natto-shoryu’ and ‘Tachinagaha’ alleles at each marker locus, respectively.

Trait value of each genotype group is shown as mean ± standard deviation.

** and *** represent statistical significance at \( P < 0.01 \) and \( 0.001 \) by Welch’s t-test, respectively; NS = not significant.
that mediate the trade-offs between SSW and NP, as well as SSW could consist of at least two independent mechanisms through the allocation of resources, then the regulation of pleiotropic properties; if these SYCs are controlled genetic factors governing the allocation of resources are ca-

These findings suggest that genetic factors governing the allocation of resources are capable of pleiotropic properties; if these SYCs are controlled through the allocation of resources, then the regulation of SSW could consist of at least two independent mechanisms that mediate the trade-offs between SSW and NP, as well as between SSW and NOP.

Despite the negative correlation between SSW and RSS (Table 1), genetic regulation of this trade-off relationship was less evident, in contrast to trade-offs between SSW and NP, and between SSW and NOP; this is possibly because the trade-off between SSW and RSS might be regulated by small-effect genetic factors. Alternatively, there may be no strict genetic control for this trade-off, but potentially heavy SSW might be more vulnerable to decline in RSS. This indicates that the large-seeded genotype carries a greater reproductively sink capacity than the small-seeded genotype but that the resource availability adjusts the actual sink size through RSS. This supposition can also explain the positive correlations between RSS and SY at Mito and between SSW and SY at Tsukuba (Table 1), given that resource availability was limited only in the Mito experimental condition. The positive effect of the E3 allele on RSS at Mito (Supplemental Table 5) may also reflect increased resource availability in the E3 genotype, while the inverse relationship at Tsukuba might indicate that RSS is susceptible to the environment to which the plant exposed. More research is needed to better understand the detail of the trade-off relationships between SYCs.

This study shows that the chromosomal regions that regulate SSW also control other SYCs, and thus selection for SSW involves changes in these other SYCs. Deeper understanding of the genetic architecture of SSW regulation would facilitate the manipulation of SSW, and help us understand the genetic basis of seed yield formation. The QTLs detected in the present study are attractive targets for functional analysis, and will contribute to elucidating a more complete picture of SSW regulation.

The QTL analysis and ANOVA suggested genetic control of the potential trade-offs between SSW and other SYCs by the SSW QTL regions, since at each locus the large-seeded allele decreased in either NP or NOP (Fig. 2, Table 2). In addition, the effects of qSw12-1 and qSw13-1 were verified by our evaluation of the RHL (Tables 3, 4). The four stable QTLs for SSW detected in the present study could be classified into two different groups according to their genetic effects on SYCs (Fig. 3). The chromosomal regions of qSw12-1 and qSw17-1 were involved in the regulation of SSW and NP, whereas the qSw13-1 and qSw20-1 regions were associated with the inverse relationship of SSW and NOP. Additionally, the chromosomal region around qSw12-1 influenced RSS and SY. However, it is not clear whether the co-regulation of these components was mediated by a single genetic factor or by independent factors located closely to each other.

The trade-off between seed/egg size and number is a well-accepted concept in both plant and animal species (Sadras 2007, Smith and Fretwell 1974, Venable 1992). The trade-off takes place under resource-limited conditions, and changes in allocation patterns of the available resources result in an inverse relationship between the size and number of seeds. The present study showed that the large-seeded allele at each QTL for SSW was associated with a decrease in either NP or NOP (Tables 2–4). These findings suggest that genetic factors governing the allocation of resources are capable of pleiotropic properties; if these SYCs are controlled through the allocation of resources, then the regulation of SSW could consist of at least two independent mechanisms that mediate the trade-offs between SSW and NP, as well as between SSW and NOP.

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