Introduction

Yield improvement is the most important objective during breeding crops. In soybean (Glycine max (L.) Merr.) cultivation, focus has been given on the growth habits of the stem as a trait affecting seed yield, as such (Bernard 1972, Cober and Morrison 2010, Hartung et al. 1981, Kilgore-Norquest and Sneller 2000). Most varieties can be classified into three growth-habit categories: indeterminate, semi-determinate, and determinate, based on the timing of termination of apical stem growth after flowering (Bernard 1972). The stem growth habit is regulated by the genes Dt1 and Dt2, which regulate stem growth habit, into three determine varieties by backcrossing and evaluated the resulting effects on yield and lodging tendency under four different growing environments. The yield and lodging degree of the semi-determinate and indeterminate lines were higher and more severe than those of the determinate lines. Despite the lower overall lodging score, the semi-determinate lines had marginally lower overall yield than that of the indeterminate lines. However, the effect of introduction of semi-determinate traits on yield and lodging degree was different in the three backgrounds, with the yield of semi-determinate lines being the highest and the difference in lodging degree between the semi-determinate and determinate lines being under 1.0 in one background. Therefore, semi-determinate growth habit has potential to develop high yielding varieties with low lodging risk.

Key Words: lodging, near isogenic line, stem growth habit, semi determinate, DNA marker, soybean.
longer than that in the determinate ones, and the main stem length of the indeterminate varieties is longer than that of the determinate varieties, particularly when varieties with similar maturities were compared (Bernard 1972, Cober and Morrison 2010, Hartung et al. 1981). Stem growth of the semi-determinate varieties is intermediate to those of the indeterminate and determinate varieties, and stem length of the semi-determinate varieties is lesser than that of the indeterminate varieties (Bernard 1972). The effects of stem growth habit on agronomic characteristics, such as yield and lodging, have been studied using near isogenic lines (NILs) developed by introducing $dt1$ and/or $Dt2$ genes into old Canadian and US indeterminate varieties, ‘Hamosy’ and ‘Clark’ ($Dt1Dt1dt2dt2$) (Bernard 1972, Hartung et al. 1981, Hicks et al. 1969, Shannon et al. 1971). These researches have shown that the yield of the semi-determinate lines was almost equal to that of the indeterminate ones, which was, however, superior to that of the determinate lines, and that the degree of lodging in the semi-determinate lines tended to be lower than that in the indeterminate ones in lodging-prone environments, where short stature is desirable (e.g., high fertility and irrigated environments), although it was the least in the determinate lines. However, the advantages of agronomic characteristics in one growth habit over those in others have been reported to vary depending on genetic background and growing environment (Chang et al. 1982, Green et al. 1977, Wilcox 1980). In particular, recent studies on allelic diversity at the molecular marker loci have demonstrated that genetic background of Japanese soybean varieties is unique and distinct from that of the exotic varieties; e.g., varieties in China and North America (Abe et al. 2003, Hwang et al. 2008, Kaga et al. 2012, Ude et al. 2003). Therefore, the comparison of agronomic characteristics among stem growth habits should be validated in Japanese genetic backgrounds and growing environments.

Since almost all Japanese soybean varieties are determinate, and Japanese breeders have rarely selected varieties with other growth habits as parents for breeding, there have been few reports describing the differences in the agricultural characteristics among the varieties with various growth habits grown under Japanese environments and/or with Japanese genetic backgrounds. To understand the yield differences between lines with indeterminate and determinate growth habits, Kato et al. (2015) cultivated recombinant inbred lines (RILs) from crosses between Japanese indeterminate and US indeterminate varieties in northern and southern locations of Japan. They demonstrated that indeterminate lines with early maturity had higher number of pods and higher yield than the determinate lines in the northern parts of Japan. However, the change from a determinate to an indeterminate growth habit increased the main stem length, and thereby increased lodging (Cober and Morrison 2010, Kato et al. 2018). Severe lodging not only decreases yield performance by reducing solar radiation interception, which is dependent on canopy architecture (Cooper 1971, Noor and Cavinness 1980, Saitoh et al. 2012, Shapiro and Flowerday 1987), but also leads to inefficient and slow mechanical harvesting and harvest losses. In addition, particularly in Japan, since soybeans have been used as foods, contamination of seed coats with soil, which is likely to occur during the combined harvest of severely lodged soybean, reduces the commercial value of the seeds. Therefore, when utilizing an indeterminate growth habit for Japanese varieties, improvements in lodging resistance must be considered. As mentioned previously, semi-determinate varieties have intermediate stem growth after flowering; between those of the indeterminate and determinate varieties. There is a possibility that utilizing the semi-determinate growth characteristics may contribute to the development of varieties with high yield and low lodging risk. However, to the best of our knowledge, there is no report describing the effect of change from determinate to semi-determinate growth habit on the latter’s agronomic characteristics, including lodging and yield, in Japan.

In this study, to evaluate yield and lodging, we developed NILs with different growth habits by introducing two genes, $Dt1$ and $Dt2$, in the determinate backgrounds adapted for cultivation in the northeast region of Japan. The effects of growth habit on the other agronomic characteristics and advantages of semi-determinate growth habit are also discussed.

**Materials and Methods**

**Plant materials**

NILs with different growth habits were developed by backcrossing three times. Two semi-determinate breeding lines, ‘PI 547717’, and ‘PI 547409’, and three determinate breeding lines adapted in the northeast region of Japan, ‘Wasesuzunari’ (Hashimoto et al. 1985), ‘Tohoku 162’, and ‘Tohoku 164’ (Kato et al. 2014), were used as donor parents and recurrent parents, respectively. The maturities of the recurrent parents ‘Wasesuzunari’ (maturity group (MG) II), ‘Tohoku 162’, (MG III) and ‘Tohoku 164’ (MG IV) were early, early-mid, and mid-maturity in the northeast region of Japan, respectively (Hashimoto et al. 1985, Kato et al. 2014). The donor parents, ‘PI 547717’, and ‘PI 547409’, were developed by introducing $Dt2$ gene in the ‘Harosoy’ and ‘Clark’ varieties, respectively, by backcrossing five times (Bernard 1972, Bernard et al. 1991).

‘Tohoku 164’ ($dt1dt1dt2dt2$) was crossed with ‘PI 547409’ ($Dt1Dt1Dt2Dt2$), while ‘Wasesuzunari’ and ‘Tohoku 162’ ($dt1dt1dt2dt2$) were crossed with ‘PI 547717’ ($Dt1Dt1Dt2Dt2$) in 2011 in the fields of Kariwano Branch of the Daisen Research Station of Tohoku Agricultural Research Center, NARO (hereafter, Kariwano). Backcrosses were repeated three times from 2011 to 2012 in the field and greenhouse in Kariwano. When backcrossing, DNA markers near the $Dt1$ and $Dt2$ loci were genotyped, and $Dt1dt1Dt2dt2$ plants were selected as pollen parents. From each genetic background, two $Dt1dt1Dt2dt2$ BC$_{2}$F$_{1}$ plants were grown in the greenhouse of the Institute of Crop
Science, NARO in 2013. Two plants with genotypes Dt1Dt1Dt2Dt2 (semi-determinate), Dt1Dt1dt2dt2 (indeterminate), or dt1dt1dt2dt2 (determinate) were selected from a BC3F3 population originating from a BC1F1 plant in each cross, in 2013 based on the alleles of DNA markers (Supplemental Table 1). In cases where two homozygous plants were not obtained in the progeny from a BC1F1 plant, we genotyped the seeds obtained from the BC1F1 plants whose alleles in the Dt1 or Dt2 locus were heterozygous, and selected the seeds in which both loci were homozygous; or used the seeds obtained from other BC1F1 plants originating from the same genetic background (Supplemental Table 1). In 2014, twelve BC3F3 lines, i.e., four Dtl Dt1Dt2Dt2, four Dtl Dl2dt2d2, and four dt1 dt2dt2d2 lines, in each genetic background were cultivated in the field. BC3F3 lines obtained by harvesting the BC1F3 plants in bulk were used as the NILs in this study.

Maker-assisted selection

The soybean genome database Wm82.a1 (Schmutz et al. 2010) was used as the reference to identify the positions of DNA markers and genes on the physical map. SSR markers located near the Dt1 gene, GmTFL1 (Glyma19g37890, 44,980–44,981 kb on Chromosome 19) (Liu et al. 2010, Tian et al. 2010), and the Dt2 gene, the dominant MADS-domain factor gene (Glyma18g30910, 59,919–59,927 kb on Chromosome 18), were genotyped for selecting the pollen parents (Ping et al. 2014). Three DNA markers, Sat_286, Sat_184, and DtlInt (Kato et al. 2015) were used for genotyping the Dt1 gene, while fourteen markers, AF162283, Sat_184, and Dt1Int (Kato 2015) were used for genotyping the Dt2 gene (Supplemental Table 2).

In addition, since four major maturity genes, E1, E2, E3, and E4 have large effects on agricultural characteristics (Cober and Morrison 2010, Curtis et al. 2000, Yamada et al. 2012) and their segregation within NILs in each genetic background complicate the comparison of their growth habits, pollen parents with alleles of the recurrent parents with the four major maturity genes (E1, E2, E3 and E4), were selected with the markers located near these genes (Supplemental Table 2). The alleles of the donor and recurrent parents for these genes were identified as per Kato et al. (2018) and Tsubokura et al. (2014). The markers were genotyped by sizing of polymerase chain reaction (PCR) fragments amplified with fluorescently labeled SSR markers with a sequencer using a DNA sequencer [3730 Genetic Analyzer DNA sequence; Applied Biosystems, CA, U.S.] as per Sayama et al. (2011) or on a 7.5% acrylamide gel (Supplemental Table 3).

Since there are two dominant alleles (Dt1), GmTfl1-a and GmTfl1-b, and four recessive alleles (dt1), GmTfl1-ta, GmTfl1-ab, GmTfl1-bb, and GmTfl1-tb at the GmTFL1 locus, we sequenced the GmTFL1 gene from the recurrent and donor parents and validated the alleles of this gene as per Tian et al. (2010).

Evaluation of agronomic characteristics and statistical analysis

The NILs, as well as their donor and recurrent parents, were planted in fields at Uenodai (39°32′N, 140°22′E) and Ishinazaka (39°32′N, 140°23′E) in Kariwano in 2015 and 2016. Experiment plots under all growing environments were arranged in split-plot designs with genetic backgrounds as the main-plots and stem growth habits as the sub-plots with two replications each. Sowing dates for Uenodai in 2015, Uenodai in 2016, Ishinazaka in 2015, and Ishinazaka in 2016 were 27th May, 23rd May, 20th May, and 19th May, respectively. Each plot consisted of a single row, 1.5–2.0 m long with a row spacing of 0.75 m and plant separation distance within each row was 0.12 m, except at Uenodai in 2015 (0.16 m plant separation distance within each row). Starter fertilizers at the Uenodai fields included 24 kg ha⁻¹ N, 160 kg ha⁻¹ P₂O₅, 80 kg ha⁻¹ K₂O, and 20 t ha⁻¹ of compost, whereas the Ishinazaka fields included 24 kg ha⁻¹ N, 120 kg ha⁻¹ P₂O₅, 80 kg ha⁻¹ K₂O, and no compost. Soils of both the experimental fields were andosol.

We measured the following fifteen characteristics: seed yield per plant, lodging score, flowering time, seed-filling period, maturing time, main stem length, number of main stem nodes, main stem internode length, number of main stem branches, lowest pod height, number of pods per plant, number of seeds per pod, 100-seed weight, protein content, and oil content as per Kato et al. (2018). Lodging score was visually evaluated in each plot as 0 (all plants were erect) to 5 (all plants were prostrate) at an interval of 1. Seed yield per plant was calculated from total seed weight and the number of harvested plants. The main stem internode length was determined by dividing the main stem length by the number of main stem nodes. The 100-seed weight was measured twice with healthy seeds randomly selected from air-dried seed samples. Seed yield and 100-seed weight were adjusted to reflect a water content of 15% by measuring the moisture content of seeds with a grain moisture tester (PM830-2; Kett Electric Laboratory, Tokyo, Japan) and a near-infrared spectrophotometer (Infratec 1241 Grain Analyzer; FOSS Hillerød, Denmark), respectively.

These traits were subjected to split-plot analysis of variance (ANOVA) using SAS software (SAS, Cary, NC, USA). Genetic background and stem growth habit were considered as the main plot and sub-plot, respectively. Stem growth habit, genetic background, location, and year were considered as fixed effects. Where the interactions between genetic background and stem growth habit were significant, the statistical significance of differences between stem growth habits was evaluated in each genetic background by the Tukey–Kramer test using EZR (Kanda 2013), a graphical user interface for R (http://www.r-project.org).
Results

Genotyping of DNA markers proximal to Dt1 and Dt2 and the four major maturity genes in the recurrent and donor parents

Nucleotide sequence of the GmTFL1 locus from −740 to +1440 (the adenosine of the start codon is numbered +1) revealed that the GmTFL1 sequences of donor parents, ‘PI 547717’, and ‘PI 547409’, were identical to that of GmTfl1-a (Dt1), and that those of recurrent parents, ‘Wasesuzunari’, ‘Tohoku 162’, and ‘Tohoku 164’, were identical to that of GmTfl1-ab (Dt1). Since the DNA marker, Dt1Int, was designated to distinguish a 6-bp length polymorphism between GmTFL1 and GmTfl1-ab (Dt1), and that those of recurrent parents, ‘Wasesuzunari’, ‘PI 547717’, and ‘PI 547409’, were identical to that of GmTfl1+1440 (the adenine of the start codon is numbered +1).

To confirm whether marker selections of Dt1 and Dt2 worked, the main stem length of NILs was compared. The lengths of the main stems in the indeterminate (Dt1Dt1dt2dt2) and semi-determinate (Dt1Dt1Dt2Dt2) lines were significantly longer than those in the determinate (Dt1Dt1dt2dt2) lines, whereas the semi-determinate lines had significantly shorter main stems than the indeterminate lines in all genetic backgrounds (Fig. 1). This result proved that marker selection for Dt1 and Dt2 was effective in all the genetic backgrounds. However, there was a significant interaction between the growth habit and genetic background (S × G) respectively. Accordingly, we genotyped the seeds obtained from the BC1F2 plants that were heterozygous at the Dt1 or Dt2 locus to identify the homozygous progeny of appropriate genotype. In BG/T162, since there was only one Dt1Dt1dt2dt2 homozygous plant originated from a single BC1F1 plant, we used three Dt1Dt1dt2dt2 homozygous plants obtained from another BC1F1 plant originating from the same genetic background (Supplemental Table 1). In 2014, 12 BC3F3 lines for each genetic background consisting of four dt1dt1dt2dt2 (determinate), four Dt1Dt1dt2dt2 (indeterminate), four Dt1Dt1Dt2Dt2 (semi-determinate) homozygous lines, were cultivated in the field. In 2015, BC3F4 seeds were obtained by harvesting each BC3F3 line in bulk.

Development of NILs with different stem growth habits

The recurrent and donor parents were crossed in 2011, and backcrosses to the recurrent parents were repeated thrice using marker assisted selection for the Dt1, Dt2, E1, E2, and E3 genes. In each of the genetic backgrounds, two BC1F1 plants, heterozygous for Dt1 and Dt2 loci for donor and recurrent parents were genotyped with a sequencer, and all the tested markers were used as dominant or codominant markers except for AF162283, Satt472 (‘Wasesuzunari’ vs ‘PI 547717’) and Gm18.60271k (‘Tohoku 162’ vs ‘PI 547717’) (Supplemental Table 3).

The genotyping of the seven DNA markers for the four major maturity genes revealed the alleles of these genes in recurrent and donor parents: ‘PI 547717’ (e1-as/e2-ns/E3-Ha/E4), ‘PI 547409’ (e1-as/E2-in/E3-Ha/E4), ‘Wasesuzunari’ (E1/e2-ns/e3-tr/E4), ‘Tohoku 162’, and ‘Tohoku 164’. The alleles for E1, E2, and/or E3 were different among the parents, but there were no differences observed for the alleles of E4.

Fig. 1. Comparison of main stem length, number of main stem nodes, seed yield, lodging, and 100-seed weight among the stem growth habits and genetic backgrounds. Error bars represent standard errors. The same letters are not significantly different at 0.05 probability level by the Tukey–Kramer test.
in determining the main stem length; furthermore, the ratio of difference between indeterminate and semi-determinate growth habits in BG/Wase was relatively larger than those in BG/T162 and BG/T164.

In addition, to evaluate the extent to which the NILs developed by backcrossing three times resembled the recurrent parents, agricultural characteristics were compared between the recurrent parents and the NILs with determinate growth habit (Supplemental Table 4). The lodging degree of determinate lines was similar to that of the recurrent parents. The seed yield per plant of determinate lines was 3.5–5.6 g lower than that of the recurrent parents, in all the genetic backgrounds. Focusing on the yield components, the number of pods in determinate lines was 8.9 less than that of the recurrent parents in BG/Wase, whereas the 100-seed weight of determinate lines was 2.8–4.7 g lower than that of the recurrent parents in BG/T162 and T164. In addition, there were 0.9%–1.9% and 0.3%–1.3% differences in protein and oil content, respectively. However, the difference in other traits, such as flowering time and main stem length, between determinate lines and recurrent parents was negligible.

**Effect of stem growth habit on yield**

Split-plot ANOVA for yield revealed that the effects of stem growth habit on yield were significant (Table 1). Overall, the seed yields of semi-determinate and indeterminate lines were higher than that of the determinate ones; and that of the semi-determinate lines was marginally lower than that of the indeterminate lines (Table 2). Since the S × G interaction for yield was significant, multiple comparisons in each genetic background were performed by Tukey–Kramer test. The yields of the semi-determinate and indeterminate lines were consistently higher than those of the determinate lines in all the genetic backgrounds (Fig. 1). The extent of differences in yield between the indeterminate and semi-determinate lines changed depending on the genetic background, although all the differences were insignificant. The yields of the semi-determinate lines in BG/Wase and BG/T162 were lower than those of the indeterminate lines, whereas that of the semi-determinate lines in BG/T164 were higher than that of the indeterminate lines.

Split-plot ANOVA for yield components revealed that the effects of stem growth habit on all the yield components were significant (Table 1). Overall, the number of seeds per pod and the number of pods of semi-determinate and indeterminate lines were more than those of determinate lines, however, the semi-determinate and indeterminate lines were similar (Table 2). The S × G interactions for the number of seeds per pod and the number of pods were insignificant. The S × G interaction for 100-seed weight was significant, and multiple comparisons demonstrated that 100-seed weight of the indeterminate lines was significantly higher than that of the determinate lines in BG/T162 and BG/T164 (Fig. 1). The 100-seed weight of semi-determinate lines was intermediate between the indeterminate and determinate lines in all the genetic backgrounds, and the differences among the NILs were 0.6–1.8 g.

Our results showed that, although the overall yield of the semi-determinate lines was marginally lower than that of the indeterminate lines, the difference in yields due to stem growth habits varied depending on the genetic background as well as did 100-seed weight.

**Effect of stem growth habit on lodging**

Split-plot ANOVA for lodging revealed that the effects of stem growth habit on lodging were significant (Table 1). Although overall lodging scores of semi-determinate and indeterminate lines were higher than that of determinate lines, that of semi-determinate lines was lower than that of indeterminate lines (Table 2). Since there was significant S × G interaction for lodging (Table 1), the lodging scores were compared amongst the growth habits in each genetic background similarly to the seed yield. The lodging scores of semi-determinate and indeterminate lines were constantly higher than that of the determinate lines, and the differences were significant in all the genetic backgrounds. On the other hand, semi-determinate lines had 0.1–0.6 lower than that of the indeterminate lines, and the difference between lodging of the semi-determinate and indeterminate lines in BG/Wase was significant, whereas those in BG/T162 and T164 were insignificant (Fig. 1).

The correlation analysis between the lodging score and main stem length in each genetic background showed positive relationships between these traits in all the genetic backgrounds, although the correlation was significant only in BG/T162 (Fig. 2). On the contrary, the regression analysis of the lodging score to main stem length in each genetic background showed that the increasing rate of lodging score accompanied with the increase in main stem length varied depending on the genetic background. Despite the main stem lengths of semi-determinate and indeterminate lines in BG/T164 being longer than those in other genetic backgrounds, respectively, the lodging scores of the semi-determinate and indeterminate lines in BG/T164 was the smallest (Fig. 2).

**Effect of stem growth habit on the other agricultural characteristics**

Split-plot ANOVA for the other agronomic characteristics revealed that there were significant differences for growth phases (flowering time and maturation), morphological traits (main stem length, number of main stem nodes, main stem internode length, number of branches, and lowest pod height), and seed components (protein and oil content) (Table 1). The numbers of main stem nodes of the indeterminate and semi-determinate lines were significantly more than that of the determinate lines in all genetic background, but the difference between the semi-determinate and indeterminate varied depending on the genetic backgrounds (Table 1). The number of main stem nodes of semi-determinate lines was significantly lesser than that of the
indeterminate lines in BG/Wase, whereas there was no significant difference between growth habits in BG/T162 and BG/T164 (Fig. 1). The main stem internode length in semi-determinate lines was between that of indeterminate and determinate lines, and there was no significant S × G interaction. Flowering and maturation of the semi-determinate lines were between the indeterminate and determinate lines, and the differences among the different growth habits were one or two days. The number of branches of the semi-determinate lines was nearly equivalent to that of the determinate ones and that was 1.0 lesser than that of the indeterminate ones. The lowest pod height of the semi-determinate lines was nearly equivalent to the indeterminate ones and were 0.9% higher and 0.4% lower than determinate ones, respectively. There was a significant S × G interaction for protein content (Table 1), and the difference between indeterminate and semi-determinate lines varied depending on the genetic background, although the content was higher than that of the indeterminate ones and were 0.9% higher and 0.4% lower than determinate ones, respectively. Therefore, it is possible to develop semi-determinate varieties with yields equivalent or superior to that of indeterminate varieties by selecting high yielding lines through yield trials. Determinate lines were always lower yielding irrespective of the genetic background and maturity (Fig. 1). Kato et al. (2015) reported that the yield advantage of the indeterminate lines as compared to the determinate lines is limited to the early-maturing genetic backgrounds corresponding to MG II; their observations were based on yield comparisons between indeterminate and determinate RILs from the crosses between Japanese determinate and US indeterminate varieties. However, our results from the NILs indicated that the advantage exists in not only early-maturing but also mid-maturing genetic backgrounds corresponding to MG III and MG IV. We are aware that the yield of developed NILs with determinate growth habit was lower than that of recurrent parents (Supplemental Table 4); therefore, we cannot conclude that it is easy to develop the semi-determinate lines with higher yield than the determinate varieties currently cultivated in Japan. Further data collection in NILs developed with more backcrossing, and multiple genetic backgrounds and growing environments is needed to determine how the change from determinate to semi-determinate and indeterminate growth habits affects yield. Our results demonstrated that semi-determinate and indeterminate growth habits have potential to develop varieties with more yield advantage than that of the determinate habits, at least in the early and mid-maturation types in the northeast region of Japan.

The comparison for yield components revealed no significant interaction between stem growth habit and genetic background with respect to the number of pods and seeds per pod (Table 1). The number of pods and seeds per pod in the semi-determinate lines were similar to those in the indeterminate lines and higher than those in the determinate lines (Table 2). Hartung et al. (1981) and Hicks et al.

### Table 1. Analysis of split-plot variance (ANOVA) of agricultural characteristics of near isogenic lines with different growth habits

| Sources of variation | Degree of Freedom | Mean of square |
|----------------------|-------------------|---------------|
|                      |                   | Lowest pod height | Seed yield | Number of pods | Number of seeds per pod | 100-seed weight | Protein content | Oil content |
| Year (Y)             | 1                 | 74.2 NS*         | 780 NS     | 8931 **       | 0.210 *              | 716.9 ***      | 167.0 **       | 220.5 *** |
| Location (L)         | 1                 | 109.8 NS         | 1063 *     | 3517 *        | 0.090 NS             | 85.8 **        | 2.6 NS         | 0.3 NS    |
| Y × L                | 1                 | 34.6 NS          | 836 NS     | 5755 *        | 0.001 NS             | 99.9 **        | 40.0 NS        | 2.5 NS    |
| Genetic background (G) | 2                 | 209.8 **         | 14670 ***  | 10574 ***     | 2.022 ***            | 1460.3 ***     | 144.3 ***      | 264.8 *** |
| Pooled error a       | 8                 | 12.9 *           | 1063 *     | 3517 *        | 0.090 NS             | 85.8 **        | 2.6 NS         | 0.3 NS    |
| S × G                | 2                 | 533.5 **         | 139 NS     | 367 *         | 0.012 *              | 64.4 *         | 4.1 *          | 0.2 *     |
| Pooled error b       | 24                | 1.1              | 72 NS      | 26 NS         | 0.006               | 1.9            | 0.5            | 0.1       |

* *, **, and *** are significant at 5%, 1%, and 0.1% probability levels, respectively. NS: not significant.
Yield and lodging resistance of semi-determinate growth habit in soybeans

Effect of stem growth habit on lodging

In our study, compared with that of the indeterminate lines, a reduction in the main stem length of the semi-determinate lines tended to increase lodging resistance (Fig. 2). The lodging score of semi-determinate varieties was smaller than that of indeterminate varieties because the main stem length of the semi-determinate varieties was shorter than that of the indeterminate varieties (Bernard 1972, Wilcox 1980); this is consistent with our results. As the reduction in main stem length varies depending on the genetic background (Fig. 1), it is necessary to pay attention to the main stem length to develop semi-determinate varieties with low lodging risk. The relationship between increased lodging score and main stem length also varies depending on the genetic background (Fig. 2). It is interesting to note that the lodging score of the semi-determinate lines in BG/T164 was the lower than that of BG/Wase and BG/T162, and the difference in lodging degree between the semi-determinate and determinate lines in BG/T164 was

(1969) showed that the introduction of Dt1 increased the number of seeds per pod even when Dt2 coexisted. Our results are consistent with these results, although the reason is unclear. Considering that the number of main stem nodes and branches of the semi-determinate lines were lesser than those of the indeterminate lines, the semi-determinate lines were thought to compensate for the reduction in the total number of nodes by maximizing the number of pods per node. Contrarily, a significant interaction was observed between the stem growth habit and genetic background with respect to 100-seed weight (Table 1). However, the 100-seed weight of semi-determinate and indeterminate lines was similar to or marginally higher than that of the determinate ones across all the genetic backgrounds (Fig. 1). The 100-seed weight is important not only for yield component but also for processing quality of soybean. Particularly in case of boiled beans, heavy seed weight is preferred. Therefore, a decrease in seed weight reduces the commercial value. Kato et al. (2018) reported that the indeterminate lines have marginally heavier seeds than that of the determinate lines when NILs displaying indeterminate and determinate growth habits were used. Our results also showed that the differences among NILs were only 0.6–1.8 g and the introduction of semi-determinate and indeterminate growth habits did not reduce seed weight (Fig. 1). Therefore, introducing indeterminate and semi-determinate growth habits in determinate varieties with large seed weight will have no detrimental effect on seed weight.

### Table 2. Mean values of agricultural characteristics for each level of near isogenic lines with different growth habits

| Factor                        | Flowering time (days) | Seed-filling period (days) | Maturation time (days) | Lodging | Main stem length (cm) | Number of main stem nodes | Main stem internode length (cm) | Number of branches |
|-------------------------------|-----------------------|---------------------------|------------------------|---------|-----------------------|----------------------------|-------------------------------|-------------------|
| **Stem growth habit**         |                       |                           |                        |         |                       |                            |                               |                   |
| Determinate                  | 61.8                  | 68.7                      | 130.5                  | 1.1     | 55.5                  | 14.7                       | 3.8                           | 4.0               |
| Semi-determinate             | 62.7                  | 69.0                      | 131.7                  | 2.4     | 86.0                  | 19.9                       | 4.3                           | 3.7               |
| Indeterminate                | 63.3                  | 69.0                      | 132.3                  | 2.7     | 95.7                  | 20.8                       | 4.6                           | 4.7               |
| **Genetic background**       |                       |                           |                        |         |                       |                            |                               |                   |
| Wasesuzunari                 | 52.5                  | 64.9                      | 117.4                  | 2.3     | 63.9                  | 16.2                       | 3.9                           | 3.9               |
| Tohoku 162                   | 65.6                  | 69.7                      | 135.3                  | 2.5     | 83.1                  | 18.7                       | 4.4                           | 4.3               |
| Tohoku 164                   | 69.8                  | 72.1                      | 141.9                  | 1.4     | 90.2                  | 20.4                       | 4.4                           | 4.3               |
| **Location**                 |                       |                           |                        |         |                       |                            |                               |                   |
| Ishinazaka                   | 62.9                  | 71.2                      | 134.1                  | 2.1     | 80.0                  | 18.5                       | 4.2                           | 3.9               |
| Uenodai                      | 62.3                  | 66.6                      | 128.9                  | 2.0     | 78.1                  | 18.3                       | 4.3                           | 4.4               |
| **Year**                     |                       |                           |                        |         |                       |                            |                               |                   |
| 2015                          | 62.5                  | 71.1                      | 133.6                  | 2.2     | 80.2                  | 18.6                       | 4.2                           | 4.0               |
| 2016                          | 62.7                  | 66.8                      | 129.4                  | 1.9     | 77.9                  | 18.3                       | 4.2                           | 4.3               |

| Factor                        | Lowest pod height (cm) | Seed yield (g/plant) | Number of pods | Number of seeds per pods | 100-seed weight (g) | Protein content (%) | Oil content (%) |
|-------------------------------|------------------------|----------------------|----------------|--------------------------|---------------------|---------------------|-----------------|
| **Stem growth habit**         |                        |                      |                |                          |                     |                     |                 |
| Determinate                  | 13.7                   | 38.1                 | 60.2           | 2.06                     | 29.7                | 41.2                | 20.8            |
| Semi-determinate             | 17.6                   | 42.2                 | 66.2           | 2.14                     | 30.2                | 42.1                | 20.4            |
| Indeterminate                | 17.9                   | 43.3                 | 65.7           | 2.14                     | 31.0                | 42.3                | 20.2            |
| **Genetic background**       |                        |                      |                |                          |                     |                     |                 |
| Wasesuzunari                 | 16.2                   | 27.0                 | 52.4           | 2.26                     | 25.8                | 40.6                | 22.0            |
| Tohoku 162                   | 15.0                   | 49.1                 | 72.9           | 1.97                     | 32.4                | 42.0                | 20.7            |
| Tohoku 164                   | 17.9                   | 47.5                 | 66.7           | 2.11                     | 32.7                | 43.0                | 18.7            |
| **Location**                 |                        |                      |                |                          |                     |                     |                 |
| Ishinazaka                   | 15.8                   | 39.3                 | 60.5           | 2.09                     | 30.9                | 42.0                | 20.5            |
| Uenodai                      | 17.0                   | 43.1                 | 67.5           | 2.13                     | 29.8                | 41.8                | 20.4            |
| **Year**                     |                        |                      |                |                          |                     |                     |                 |
| 2015                          | 15.9                   | 42.8                 | 69.6           | 2.08                     | 31.9                | 42.6                | 19.6            |
| 2016                          | 16.9                   | 39.6                 | 58.5           | 2.14                     | 28.7                | 41.1                | 21.3            |
significant S × G interaction for main stem internode length between indeterminate and determinate lines, and there was no stem internode length of semi-determinate lines was be-
port those of Hartung (1981). Contrarily, the main
ty date among the three recurrent parents, our results sup-
, and
E2
ty genes when NILs with different combinations of
, E1
determinate lines were influenced by a combination of ma-
that the differences between the indeterminate and semi-
determinate lines was less than that of the
Table 2). The differ-
eum among the various stem growth habits depended on the genetic background, including the combi-
nation of the maturation genes (Bernard 1972, Chang et al.
1982, Hartung et al. 1981). As, the overall difference in
maturity between the growth habits was just within 2 days
in the present study, the effect of introduction of semi-
determinate growth habit on the maturation was considered
to be negligible, when some determinate varieties in the
northeast region of Japan are used as recurrent parents.

The lowest pod height of the semi-determinate lines was
similar to that of the indeterminate lines, but was higher
than that of the determinate lines (Table 2). These results
are consistent with those of Hartung et al. (1981) and Hicks
et al. (1969). High lowest pod height minimizes yield losses
due to machine harvesting (Kang et al. 2017, Martin and
Wilcox 1973) and reduces the risk of soil contamination in
the seeds using a low cutter bar. This height is imperative in
areas where soybeans are cultivated on the ridges. There-
fore, the change from determinate to semi-determinate
growth habit has an advantage in terms of the lowest pod
height.

In conclusion, our results demonstrated that the semi-
determinate growth habit has potential to develop varieties
with high yield and low lodging risk without reducing seed
size and protein.

Acknowledgments

We thank Dr. Setsuzu Yamoto and Dr. Kenichiro Fujii for
critical reading, and Hitoshi Sato, Osamu Fujii, Yuko Sato,
Akihiro Takahashi, Hisaho Takagai, Kenei Takahashi,
Toshiyuki Sato, and Tsuyoshi Chiba (NARO) for the field
management of yield trials. This study was supported by
grants from the Ministry of Agriculture, Forestry, and
Fisheries of Japan [Genomics-Based Technology for Agri-
cultural Improvement (SFC-1001)]. We would like to thank
Editage (www.editage.jp) for English language editing.

Literature Cited

Abe, J., D.H. Xu, Y.Suzuki, A.Kanazawa and Y.Shimamoto (2003)
Soybean germplasm pools in Asia revealed by nuclear SSRs.
Theor. Appl. Genet. 106: 445–453.
Bernard, R.L. (1972) Two genes affecting stem termination in soy-
beans. Crop Sci. 12: 235–239.
Bernard, R.L., R.L. Nelson and C.R. Cremeens. (1991) USDA Soybean
Genetic Collection: Isoline Collection. Soyb. Genet. News. 18:
27–57.
Yield and lodging resistance of semi-determinate growth habit in soybeans

Chang, J.F., D.E. Green and R. Shibles (1982) Yield and agronomic performance of semi-determinate and indeterminate soybean stem types. Crop Sci. 22: 97–101.

Cober, E.R. and M.J. Morrison (2010) Regulation of seed yield and agronomic characters by photoperiod sensitivity and growth habit genes in soybean. Theor. Appl. Genet. 120: 1005–1012.

Cooper, R.L. (1971) Influence of early lodging on yield of soybean \textit{(Glycine max (L.) Merrill.)} Agron. J. 63: 449–450.

Curtis, D.F., J.W. Tanner, B.M. Luzzi and D.J. Hume (2000) Agronomic and phenological differences of soybean isolines differing in maturity and growth habit. Crop Sci. 40: 1624–1629.

Green, D.E., P.F. Burlamaqui and R. Shibles (1977) Performance of randomly selected soybean lines with semideterminate and indeterminate growth habits. Crop Sci. 17: 335–339.

Hartung, R.C., J.E. Specht and J.H. Williams (1981) Modification of soybean plant architecture by genes for stem growth habit and maturity. Crop Sci. 21: 51–56.

Hashimoto, K., T. Nagasawa, S. Murakami, K. Kokubun, S. Nakamura, T. Koyama, S. Matsumoto, K. Sasaki and A. Okabe (1985) A new soybean variety ‘Wasesuzuzunari’. Bull. Tohoku Natl. Agric. Exp. Stn. 71: 23–42.

Hicks, D.R., J.W. Pendleto, R.L. Bernard and T.J. Johnston (1969) Response of soybean plant types to planting patterns. Agron. J. 61: 290–293.

Hwang, T.Y., Y. Nakamoto, I. Kono, H. Enoki, H. Funatsuki, K. Kitamura and M. Ishimoto (2008) Genetic diversity of cultivated and wild soybeans including Japanese elite cultivars as revealed by length polymorphism of SSR markers. Breed. Sci. 58: 315–323.

Kaga, A., T. Shimizu, S. Watanabe, Y. Tsubokura, Y. Katayose, K. Harada, D.A. Vaughan and N. Tomooka (2012) Evaluation of soybean germplasm conserved in NIAS genebank and development of mini core collections. Breed. Sci. 61: 566–592.

Kanda, Y. (2013) Investigation of the freely available easy-to-use software ‘EZR’ for medical statistics. Bone Marrow Transplant. 48: 452–458.

Kang, B.K., H.T. Kim, M.S. Choi, S.C. Koo, J.H. Seo, H.S. Kim, S.O. Shin, H.T. Yun, J.S. Oh, K.P. Kulkarni et al. (2017) Genetic and environmental variation of first pod height in soybean \textit{(Glycine max (L.) Merr.)}. Plant Breed. Biotechnol. 5: 36–44.

Kato, S., A. Kikuchi, S. Shimamura, Y. Kono, S. Yumoto, Y. Takada, S. Shimada, T. Sakai, H. Shimada, K. Takahashi et al. (2014) A new soybean cultivar, “Akimiyabi,” with resistance to soybean mosaic virus and lodging. Bull. Tohoku Agric. Res. Cent. 116: 13–27.

Kato, S., K. Fujii, S. Yumoto, M. Ishimoto, T. Shiraiva, T. Sayama, A. Kikuchi and T. Nishio (2015) Seed yield and its components of indeterminate and determinate lines in recombinant inbred lines of soybean. Breeding Sci. 65: 154–160.

Kato, S., T. Sayama, M. Ishimoto, S. Yumoto, A. Kikuchi and T. Nishio (2018) The effect of stem growth habit on single seed weight and seed uniformity in soybean \textit{(Glycine max (L.) Merrill.)} Breed Sci. 68: 352–359.

Kilgore-Norquesta, L. and C.H. Sneller (2000) Response of soybean plant types to planting patterns. Crop Sci. 40: 83–90.

Liu, B.H., S. Watanabe, T. Uchiyama, F.J. Kong, A. Kanazawa, Z.J. Xia, A. Nagamatsu, M. Arai, T. Yamada, K. Kitamura et al. (2010) The soybean stem growth habit gene \textit{Dt1} is an ortholog of Arabidopsis \textit{TERMINAL FLOWER1}. Plant Physiol. 153: 198–210.

Liu, Y., D. Zhang, J. Ping, S. Li, Z. Chen and J. Ma (2016) Innovation of a regulatory mechanism modulating semi-determinate stem growth through artificial selection in soybean. PLoS Genet. 12: e1005818.

Martin, R.J. and J.R. Wilcox (1973) Heteritasty of lowest pod height in soybeans. Crop Sci. 13: 201–203.

Noor, R.B.M. and C.E. Caviness (1980) Influence of lodging on pod distribution and seed yield in soybeans. Agron. J. 72: 904–906.

Ping, J., Y. Liu, L. Sun, M. Zhao, Y. Li, M. She, Y. Sui, F. Lin, X. Liu, Z. Tang et al. (2014) \textit{Dt2} is a gain-of-function MADS-domain factor gene that specifies semideterminacy in soybean. Plant Cell. 26: 2831–2842.

Saitoh, K., N. Nishimura and T. Kitahara (2012) Effect of lodging on seed yield of field-grown soybean: Artificial lodging and lodging preventing treatments. Jpn. J. Crop Sci. 81: 27–32.

Sayama, T., T.Y. Hwang, K. Komatsu, Y. Takada, M. Takahashi, S. Kato, H. Sasama, A. Higashi, Y. Nakamoto, H. Funatsuki et al. (2011) Development and application of a whole-genome simple sequence repeat panel for high-throughput genotyping in soybean. DNA Res. 18: 107–115.

Schmutz, J., S.B. Cannon, J. Schlueeter, J. Ma, T. Mitros, W. Nelson, D.L. Hyten, Q. Song, J.J. Thelen, J. Cheng et al. (2010) Genome sequence of the palaeopolyploid soybean. Nature 463: 178–183.

Shannon, J.G., J.R. Wilcox and A.H. Probst (1971) Response of soybean genotypes to spacing in hill plots. Crop Sci. 11: 38–40.

Shapiro, C.A. and A.D. Flowerday (1987) Effect of simulated lodging on soybean yield. J. Agron. Crop Sci. 158: 8–16.

Tian, Z.X., X.B. Wang, R. Lee, Y.H. Li, J.E. Specht, R.L. Nelson, P.E. McClean, L.J. Qiu and J.X. Ma (2010) Artificial selection for determinate growth habit in soybean. Proc. Natl. Acad. Sci. USA 107: 8563–8568.

Tsubokura, Y., S. Watanabe, Z. Xia, H. Kanamori, H. Yamagata, A. Kaga, Y. Katayose, J. Abe, M. Ishimoto and K. Harada (2014) Natural variation in the genes responsible for maturity loci \textit{E1}, \textit{E2}, \textit{E3} and \textit{E4} in soybean. Ann. Bot. 113: 429–441.

Ude, G.N., W.J. Kenworthy, J.M. Costa, P.B. Cregan and J. Alvernaz (2003) Genetic diversity of soybean cultivars from China, Japan, North America, and North American ancestral lines determined by amplified fragment length polymorphism. Crop Sci. 43: 1858–1867.

Wilcox, J.R. (1980) Comparative performance of semideterminate and indeterminate soybean lines. Crop Sci. 20: 277–280.

Yamada, T., M. Hajika, N. Yamada, K. Hirata, A. Okabe, N. Oki, K. Takahashi, K. Seki, K. Okano, Y. Fujita et al. (2012) Effect on flowering and seed yield of dominant alleles at maturity loci \textit{E2} and \textit{E3} in a Japanese cultivar, Enrei. Breed. Sci. 61: 653–660.