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## Effect of thinning on cultivar differences of green stem disorder in soybean

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### ABSTRACT

Green stem disorder (GSD) in soybean (*Glycine max* (L.) Merrill) negatively affects harvest efficiency and seed appearances. Breeding GSD-insensitive cultivars is expected to be an effective countermeasure to GSD. However, it is difficult to stably detect cultivar differences in GSD under conventional field conditions because the occurrences of GSD largely vary by location and year. The thinning effect, which had been reported to promote GSD, may help accurate phenotyping for occurrences of GSD in breeding. To verify this possibility, the thinning treatment was applied to four cultivars, the GSD severity values of which were evaluated in an independent study by another group. As a result, the cultivar differences in GSD severity were generally comparable between the present and previous studies. However, the difference was more evident, with the thinning treatment exhibiting the GSD score of 2.8 of 'Hatsusayaka' compared with the GSD score of 3.6 of 'Sachiyutaka', while the scores of those cultivars were similar without the thinning treatment. A positive correlation between GSD severity and N concentration in the main stem could be seen but the increasing rate of GSD score with the N concentration in the main stem differed between cultivars. Thus, although more cultivars need to be tested to prove, the thinning treatment could be useful as a phenotyping technique in the breeding of GSD-insensitive cultivars.

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## 1. Introduction

Green stem disorder (GSD) in soybean is defined as the symptom in which stems and leaves stay green and retain some moisture even when pods normally become matured (Harbach et al., 2016; Hobbs et al., 2006). GSD is a problem for farmers because it negatively affects seed appearances and harvest efficiency (Hill et al., 2006; Ogiwara, 2002).

It has been reported that there are differences in GSD occurrences between cultivars (Fujii et al., 2015; Furuya & Umezaki, 1993; Hill et al., 2006; Isobe et al., 2015; Matsumoto et al., 1986; Mochizuki et al., 2005; Pierce et al., 1984; Yamada et al., 2014). Thus, breeding GSD-insensitive cultivars is expected to be an effective solution to GSD.

During breeding, comparisons of the GSD severity between many cultivars or lines are needed. However, it is difficult to accurately detect the differences in GSD severity between cultivars or lines because the occurrences of GSD largely vary by field location and cultivation year (Fujii et al., 2015; Hill et al., 2006). In particular, it is generally difficult for breeders to find differences in potential GSD sensitivity between cultivars or lines when GSD occurrences are somehow suppressed.

Thus, it is desirable to establish an effective experimental treatment that promotes GSD occurrences in

order to stably detect the differences between cultivars or lines.

Depodding, which has often been used in previous studies, promotes the delayed senescence of leaves and stems (Crafts-Brandner et al., 1984; Crafts-Brandner & Egli, 1987; Egli & Bruening, 2006; Htwe et al., 2011; Leopold et al., 1959; Mondal et al., 1978; Wittenbach, 1982) and has been thought to imply that GSD is related to source–sink balance, that is, a relative increase in source levels resulting from sink limitation (Egli & Bruening, 2006; Wittenbach, 1983a, 1983b). However, depodding is a time- and labor-consuming method and is difficult to do, especially in a field experiment. Depodding also has a problem as an experimental technique, because cutting organs induces the expression of stress-response genes and metabolic shifts in distant leaves, which may lead to unintended consequences as well as sink limitation (Turner et al., 2012).

In our previous study, it was found that thinning at the R5 growth stage, which can be performed more easily than depodding at the field scale, promoted GSD occurrences in soybean (Yamazaki et al., 2018). Yamazaki et al. (2018) discussed that the improved light availability at R5 stage by thinning enhanced source relative to sink, and then promoted the

occurrence of GSD. Thus, GSD occurrences promoted by thinning also could be related to source–sink balance as well as depodding.

However, in the previous study, the cultivar used in the experiment was only ‘Sachiyutaka’, the leading cultivar in the region including the experimental field.

Thus, the effectiveness of the thinning treatment as the experimental technique for breeding needs to be validated by confirming (1) whether thinning promotes the GSD occurrences of other cultivars in addition to ‘Sachiyutaka’ and (2) whether differences in GSD severity between cultivars are clarified by thinning.

To confirm these, in the current study, we applied the thinning treatment on four cultivars, which were evaluated in terms of their GSD severity in an independent study by another group (Saruta et al., 2012), and we compared the GSD severity and agronomical traits to draw genotypically different characteristics.

## 2. Materials and methods

### 2.1. Plant materials and experimental site

Two experiments were conducted in the experimental fields of NARO, Western Region Agricultural Research Center, Hiroshima, Japan (lat. 34°30’N, long. 133°23’ E, and 2 m elevation; Typic Fluvaquents soil type). Exp. 1 was conducted in 2015. Exp. 2 was conducted in 2016 and in 2017. In the experimental fields, the ground-water level was maintained at 30 cm below the

ground surface by the farm-oriented enhanced aquatic system FOEAS (Wakasugi & Fujimori, 2009). Inorganic fertilizers were applied at 3 g m<sup>-2</sup> of N, 10 g m<sup>-2</sup> of P<sub>2</sub>O<sub>5</sub>, and 10 g m<sup>-2</sup> of K<sub>2</sub>O a day before sowing. Insecticides and fungicides were applied properly to avoid biotic stresses. In 2016, three cultivars, namely, ‘Tachinagaha’ (Maturity group 5, Ude et al., 2003), ‘Sachiyutaka’ (Maturity group 6, Fatichin et al., 2013), and ‘Hatsusayaka’ (equivalent to Maturity group 5) were used (Table 1). In 2017, four cultivars, namely, ‘Tachinagaha’, ‘Tamahomare’ (Maturity group 6, Fatichin et al., 2013), ‘Sachiyutaka’, and ‘Hatsusayaka’ were used (Table 1). In these cultivars, the previously judged GSD score (on a 6-point scale) was reported by Saruta et al. (2012) (Table 1).

### 2.2. Treatments

#### 2.2.1. Exp. 1: the effect of the timing of thinning on GSD in ‘Sachiyutaka’

The sowing date was 24 June 2015. There were 12 plots (6 treatments and 2 replications). The size of each plot was 3.0 m × 3.3 m. The planting density was either dense (22.2 plants m<sup>-2</sup>, 0.3 m row, and 0.15 m plant spacing) or sparse (5.56 plants m<sup>-2</sup>, 0.6 m row, and 0.3 m plant spacing). There were also plots in which thinning treatments were conducted at R1, R1 + 14 d, R5, and R5 + 14 d (7 August, 21 August, 4 September, and 18 September). In the thinning treatment, the planting density changed from dense (22.2 plants m<sup>-2</sup>) to sparse (5.56 plants m<sup>-2</sup>) by performing thinning

Table 1. Dates of growth stage and treatments.

Year	Cultivar	Previously evaluated GSD score <sup>a</sup>	Treatment <sup>b</sup>	The date of the growth stage or treatment				
				Sowing	R1	R5	Thinning	R8
2016	Tachinagaha	4.8	Dense	27 June	30 July	18 August	-	27 October
			Thinning	27 June	30 July	19 August	26 August	28 October
	Sachiyutaka	2.0	Dense	27 June	4 August	29 August	-	1 November
			Thinning	27 June	4 August	28 August	5 September	1 November
	Hatsusayaka	0.7	Dense	27 June	5 August	3 September	-	2 November
			Thinning	27 June	5 August	3 September	10 September	3 November
2017	Tachinagaha	4.8	Dense	22 June	28 July	20 August	-	1 November
			Thinning	22 June	28 July	21 August	27 August	5 November
			Sparse	22 June	29 July	18 August	-	31 October
	Tamahomare	2.4	Dense	22 June	30 July	25 August	-	5 November
			Thinning	22 June	30 July	26 August	1 September	8 November
			Sparse	22 June	31 July	25 August	-	5 November
	Sachiyutaka	2.0	Dense	22 June	3 August	28 August	-	1 November
			Thinning	22 June	3 August	28 August	4 September	1 November
			Sparse	22 June	1 August	24 August	-	1 November
	Hatsusayaka	0.7	Dense	22 June	3 August	30 August	-	2 November
			Thinning	22 June	4 August	30 August	6 September	3 November
			Sparse	22 June	3 August	29 August	-	1 November

Notes: <sup>a</sup>Previously evaluated GSD score means GSD score evaluated by 6-grade system (0–5) in Saruta et al. (2012).

<sup>b</sup>Dense: plant population density was kept at 22.2 plants m<sup>-2</sup>. Thinning: thinning was conducted at R5. Sparse: plant population density was kept at 5.56 plants m<sup>-2</sup>.

activities. Thinning involved cutting off all the above-ground parts of the plant in every other row of the plot and every other plant in the remaining rows. All data were recorded by sampling individual plants randomly selected from each plot, excluding plants on the border of the plot. The number of plants selected was six. The value for each plot was the average score of the recorded plants, and the mean of the replications was the representative score for each treatment group.

### 2.2.2. Exp. 2: the effect of thinning on cultivar differences in GSD

The sowing dates were 27 June 2016 and 22 June 2017 (Table 1). There were 12 plots (3 cultivars, 2 treatments, and 2 replications) in 2016 and 24 plots (4 cultivars, 3 treatments, and 2 replications) in 2017. The size of each plot was 3.0 m × 2.1 m in both years. There were three treatments that differed in their planting density: dense, sparse, and thinning. In the dense and sparse treatments, the planting density was maintained at 22.2 and 5.56 plants m<sup>-2</sup>, respectively, from sowing until R8. In the thinning treatment, thinning was conducted at 6 or 7 d after R5 for each cultivar (Table 1). All data were recorded by sampling individual plants randomly selected from each plot, excluding the plants on the border of the plot. The number of plants selected was six in 2016 and nine in 2017. The value for each plot was the average score of the recorded plants, and the mean of the replications was the representative score for each treatment group.

### 2.3. Measurements

The dates of growth stages R1, R5, and R8 were recorded for each plant, following the method of Fehr and Caviness (1977). The severity of GSD was assessed for each plant at the R8 stage using a scoring method slightly modified from that of Furuya and Umezaki (1993) (Yamazaki et al., 2018). In the scoring method, the GSD score (1–5) was assigned based on the stem color and number of leaves left on the stem at R8. A high GSD score represents severe GSD symptoms. In Exp. 2, seed weight was measured for each plant sampled after R8 stage. Pod number per node was calculated by the number of pods and the number of total nodes counted for each plant sampled after R8 stage. The dry matter N concentration of the main stem was measured using a Vario MAX CN (Elementar Analysensysteme GmbH, Langenselbold, Germany). Three nodes at the center of the main stem were sampled and analyzed. In Exp. 2, several plants were randomly selected from each plot in the plants thinned after R5, excluding the plants on the border of the plot,

except ‘Tamahomare’ in 2017, in order to investigate cultivar differences of plants in dense plant population at R5 growth stage in leaf area, the number of leaves, dry weight of leaflets, and the dry matter N concentration of the main stem. The number of plants selected was six in 2016 and nine in 2017. Leaf area was measured using a LI-3100C (LI-COR, Nebraska, USA). Dry weight of leaflets was measured after drying at 80°C for 3 d.

### 2.4. Statistical analysis

Experiments were conducted in a completely randomized design. Analysis of variance (ANOVA) and Tukey’s test or *t*-test were used to test the differences in values and compare the means between the treatment groups or cultivars ( $p < 0.05$  or  $p < 0.01$ ). GSD scores were analyzed after Box-Cox transformation. The correlation coefficient of the GSD score and the N concentration of the main stem for each plant sampled were calculated. All analyses were performed using the statistical software BellCurve for Excel (Social Survey Research Information Co., Ltd., Tokyo, Japan).

## 3. Results

### 3.1. Effect of the growth stage of the thinning treatment on the severity of GSD

The GSD scores of the treatment of thinning at R5 (3.7) and R5 + 14 d (3.5) were significantly higher than those of the treatment kept dense or the sparse plant population (2.3) (Table 2). The GSD scores of the thinning treatments at R1 and R1 + 14 d were not significantly different from those of the dense or sparse treatments or from the thinning treatments at R5 and R5 + 14 d, showing intermediate values (Table 2). There was no

**Table 2.** The effects of the timing of thinning on GSD in ‘Sachiyutaka’.

Cultivar	Treatment	The growth stage of thinning	GSD score
Sachiyutaka	Dense	-	2.3 <sup>b</sup>
		R1	2.8 <sup>ab</sup>
	Thinning	R1 + 14 d	3.1 <sup>ab</sup>
		R5	3.7 <sup>a</sup>
		R5 + 14 d	3.5 <sup>a</sup>
Sparse	-	2.3 <sup>b</sup>	
ANOVA			*

Notes: Within columns, means followed by the same letter are not significantly different according to Tukey’s test (0.05).

\*Significant at the 0.05 probability level. The GSD scores of dense, thinning at R1, thinning at R5, and sparse treatment were the data used in Yamazaki et al. (2018).

difference in GSD score between the treatments with thinning at R5 and thinning at R5 + 14 d (Table 2).

### 3.2. Differences in severity of GSD between treatments in each cultivar and between cultivars in each treatment

In the average of the 2 years, the GSD scores of the thinning treatment were significantly higher than those of the dense treatment in both 'Sachiyutaka' and 'Hatsusayaka' (Table 3). Meanwhile, in 'Tachinagaha', there were no significant differences in GSD scores between the thinning treatment and the dense treatment.

In the comparison between the three planting density treatments in 2017 (Table 5), there were no significant differences in GSD score between the dense treatment and the sparse treatment within each cultivar. The thinning treatment, however, showed significantly higher GSD scores than the other treatments in all cultivars except 'Tachinagaha'.

In the dense treatment, the GSD score of 'Tachinagaha' (4.0) was significantly higher than those of the other two cultivars (Table 3). There were no significant differences between 'Sachiyutaka' (2.1) and 'Hatsusayaka' (1.8) in terms of their GSD score.

In the thinning treatment, 'Tachinagaha' showed the highest GSD score (4.3), and the next was 'Sachiyutaka' (3.6), and 'Hatsusayaka' showed the lowest (2.8) (Table 3). This order is the same as the order of the previously evaluated GSD scores (Table 1).

In the results of three-way ANOVA (Table 3), there were no significant differences by year, while there were significant differences in the cultivars, treatments, and interactions of cultivar and treatment.

### 3.3. Cultivar differences in seed weight, number of pods, and leaf growth at R5

There were no significant differences between cultivars in terms of the seed weight per plant and the number of pods per node in the dense treatment (Table 3). In the thinning treatment, the seed weight per plant of 'Sachiyutaka' was significantly larger than any those of the other cultivars, and the number of pods per node did not differ between cultivars (Table 3). The seed weight per plant in the thinning treatment was significantly higher than that in the dense treatment only in 'Sachiyutaka', although the seed weight per plant tended to increase in the thinning treatment compared to the dense treatment in the other two cultivars (Table 3). The increasing rates of seed weight per plant by thinning were 23.6% in 'Tachinagaha' (22.0–17.8 g plant<sup>-1</sup>), 58.0% in 'Sachiyutaka' (32.7–20.7 g plant<sup>-1</sup>), and 31.9 % in 'Hatsusayaka' (26.9–20.4 g plant<sup>-1</sup>) (Table 3). The numbers of pods per node were significantly higher in the thinning treatment than in the dense treatment in 'Tachinagaha' and 'Sachiyutaka' (Table 3).

There was no statistically significant difference between cultivars in terms of their leaf area, number of leaves, or dry weight of leaflets at the R5 growth stage (Table 4).

### 3.4. N concentration of the main stem

The N concentrations of the main stem sampled at R5 stage in the dense plant population were not different between the three cultivars or between 2 years (Table 4). The N concentration in the main stem sampled after the R8 stage in 'Tachinagaha' and 'Sachiyutaka' with thinning was significantly higher than that in the dense treatment (Table 3). The dense treatment of 'Tachinagaha' also tended to have higher N concentrations when compared

**Table 3.** The effects of thinning on GSD, growth duration, N concentration, and sink size (mean of 2-year experiment).

Cultivar	Treatment <sup>a</sup>	GSD score <sup>b</sup>	R8 (DAS)	N (mg g <sup>-1</sup> ) <sup>c</sup>	Seed weight (g plant <sup>-1</sup> )	Pod number per node
Tachinagaha	Dense	4.0 <sup>ab</sup>	127	5.8 <sup>bc</sup>	17.8 <sup>b</sup>	1.25 <sup>bc</sup>
	Thinning	4.3 <sup>a</sup>	130	9.8 <sup>a</sup>	22.0 <sup>b</sup>	1.75 <sup>a</sup>
Sachiyutaka	Dense	2.1 <sup>cd</sup>	130	3.8 <sup>c</sup>	20.7 <sup>b</sup>	1.19 <sup>c</sup>
	Thinning	3.6 <sup>b</sup>	130	9.5 <sup>ab</sup>	32.7 <sup>a</sup>	1.63 <sup>ab</sup>
Hatsusayaka	Dense	1.8 <sup>d</sup>	131	3.0 <sup>c</sup>	20.4 <sup>b</sup>	1.18 <sup>c</sup>
	Thinning	2.8 <sup>c</sup>	132	3.8 <sup>c</sup>	26.9 <sup>b</sup>	1.47 <sup>abc</sup>
ANOVA	Year	ns	**	ns	ns	**
	Cultivar	**	ns	**	*	ns
	Treatment	**	ns	**	**	**
	Year*Cultivar	ns	*	ns	ns	ns
	Year*Treatment	ns	ns	ns	ns	ns
	Cultivar*Treatment	**	ns	*	ns	ns
	Year*Cultivar*Treatment	ns	ns	ns	ns	ns

Notes: Within columns, means followed by the same letter are not significantly different according to Tukey's test (0.05).

\*\*Significant at the 0.01 probability level; \*Significant at the 0.05 probability level. ns, nonsignificant at the 0.05 probability level.

<sup>a</sup>Dense: plant population density was kept at 22.2 plants m<sup>-2</sup>. Thinning: the thinning treatment was conducted at R5.

<sup>b</sup>ANOVA and Tukey's test were conducted after Box-Cox transformation. A part of the data in Yamazaki et al. (2018) was used as the GSD scores of 'Sachiyutaka'.

<sup>c</sup>N (mg g<sup>-1</sup>) is dry matter N concentration in main stem.

**Table 4.** Leaf growth at R5 of each cultivar (mean of 2-year experiments).

Cultivar	Leaf area (cm <sup>2</sup> plant <sup>-1</sup> )	Leaf number (plant <sup>-1</sup> )	Dry weight of leaflets (g plant <sup>-1</sup> )	N (mg g <sup>-1</sup> ) <sup>a</sup>
Tachinagaha	2463	15.8	9.2	11.5
Sachiyutaka	2724	18.1	9.7	11.9
Hatsusayaka	2526	17.0	9.4	11.8
ANOVA Cultivar	ns	ns	ns	ns
Year	ns	ns	ns	ns
Interaction	ns	ns	ns	ns

Note: ns, nonsignificant at the 0.05 probability level.

<sup>a</sup>N (mg g<sup>-1</sup>) is dry matter N concentration in main stem.

**Table 5.** The effect of thinning on GSD in Exp. 2 in 2017.

Cultivar	Treatment <sup>a</sup>	GSD score
Tachinagaha	Dense	4.0 <sup>a</sup>
	Thinning	4.2 <sup>a</sup>
	Sparse	4.2 <sup>a</sup>
Tamahomare	Dense	2.1 <sup>cd</sup>
	Thinning	3.9 <sup>a</sup>
	Sparse	2.3 <sup>cd</sup>
Sachiyutaka	Dense	1.8 <sup>d</sup>
	Thinning	3.6 <sup>ab</sup>
	Sparse	2.0 <sup>cd</sup>
Hatsusayaka	Dense	1.7 <sup>d</sup>
	Thinning	3.0 <sup>bc</sup>
	Sparse	1.6 <sup>d</sup>
ANOVA	Cultivar	**
	Treatment	**
	Cultivar*Treatment	**

Notes: Within columns, means followed by the same letter are not significantly different according to Tukey's test (0.05).

\*\*Significant at the 0.01 probability level.

<sup>a</sup>Dense: plant population density was kept at 22.2 plants m<sup>-2</sup>. Thinning: the thinning treatment was conducted at R5. Sparse: plant population density was kept at 5.56 plants m<sup>-2</sup>.

with 'Hatsusayaka' (Table 3). These three groups which showed high N concentration in the main stem

corresponded to the groups which showed significantly high GSD scores (Table 3).

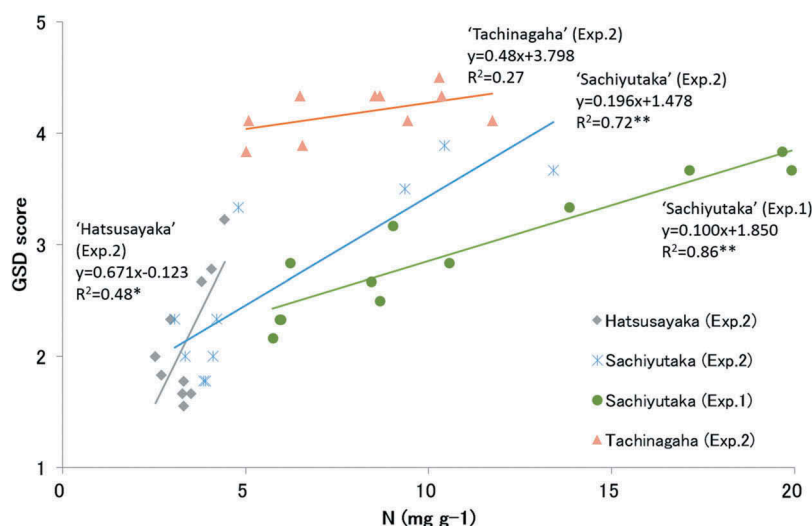
The regression lines of the correlation between the N concentration in the main stem and GSD score are shown in Figure 1. 'Sachiyutaka' in Exp. 1 and in Exp. 2 and 'Hatsusayaka' showed significant coefficients of determination (R<sup>2</sup>) (Figure 1). The inclinations of the regression lines were smaller in 'Tachinagaha' (0.048) and larger in 'Hatsusayaka' (0.671) compared to those in 'Sachiyutaka' in Exp. 2 (0.196) and Exp. 1 (0.100) (Figure 1).

## 4. Discussion

### 4.1. Thinning generally promotes GSD occurrences in soybean cultivars

In the previous study (Yamazaki et al., 2018), thinning did not affect the developmental progression of the reproductive stage, which was evaluated based on pod maturation, suggesting that GSD promoted by thinning was characterized by the delayed maturation of the leaves and stems. In the present results, the days from sowing to R8 were also not significantly different between treatments and between cultivars (Table 3), suggesting that the GSD occurrences promoted by thinning or cultivar differences in our experiments were characterized by the delayed maturation of the leaves and stems.

As a result of the 2-year experiment, not only 'Sachiyutaka' but also 'Hatsusayaka' showed significantly severe GSD symptoms in the thinning treatment compared to those in the dense treatment (Table 3). In



**Figure 1.** The correlation between N concentration in the main stem and GSD scores of 'Sachiyutaka' in Exp. 1 ( $n = 12$ ), 'Tachinagaha' ( $n = 10$ ), 'Sachiyutaka' ( $n = 10$ ), and 'Hatsusayaka' ( $n = 10$ ) in 2-year experiments in Exp. 2.

Notes: Each point in the figure indicates the average score of 6 or 9 plants in a replication.

'Tamahomare', the thinning treatment showed severe GSD symptoms compared to the dense or sparse treatment, which were the same as those shown by 'Sachiyutaka' and 'Hatsusayaka', although that was the result of only 1 year (Table 5). These results suggest that the phenomenon that thinning promotes GSD occurrences is not limited to 'Sachiyutaka' but is seen generally in soybean cultivars. However, 'Tachinagaha' without thinning treatment showed significantly severe GSD symptoms compared to the other cultivars, and there were no differences between treatments (Table 3). This fact suggests that in 'Tachinagaha', there was another factor unrelated to the thinning treatment that promotes GSD occurrences, due to which the effect of thinning on GSD severity could not be detected as statistically significant differences.

#### **4.2. Thinning clarified differences in GSD severity between cultivars**

In Exp. 2, there was no significant difference in GSD severity without thinning between 'Sachiyutaka' and 'Hatsusayaka' (Table 3). On the other hand, in the thinning treatment group, there was a significant difference between 'Sachiyutaka' and 'Hatsusayaka'. In addition, the order of GSD severity of cultivars corresponded to the order of the previously judged GSD severity (Saruta et al., 2012). These facts indicated that thinning treatment magnifies cultivar differences that could not be easily detected without thinning treatment. Thus, although more cultivars need to be tested to prove, the thinning treatment could be used as a useful experimental technique to stably detect cultivar differences in breeding, especially when GSD occurrences are generally scarce and there are little differences between cultivars.

#### **4.3. The timing of thinning and cultivar differences in GSD**

In Exp. 2, the timing of thinning differed between cultivars by 15 d in 2016 and 10 d in 2017 because the date of the R5 growth stage differed between cultivars (Table 1). Thus, we cannot deny the possibility that the differences in GSD scores between cultivars in the thinning treatment at R5 were caused by the different timing of thinning, which possibly lead to unequal meteorological conditions. However, in Exp. 1, both thinning at R5 and thinning at R5 + 14 d significantly promoted GSD occurrences compared to the dense or sparse groups, and there was no significant difference in GSD occurrences between the former two treatments (Table 2). These results suggest that the effect of thinning on GSD severity does not significantly change, at least during the 14 d after R5.

Although there were significant differences in GSD score between 'Sachiyutaka' and 'Hatsusayaka' subjected to the thinning treatment, the differences in the timing of thinning were only 5 d in 2016 and 2 d in 2017 (Table 1). This result suggests that the differences in GSD score between 'Sachiyutaka' and 'Hatsusayaka' subjected to the thinning treatment were not caused by the timing of the thinning treatment but rather by some genotypic factors.

#### **4.4. Sink and source size and the cultivar differences in GSD**

As the mechanism of GSD occurrences has been suggested to be related to source–sink balance (Egli & Bruening, 2006; Wittenbach, 1983a, 1983b), cultivar differences in GSD may be caused by differences in source–sink balance, although there has been no study of the relation between the cultivar differences of GSD and source–sink balance. In other words, it could be that cultivars that have a smaller sink size or a larger source ability may show higher GSD scores.

However, in the present study, there were no significant differences in the sink size between cultivars in the dense treatment group, which was shown as the seed weight per plant or the number of pods per node, although there were significant differences in GSD score between cultivars (Table 3). In the thinning treatment group, 'Sachiyutaka' had significantly larger seed weight per plant than 'Hatsusayaka', although 'Sachiyutaka' showed significantly higher GSD scores than 'Hatsusayaka' (Table 3). These results suggested that different sink size itself was not the cause of differences in GSD severity among cultivars. The results agreed with the reports of Isobe et al. (2015), which demonstrated no correlation between growth parameters and GSD severity in several cultivars.

Meanwhile, the size of photosynthetically assimilative organs (leaf area, the number of leaves, and dry matter of leaflets) at R5, which partly contributes to source ability (Saeki, 1960), showed no difference between cultivars (Table 4). Thus, it should be examined whether leaf photosynthetic ability, which is also related to source ability (Long et al., 2006; Zhu et al., 2010), as well as the nutrient absorption ability of root and assimilation translocation activities are the cause of the cultivar differences in GSD.

From the viewpoint of leaf photosynthetic ability, the result that the increasing ratio of seed weight per plant by the thinning treatment in 'Sachiyutaka' tended to be higher than that of 'Hatsusayaka' is also notable (Table 3). Given that the increasing

ratio of seed weight per plant indicated enhanced photosynthetic ability by light enrichment caused by thinning (Schou et al., 1978), the positive effect of thinning on photosynthetic ability may be larger in 'Sachiyutaka' than in 'Hatsusayaka'. This may have caused the thinning treatment to magnify the differences in GSD severity between 'Sachiyutaka' and 'Hatsusayaka'.

#### 4.5. Cultivar differences in the correlation between the N concentration in the main stem and GSD severity

In our previous study (Yamazaki et al., 2018), the N concentration in the main stem at maturity was significantly increased by the thinning treatment at R5, and it was positively and significantly correlated to the GSD score in 'Sachiyutaka'. This result corresponded to the report that depodded GSD soybean plants tend to have high dry matter N concentration in the stem compared to controls (Egli & Bruening, 2006).

In the present study, the N concentrations in the main stem also showed the same tendency in each cultivar in Exp. 1 and in Exp. 2. Especially, there were statistically significant positive correlations in 'Sachiyutaka' in both Exp. 1 and Exp. 2 and 'Hatsusayaka' (Figure 1), and the thinning treatment significantly increased the N concentrations in the main stem in 'Tachinagaha' and 'Sachiyutaka' in Exp. 2 (Table 3).

In the comparisons of cultivars in the same treatment in Exp. 2, the N concentration in the main stem increased as the GSD score increased (Table 3). In addition, there were no differences between cultivars in terms of the N concentration in the main stem at the R5 growth stage, and the values at R5 (11.5–11.9 mg g<sup>-1</sup>) were higher than the values at R8 (3.0–9.8 mg g<sup>-1</sup>) in each cultivar, regardless of the thinning treatment (Tables 3 and 4). These results suggested that a positive correlation between GSD severity and N concentration in the main stem can generally be seen in soybean cultivars, and differences in N concentration in the main stem were characterized by decreased N concentrations from the R5 growth stage to the R8 growth stage.

However, the increasing rate of GSD score with an increase in N concentration in the main stem indicated by the inclination of the regression line in Figure 1 differed between cultivars. The increasing rate of the GSD score with N concentration in the main stem was smaller in 'Tachinagaha' than in 'Sachiyutaka' and was larger in 'Hatsusayaka' than in 'Sachiyutaka'. These results imply that the severity of the GSD response to N concentrations in the main stem differed between cultivars. Depodded soybean showed expression of vegetative storage

proteins in vegetative organs, suggesting that a surplus of assimilation products accumulate (Ogiwara & Ishikura, 1994; Wittenbach, 1983a, 1983b) and lead to high N concentrations in the main stem in vegetative organs in GSD plants. Thus, the maturation of the leaf and stem is seemingly delayed with the surplus accumulation in 'Sachiyutaka', while the surplus might be critical to the delay of maturation in 'Hatsusayaka'. In 'Tachinagaha', the delay of maturation was induced regardless of increasing N concentration in the main stem by thinning treatment. This may indicate that there is another factor to induce the delay which is not related to the N surplus in 'Tachinagaha'. Zhao et al. (2014) reported that there were cultivar differences in nitrogen redistribution and its contribution to seed yield. This cultivar differences may be related to the differences in relationships between N surplus and GSD severity.

There was a slight difference in the inclination of the regression line between Exp. 1 and Exp. 2 in 'Sachiyutaka', suggesting that some environmental factors influence the severity of the GSD response to N surplus.

## 5. Conclusions

The thinning treatment at R5 growth stage magnified cultivar differences in GSD severity that could not be detected without this treatment. Thus, although more cultivars need to be tested to prove, the thinning treatment could be used as an experimental technique for breeding GSD-insensitive cultivars to stably detect cultivar differences in GSD severity.

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