The Effects of Developmental Traits on Genetic Variation of Green Stem Disorder in Soybean [*Glycine max* (L.) Merr.]

2015

Kenichiro Fujii

Contents

Summary

Soybean [*Glycine max* (L.) Merr.] is a typical monocarpic plant species that loses green color and stem moisture as the seeds mature. However, these plants occasionally maintain stem greenness, stem moisture and leaf color at seed maturity, and this phenomenon was termed as green stem disorder (GSD). The severity level in plants has been described from entirely green plants to leafless plants with a yellow-green stem. Because GSD plant causes a reduction of harvesting efficiency and negatively impacts seed appearance when combine harvester is employed, GSD is regarded as an unfavorable phenomenon that should be prevented. The occurrence of GSD seems to be induced by biotic and abiotic stress. But, the reproducibility of experimental result is uncertain, and GSD plants appear even under appropriately managed condition. Hence, any reliable countermeasures through modifying cultivation methods have not been established. On the other hand, variability in the severity of GSD in soybean cultivars has been reported, and thus use of GSD resistant cultivars would be effective to prevent the occurrence of GSD. This study identified inherited plant characteristics related to GSD, and analyzed how the heritable traits are associated with the severity of GSD to provide a reliable countermeasure.

Assessment of variability of GSD severity in soybean cultivars is fundamental for the breeding of cultivars resistant to GSD. But, the evaluation using a wide variation of soybean genotypes has not been conducted. In Japan, introduction of combine harvester has started in 1970s, while combine has been utilized since early twentieth century in USA. Therefore, US soybean cultivars may accumulate suitable plant properties for mechanical harvesting. In this chapter, various Japanese and US cultivars were used, and the severity of GSD was evaluated with developmental traits at the same location (Kyoto) over two years (2009 and 2011). The severity of GSD was recorded using a 5-point scale evaluation method proposed by Furuya and Umezaki (1993), which evaluates the degree of synchronous senescence (DSS) between vegetative and reproductive organs. A lower DSS represents more sever symptom of GSD. Both the Japanese and US cultivars exhibited wide variations of DSS. Means of DSS were significantly different between 2009 and 2011, but the DSS values showed correlation between

the years, suggesting that the severity of GSD was genetically controlled. Mean DSS of the US cultivars was significantly higher than that of the Japanese cultivars, and thus the US cultivars likely have favorable properties against GSD. Late flowering cultivars with determinate stem growth habit (D-type) tended to have high DSS compared to early flowering cultivars, while DSS of cultivars with indeterminate stem growth habit (I-type) was much higher than that of the D-type cultivars with the same flowering time as I-type cultivars. In the US cultivars, early flowering cultivars were with I-type stem growth habit and late cultivars had D-type stem growth, which seemed to be the cause of DSS difference between the Japanese and US cultivars. This analysis suggested that developmental traits such as stem determination type and earliness of flowering have significant effect on DSS in a wide variation of cultivars.

Developmental trait such as stem determination and earliness of flowering were suggested as major genetic factors which influence DSS. But, the cultivation area of I-type cultivars is entirely different from that of D-type cultivars, as well as earliness of flowering. Hence, it is suspicious these traits certainly influence DSS. To verify their effects on DSS, two sets of recombinant inbred line (RIL) populations segregating stem determination and flowering time were tested at two different locations (Kyoto and Akita) over two years. One population was developed from a cross between the Stressland (US I-type) and Tachinagaha (Japanese D-type) cultivars, and the other was derived from a cross between the Ohsuzu (Japanese D-type) and Athow (US I-type) cultivars. Wide variations in DSS were observed for ST and OA RILs, but many lines normally senesced in Akita. Although DSS of each line differed considerably between the locations, the scores showed significant correlations among the environments. Therefore, the susceptibility to GSD is relatively consistent even at different locations. Quantitative trait locus (QTL) analysis revealed a strong and consistent QTL for GSD severity in ST RILs across the environments near the *Dt1* locus, which governs stem determination, and the determinate growth genotypes showed more evident symptoms of GSD. However, QTLs were not detected near the *Dt1* locus in OA RILs. Thus, it was unclear if the responsible gene was identical to the stem determination gene. The early flowering lines showed more severe symptom of GSD in both populations, but this trend was evident only in the D-type lines. The effect of another QTL detected in OA RILs also depended on the allele near the *Dt1* locus. Thus,

the genetic factor near the *Dt1* locus seemed to interact with the other genetic factors in relation to DSS. These results indicated that the genetic factor at the *Dt1* locus and the factor controlling flowering time influenced DSS under every environment, and that their effects and interaction complicated the genetic control of GSD.

In soybean, depodding is known to delay the senescence of vegetative organs, and thus imbalance of reproductive sink organs and assimilate supply (source) is believed to be a possible cause of GSD. Therefore, the effect of the genetic factor near the *Dt1* locus was investigated from a viewpoint of source and sink balance using near isogenic lines (NILs) segregating stem growth habits. These NILs were derived from the Clark, Harosoy, Williams and Elf cultivars. Because the amount of vegetative growth differs between I-type and D-type genotypes, source and sink balance was standardized in the node-basis. The D-type showed more sever symptom of GSD than the corresponding I-type in all of the genetic backgrounds, the allele near the *Dt1* locus which induced GSD symptom was compatible with the D-type. Leaf area index (LAI) at pod setting stage of the I-type was about twice as large as that of the D-type. It is reported that photo-assimilate supply to reproductive organs during flowering and pod setting positively correlates with the number of pods and seeds. The amount of available source per node in the D-type would be larger than the I-type due to smaller LAI. Because the number of pods per node, the number of seeds per node and seed weight per node at maturity were not different between the stem determination types, the D-type seemed to be under source and sink imbalance. The D-type has less small number of flowers per node, and this mainly resulted from decreased number of flowers per primary raceme. The responsible gene of stem determination turned out to be an ortholog of arabidopsis *TERMINAL FLOWER1*, which is known to associate with the continuation of stem apical meristem and inflorescence development. Therefore, the stem determination gene of soybean may also affect the morphology of inflorescence. This result suggested that the I-type stem growth habit secured the number of flowers per node which prevent the occurrence of source and sink imbalance. If the genetic variation in the severity of GSD is associated with the number of flowers per node, the genetic factor near the *Dt1* locus influencing DSS might be identical to the stem determination gene.

A genetic factor near the *Dt1* locus was suggested to influence source and sink balance through the number of flowers per node, and to be associated with the severity of green stem disorder (GSD) of soybean. But, further investigation is needed to verify the relationship between the number of flowers per node and the severity of GSD. Here, the relationship between the number of flowers per node and DSS were analyzed at two different environments. Selected lines from ST and OA RILs were used, and they segregated for DSS. Wide variation in the number of flowers per node was observed in ST and OA RILs, and the number was environmentally stable, and thus the number of flowers per node would be genetically controlled. The line with large number of flowers per node tended to have high DSS score. Therefore, it was suggested that the number of flowers per node influenced DSS. The variation of the number of flowers per node was wide in the D-type lines of ST and OA RILs compared with that in the I-type lines, but the number of flower per node was relatively high in the I-type lines. In addition, the late flowering lines tended to have large number of flowers per node as compared with early flowering lines especially in the D-type lines. The genes influencing flowering time are associated with the maintenance of stem apical meristem (SAM) activity. This result suggested that these genes also associated with the continuation of inflorescence meristem activity. These associations in the flower production might explain the variation of DSS caused by the genetic factor near the *Dt1* locus and earliness of flowering.

The genetic factor near the *Dt1* locus and its interaction with flowering time genes are considered as major determinant factors to cause genetic variation in DSS in soybean. This study also revealed these genetic factors would govern the number of flowers per node. Excess source supply relative to sink size is known to induce the symptom of GSD. Increased flower number was suggested to ameliorate the source and sink imbalance. The other genetic factor(s) which increases flower production would contribute to the improvement of GSD characteristics without altering stem growth habit and flowering time.

Chapter 1

Introduction

Mechanization in agricultural system improves productivity by saving time and labors, and also enables large-scale cultivation. In soybean [*Glycine max* (L.) Merr.] seed production, dedicated machines are introduced in the processes of seed sowing, tilling, watering and harvesting as well as soil mixing using tractors. Especially, introduction of combine harvester facilitates the harvesting and threshing processes which occupy approximately 40% of laboring time for the seed production (Nakamura, 1993). Cultivars with suitable property, as well as adjusting production system, is required for mechanization. Lodging, lowest pod-setting height, pod dehiscence and uniformity of seed maturation are target plant traits intended for the combine harvesting. Anti-lodging, high lowest pod-setting height and resistance to pod dehiscence reduce harvesting loss. The uniformity of maturation is needed to harvest at an appropriate time for every plants, and missing the time decreases seed germination ability and seed quality (Howell et al., 1959; Wilcox et al., 1974; TeKrony et al., 1984).

There is another nuisance for combine harvesting, which is related to the uniformity of maturation. Soybean is a typical monocarpic plant species that loses green color and stem moisture as the seeds mature. However, these plants occasionally maintain stem greenness, stem moisture and leaf color (green or yellow-green) at seed maturity. Phillips et al. (1984) described this phenomenon as "delayed leaf senescence", and Furuya et al. (1988) and Hobbs et al. (2006) termed this phenomenon as "inharmonious maturation" and "green stem disorder (GSD)", respectively, after unsynchronized senescence between vegetative and reproductive organs. The severity level in plants has been described from entirely green plants to leafless plants with a yellow-green stem. GSD plants are difficult to cut, which places an extra load on combine harvesters (Malvick, 2001). Furthermore, GSD contaminates seeds with the sap of green and wet tissues during the threshing process, which reduces the appearance quality and storability of seeds (Ogiwara, 2002; Morita et al., 2006). The appearance quality affects market value especially in Japanese where whole soybean seeds are cocked. Therefore, GSD is a

phenomenon to be prevented.

The occurrence of GSD seems to be induced by biotic and abiotic factors. Furuya and Kato (1963) indicated water stress during seed filling period was a cause of GSD. Takeda et al. (2003) reported similar result. Mochizuki et al. (2005) suggested high temperature stress induced the incidence of GSD. The damage by pests is another possible cause of GSD. Yamazaki and Inoue (1993) suggested that stinkbug feeding induced the occurrence of GSD, where Ojima et al (2001) indicated the relationship between the damage by leaf beetle and the symptom. Schwenk and Nickell (1980) implicated *Bean pod mottle virus* as the cause of GSD. However, GSD plants appear even in appropriately cultivated fields, and the reproducibility of experimental results is not certain.

Several researchers reported variability of the severity of GSD in soybean cultivars (Matsumoto et al., 1986; Mochizuki et al., 2005; Hill et al., 2006). Because any reliable countermeasures through modifying cultivation methods have not been established, introduction of anti-GSD cultivars seems to be effective to prevent the occurrence of GSD. Identification of genetic factor influencing GSD facilitates the breeding of GSD resistant cultivars, and contributes the elucidation of the process leading to the occurrence of GSD.

This study focused on the genetic variation of GSD severity as the countermeasure against the occurrence of GSD. For the breeding of GSD resistant cultivars, identification of inherited characteristics influencing the incidence of GSD is essential. Besides, to verify the stability of their effects on GSD against genetic background and environment is required. This study evaluated variability of GSD severity in various soybean cultivars and identified genetic factors related to the occurrence of GSD. In addition, this study analyzed how the heritable traits are associated with the occurrence of GSD to provide a reliable countermeasure.

Chapter 2

Genetic Variation of the Susceptibility to Green Stem Disorder in Japanese and US soybean cultivars

2.1 Introduction

Assessment of variability of GSD severity in soybean cultivars is fundamental for the breeding of cultivars resistant to GSD. Several studies have demonstrated the variability of GSD severity in soybean cultivars (Matsumoto et al., 1986; Mochizuki et al., 2005; Hill et al., 2006). Matsumoto et al. (1986) and Mochizuki et al. (2005) observed the varietal difference in GSD severity in Japanese cultivars. Hill et al. (2005) evaluated the sensitivity among commercial or near-commercial US cultivars and observed the genetic variability. But, these studies evaluated only limited genetic variation. Abe et al. (2002) and Kaga et al. (2012) indicated that Japanese soybean varieties belonged to different germplasm pool from those from the other regions such as China, Korea and USA. In USA, combine harvester has been utilized since early twentieth century while introduction of combine has started in 1970s in Japan. Therefore, the US cultivars seemed to accumulate suitable plant properties for combine harvesting.

Pierce et al. (1984) demonstrated the relationship between this phenomenon and stem growth habits dominated by the *Dt1* locus using near isogenic lines, and reported that determinate genotypes (D-type) showed severe GSD phenotypes in comparison with indeterminate genotypes (I-type). The I-type plant continues increasing the node number after flowering, while the D-type plant suddenly ceases node production. These authors also suggested the involvement of the earliness of maturity or flowering in the occurrence of GSD.

In the present study, various soybean cultivars derived from Japan and North America were cultivated at the same location over two year, and their GSD severities were evaluated to seek prominent cultivars or effective characteristics to prevent the occurrence of GSD. In addition, the contribution of stem growth habit and earliness to variability of GSD severity was estimated.

For the assessment of GSD severity, a 5-point scale evaluation was utilized. This method

was developed by Furuya and Umezaki (1993) to describe the plant status of different GSD severities minutely and readily, and was therefore useful for the rapid assessment of a large number of samples. The score of the GSD severity refers to the change of plant greenness during the process of senescing, and was regarded as a continuous scale. This scale was expected to detect small varietal differences in GSD susceptibility as compared to the frequency of GSD occurrence.

2.2 Materials and Methods

2.2.1 Plant materials and cultivation conditions

A total of seventy five cultivars were tested (Table 2.1). Of them, thirty eight cultivars were derived from Japan, thirty five cultivars from USA, and two cultivars from Canada. In this analysis, two Canadian cultivars were treated as the US cultivars. The US cultivars consisted of fifteen I-type and twenty two D-type genotypes, while the Japanese cultivars had only D-type growth habit. In 2009, twenty seven Japanese cultivars and thirty five US cultivars were tested. In 2011, thirty eight Japanese and thirty six US cultivars were tested. Twenty seven Japanese cultivars, thirteen US I-type and twenty two US D-type cultivars were tested in both years.

These cultivars were cultivated at the Kyoto Experimental Farm of the Graduate School of Agriculture, Kyoto University, Kyoto, Japan (Lat. 35°02’ N, Long. 135°47’ E and 65 m altitude; Alluvial sandy loam soil). Seeds were sown on June 19 in 2009, and on June 23 in 2011. Plots were composed of seven plants in single row. Cultivation was conducted with two replications in a randomized complete block design. The row and plant spacing distances were 0.7 and 0.15 m, respectively.

During the cultivation, irrigation was conducted to avoid drought stress, and pesticides were sprayed because biotic and abiotic stresses complicates the varietal difference of GSD severity.

Table 2.1. Information of the Japanese and US cultivars used in this study.

Origin	Variety	PI number	Stem growth habit	Maturity group	Developmental site	Registration or development
Japan	Akasaya	-	Determinate	-	-	Native
Japan	Akisengoku	PI 423956	Determinate	VIII	Kumamoto, Japan	1962
Japan	Akita	-	Determinate	-	Akita, Japan	1910
Japan	Ayakogane	-	Determinate	-	Nagano, Japan	1999
Japan	Bonminori	PI 360835	Determinate	II	Ibaraki, Japan	1961
Japan	Enrei	PI 385942	Determinate	IV	Nagano, Japan	1971
Japan	Fujimijiro	PI 342005	Determinate	IV	Nagano, Japan	1964
Japan	Fukuibuki	-	Determinate	III	Akita, Japan	2002
Japan	Fukusen nari	PI 423904	Determinate	V	Niigata, Japan	1970
Japan	Fukuyutaka	PI 506675	Determinate	VI	Kumamoto, Japan	1980
Japan	Fusanari	PI 416872	Determinate	IV	Niigata, Japan	1966
Japan	Hatayutaka	-	Determinate	III	Akita, Japan	1999
Japan	Hyuga	PI 423962	Determinate	VIII	Kumamoto, Japan	1969
Japan	Ippon-Sangoh	-	Determinate	-	Ibaraki, Japan	Native
Japan	Kakushin 1	PI 423910	Determinate	IV	Fukushima, Japan	1953
Japan	Kariyutaka	PI 593971	Determinate	I	Hokkaido, Japan	1991
Japan	Kogane daizu	PI 417050	Determinate	II	Saga, Japan	1958
Japan	Kotoyutaka	-	Determinate	-	Kumamoto, Japan	2006
Japan	Misuzu daizu	PI 423912	Determinate	V	Nagano, Japan	1968
Japan	Miyagi oojiro	PI 594219	Determinate	VI	Nagano, Japan	1978
Japan	Mizukuguri	-	Determinate	-	Shiga, Japan	Native
Japan	Nakasennari	PI 507079	Determinate	V	Nagano, Japan	1978
Japan	Nemashirazu	PI 342004	Determinate	IV	Akita, Japan	1961
Japan	Norin-I-go	PI 205088	Determinate	IV	Ibaraki, Japan	1939
Japan	Norin-II-go	PI 205089	Determinate	IV	Ibaraki, Japan	1940
Japan	Ohsuzu	-	Determinate	-	Akita, Japan	1998
Japan	Ootsuru	PI 594250	Determinate	IV	Nagano, Japan	1988
Japan	Ouu 13	PI 594255	Determinate	IV	Akita, Japan	1947
Japan	Rikuu 27	PI 423977	Determinate	IV	Akita, Japan	1922
Japan	Ryuho	-	Determinate	-	Akita, Japan	1995
Japan	Sachiyutaka	-	Determinate	-	Kumamoto, Japan	2001
Japan	Shakkinnashi	PI 417286	Determinate	VII	Saitama, Japan	Native
Japan	Shirotae	PI 423921	Determinate	VI	Nagano, Japan	1965
Japan	Suzukari	PI 594283	Determinate	IV	Akita, Japan	1985
Japan	Suzuyutaka	PI 561395	Determinate	V	Akita, Japan	1982
Japan	Tachinagaha	PI 561396	Determinate	V	Nagano, Japan	1986
Japan	Tamahomare	PI 507327	Determinate	VI	Nagano, Japan	1980
Japan	Ugo daizu	PI 594308	Determinate	IV	Akita, Japan	1952
USA	5002T	PI 634193	Determinate	V	Tennessee, United States	2003
USA	5601T	PI 630984	Determinate	V	Tennessee, United States	2002
USA	Athow	PI 595926	Indeterminate	III	Indiana, United States	1996
USA	Boggs	PI 602597	Determinate	VI	Georgia, United States	1998
USA	Clark	PI 548533	Indeterminate	IV	Illinois, United States	1952
USA	CNS	PI 548445	Determinate	VII	South Carolina, United States	1943
USA	Elf	PI 548556	Determinate	III	Illinois, United States	1977
USA	Essex	PI 548667	Determinate	V	Virginia, United States	1972
USA	Forrest	PI 548655	Determinate	V	Mississippi, United States	1972
USA	Freedom	PI 636463	Determinate	V	Mississippi, United States	2004
USA	Graham	PI 594922	Determinate	V	North Carolina, United States	1996
Canada*	Harosoy	PI 548573	Indeterminate	II	Ontario, Canada	1956
USA	Hill	PI 548654	Determinate	V	Mississippi, United States	1959
USA	Hutcheson	PI 518664	Determinate	V	Virginia, United States	1988
USA	INA	PI 606749	Indeterminate	IV	Illinois, United States	1998
USA	Jack	PI 540556	Indeterminate	II	Illinois, United States	1988
USA	LD00-3309	PI 639740	Indeterminate	IV	Illinois, United States	2005
USA	Lee	PI 548656	Determinate	VII	Mississippi, United States	1954
USA	LS90-1920	PI 604100	Indeterminate	IV	Illinois, United States	1998
USA	LS94-3207	PI 634335	Indeterminate	IV	Illinois, United States	2003
USA	Mack	PI 559370	Determinate	V	Arkansas, United States	1972
Canada*	Mandarin	PI 548379	Indeterminate	I	Ontario, Canada	1956
USA	Manokin	PI 559932	Determinate	IV	Maryland, United States	1991
USA	Narow	PI 553052	Determinate	V	Arkansas, United States	1985
USA	NC-Roy	PI 617045	Determinate	VI	North Carolina, United States	2001
USA	Ogden	PI 548477	Determinate	VI	Tennessee, United States	1953
USA	Omaha	PI 597382	Indeterminate	IV	Illinois, United States	1997
USA	OSAGE	PI 648270	Determinate	V	Arkansas, United States	2007
USA	OZARK	PI 633970	Determinate	V	Arkansas, United States	2003
USA	S-100	PI 548488	Indeterminate	V	Missouri, United States	1938
USA	S99-3181	PI 635039	Indeterminate	V	Missouri, United States	2004
USA	Spry	PI 553051	Determinate	IV	Illinois, United States	1991
USA	Stressland	PI 593654	Indeterminate	IV	Ohio, United States	1995
USA	TN 5-85	PI 548991	Determinate	V	Tennessee, United States	1986
USA	UA 4805	PI 639187	Determinate	IV	Arkansas, United States	2005
USA	Williams	PI 548631	Indeterminate	III	Illinois, United States	1970
USA	Williams 82	PI 518671	Indeterminate	III	Illinois, United States	1980

* Developed in Canada, but they were included into US variey group.

2.2.2 Trait evaluations and data recording

The dates of the beginning of flowering (R1 stage) and full maturity (R8 stage) were recorded according to Fehr et al. (1971). At R8, the severity of GSD was recorded by using a simple scoring method proposed by Furuya and Umezaki (1993), which evaluates the degree of synchronous senescence (DSS) between vegetative and reproductive organs with a 5-point scale from DSS1 to DSS5 (Figure. 2.1). At DSS1, the stem is green and green or yellow-green leaves remain at more than one-third of the nodes of the plant. At DSS2, the stem is green or green-yellow and green or yellow-green leaves remain at less than one-third of the nodes of the plant. At DSS3, the stem is light green and contains some moisture and chlorophyll, and several leaves contain some moisture or several petioles without leaves remain at R8. At DSS4, the stem is yellow and retains some moisture, and occasionally a few leaves are yellow to yellow-green. At DSS5, the stem is dry with gray or brown color, and all leaves have abscised. Plants exhibiting intermediate symptoms were assigned intermediate scores. Thus, a severe symptom of GSD corresponds to a low value. In general, we regard the plant status categorized into less than DSS3 as GSD.

Figure 2.1. Degree of synchronous senescence of vegetative and reproductive organs (DSS) proposed by Furuya and Umezaki (1993) and its plant status at full maturity (R8).

For each trait, the average values for the plants in the plot, excluding border plants at both ends of the plot were recorded, and the mean of the replications was adopted as the representative value of each cultivars.

2.2.3 Statistical analysis

Statistical analysis was performed with statistics software R version 3.1.2 for Windows. The statistical significance of differences was evaluated by analysis of variance (ANOVA). Tukey's test was conducted to evaluate the mean difference among the groups. The association of two traits ware evaluated by Pearson's correlation test.

2.3 Results

2.3.1 Variations in DSS

Both in 2009 and 2011, wide variations of DSS in the Japanese and the US soybean cultivars were observed (Figure 2.2 and Table 2.2). In 2009, DSS variation of the Japanese cultivars ranged from 2.00 in Akita to 3.88 in Akazaya, and the average value was 2.86. DSS variation of the US indeterminate cultivars ranged from 2.38 in S-100 to 3.50 in Stressland whereas that of the US determinate cultivars did from 2.50 in Mack to 3.88 in Hill. The average values of the indeterminate and determinate cultivars were 2.95 and 3.17, respectively. In 2011, the variation of the Japanese cultivars ranged from 1.75 in Kariyutaka to 3.88 in Fukuyutaka, and the average value was 3.02. DSS variation of the US indeterminate cultivars ranged from 2.63 in S99-3181 to 4.50 in Harosoy while that of the US determinate cultivars did from 2.50 in Elf to 4.25 in Hill. The average values of DSS in the US indeterminate and determinate cultivars were 3.47 and 3.22, respectively.

ANOVA revealed significant differences in mean of DSS between the origins of the cultivars (Japan and US) and between the years (2009 and 2011), and also indicated interaction between stem growth habit and year in relation to DSS (Table 2.2). Mean DSS of the US cultivars was higher than that of the Japanese cultivars, and the mean DSS in 2011 was higher than that in

2009. The mean DSS of the Japanese cultivars was lower than that of the US D-type cultivars in 2009, and it was significantly lower than that of the US I-type cultivars in 2011 by Tukey's test. Significant correlation of DSS between the years was observed, and the correlation coefficient was 0.380 ($P<0.01$, Figure 2.3).

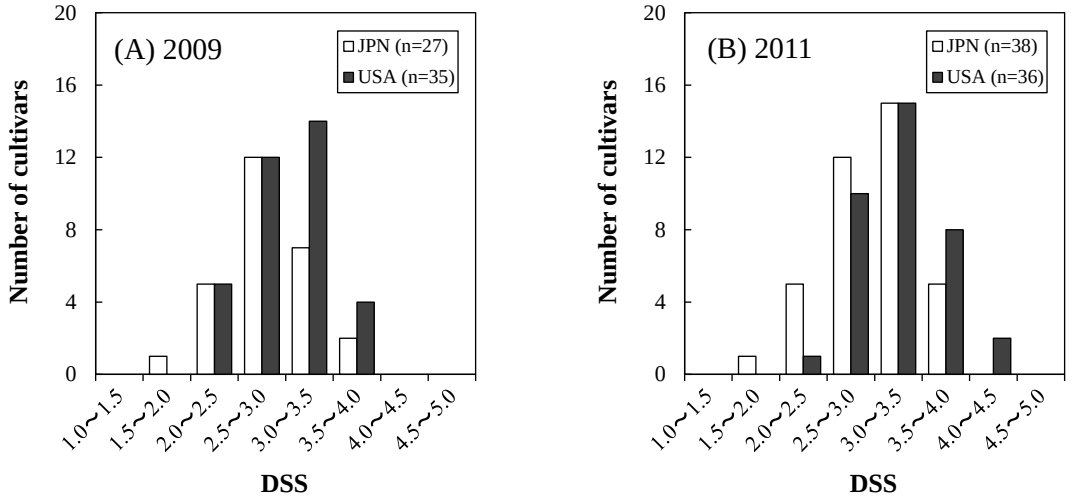


Figure 2.1. Variations of DSS in Japanese and the USA cultivars in (A) 2009 and (B) 2011. Open and closed bars represent Japanese and US cultivars, respectively.

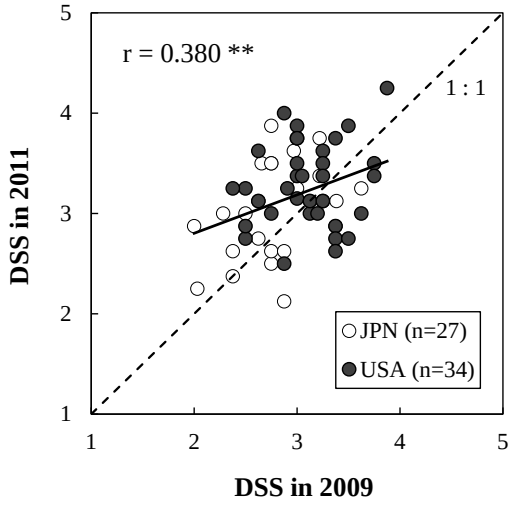


Figure 2.2. Relationship between DSS in 2009 and 2011. Open and closed circles represent Japanese and US cultivars, respectively.

Table 2.2. General statistics for days from sowing to R1 and R8 and DSS of the Japanese and US cultivars in 2009 and 2011.

Year	Origin	I/D	n	R1	R8	DSS
2009	JPN	D	27	39.9 ± 8.0 b	113.0 ± 11.5 b	2.86 ± 0.45 b
	USA	I	13	32.6 ± 4.6 c	111.2 ± 6.9 b	2.95 ± 0.32 ab
		D	22	46.1 ± 6.5 a	123.8 ± 8.1 a	3.17 ± 0.40 a
2011	JPN	D	38	39.9 ± 6.4 b	115.9 ± 11.0 b	3.02 ± 0.48 b
	USA	I	14	31.9 ± 4.8 c	110.2 ± 6.5 b	3.47 ± 0.48 a
		D	22	44.5 ± 5.3 a	123.7 ± 7.2 a	3.22 ± 0.41 ab
ANOVA		Origin		***	***	**
		I/D		***	***	n.s.
		Year		n.s.	n.s.	*
		Origin×Year		n.s.	n.s.	n.s.
		I/D×Year		n.s.	n.s.	*

*, ** and *** represent statistical significance at P<0.05, 0.01 and 0.001, respectively.

n.s. represents not significant.

Values with different letters are statistically different at P<0.05 by Tukey HSD test.

2.3.2 Variations of days to R1 and days to R8

Variations of the duration from sowing to R1 and from sowing to R8 were observed both in 2009 and in 2011 (Table 2.2). The days from sowing to R1 in the Japanese cultivars ranged from 32.2 to 75 in 2009 and from 30 to 68.5 in 2011, and the average days were 39.9 in both years. The durations in the US I-type cultivars ranged from 27 to 43.5 days in 2009 and from 25.0 to 43 days in 2011, and the means in 2009 and 2011 were 32.6 and 31.9 days, respectively. The durations in the US D-type cultivars ranged from 31 to 67 days in 2009 and 30.5 to 62 days in 2011, and the means in 2009 and 2011 were 46.1 and 44.5 days respectively.

The days from sowing to R8 in the Japanese cultivars ranged from 90 to 134 in 2009 and from 97.5 to 146 in 2011, and the average days in 2009 and 2011 were 113 and 116, respectively. The durations in the US I-type cultivars ranged from 105 to 127 days in 2009 and from 99 to 125 days in 2011, and the means in 2009 and 2011 were 111 and 110, respectively. The durations in the US D-type cultivars ranged from 104 to 139 days in 2009 and 113 to 141 days in 2011, and the means were 124 days in both years.

The durations from sowing to R1 and from sowing to R8 were significantly different between the origins of cultivars and between stem growth habits of the cultivars.

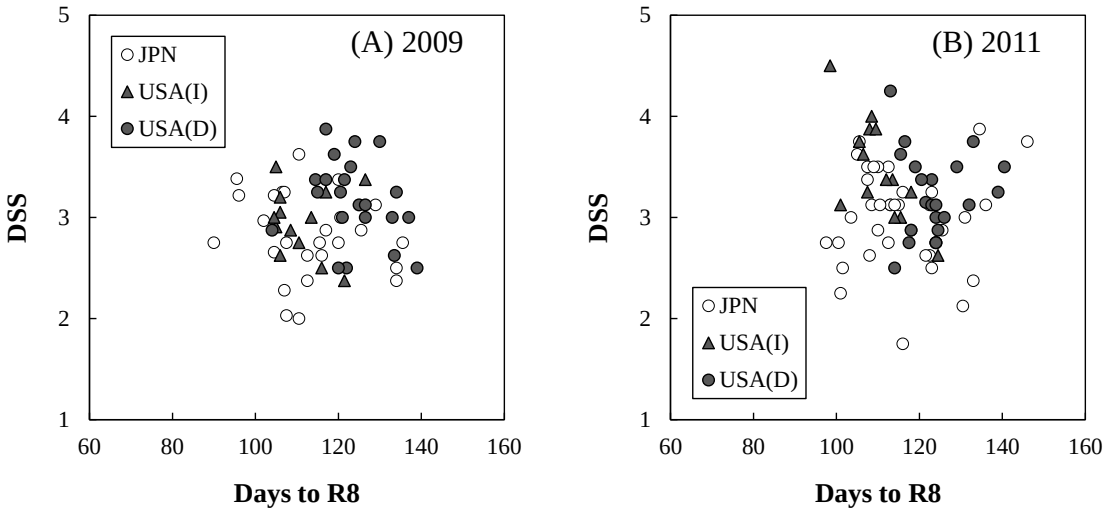


Figure 2.3. Relationship between days to R8 and DSS in (A) 2009 and (B) 2011. Open and closed circles represent Japanese and US D-type cultivars, respectively, and closed triangle represents US I-type cultivars.

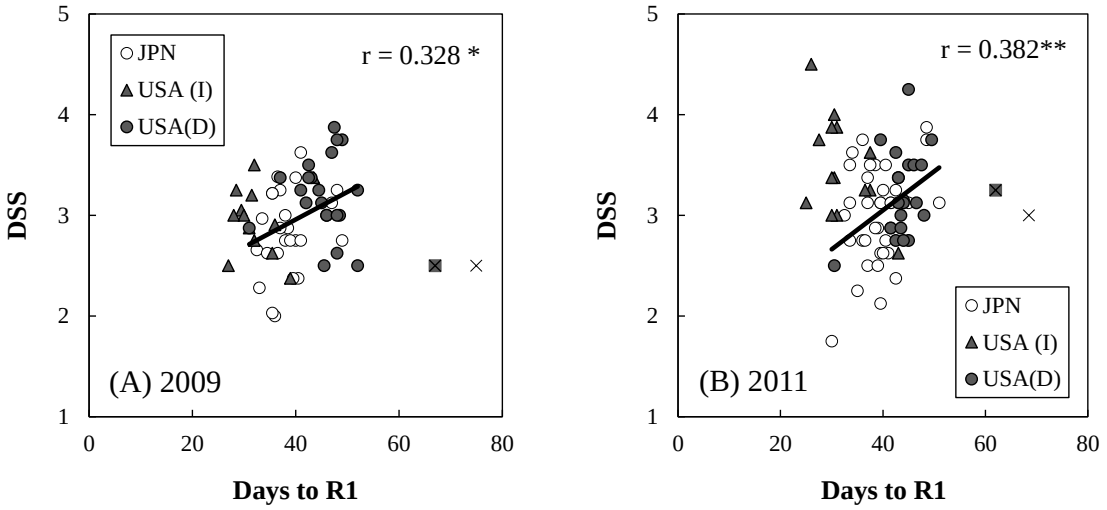


Figure 2.4. Relationship between days to R1 and DSS in (A) 2009 and (B) 2011. Open and closed circles represent Japanese and US D-type cultivars, respectively, and closed triangle represents US I-type cultivars. Trend lines were drawn using the values of D-type cultivars. Cross marks are cultivars excluded from regression.

2.3.3 Relationship between DSS and earliness

Relationships between DSS and the duration from sowing to R1 or R8 were analyzed by Pearson' correlation test (Figures 2.4 and 2.5). The correlations between DSS and the days to R8 were not significant in both the Japanese and the US cultivars, and in both stem growth habits. The correlations between DSS and the days to R1 were not significant, but excluding two late flowering cultivars, Akisengoku and CNS, DSS in the D-type cultivars showed positive correlations with the duration from sowing to R1 in both years.

2.4 Discussion

Various Japanese and US (including two Canadian cultivars) cultivars were cultivated under appropriately managed condition, and the severity GSD were evaluated using a five-point scale evaluation method (DSS) at the same location over two years. Both the Japanese and the US cultivars exhibited wide variations of DSS both in 2009 and in 2011 (Figure 2.2). Means of DSS were significantly different between the years (Table 2.2), but the DSS of the cultivars showed significant correlation between the years, suggesting that the severity of GSD was genetically controlled (Figure 2.3). Therefore, breeding of the cultivars resistant to GSD was thought to be possible.

Mean DSS of the US cultivars was significantly higher than that of the Japanese cultivars (Table 2.2). In USA, combine harvester has utilized since early twentieth century. Morrison et al. (2000) suggested the improvement of lodging tolerance in North American soybean cultivars, but there were no reports related to the resistance to GSD. This result suggested that the US cultivars took advantage of the genetic factors resistant to GSD, and there was room for improvement in the Japanese cultivars.

Pirece et al. (1984) reported that D-type genotype frequently exhibited GSD symptom as compared with I-type genotype, and the D-type with early flowering or maturity genotype tended to show severe symptom of GSD. This analysis revealed the relationship between DSS and the earliness of flowering, rather than maturity, in D-type cultivars (Figures 2.4 and 2.5). It followed that flowering genes themselves or difference of surroundings, to which the plants were exposed, might influence DSS. To certify the effect of earliness of flowering, the

evaluation under controlled condition is needed. Flowering date of the US D-type was later than that of the Japanese cultivars, and it might reflect the difference of DSS between these cultivars. Although the result of ANOVA did not indicated the advantage of I-type against D-type, the I-type cultivars had higher DSS as compared to the D-type cultivars with the same flowering dates. In the US cultivars, early flowering cultivars were derived from north region of USA and had I-type stem growth habit, while late flowering cultivars were from south region and had D-type stem growth habit. Theses combination of earliness of flowering and stem growth habit in the US cultivars might resulted in the significant difference in DSS between the Japanese and the US cultivars.

This analysis showed variability of DSS in the Japanese and the US cultivars, and stem growth habit and earliness of flowering were associated with DSS. Especially, stem determination seemed to have remarkable effect on DSS. I-type stem growth habit would contribute the improvement of DSS in early flowering genotype.

Chapter 3

Verification of the Effects of Stem Determination and Earliness of Flowering on Green Stem Disorder of Soybean against Genetic Background and Environment

3.1 Introduction

The results of the previous chapter suggested that developmental traits such as stem growth habit and earliness of flowering were major genetic factors which influence the severity of green stem disorder (GSD) of soybean, and the cultivars with indeterminate stem growth habit (I-type) tended to have lower severity of GSD compared with those with determinate stem growth habit (D-type). But, cultivation area of the I-type cultivars were entirely different from that of the D-type cultivars, and thus it is uncertain whether the responsible factor influencing the severity of GSD is stem growth habit itself. In addition, Abe et al. (2002) and Kaga et al. (2012) revealed that Japanese soybean varieties belonged to different germplasm pool from those from USA, and I-type stem growth habit has not been utilized in Japan. Therefore, it is suspicious that I-type stem growth habit improves the resistance to GSD in Japanese soybean germplasm. Because these genetic factors influence important agronomical traits such as yield, maturity and lodging (Bernard, 1972; Foley et al., 1986; Curtis et al., 2000; Kilgore-Norquesta and Sneller, 2000; Cober and Voldeng, 2001), understanding of the effect of these genetic factors on GSD is necessary before the alleles of these loci can be modified.

To verify the effects of stem determination and earliness of flowering on GSD, this study examined two sets of recombinant inbred populations derived from crosses between Japanese D-type and US I-type cultivars at two different locations (Kyoto and Akita). One population was derived from the Tachinagaha cultivar for the determinate parent, which is well-known for the frequent occurrence of GSD (Ookawa et al., 1999; Mochizuki et al., 2005). The GSD of the determinate parent of the other population, the Ohsuzu cultivar, has not been previously reported. QTLs involved in the severity of GSD were identified for both populations, and the genetic effects, including stem growth habit and earliness of flowering, were analyzed.

3.2 Materials and Methods

3.2.1 Plant materials and linkage map construction

Two sets of recombinant inbred lines (RILs) were used in the present study. One population was derived from a cross between cvs. Stressland and Tachinagaha (ST RILs), and the other population was derived from a cross between cvs. Ohsuzu and Athow (OA RILs). The Stressland (MG V) and Athow (MG IV) cultivars are from USA, and they have indeterminate stem growth habit (I-type). The Tachinagaha (MG V) and Ohsuzu (MG IV) cultivars are from Japan, and they have determinate stem growth habit (D-type). These two populations were developed via single seed descent to the F6 (ST RILs) or F7 (OA RILs) generation.

Total DNA was extracted from 10 mg of seed powder using an automated purification system (BioSprint 96 DNA Plant Kit, Qiagen, Germany). The DNA sample of ST and OA RILs was collected from five seeds of the single plant in their F7 and F6 generations, respectively. Genotyping was performed according to Sayama et al. (2011) because the methodology is systematic and rapid. Briefly, polymerase chain reaction (PCR) was performed using a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems) in a reaction mixture containing multiple simple sequence repeat (SSR) primers labeled with different florescent dyes (6-FAM, VIC, NED and PET), and the SSR fragment length in the product were measured using a DNA analyzer (3730 DNA Analyzer, Applied Biosystems, USA) instead of general gel electrophoresis. The SSR markers used here were developed by USDA-ARS (United States Department of Agriculture, Agriculture Research Service), Chiba University and Kazusa DNA Research Institute (Cregan et al., 1999; Song et al., 2004; Hisano et al., 2007; Xia et al., 2007). Genetic linkage maps with 20 linkage groups were constructed with Kosambi's map function (Kosambi, 1943) using MAPMAKER/EXP VER. 3.0 software (Lincoln et al., 1993). A total of 169 SSR markers were used for ST RILs, and the map covered 2633.9 cM with an average distance between markers of 17.7 cM. The linkage map of OA RILs covered 2629.8 cM with an average distance of 15.1 cM using a total of 194 SSR markers.

3.2.2 Experimental sites and design

In 2009, Stressland, Tachinagaha and 78 lines of ST RILs were planted at the Kyoto Experimental Farm of the Graduate School of Agriculture, Kyoto University, Kyoto, Japan (Lat. 35°02’ N, Long. 135°47’ E and 65 m altitude; Alluvial sandy loam soil). In 2010, 118 lines of both ST and OA RILs along with parental cultivars were planted at the Kariwano experimental site of the Tohoku Agricultural Center, Daisen, Akita (Lat. 39°32’ N, Long. 140°22’ E and 30 m altitude; High-humic Andosol soil) and at the Kyoto field. At Kyoto, the sowing dates were June 19 in 2009 and June 28 in 2010. At Akita in 2010, ST and OA RIL seeds were sown on June 15 and June 23, respectively. To ensure the heritability of the degree of GSD, 18 randomly selected lines from each population and parental variety, consisting of early, middle and late flowering lines with different stem growth habits, were tested at Kyoto in 2011, and the seeds were sown on June 23. In all experiments, plots were single row with two replications in a randomized complete block design. The row and plant spacing distances were 0.7 and 0.15 m, respectively. For each plot, samples were composed of 8 (in 2009) and 10 (in 2010 and 2011) randomly selected non-sequential plants in a single row.

3.2.3 Trait evaluations and data recording

The dates of the beginning of flowering (R1 stage) and full maturity (R8 stage) were recorded according to Fehr et al. (1971). The node number of the main stem was counted at R1 and R8 at Kyoto in 2010 to certify the accurate chromosomal position of the *Dt1* locus, which dominates stem determination. At R8, the severity of GSD was recorded by using a simple scoring method proposed by Furuya and Umezaki (1993), which evaluates the degree of synchronous senescence (DSS) between vegetative and reproductive organs with a 5-point scale from DSS1 to DSS5. For each trait, the average values for the plants in the plot, excluding border plants at both ends of the plot were recorded, and the mean of the replications was adopted as the representative value of each line.

3.2.4 Statistical and QTL analysis

Analysis of variance (ANOVA) was conducted to test the differences of trait values among location, year and genotype. The Tukey's test at $P<0.05$ was used for the comparison of means. The Steel-Dwass test was adopted when the Tukey's test was not applicable. The correlation of DSS for the location and year and the relationship between the traits were statistically analyzed by Pearson's correlation test. Spearman's rank correlation coefficient was also shown for fear that parametric approach was not applied to DSS. QTL analysis was performed using Windows QTL Cartographer 2.5 (Wang et al., 2007; http://statgen.ncsu.edu/qtlcart/WQTLCart.htm). The composite interval mapping (CIM) method with a walk speed of 1.0 cM was adopted for the detection of chromosomal positions and additive effects of QTLs for each trait. The threshold value of the logarithm of odds (LOD) for the detection of QTL was calculated by the permutation test (1000 runs, $P=0.05$).

3.3 Results

3.3.1 Variations and distributions of DSS

Wide variations of DSS were observed in ST RILs with the location and year (Table 3.1 and Figure 3.1). The DSS ranged from 1.0 to 4.5, with an average of 2.9, at Kyoto in 2009. The scores in Stressland (maternal parent) and Tachinagaha (paternal parent) were 3.1 and 2.2, respectively. At Kyoto in 2010, the DSS range was from 1.3 to 4.0, with an average of 2.8, and the scores in Stressland and Tachinagaha were 3.1 and 2.0, respectively. At Akita in 2010, the two latest lines did not reach R8 due to frost, so the lines were not included in the analyses. The DSS range was from 1.0 to 5.0, but the average value was as high as 4.4 because the frequency did not exhibit normal distribution. The scores in maternal and pollen parents were 5.0 and 3.8, respectively. When the selected 20 genotypes were tested at Kyoto in 2011, the range of DSS was between 1.4 and 3.9, and the scores in Stressland and Tachinagaha were 3.9 and 2.8, respectively. The score in Tachinagaha was lower than that in Stressland in every test.

Variations of DSS were also observed in OA RILs (Table 3.1 and Figure 3.2). DSS ranged from 1.8 to 4.6 with an average of 3.2 at Kyoto in 2010, and the scores in Ohsuzu and Athow

were 3.8 and 3.7, respectively. At Akita in 2010, the DSS range was from 2.0 to 5.0, and the average value was 4.5. The distribution of OA RILs showed the same trend as that of ST RILs in Akita. The scores of Ohsuzu and Athow were 4.8 and 5.0, respectively. At Kyoto in 2011, the DSS range of the selected 20 genotypes tested was from 1.3 to 4.5, and the scores in Ohsuzu and Athow were 3.5 and 3.8, respectively.

Correlation coefficients of the DSS of each line for the location and year were analyzed by Pearson's correlation test and Spearman's rank correlation test (Tables 3.2 and 3.3). The relationships were close even between the different locations except for the relationship in OA RILs between 2010 and 2011 at Kyoto (Table 3.3). Excluding the data of a line, OA_156, however, the correlation coefficient was statistically significant at $P<0.001$.

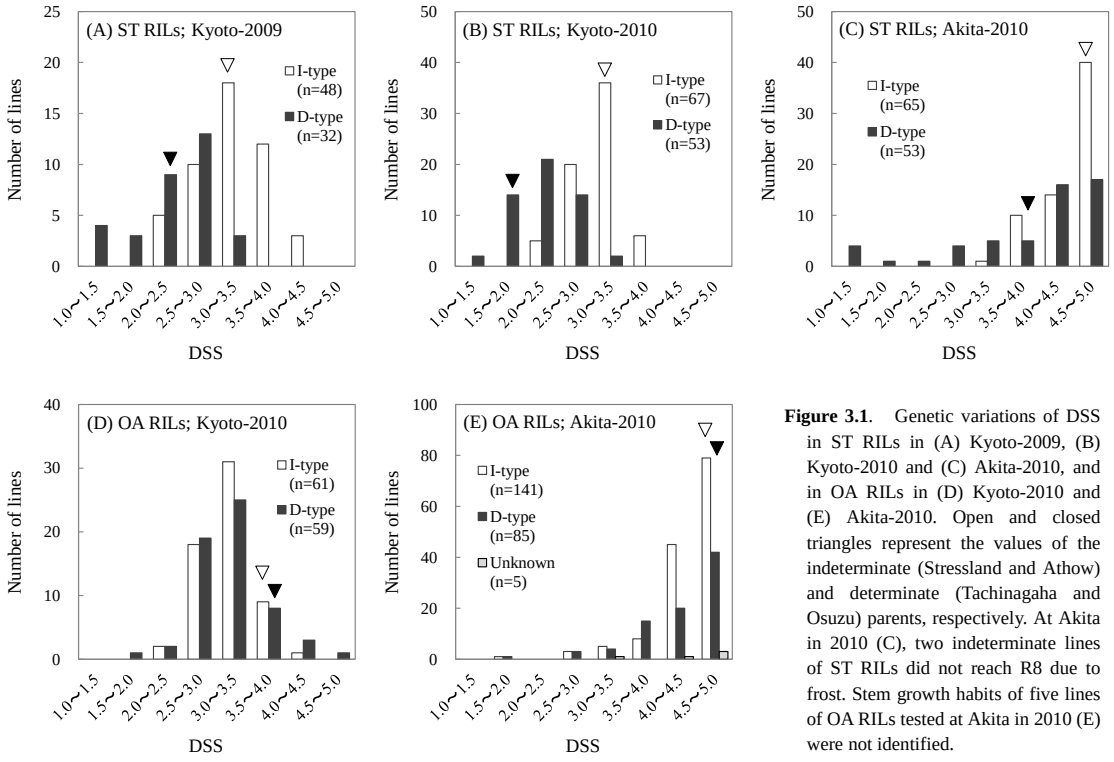


Figure 3.1. Genetic variations of DSS in ST RILs in (A) Kyoto-2009, (B) Kyoto-2010 and (C) Akita-2010, and in OA RILs in (D) Kyoto-2010 and (E) Akita-2010. Open and closed triangles represent the values of the indeterminate (Stressland and Athow) and determinate (Tachinagaha and Osuzu) parents, respectively. At Akita in 2010 (C), two indeterminate lines of ST RILs did not reach R8 due to frost. Stem growth habits of five lines of OA RILs tested at Akita in 2010 (E) were not identified.

Table 3.2. Correlation coefficients for DSS of ST RILs among different environments. Upper and lower tables were the results of Pearson's correlation analysis and Spearman's rank-correlation analysis, respectively.

Location	Year	Kyoto			Akita
		2009	2010	2011	2010
Kyoto	2009	-	0.78 ***	0.74 ***	0.54 ***
Kyoto	2010	n = 80	-	0.86 ***	0.61 ***
Kyoto	2011	n = 19	n = 20	-	0.56 **
Akita	2010	n = 79	n = 118	n = 20	-
Kyoto	2009	-	0.80 ***	0.78 ***	0.49 ***
Kyoto	2010	n = 80	-	0.86 ***	0.51 ***
Kyoto	2011	n = 19	n = 20	-	0.56 **
Akita	2010	n = 79	n = 118	n = 20	-

** and *** represent statistical significance at P<0.01 and 0.001, respectively.

Table 3.3. Correlation coefficients for DSS of OA RILs among different environments. Upper and lower tables were the results of Pearson's correlation analysis and Spearman's rank-correlation analysis, respectively.

	Location	Year	Kyoto		Akita
			2010	2011	2010
All lines	Kyoto	2010	-	0.30 n.s.	0.27 **
	Kyoto	2011	n = 20	-	0.54 *
	Akita	2010	n = 120	n = 20	-
	Kyoto	2010	-	0.50 *	0.31 ***
	Kyoto	2011	n = 20	-	0.61 **
	Akita	2010	n = 120	n = 20	-
Excluding OA_156 at Kyoto in 2010	Kyoto	2010	-	0.82 ***	0.28 **
	Kyoto	2011	n = 19	-	0.54 *
	Akita	2010	n = 119	n = 20	-
	Kyoto	2010	-	0.75 ***	0.31 ***
	Kyoto	2011	n = 19	-	0.61 **
	Akita	2010	n = 119	n = 20	-

*, ** and *** represent statistical significance at P<0.05, 0.01 and 0.001, respectively, and n.s. represents not significant.

3.3.2 Variations of duration from sowing to flowering

At Kyoto, the durations from sowing to R1 were relatively constant in both years and ranged among lines from 25 to 48 days and from 24 to 42 days in ST and OA RILs, respectively (Table 3.1 and Figure 3.2). At Akita, the durations were longer than those at Kyoto and ranged from 32 to 66 days and from 30 to 49 days in ST and OA RILs, respectively. The difference in the duration between the locations was larger in ST RILs in comparison with OA RILs. The durations from sowing to R1 in Stressland and Tachinagaha were 34 and 33 days at Kyoto in 2009, 33 and 34 days at Kyoto in 2010, 43 and 50 days at Akita in 2010, and 30 and 33 days at Kyoto in 2011, respectively. In Ohsuzu and Athow, the duration was 32 and 29 days, respectively, at Kyoto in 2010, 39 and 35 days, respectively, at Akita in 2010, and 33 and 27 days, respectively, at Kyoto in 2011.

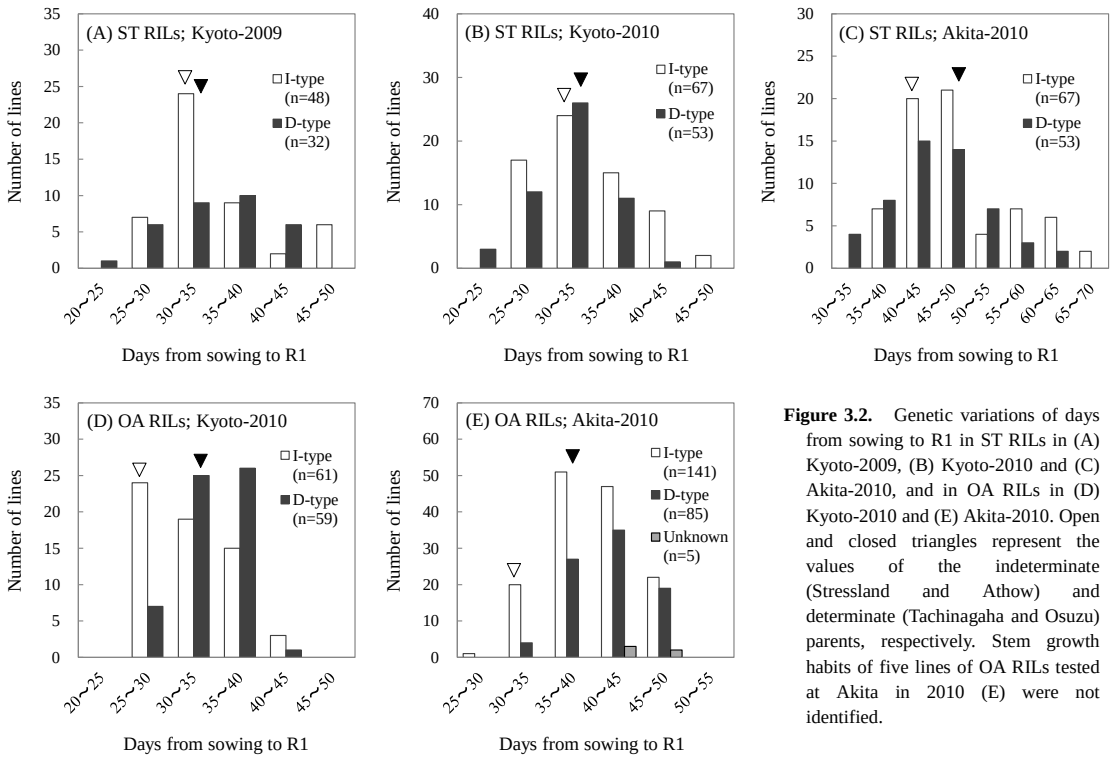


Figure 3.2. Genetic variations of days from sowing to R1 in ST RILs in (A) Kyoto-2009, (B) Kyoto-2010 and (C) Akita-2010, and in OA RILs in (D) Kyoto-2010 and (E) Akita-2010. Open and closed triangles represent the values of the indeterminate (Stressland and Athow) and determinate (Tachinagaha and Osuzu) parents, respectively. Stem growth habits of five lines of OA RILs tested at Akita in 2010 (E) were not identified.

Table 3.1. General statistics for DSS and days from sowing to R1 of ST and OA RILs.

Population	Location	Year	I/D-type	n	DSS			Days from sowing to R1		
					mean ± s.d.[a), b)]	Maternal[c)]	Paternal[c)]	mean ± s.d.[a), b)]	Maternal[c)]	Paternal[c)]
ST RILs	Kyoto	2009	Both	80	2.89 ±0.69 CD	3.09	2.24	34.8 ±5.4 C	33.5	33.0
			I	48	3.24 ±0.49 d	3.09	-	35.0 ±5.6 d	33.5	-
			D	32	2.36 ±0.61 e	-	2.24	34.5 ±5.1 d	-	33.0
	Kyoto	2010	Both	120	2.76 ±0.58 D	3.13	2.00	33.8 ±4.8 C	32.5	34.0
			I	67	3.12 ±0.38 d	3.13	-	34.7 ±5.2 d	32.5	-
			D	53	2.30 ±0.45 e	-	2.00	32.7 ±4.1 d	-	34.0
	Kyoto	2011	Both	20	2.71 ±0.77 BCD	3.88	2.75	32.8 ±5.8 C	30.0	33.0
			I	9	3.36 ±0.48 cd	3.88	-	34.7 ±6.8 cd	30.0	-
			D	11	2.17 ±0.50 e	-	2.75	31.3 ±4.6 d	-	33.0
	Akita	2010	Both	118 / 120[d)]	4.35 ±0.87 A	5.00	3.75	47.3 ±7.8 A	43.0	50.0
			I	65 / 67[d)]	4.65 ±0.40 a	5.00	-	48.4 ±8.0 a	43.0	-
			D	53	3.98 ±1.12 bc	-	3.75	45.8 ±7.4 ab	-	50.0
OA RILs	Kyoto	2010	Both	120	3.20 ±0.45 B	3.81	3.69	33.7 ±4.0 C	32.0	28.5
			I	61	3.21 ±0.41 d	-	3.69	33.0 ±4.3 d	-	28.5
			D	59	3.18 ±0.49 d	3.81	-	34.5 ±3.5 d	32.0	-
	Kyoto	2011	Both	20	3.17 ±0.78 BC	3.50	3.75	32.9 ±5.8 C	33.0	27.0
			I	9	3.57 ±0.50 cd	-	3.75	33.2 ±5.4 d	-	27.0
			D	11	2.84 ±0.84 de	3.50	-	32.5 ±6.3 d	33.0	-
	Akita	2010	Both	231[e)]	4.54 ±0.56 A	4.75	5.00	40.9 ±4.4 B	39.0	34.5
			I	141	4.60 ±0.51 a	-	5.00	40.2 ±4.6 c	-	34.5
			D	85	4.45 ±0.62 ab	4.75	-	41.9 ±3.9 bc	39.0	-
ANOVA[f)]	Kyoto and Akita in 2010		Location		***			***		
			Population		***			***		
			I/D-type		***			n.s.		
			Location×Population		**			***		
			Location×I/D-type		n.s.			n.s.		
			Population×I/D-type		***			***		
			Location×Population×I/D-type		n.s.			n.s.		
	Kyoto in 2010 and 2011		Year		n.s.			n.s.		
			RILPopulation		***			n.s.		
			I/D-type		***			n.s.		
			Year×Population		n.s.			n.s.		
			Year×I/D-type		***			n.s.		
			Population×I/D-type		***			*		
			Year×Population×I/D-type		n.s.			n.s.		

a) s.d. represents standard deviation. b) Values with different letters are significantly different at P<0.05 by Steel-Dwass test, and small and capital letters are applied to the comparisons with and without classification by stem determination, respectively. c) Maternal and paternal represent the seed and pollen parents of each populations. d) Two I-type lines did not reach R8 due to frost, and their DSS were not recorded. e) Stem growth habits of five lines were not identified. f) *, ** and *** represent statistical significance at P<0.05, 0.01 and 0.001, respectively, and n.s. represents not significant.

3.3.3 QTL analyses for DSS, days from sowing to R1 and node production after R1

Two QTLs for DSS were detected in every test in ST RILs (Table 3.4) although not the same two QTLs in each test. However, one QTL for DSS was consistently mapped at the chromosomal position of 95.4 cM on Chr. 19, which was coincident with the *Dt1* locus. The Stressland (I-type) cultivar had a higher value of DSS. The other QTL mapped close to the *Dt1* locus found at Kyoto in 2009 was assumed to be due to the effect of the QTL at the *Dt1* locus

and the recombination pattern of the lines used. Other QTLs were detected on Chr. 13 and 12 at Kyoto and Akita, respectively, in 2010, but they were inconsistent between experiments and explained only minor proportions of the total variance. In OA RILs, one QTL for DSS were mapped on Chr. 3 (LG_N) in each test at Kyoto and Akita in 2010. These QTLs did not co-locate, but their additive effects were similar.

Table 3.4. Putative QTL, map position and genetic contribution for DSS in ST and OA RILs

Population	Location	Year	Chromosome number	Linkage group	Peak position (cM)	Flanking[a)] markers	LOD score	Additive[b)] effect	R^2
ST RILs	Kyoto	2009	19	L	95.4	**CSSR116** － Sat_286	10.35	0.42	0.35
			19	L	96.8	**Sat_286** — Satt229	9.91	0.42	0.35
		2010	13	F	155.3	**Sat_197** — Sat_417	3.20	-0.21	0.13
			19	L	95.4	**CSSR116** — Sat_286	20.03	0.40	0.44
	Akita	2010	12	H	68.6	**Sat_206** — Satt302	3.01	0.24	0.08
			19	L	95.4	**CSSR116** — Sat_286	5.11	0.33	0.14
OA RILs	Kyoto	2010	3	N	77.5	**Satt521** — Satt237	4.86	0.18	0.15
	Akita	2010	3	N	45.3	**Sat_208** — Sat_033	3.59	0.16	0.08

a) Bold font represents a marker closer to peak position.

b) Additive effect represents the effect of allele of maternal parent against that of paternal parent.

There were several QTLs for days from sowing to R1 detected in both RILs (Table 3.5). In ST RILs, the consistent QTLs across the environments were located on chromosomes (Chr.) 6, 10 and 19, corresponding to linkage groups (LG) C2, O and L, respectively, and these QTLs explained 65.9, 15.1 and 15.6% on average, respectively, of the total phenotypic variation in days from sowing to R1. In OA RILs, the consistent QTLs were mapped on Chr. 6 and 10, which accounted for 64.8 and 11.8%, respectively, of the total phenotypic variation at Kyoto, and 27.7 and 14.7%, respectively, of total phenotypic variation at Akita. The additive effects of the Tachinagaha and Ohsuzu alleles were positive for the QTL on Chr. 6 and negative for the QTL on Chr.10. The additive effect of the QTL on Chr. 19 was positive with the Stressland-derived allele in ST RILs. The QTLs of both RILs on Chr. 6 and Chr. 10 were commonly linked to markers, Satt557 and GMES4019, respectively. The QTL of ST RILs on Chr. 19 was mapped beside Satt229. The QTLs on Chr. 6, 10 and 19 were co-located with the *E1*, *E2* and *E3*, respectively (Bernard, 1971; Buzzell, 1971).

Table 3.5. Putative QTL, map position and genetic contribution for days from sowing to R1 and node number produced after R1 in ST and OA RILs.

Trait	RIL	Location	Year	Chromosome number	Linkage group	Peak position (cM)	Flanking[a)] markers	LOD score	Additive[b)] effect	R^2
Days to R1	ST RILs	Kyoto	2009	6	C2	75.0	Satt277—**Satt557**	23.17	-5.13	0.68
				10	O	116.5	**GMES4019**—Satt243	4.90	1.57	0.08
				19	L	95.4	**CSSR116**—Sat_286	10.05	2.80	0.19
			2010	6	C2	66.1	Satt457—**Satt277**	26.82	-3.81	0.59
				6	C2	75.0	Satt277—**Satt557**	46.96	-3.99	0.65
				10	O	116.5	**GMES4019**—Satt243	21.41	1.96	0.16
				19	L	104.8	Sat_286—**Satt229**	19.90	1.99	0.16
		Akita	2010	6	C2	67.1	Satt457—**Satt277**	23.58	-6.09	0.57
				6	C2	75.0	Satt277—**Satt557**	45.61	-6.55	0.65
				10	O	115.2	Satt592—**GMES4019**	25.28	3.72	0.22
				19	L	104.8	Sat_286—**Satt229**	14.89	2.73	0.12
				19	L	108.4	**Satt229**—Satt373	14.52	2.64	0.11
	OA RILs	Kyoto	2010	6	C2	109.4	Satt322—**Satt277**	7.67	2.73	0.41
				6	C2	126.8	**Satt557**—Satt307	20.81	3.99	0.65
				10	O	111.6	Satt592—**GMES4019**	5.58	-1.50	0.12
				10	O	114.0	**GMES4019**—Sat_038	5.41	-1.54	0.12
		Akita	2010	6	C2	109.4	Satt322—**Satt277**	8.58	2.21	0.24
				6	C2	122.8	**Satt557**—Satt307	18.27	2.62	0.28
				8	A2	49.7	**AW132402**—CSSR420	4.13	1.20	0.07
				10	O	102.4	Satt477—**Satt592**	4.19	-1.17	0.06
				10	O	111.6	Satt592—**GMES4019**	9.99	-1.84	0.15
				10	O	114.0	**GMES4019**—Sat_038	9.18	-1.78	0.14
Node production after R1	ST RILs	Kyoto	2010	19	L	95.4	**CSSR116**—Sat_286	72.29	5.37	0.89
	OA RILs	Kyoto	2010	19	L	112.3	**Sat_286**—Sat_184	75.05	-4.85	0.82
				19	L	114.7	**Sat_184**—Satt229	55.72	-4.74	0.79

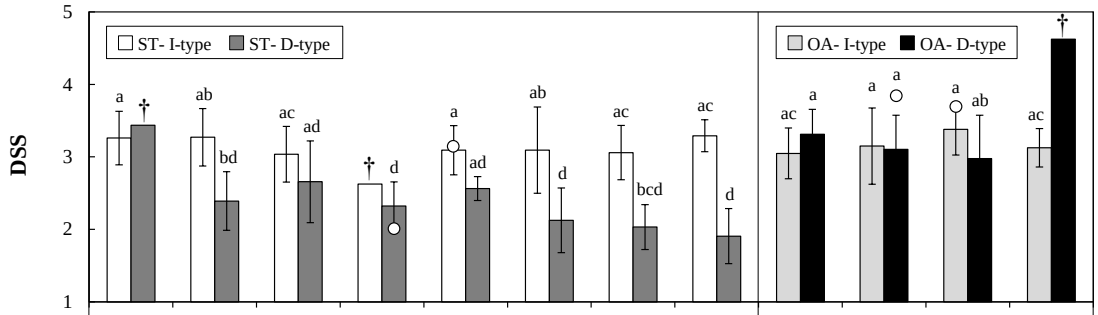
a) Bold font means a marker closer to peak position. b) Additive effect means the effect of allele of maternal parent against that of paternal parent.

A QTL for node production after R1 was detected on Chr. 19 in both RIL populations, which indicated the accurate chromosomal positions of the *Dt1* locus (Table 3.5). The peak position of LOD for the unique QTL was at 95.4 cM in ST RILs, and this was in accordance with the LOD peak of a QTL for DSS. Moreover, 2 contiguous peaks were detected in OA RILs, but the LOD peaks were shaped by the effect of a single locus according to the directions and the additive effects of these QTLs. These QTL were closely linked to CSSR116 and Sat_286 for ST and OA RILs, respectively, and the markers were located close to the tagging marker (Satt006) for the *Dt1* locus by Molnar et al. (2003).

3.3.4 Relationship between days from sowing to R1 and DSS

After the classification of the genotype of each line on the *E1* (*E1/e1*), *E2* (*E2/e2*), *E3* (*E3/e3*) and *Dt1* (*Dt1/dt1*, I/D-type) loci, DSS in each genotype group at Kyoto in 2010, where

the frequency exhibited normal distribution, was compared (Figure 3.3). The genotype of each line was determined according to the alleles of Satt557, GMES4019 and Satt229 for the *E1*, *E2* and *E3* loci, respectively. The alleles with positive effects were represented with capital letters, and the alleles with negative effects were with lower-case letters. Stem determination type was determined based on the phenotypic value of node number produced after R1, namely the lines produced more than 5 nodes after R1 were classified into I-type. In ST RILs, I-type lines had large values of DSS compared with D-type lines, and the difference was evident in early flowering genotype. On the other hand, the difference of DSS between the stem determination types was not significant in OA RILs. DSS of OA RILs with D-type growth habit was larger than that of ST RILs with D-type growth habit even with the same flowering time.



	E1E2E3	*E1E2e3*	*E1e2E3*	*E1e2e3*	*e1E2E3*	*e1E2e3*	*e1e2E3*	*e1e2e3*	*E1E2*	*E1e2*	*e1E2*	*e1e2*
Days to R1												
I-type	43.1 ± 2.2 (n = 13)	37.5 ± 0.9 (n = 3)	36.6 ± 1.7 (n = 12)	34.0 ± — (n = 1)	33.4 ± 1.6 (n = 15)	30.4 ± 2.0 (n = 6)	29.7 ± 1.1 (n = 14)	27.8 ± 1.8 (n = 3)	37.3 ± 3.7 (n = 17)	34.0 ± 1.3 (n = 13)	29.8 ± 2.9 (n = 24)	31.4 ± 4.0 (n = 7)
D-type	42.5 ± — (n = 1)	36.3 ± 1.7 (n = 12)	37.5 ± 2.6 (n = 4)	33.5 ± 1.6 (n = 15)	31.2 ± 1.0 (n = 3)	29.3 ± 1.6 (n = 10)	29.3 ± 2.5 (n = 2)	26.2 ± 1.7 (n = 6)	37.6 ± 1.2 (n = 20)	33.5 ± 2.4 (n = 30)	31.4 ± 4.9 (n = 8)	26.5 ± — (n = 1)

Figure 3.3. DSS (upper figure) and days from sowing to R1 (lower table) of genotypes of ST and OA RILs segregating the alleles at the *E1*, *E2*, *E3* and *Dt1* (I/D-type) loci at Kyoto in 2010. Genotype of each line was determined based on the alleles of SSR markers closest to the loci. The values of DSS and days from sowing to R1 are shown as mean±standard deviation. Circles represent DSS values of the parental cultivars. †; Only one line belonged to the genotype group. The DSS values with different letters are significantly different at P<0.05 by Tukey's test.

The correlation between days from seed sowing to R1 and DSS was examined (Tables 3.6 and 3.7). These analyses showed positive correlations between the traits in D-type lines in both RIL populations in most of the tests. The stem growth habit of each line was determined based on the phenotypic value of node production after R1, or the SSR allele linked to this QTL for the lines that were not tested at Kyoto in 2010. Although the correlation in D-type lines of OA RILs was not statistically significant at Kyoto in 2010, the relationship became evident when

the OA_156 line data was removed. In contrast, the I-type lines of both RIL populations showed indistinct relations in most of the tests.

Table 3.6. Pearson's correlation coefficients and Spearman's rank correlation coefficients between days from seed sowing to R1 and DSS in ST RILs.

Location	Year	I/D-type	n	Pearson's [a)] correlation coefficient	Spearman's [a)] rank correlation coefficient
Kyoto	2009	Both	80	0.27 *	0.18 n.s.
		I-type	48	0.22 n.s.	0.26 n.s.
		D-type	32	0.42 *	0.38 *
Kyoto	2010	Both	120	0.36 ***	0.31 ***
		I-type	67	0.15 n.s.	0.16 n.s.
		D-type	53	0.51 ***	0.39 **
Kyoto	2011	Both	20	0.16 n.s.	0.17 n.s.
		I-type	9	-0.41 n.s.	-0.51 n.s.
		D-type	11	0.19 n.s.	0.19 n.s.
Akita	2010	Both	118 [b)]	0.29 **	0.16 n.s.
		I-type	65 [b)]	-0.08 n.s.	-0.03 n.s.
		D-type	53	0.45 ***	0.36 **

a) *, ** and *** represent statistical significance at $P<0.05$, 0.01, 0.001, respectively, and n.s. represents not significant. b) Two indeterminate lines did not reach R8.

Table 3.7. Pearson's correlation coefficients and Spearman's rank correlation coefficients between days from seed sowing to R1 and DSS in OA RILs.

Location	Year	I/D-type	n	Pearson's [a)] correlation coefficient	Spearman's [a)] rank correlation coefficient
Kyoto	2010	Both	120	0.01 n.s.	0.01 n.s.
		I-type	61	-0.19 n.s.	-0.19 n.s.
		D-type	59	0.23 n.s.	0.31 *
Kyoto (Excluding OA_156)	2010	Both	119	0.06 n.s.	0.04 n.s.
		D-type	58	0.39 **	0.38 **
Kyoto	2011	Both	20	0.55 *	0.52 *
		I-type	9	0.06 n.s.	-0.10 n.s.
		D-type	11	0.82 **	0.78 **
Akita	2010	Both	231	0.28 ***	0.33 ***
		I-type	141	0.25 **	0.28 ***
		D-type	85	0.46 ***	0.50 ***

a) *, ** and *** represent statistical significance at $P<0.05$, 0.01, 0.001, respectively, and n.s. represents not significant.

3.4 Discussion

3.4.1 DSS variation and environmental effects

This study evaluated DSS, a measure of GSD severity, for two RIL (ST and OA RILs) at two different locations, Kyoto and Akita. The results showed that DSS of each line differed with the location and that most of the lines of both RILs had higher values at Akita compared to those at Kyoto (Table 3.1 and Figure 3.1). Because many lines reached the maximum value of DSS (DSS5) at Akita and thus the frequency of DSS showed a skewed distribution, it was

assumed that it was difficult to detect the genotypic differences of DSS there. In addition, the fact that many lines normally senesced at Akita was considered to cause the interaction between location and population, and between location and I-D type. Konaka and Takahashi (1965) and Furuya et al. (1988) reported that cultivars developed in the northern region of Japan showed GSD symptoms when they are cultivated in the southern region of Japan. The experimental site at Akita (lat. 39°32' N) is located approximately 4°1' north of Kyoto (lat. 35°02' N). The results of the present study agreed with these previous results because the genotypes adapted to the latitude of Akita, as shown by the reduced DSS scores at Kyoto. However, the susceptibility to GSD of each line was consistent according to the correlation and rank-correlation analyses for location and year (Tables 3.2 and 3.3). These results suggested that the genetic approach was effective for the improvement of resistance to GSD.

DSS of the I-type parent of ST RILs, Stressland, was higher than that of the D-type parent, Tachinagaha, in every test (Table. 3.1). In OA RILs, DSS values of the I-type parent, Athow, and the D-type parent, Ohsuzu, were similar, and were as high as that of Stressland. D-type plants are known to exhibit more severe symptom of GSD than I-type plants (Pierce et al., 1984). If the effect of stem determination, which was governed by the *Dt1* locus, on DSS was consistent, it was presumed that the lower DSS of Tachinagaha was caused by the allele, and Ohsuzu possessed the genetic factors that modified the adverse effect of the D-type allele. The variations of DSS in both ST and OA RILs were considerably wide in every test as compared with the differences of DSS between the parental cultivars, thus both populations showed transgressive segregation. Therefore, it is likely that the susceptibility to GSD is governed by multiple loci and that the parental cultivars carried alleles that both positively and negatively affect DSS.

3.4.2 QTL mapping for flowering time and node number produced after R1

Three consistent QTLs for days from sowing to R1 were detected on Chr. 6, 10 and 19 in ST RILs, and two consistent QTLs for days from sowing to R1 were detected on Chr. 6 and 10 in OA RILs (Table 3.5). QTLs mapped on the same chromosomes were assumed to be common loci between the two RIL populations. Another consistent QTL was mapped on Chr. 19 in ST RILs. Several QTLs for days to R1 have been previously reported (Cober et al., 1996 ; Mansur

et al., 1996; Yamanaka et al., 2000; Tasma et al., 2001; Abe et al., 2003). The QTLs detected on Chr. 6, 10 and 19 corresponded to *E1*, *E2* and *E3* loci, respectively (Bernard, 1971; Buzzell, 1971). The genes on these loci have been genetically and molecularly identified. *E1* and *E3* gene are involved in photoperiod response, and a clock-related protein is encoded on the *E2* locus (Watanabe et al., 2009; Watanabe et al., 2011; Xia et al., 2012). Thus, phenotypic polymorphism was recognized for *E1*, *E2* and *E3* in ST RILs and for *E1* and *E2* in OA RILs. These QTLs explained 96.6% of the total phenotypic variance on average in ST RILs, and 76.6 and 42.4% in Kyoto and Akita in OA RILs. The additive effects of the *E1* and *E2* hardly differed with the location in OA RILs, while all of the consistent QTLs of ST RILs had larger additive effects at Akita. This result is in accord with the significant interaction between population and location by ANOVA (Table 3.1). A major difference between the populations was the segregation at the *E3* locus and this segregation might provide the cause of the interaction.

Stem growth habits segregated into I- and D-types in both RIL populations. A QTL for node number produced after R1 was clearly mapped in each population on Chr. 19 with an R^2 value of 0.8 or higher, and this QTL corresponded to the *Dt1* locus (Table 3.5). At this locus, an ortholog of arabidopsis *TERMINAL FLOWER1*, *GmTfl1b*, is present, which controls the emergence of the terminal raceme at the shoot apical meristem (Liu et al., 2010).

3.4.3 Stem determination and DSS

A strong QTL for DSS was mapped at the *Dt1* locus in ST RILs, and the DSS scores of the I-types were higher than those of the D-types both at Kyoto and Akita (Tables 3.4 and 3.5), which agreed with the result reported by Pierce et al. (1984). In OA RILs, however, no QTLs were detected near the *Dt1* locus even though this population showed segregation in stem growth habits. These results were also observed as an interaction between population and I/D-type by ANOVA (Table 3.1). Regarding these inconsistent results, three different possibilities can be hypothesized. The first possibility is that the responsible gene is not *GmTfl1b* but rather another closely linked gene, which would suggest that stem growth habit does not affect GSD and that the GSD trait of Tachinagaha can be improved by recombination

of this allele without conversion to I-type. The second possibility is that *GmTfl1b* interacts with other genetic factor(s) in relation to the occurrence of GSD. The third possibility is that *GmTfl1b* has an additional function other than the segregation of stem growth habit. The *Dt1* locus is also known to influence various traits such as leaf morphological traits (Tanaka et al., 2009), and expression of *GmTfl1b* is also observed in the root (Liu et al., 2010). Therefore, it is likely that some mutation within this gene acts on DSS without the conversion of stem growth habit. Progeny of a cross between Tachinagaha and Ohsuzu or plants transgenic for *GmTfl1b* should be analyzed to verify these hypotheses. These results suggest that the parental cultivars of the OA RILs carried the effective genetic factor(s) and it was possible to improve resistance to GSD without conversion of stem growth habit even if the responsible gene on *Dt1* locus was identical to *GmTfl1b*.

3.4.4 Earliness of flowering and DSS

Pierce et al. (1984) reported that D-type genotypes with early flowering alleles (*e1e1/e2e2/dt1dt1*) show severe symptoms of GSD. In the present study, DSS was used as a measure of GSD, but we did not detect any QTLs for DSS at flowering-time loci, such as *E1*, *E2* and *E3*, in any test. However, the classification by the alleles at the *E1*, *E2*, *E3* and *Dt1* (I/D-type) loci, which was based on the alleles of the flanking markers, showed the trend that D-type with early flowering allele had lower DSS since the difference of DSS between early and late flowering genotypes was not significant by the Tukey's test (Figure 3.3). Moreover, our results illustrated the successive change of DSS along with earliness of flowering in D-type lines, although this trend was not general in I-type lines (Tables 3.6 and 3.7). This result indicated that each locus controlling flowering time would have a weak effect on the total variance of DSS and that other weak QTLs and/or environmental factors were concealed. Furthermore, it is likely that the responsible gene at the *Dt1* locus and earliness of flowering interact with each other in relation to the occurrence of GSD. Interestingly, this particular relationship was observed even in OA RILs even though no QTLs were detected at the *Dt1* locus. Therefore, the OA RILs population was assumed to have a similar genetic factor influencing the variation of DSS at this locus. Although this interactive effect was observed in

both RIL populations, we could not determine whether *GmTfl1b* itself or a closely linked gene is involved in the occurrence of GSD.

The date of R1 in Ohsuzu, D-type parent of OA RILs, was not very different from that of Tachinagaha, D-type parent of ST RILs, but DSS in Ohsuzu was markedly greater than that in Tachinagaha (Table 3.1). Similarly, OA RILs tended to have higher values of DSS compared to ST RILs with the same flowering period though earliness of flowering influenced DSS of the determinate genotypes in both ST and OA RILs (Figure 3.3). These results indicated that the effective genetic factor(s) of OA RILs was independent of flowering time, and thus it enabled enhancement of the DSS of D-type genotypes without altering flowering time.

The flowering date in short-day plant species is delayed in higher latitude areas. At Akita, the pre-flowering period in each line was longer compared with that at Kyoto because Akita is located at a higher latitude than Kyoto. The delay of flowering date might explain the difference in DSS in D-type lines with the location, it is unlikely that this effect can be applied to indeterminate lines. Because early planting is likely to cause GSD (Isobe et al., 2011), geographical or environmental effects, such as planting date and day-length, may have an impact on the occurrence of GSD.

3.4.5 OA_156 is a unique line in OA RILs

The OA_156 line was considered a unique line in this study because it had an unusually high DSS at Kyoto in 2010 even though it was a D-type line with an early flowering genotype (*e1e2dt1*, Figure 3.3). However, the DSS score of the OA_156 line was only 1.3 in an additional test in 2011, which was much lower than the score at Kyoto in 2010. Lethal yellowing diseases, such as Phytophthora stem rot, can blight the soybean plant (Dorrance et al., 2003). Thus, it is likely that the score in 2010 may have been increased by some undetected disease. Excluding this line, the correlation coefficients of DSS of each line with the location and year were higher, and the relationship between DSS and earliness of flowering became more apparent (Tables 3.3 and 3.7). Although this irregular procedure did not affect the QTL analysis result for DSS, attention should be paid to the influence of diseases to avoid overlooking important signals that lead to improvement of the GSD trait.

3.4.6 Effects of other QTLs on DSS

In ST RILs, QTLs were detected on Chr. 13 (Kyoto-2010) and Chr. 12 (Akita-2010) other than the *Dt1* locus, and they were linked to the marker loci, Sat_197 and Sat_206, respectively (Table 3.4). In these regions, several QTLs for plant height and seed yield have been previously reported (Specht et al., 2001; Reinprecht et al., 2006; Abdel-Haleem et al., 2012). In OA RILs, QTLs found on Chr. 3 were associated with Satt521 and Sat_208. Several researchers have reported QTLs related to seed yield in these regions (Kabelka et al., 2004; Chen et al., 2007). Leopold et al. (1959) demonstrated that the removal of reproductive organs delayed plant senescence, and many researchers reported similar results (Wittenbach, 1982; Crafts-Brandner et al., 1984). Furthermore, Noodén (1980) suggested the presence of senescence factor produced in the seed. Sinclair and de Wit (1975) related the high nitrogen demand of seeds to 'self-destruction' of vegetative organs to produce translocatable nitrogen. Because the QTLs for DSS found in the present study co-located with yield-related QTLs, seed or pod production might have affected the degree of GSD in a similar way.

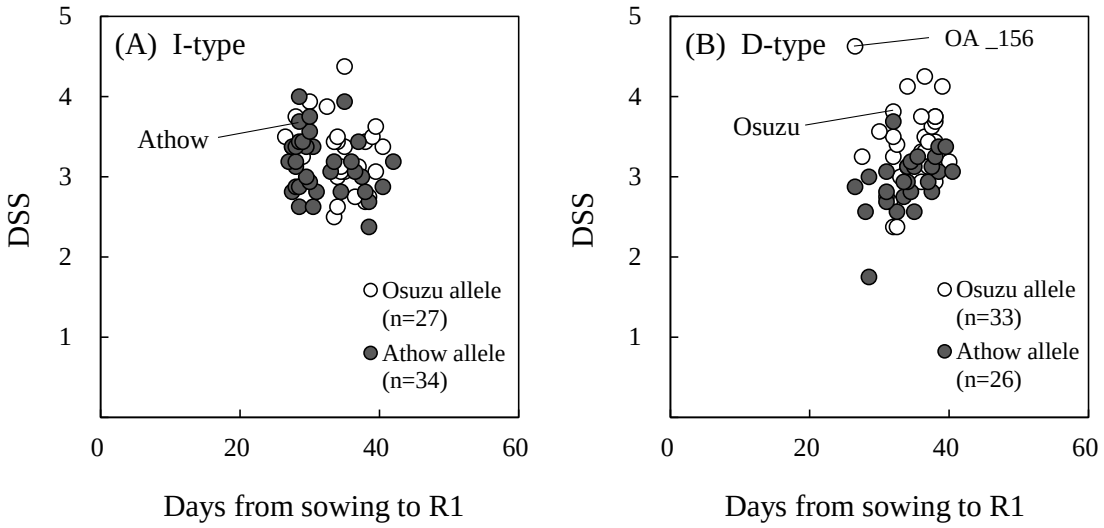


Figure 3.4. Relationship between days from seed sowing to R1 and DSS in (A) I-type and (B) D-type of OA RILs at Kyoto in 2010. Open and closed circles represent the genotypes with Osuzu and Athow-derived allele at Satt521, respectively.

The effect of a QTL for DSS detected at Kyoto in 2010 in OA RILs was analyzed after classification based on the allele of the Satt521 marker, which was tightly linked to this QTL. The effect of the QTL was evident only in D-type lines though the interaction between stem determination and this QTL allele was not significant (P=0.11 without OA_156) by ANOVA (Figure 3.4). Although the effect of the genetic factor at the *Dt1* locus was not confirmed in OA RILs by the QTL analysis for DSS, the QTL on Chr. 3, as well as flowering time, may interact with the genetic factor at the *Dt1* locus. Moreover, the segregation pattern of DSS by this marker allele had a commonality with the difference between ST and OA RILs. Such a QTL might explain the difference of DSS between ST and OA RILs as well as between Tachinagaha and Ohsuzu, and might influence the effect of the stem determination gene on DSS. In the case where *GmTfl1b* certainly engages in the occurrence of GSD, this kind of genetic factor may explain the inconsistent results of the QTL analyses between the populations segregating stem growth habits.

The QTL analysis for DSS separately for I- and D-types within each population was incrementally conducted (data not shown). Several QTLs were detected except for the I-type of ST RILs at Kyoto in 2010 and D-type of OA RILs at Akita in 2010. However, no QTLs were co-located between the stem determination types. A QTL in the D-type of ST RILs found at Kyoto in 2010 and at Akita in 2010 was located near the *E1* locus. A QTL from the OA RILs was identified on Chr. 3 at Kyoto in 2010 using all lines tested but was only found in D-type lines. These results suggested the interactions between the genetic factor at or near the *Dt1* locus and other genetic factors in other places in the genome affect the occurrence of GSD. Some QTLs detected using all lines were not found in both I- and D-types. Possibly, the reduction of population size by separating the stem determination types was the reason for this. On the other hand, in the D-type ST RILs, a consistent QTL at Kyoto and Akita was detected on Chr. 13, which was not found by the normal QTL analysis at Akita in 2010. Therefore, to investigate the genetic factors that influence DSS of both I- and D-types and that involve complex interactions, a greater number of lines need to be evaluated. However, in populations not segregating for stem determination and flowering time, evaluation of fewer lines may be effective in detecting QTLs for the resistance to GSD.

Chapter 4

Analysis of the Effect of the Genetic Factor near the *Dt1* Locus on Source and Sink Balance and Its Relationship to the Severity of Green Stem Disorder of Soybean

4.1 Introduction

In soybean, depodding is known to delay the senescence of vegetative organs (Noodén, 1984; Crafts-Brandner and Egli, 1987), and thus imbalance of reproductive sink organs and assimilate (source) supply is believed to be a possible cause of green stem disorder. Takeda et al. (2002) reported a case that GSD plants had less pod number as compare to normally sensed plants. But, this relation has not been consistent in field observations. The reason for this seemed to be that GSD symptom is difficult to duplicate, and the treatment to induce GSD symptom affected both source supply and sink organ production. Hence, the relationship between source and sink balance and the occurrence of GSD has not determined.

Genetic variation in the susceptibility to GSD may be determined through source and sink balance. Chapter 2 and 3 identified a genetic factor located near the *Dt1* locus which dominates stem determination as a consistent and strong factor influencing the occurrence of GSD, although its effect might depend on genetic background. In this chapter, the effect of the genetic factor was investigated from a viewpoint of source and sink balance using near isogenic lines (NILs) segregating at the *Dt1* locus. Thus, one genotype has indeterminate growth habit (I-type) and the other has determinate stem growth habit (D-type) for each genetic background. Utilization of NIL can conceal the effects of the other genetic region. Because the amount of vegetative growth differs between I-type and D-type genotypes, source and sink balance was standardized in the node-basis.

4.2 Materials and Methods

4.2.1 Plant materials and cultivation conditions

The soybean cultivars, Clark, Harosoy, Williams, Elf and their NILs segregating at the *Dt1* locus, or stem determination were tested (Bernard et al. 1991). These eight genotypes were cultivated at the Kyoto Experimental Farm of the Graduate School of Agriculture, Kyoto University, Kyoto, Japan (Lat. 35°02' N, Long. 135°47' E and 65 m altitude; Alluvial sandy loam soil). Seeds were sown on June 23 in 2011. Plots were composed of ten plants in single row. Cultivation was conducted with two replications in a randomized complete block design. The row and plant spacing distances were 0.7 and 0.15 m, respectively.

During the cultivation, irrigation and spraying pesticides were conducted to avoid biotic and abiotic stresses which complicate the varietal difference of the GSD severity.

4.2.2 Trait evaluations and data recording

At the dates of the beginning of seed filling (R5 stage, Fehr et al., 1971) and full maturity (R8 stage), two plants in each plot were harvested. At R5, the number of nodes per plant, leaf dry weight and leaf area index (LAI) were measured. At R8, the number of nodes per plant, the number of pods per plant, the number of seeds per plant and seed dry weight were measured. The number of flower scars and pods on each inflorescence were also measured at R8, and pod-set ratio was calculated. The inflorescences of soybean were classified into primary raceme, secondary raceme, terminal raceme and higher-ordered raceme according to Shibles et al. (1975, Figure 4.1).

At R8, the severity of GSD was recorded using a simple scoring method proposed by Furuya and Umezaki (1993), which evaluates the degree of synchronous senescence (DSS) between vegetative and reproductive organs with a 5-point scale from DSS1 to DSS5. At DSS1, the stem is green and green or yellow-green leaves remain at more than one-third of the nodes of the plant. At DSS2, the stem is green or green-yellow and green or yellow-green leaves remain at less than one-third of the nodes of the plant. At DSS3, the stem is light green and contains some moisture and chlorophyll, and several leaves contain some moisture or several petioles without leaves remain at R8. At DSS4, the stem is yellow and retains some moisture, and occasionally a few leaves are yellow to yellow-green. At DSS5, the stem is dry with gray or

brown color, and all leaves have abscised. Plants exhibiting intermediate symptoms were assigned intermediate scores.

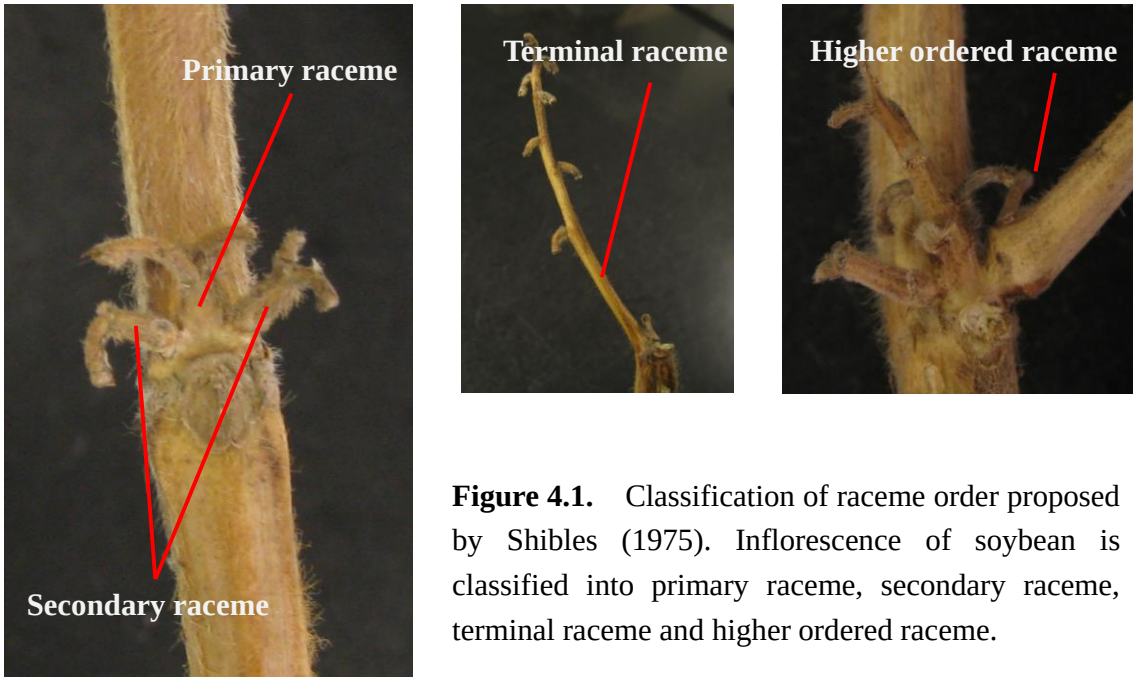


Figure 4.1. Classification of raceme order proposed by Shibles (1975). Inflorescence of soybean is classified into primary raceme, secondary raceme, terminal raceme and higher ordered raceme.

4.2.3 Statistical analysis

Statistical analysis was performed with statistics software R version 3.1.2 for Windows. The statistical significance of differences was evaluated by analysis of variance (ANOVA).

4.3 Results

In all of the genetic backgrounds (Clark, Harosoy, Williams and Elf), DSS scores of the D-types were lower than those of the I-types, and the means of DSS were 1.81 in D-type and 3.69 in I-type (Table 4.1). The D-type had smaller number of nodes per plant than the I-type, and hence LAI, measured at R5, of the D-type was approximately half as high as that of the I-type. On the other hands, leaf weight per node did not differ between the stem growth habits.

The numbers of pods and seeds per node were not statistically different between the I- and D-types, and the difference in seed weight per node was not significant between the stem determination types, too.

Table 4.1. General statistics for DSS , total node number at R8, LAI at R5, leaf weight per node at R5, pod number per node at R8, seed number per node at R5, seed weight per node at R8, flower number per node and pod-set ratio at R8 of Clark, Harosoy, Williams, Elf and their NILs.

I/D-type	Background	DSS	Total node number	LAI ($m\ m^{-1}$)	Leaf weight per node	Pod number per node	Seed number per node	Seed weight per node	Flower number per node	Pod-set ratio
I-type	Clark	3.00 ±0.50	49.5 ±6.8	5.04 ±0.11	0.367 ±0.025	2.04 ±0.43	3.54 ±0.22	0.62 ±0.06	5.00 ±0.38	0.41 ±0.08
	Harosoy	4.50 ±0.25	50.3 ±13.2	2.68 ±0.32	0.298 ±0.028	2.42 ±0.22	5.05 ±0.38	0.86 ±0.08	4.72 ±0.29	0.51 ±0.03
	Williams	4.00 ±0.00	36.3 ±9.3	3.23 ±0.39	0.258 ±0.000	1.94 ±0.29	3.72 ±0.99	0.59 ±0.16	5.56 ±0.48	0.35 ±0.03
	Elf	3.25 ±0.50	69.0 ±12.0	5.62 ±1.20	0.269 ±0.011	1.64 ±0.04	3.24 ±0.05	0.52 ±0.01	5.10 ±0.24	0.32 ±0.01
D-type	Clark	1.50 ±0.00	35.3 ±5.4	2.87 ±0.30	0.326 ±0.041	1.75 ±0.12	4.03 ±0.32	0.81 ±0.08	2.87 ±0.12	0.61 ±0.04
	Harosoy	2.00 ±0.00	18.3 ±3.6	1.29 ±0.11	0.342 ±0.025	2.62 ±0.59	5.61 ±1.14	1.07 ±0.19	3.44 ±0.61	0.76 ±0.08
	Williams	1.25 ±0.25	31.8 ±2.7	1.93 ±0.12	0.397 ±0.016	1.89 ±0.23	4.11 ±0.44	0.77 ±0.08	2.91 ±0.27	0.65 ±0.03
	Elf	2.50 ±0.00	35.0 ±4.1	2.46 ±0.08	0.277 ±0.015	1.90 ±0.21	3.73 ±0.81	0.63 ±0.18	3.67 ±0.18	0.52 ±0.06
I-type		3.69 ±0.21	51.3 ±2.5	4.14 ±0.41	0.298 ±0.011	2.01 ±0.14	3.89 ±0.36	0.65 ±0.05	5.09 ±0.09	0.40 ±0.02
D-type		1.81 ±0.11	30.1 ±1.0	2.14 ±0.09	0.335 ±0.010	2.04 ±0.18	4.37 ±0.32	0.82 ±0.05	3.22 ±0.19	0.63 ±0.02
I/D ratio		2.03	1.70	1.94	0.89	0.98	0.89	0.79	1.58	0.63
ANOVA	I/D-type	*	*	*	n.s.	n.s.	n.s.	n.s.	***	***
	Background	n.s.	*	n.s.	n.s.	*	*	**	*	***
	Interaction	*	*	n.s.	*	n.s.	n.s.	n.s.	*	n.s.

*,** and *** represent statistical significance at P<0.05, 0.01 and 0.001, respectively, and n.s. represents not significant.

The number of flowers per node was smaller in the D-type as compared with that in the I-type, but the pod-set ratio was higher in the D-type instead (Table 4.1). The smaller number of flowers per node in the D-type resulted from the smaller number of flowers on primary and secondary racemes per node, but the number of flowers on higher-ordered racemes per node were larger in the D-type (Table 4.2). The number of pods on the primary and the secondary racemes per node was considerably smaller in the D-type, and the number of pods on the higher-ordered racemes per node was higher in the D-type. The number of flowers per primary raceme of the D-type was about half as large as that of the I-type, and the number of pods per primary raceme of the D-type was smaller than that of the I-type genotype although the D-type genotype had a higher pod-set ratio (Table 4.3). The number of flowers per terminal raceme was also smaller in the D-type, but the number of pods per terminal raceme was not significantly different between the stem determination types. The number of flowers, the number of pods and

the pod-set ratio per secondary raceme did not differ between the I- and D-types. The number primary racemes per node was smaller in the D-type, and the number of secondary racemes per node of the D-type was less than half that of I-type. The number of primary and secondary racemes per node is also decreased by the emergence of branch and sub-branch.

Table 4.2. General statistics for pod number, flower number and pod-set ratio on primary, secondary and higher ordered raceme per node of Clark, Harosoy, Williams, Elf and their NILs.

I/D-type	Background	On primary raceme per node			On secondary racemes per node			On higher ordered racemes per node		
		Pod number	Flower number	Pod-set ratio	Pod number	Flower number	Pod-set ratio	Pod number	Flower number	Pod-set ratio
I-type	Clark	1.25 ± 0.23	2.65 ± 0.14	0.47 ± 0.06	0.63 ± 0.24	1.91 ± 0.26	0.33 ± 0.11	0.00 ± 0.00	0.00 ± 0.00	—
	Harosoy	1.39 ± 0.05	2.23 ± 0.39	0.64 ± 0.09	0.89 ± 0.19	2.10 ± 0.49	0.43 ± 0.02	0.01 ± 0.01	0.01 ± 0.01	1.00 ± 0.00
	Williams	1.22 ± 0.13	2.78 ± 0.20	0.44 ± 0.02	0.64 ± 0.23	2.41 ± 0.29	0.26 ± 0.07	0.00 ± 0.00	0.03 ± 0.04	0.00 ± 0.00
	Elf	0.75 ± 0.07	2.16 ± 0.20	0.35 ± 0.00	0.72 ± 0.04	2.38 ± 0.01	0.31 ± 0.02	0.01 ± 0.01	0.05 ± 0.03	0.07 ± 0.07
D-type	Clark	0.84 ± 0.04	1.12 ± 0.03	0.75 ± 0.03	0.30 ± 0.06	0.81 ± 0.17	0.37 ± 0.05	0.14 ± 0.10	0.31 ± 0.16	0.48 ± 0.23
	Harosoy	0.95 ± 0.17	1.01 ± 0.08	0.93 ± 0.10	0.81 ± 0.41	1.17 ± 0.29	0.64 ± 0.23	0.26 ± 0.15	0.60 ± 0.26	0.40 ± 0.27
	Williams	0.82 ± 0.10	1.04 ± 0.10	0.79 ± 0.04	0.33 ± 0.10	0.72 ± 0.07	0.48 ± 0.20	0.23 ± 0.15	0.44 ± 0.24	0.47 ± 0.16
	Elf	0.86 ± 0.05	1.10 ± 0.02	0.78 ± 0.05	0.35 ± 0.10	1.08 ± 0.22	0.32 ± 0.05	0.13 ± 0.09	0.53 ± 0.18	0.25 ± 0.16
I-type		1.15 ± 0.07	2.45 ± 0.10	0.47 ± 0.04	0.72 ± 0.08	2.20 ± 0.17	0.33 ± 0.04	0.00 ± 0.01	0.02 ± 0.02	—
D-type		0.87 ± 0.05	1.07 ± 0.03	0.81 ± 0.03	0.45 ± 0.14	0.94 ± 0.08	0.45 ± 0.08	0.19 ± 0.03	0.47 ± 0.04	0.40 ± 0.04
I/D ratio		1.33	2.30	0.58	1.62	2.33	0.73	0.02	0.05	—
ANOVA	I/D-type	**	***	***	n.s.	***	n.s.	n.s.	*	—
	Background	n.s.	n.s.	*	*	n.s.	*	n.s.	n.s.	—
	Interaction	*	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	—

*,** and *** represent statistical significance at P<0.05, 0.01 and 0.001, respectively, and n.s. represents not significant.

Table 4.3. General statistics for pod number, flower number and pod-set ratio per primary, secondary and terminal raceme of Clark, Harosoy, Williams, Elf and their NILs.

I/D-type	Background	Per primary raceme			Per secondary raceme			Per terminal raceme		
		Pod number	Flower number	Pod-set ratio	Pod number	Flower number	Pod-set ratio	Pod number	Flower number	Pod-set ratio
I-type	Clark	1.34 ± 0.25	2.84 ± 0.16	0.47 ± 0.06	0.38 ± 0.13	1.16 ± 0.06	0.33 ± 0.11	1.88 ± 1.08	5.13 ± 1.51	0.33 ± 0.12
	Harosoy	1.46 ± 0.05	2.34 ± 0.31	0.64 ± 0.09	0.76 ± 0.04	1.79 ± 0.15	0.43 ± 0.02	0.82 ± 0.25	2.35 ± 0.40	0.37 ± 0.13
	Williams	1.29 ± 0.12	2.93 ± 0.19	0.44 ± 0.02	0.39 ± 0.14	1.48 ± 0.21	0.26 ± 0.07	1.15 ± 0.60	4.25 ± 0.43	0.27 ± 0.15
	Elf	0.81 ± 0.08	2.35 ± 0.23	0.35 ± 0.00	0.40 ± 0.02	1.33 ± 0.00	0.31 ± 0.02	1.52 ± 0.65	4.98 ± 0.15	0.31 ± 0.14
D-type	Clark	0.96 ± 0.04	1.28 ± 0.04	0.75 ± 0.03	0.49 ± 0.03	1.34 ± 0.12	0.37 ± 0.05	1.60 ± 0.33	2.11 ± 0.22	0.75 ± 0.09
	Harosoy	1.18 ± 0.24	1.26 ± 0.14	0.93 ± 0.10	1.41 ± 0.60	2.12 ± 0.31	0.64 ± 0.23	2.15 ± 0.65	2.40 ± 0.66	0.90 ± 0.11
	Williams	0.99 ± 0.11	1.25 ± 0.08	0.79 ± 0.04	0.69 ± 0.30	1.43 ± 0.10	0.48 ± 0.20	1.63 ± 0.47	2.26 ± 0.51	0.72 ± 0.13
	Elf	1.02 ± 0.06	1.30 ± 0.05	0.78 ± 0.05	0.55 ± 0.13	1.68 ± 0.16	0.32 ± 0.05	1.83 ± 0.32	3.08 ± 0.20	0.60 ± 0.13
I-type		1.23 ± 0.08	2.62 ± 0.06	0.47 ± 0.04	0.48 ± 0.05	1.44 ± 0.08	0.33 ± 0.04	1.34 ± 0.29	4.18 ± 0.52	0.32 ± 0.01
D-type		1.04 ± 0.08	1.27 ± 0.04	0.81 ± 0.03	0.78 ± 0.22	1.64 ± 0.08	0.45 ± 0.08	1.80 ± 0.14	2.46 ± 0.20	0.74 ± 0.02
I/D ratio		1.18	2.05	0.58	0.62	0.88	0.73	0.75	1.69	0.43
ANOVA	I/D-type	**	***	***	n.s.	n.s.	n.s.	n.s.	***	***
	Background	n.s.	n.s.	*	***	***	*	n.s.	n.s.	n.s.
	Interaction	*	*	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.

*,** and *** represent statistical significance at P<0.05, 0.01 and 0.001, respectively, and n.s. represents not significant.

4.4 Discussion

Because the D-type showed more sever symptom of GSD than the corresponding I-type in all of the genetic backgrounds derived from the Clark, Harosoy, Williams and Elf cultivars, the allele at the *Dt1* locus which induced GSD symptom was compatible with the D-type stem growth habit (Table 4.1). LAI, measured at R5, of the I-type was twice as high as that of the D-type, which was thought to be due to increased node number of the I-type. Leaf weight per node at R5 did not differ between the D- and I-types. The number of pods, seeds per node and seed weight per node were not different between the stem determination types. Hardman and Brun (1971) suggested that photosynthesis during flowering and pod set was associated with the number of pods and seeds. Schou et al. (1978), Egli and Zhen-wen (1991) and Jiang and Egli (1995) reported similar results. Generally, photosynthate is affected by the amount of light received. Because mutual leaf shielding was limited in the D-type due to lower LAI, the amount of light received per node seemed to be larger in the D-type compared with the I-type. In addition, the amount of the photosynthate used for vegetative growth during flowering and pod set would be smaller in the D-type. Thus, the D-type had more photosynthate (source) supply used for reproductive organ productions (sink). However, the number of pods and seeds of the D-type was comparable to that of the I-type. Therefore, it is presumed that the D-type was in source and sink imbalance, and which might be related to the severity of GSD, or DSS.

The number of pods per node is the product of the number of flowers per node and pod-set ratio. The number of flowers per node was considerably small in the D-type in comparison to the I-type, while pod-set ratio of the D-type was higher than that of the I-type (Table 4.1). Saitoh et al. (1998) suggested that the number of pods was more sensitive to the number of flowers. Soybean produces multiple types of inflorescence on each node, and they are classified into primary, secondary and higher-ordered racemes (Shibles et al, 1975). In addition, terminal raceme occurs at apexes of the stems. The I-type had the larger number of flowers per derived from primary and secondary racemes (Table 4.2). The larger number of flower on primary raceme per node in the I-type was mainly due to the number of flowers per primary raceme rather than the number of primary racemes per node (Table 4.3). The primordia of primary and

secondary raceme is also the primordia of branches and sub-branches. The number of branches and sub-branches was larger in the D-type, which led to the decreased number of flowers on primary and secondary racemes per node.

The responsible gene controlling the stem determination of soybean turned out to be an ortholog of arabidopsis *TERMINAL FLOWER1* (*TFL1*), and termed as *GmTFL1b* (Liu et al., 2010). The *TFL1* gene is known to associate with the continuation of stem apical meristem and inflorescence development in *Arabidopsis thaliana* (Alvarez et al, 1992). The number of flowers per primary and terminal raceme were significantly larger in the I-type compared to that of the D-type (Table 4.3). Therefore, the *GmTFL1b* may also affect inflorescence morphology. This result suggested that the I-type stem growth habit secured the number of flowers per node which prevent the occurrence of source and sink imbalance. If the genetic variation in the severity of GSD is associated with the number of flowers per node, the genetic factor at the *Dt1* locus influencing DSS might be identical to the *GmTFL1b*.

Chapter 5

Verification of the Impact of Genetic Variation in Flower Production on the Severity of Green Stem Disorder in Soybean

5.1 Introduction

In Chapter 4, a genetic factor near the *Dt1* locus, which dominates stem determination, was suggested to influence source and sink balance through the number of flowers per node, and to be associated with the severity of green stem disorder (GSD) of soybean. But, further investigation is needed to verify the relationship between the number of flowers per node and the severity of GSD. Excision of flowers is likely conventional method to control the number of flowers. But, it is difficult to manipulate the flower number at appropriate timing, and the excision of flower itself is suggested to induce the incidence of GSD. Therefore, the relationship between the genetic variations in the severity of GSD and in the number of flowers were analyzed.

Two sets of recombinant inbred line (RIL) population, Stressland×Tachinagaha RILs and Ohsuzu×Athow RILs, were used. These RIL populations were segregated for the severity of GSD as reported in Chapter 3. The utilization of RIL population can remove the complicated genetic effect and interactions which influence the severity of GSD.

5.2 Materials and Methods

5.2.1 Plant materials and cultivation conditions

Two sets of recombinant inbred line (RIL) populations segregating stem growth habit and earliness of flowering were tested. One population was developed from a cross between the Stressland and the Tachinagaha cultivars, while the other was from a cross between the Ohsuzu and the Athow cultivars. These populations were also used in Chapter 3. Randomly selected 18

lines from each population and parental cultivars were planted at the Kyoto Experimental Farm of the Graduate School of Agriculture, Kyoto University, Kyoto, Japan (Lat. 35°02’ N, Long. 135°47’ E and 65 m altitude; Alluvial sandy loam soil) in 2011. Of the RILs, eight lines were with indeterminate stem growth habit (I-type), and the other ten lines were with determinate stem growth habit (D-type) for each population. In 2012, selected seven D-type lines from each population and the D-type parents (Tachinagaha and Ohsuzu) were re-analyzed at the Takatsuki Experimental Farm of the Graduate School of Agriculture Kyoto University, Takatsuki, Japan (Lat. 34°51’ N, Long. 135°37’ E and 8 m altitude; Alluvial sandy loam soil). Seeds were sown on June 23 in Kyoto-2011 and on July 19 in Takatsuki-2012. Plots were composed of ten plants in single row. Cultivation was conducted with two replications in a randomized complete block design. The row and plant spacing distances were 0.7 and 0.15 m, respectively.

During the cultivation, irrigation was conducted to avoid drought stress, and pesticides were sprayed because biotic and abiotic stresses complicates the varietal difference of GSD severity.

5.2.2 Trait evaluations and data recording

According to Fehr et al. (1971), the date of the beginning of flowering (R1) was recorded. At full maturity (R8), the severity of GSD was recorded using a simple scoring method proposed by Furuya and Umezaki (1993), which evaluates the degree of synchronous senescence (DSS) between vegetative and reproductive organs with a 5-point scale from DSS1 to DSS5. At DSS1, the stem is green and green or yellow-green leaves remain at more than one-third of the nodes of the plant. At DSS2, the stem is green or green-yellow and green or yellow-green leaves remain at less than one-third of the nodes of the plant. At DSS3, the stem is light green and contains some moisture and chlorophyll, and several leaves contain some moisture or several petioles without leaves remain at R8. At DSS4, the stem is yellow and retains some moisture, and occasionally a few leaves are yellow to yellow-green. At DSS5, the stem is dry with gray or brown color, and all leaves have abscised. Plants exhibiting intermediate symptoms were assigned intermediate scores.

At R8, two plants in each plot were harvested, and the number of nodes per plant, the number of flower scars and pods on each inflorescence were also measured. Pod-setting ratio

was also calculated. The inflorescences of soybean were classified into primary raceme, secondary raceme, terminal raceme and higher-ordered raceme according to Shibles et al. (1975).

5.2.3 Statistical analysis

Statistical analysis was performed with statistics software R version 3.1.2 for Windows. The association of two traits ware evaluated by Pearson's correlation test.

5.3 Results

5.3.1 Variation of DSS

Wide variation of DSS were observed in ST RILs. At Kyoto in 2011, the DSS of ST RILs with I-type stem growth habit ranged from 2.50 to 3.88, with an average of 3.36. The DSS of ST RILs with D-type stem growth habit ranged from 1.38 to 2.88, with an average of 2.17. The scores in Stressland (I-type parent) and Tachinagaha (D-type parent) were 3.88 and 2.75, respectively. At Takatsuki in 2012, the DSS of ST RILs with D-type stem growth habit ranged from 1.88 to 3.75, with an average of 2.54. The score of Tachinagaha was 3.00.

Variation of DSS were also observed in OA RILs. At Kyoto in 2012, the DSS of OA RILs with I-type stem growth habit ranged from 2.75 to 4.50 with an average of 3.57. The DSS of OA RILs with D-type growth habit ranged from 1.25 to 3.88 with an average of 2.87. The scores in Ohsuzu (D-type parent) and Athow (I-type parent) were 3.50 and 3.75, respectively. At Takatsuki in 2012, the DSS of OA RILs with D-type stem growth habit ranged from 1.44 to 4.31 with an average of 3.52. The DSS of Osuzu was 4.13.

The DSS score was closely correlated between the two environments, and the correlation coefficient was 0.936 (P<0.001) including both ST and OA RILs with D-type stem growth habit (Figure 5.1)

5.3.2 Variation of duration from sowing to flowering

At Kyoto in 2011, the variation of the days from sowing to R1 in ST RILs ranged from 27.0 to 45.5. The duration from sowing to R1 in OA RILs ranged from 25.0 to 40.0. At Takatsuki in 2012, the days from sowing to R1 in ST RILs ranged 23.0 to 34.5 while the duration in OA RILs ranged from 25.0 to 35.5.

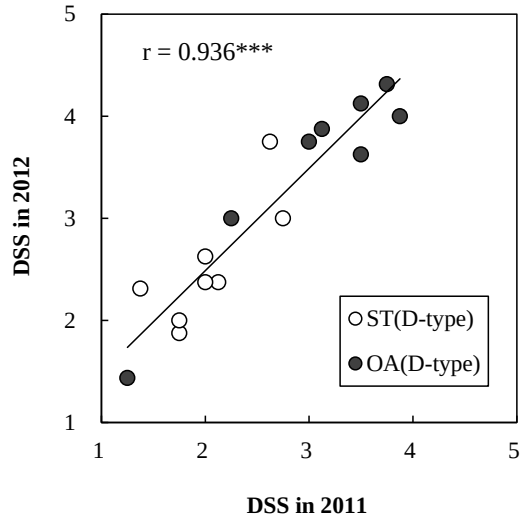


Figure 5.1. Correlation between DSS in Kyoto-2011 and Takatsuki-2012 of D-type ST and OA RILs. Open and closed circles represent D-type ST and OA RILs, respectively. *** represents statistical significance at P<0.001.

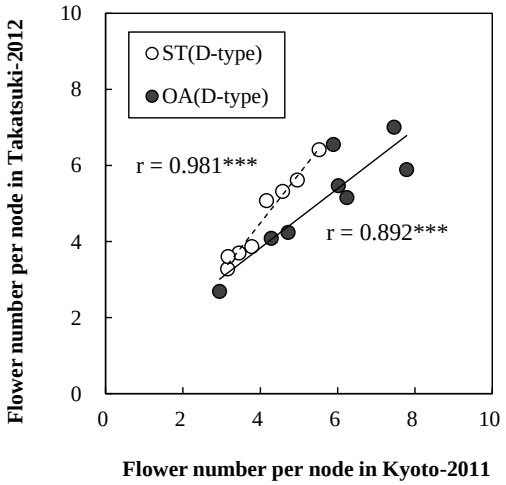


Figure 5.2. Relationship between DSS in 2011 and 2012 of determinate ST and OA RILs. Open and closed circles represent determinate ST and OA RILs, respectively. Broken and solid lines were trend lines od ST and OA RILs, respectively. *** represents statistical significance at P<0.001.

5.3.3 Variation of flower number per node

At Kyoto in 2011, the number of flowers per node in ST RILs with I-type growth habit ranged from 4.26 to 6.93, while that in ST RILs with D-type growth habit ranged from 3.16 to 5.52. The number of flowers per node in OA RILs with I-type growth habit ranged from 4.65 to 5.50, whereas that in OA RILs with D-type growth habit ranged from 2.95 to 7.79. At Takatsuki in 2012, the number of flower in ST RILs with D-type stem growth habit ranged from 3.28 to 6.41, while that in OA RILs with D-type growth habit ranged from 2.69 to 7.01. The number of flowers per node at Kyoto in 2011 was closely correlated with that at Takatsuki in 2011, and the correlation coefficient was statistically significant at $P<0.001$ (Figure 5.2).

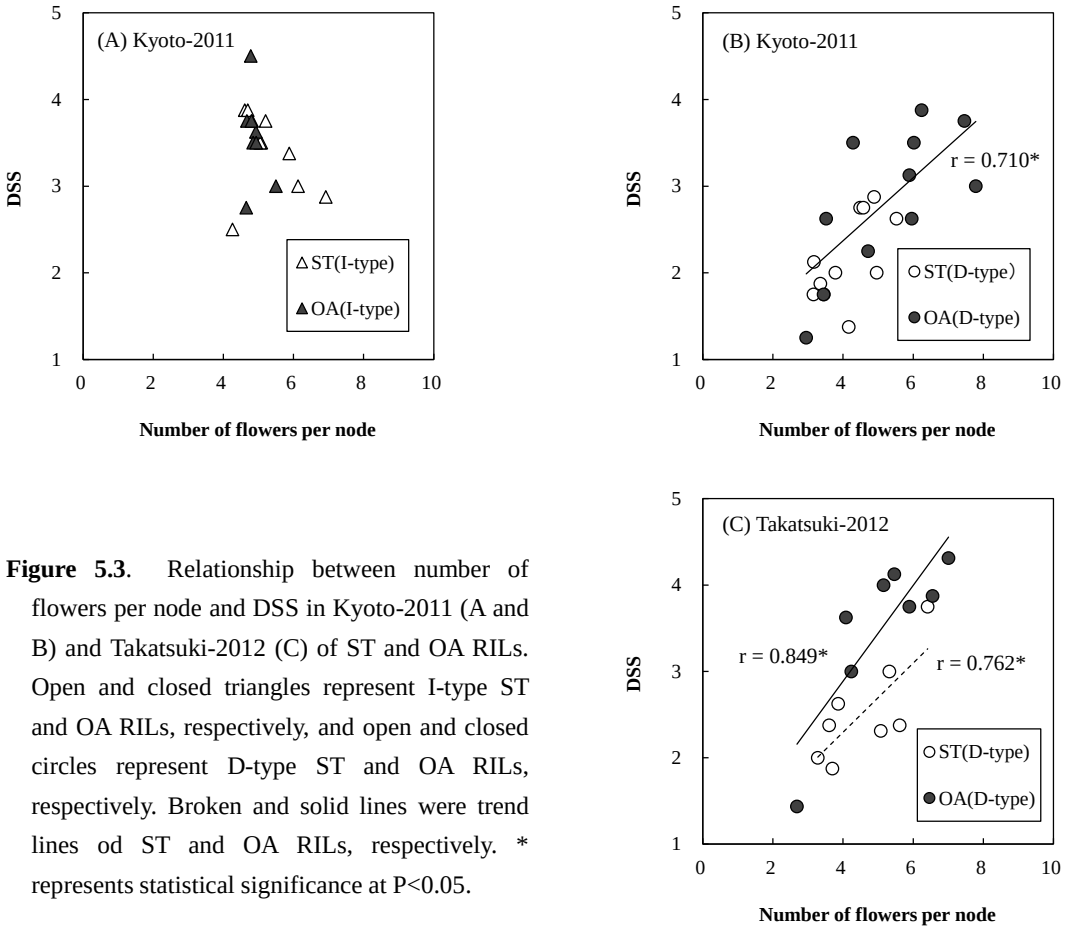


Figure 5.3. Relationship between number of flowers per node and DSS in Kyoto-2011 (A and B) and Takatsuki-2012 (C) of ST and OA RILs. Open and closed triangles represent I-type ST and OA RILs, respectively, and open and closed circles represent D-type ST and OA RILs, respectively. Broken and solid lines were trend lines od ST and OA RILs, respectively. * represents statistical significance at P<0.05.

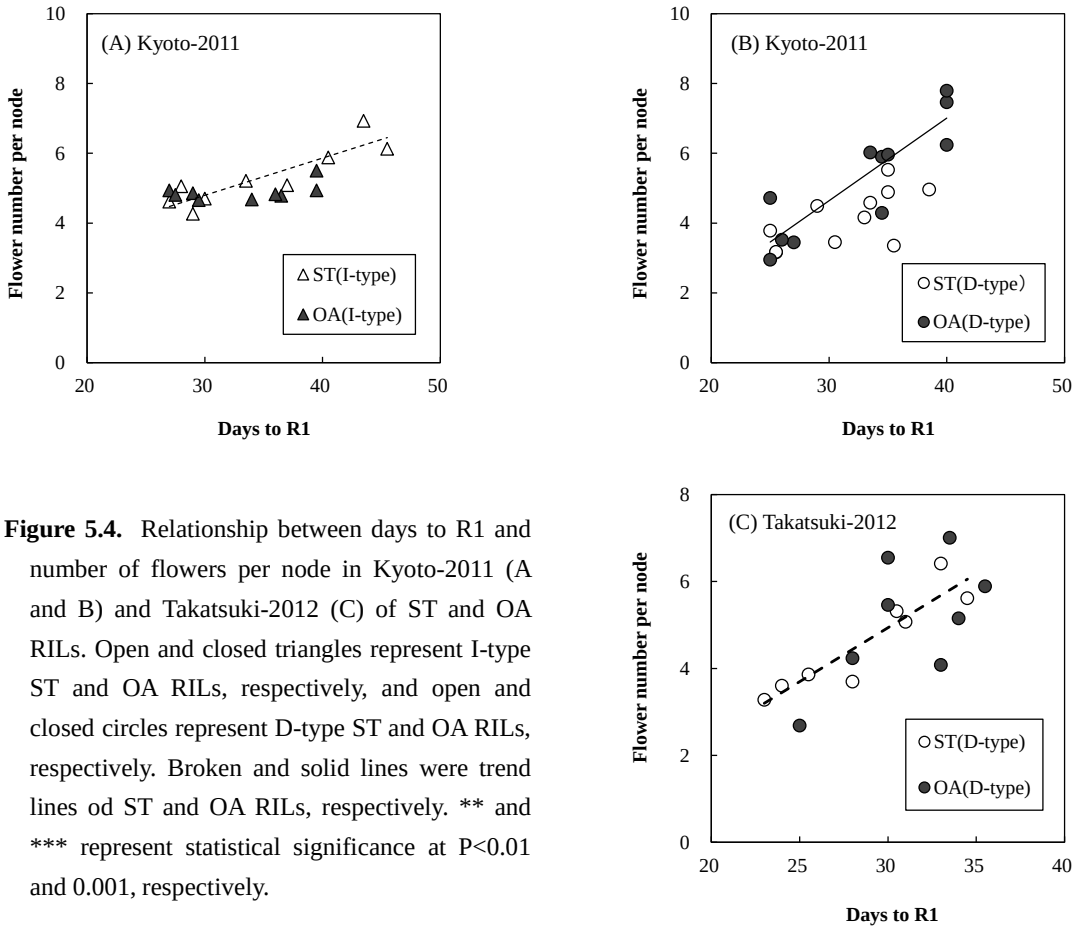


Figure 5.4. Relationship between days to R1 and number of flowers per node in Kyoto-2011 (A and B) and Takatsuki-2012 (C) of ST and OA RILs. Open and closed triangles represent I-type ST and OA RILs, respectively, and open and closed circles represent D-type ST and OA RILs, respectively. Broken and solid lines were trend lines od ST and OA RILs, respectively. ** and *** represent statistical significance at P<0.01 and 0.001, respectively.

5.3.3 Relationship between DSS and the number of flowers per node

At Kyoto in 2011, DSS did not showed significant correlation with the number of flowers per node in the I-type lines of ST and OA-RILs (Figure 5.3). But, the relations were statistically significant in the D-type lines of ST and OA RILs, and the correlation coefficients in ST and OA RILs were 0.707 (P<0.05) and 0.614 (P<0.05), respectively. For all of the lines, including the two RIL populations and both stem growth habits, the correlation coefficient was 0.524 (P<0.001, data not shown). At Takatsuki in 2012, DSS showed positive correlation with the number of flowers per node in both ST and OA RILs with the D-type stem growth habit.

5.3.4 Relationship between duration from sowing to R1 and the number of flowers per node

At Kyoto in 2011, the number of flowers per node was positively correlated with the

duration from sowing to R1 for ST RILs with the I- and D-types (Figure 5.4). The relationship was also positive for OA RILs with the D-type stem growth habit, but the number of flowers per node did not show correlation with the earliness of flowering for OA RILs with the I-type growth habit. At Takatsuki in 2012, the correlation between the duration from sowing to R1 and the number of flowers per node was strong in ST RILs with the D-type stem growth habit, but that was not evident in OA RILs with D-type stem growth habit.

5.4 Discussion

Wide variation of the severity of GSD, as measured with DSS, was observed in ST and OA RILs, and the score was environmentally stable (Figure 5.1). The variation of the number of flowers per node was wide in the D-type lines of ST and OA RILs compared with that in the I-type lines, but the number of flower per node was relatively high in the I-type lines (Figure 5.3). Although the correlation between the number of flowers per node and DSS was not observed in the I-type lines of ST and OA RILs, the line with large number of flowers per node tended to have high DSS score. Therefore, it was suggested that the number of flowers per node influenced the severity of GSD.

The number of flowers per node would be genetically controlled because of the positive correlation of the number between the two environments (Figure 5.2). The number of flowers per node was relatively higher in the I-type lines (Figure 5.3). This observation agreed with the result of Chapter 4, where the genetic factor near the *Dt1* locus, which dominates the stem determination, influenced the number of flowers per node. In addition, this analysis indicated that the earliness of flowering is another possible factor which influenced the number of flowers per node (Figure 5.4). Saitoh et al. (2003) reported a similar result using some Japanese and US conventional cultivars. The genetic factors influencing flowering time are associated with the maintenance of stem apical meristems (SAMs) which produce vegetative organs. ST RILs were segregated at the *E1*, *E2* and *E3* loci, while OA RILs were segregated at the *E1* and *E2* loci as shown in Chapter 3. This result suggested that these loci are associated with the continuation of

inflorescence meristems, and the late flowering genotype secured the number of flowers per node which prevent the occurrence of source and sink imbalance. Increased flower number was suggested to ameliorate the source and sink imbalance. The other genetic factor(s) which increases flower production would contribute to the improvement of GSD characteristics without altering stem growth habit and flowering time.

Chapter 6 General Discussion

In a series of analyses, the severity of green stem disorder (GSD) of soybean was evaluated using a scoring method proposed by Furuya and Umezaki (1993), which evaluates the degree of synchronous senescence (DSS) between vegetative and reproductive organs with a 5-point scale from DSS1 to DSS5. This scoring method facilitates the description of the GSD severity and enables the evaluation of many plant samples.

In Chapter 2, the old and modern soybean cultivars from Japan and USA were observed, and DSS was determined in two seasons along with various agronomic traits. The consistency of DSS results between two years indicated that DSS is under genetic control to a considerable extent. The results suggested that stem determination and earliness of flowering are major plant factors associated with variation of DSS among the cultivars. Only determinate stem growth type (D-type) has been exclusively used in Japanese soybean production and on average indeterminate stem growth type (I-type) showed greater DSS values than did D-type. This suggested that introducing I-type in commercial cultivars would help to mitigate the occurrence of GSD in Japan.

The genetic factors for DSS was examined in Chapter 3 with special interest in involvement of the stem determination. Two sets of recombinant inbred line populations (ST RILs and OA RILs) were studied. They were derived from crosses between Japanese D-type (Tachinagaha and Ohsuzu) and US I-type cultivars (Stressland and Athow). A QTL was detected at the chromosomal position close to the *Dt1* locus that governs the stem determination. But, it was true only in ST RILs and not in OA RILs. Therefore, the direct involvement of the stem determination-gene in the severity of DSS was not concluded. However, in both the ST and OA RILs, the effect of two other genetic factors on DSS was dependent on the *Dt1* genotypes, and this fact suggested indirect influence of the genetic factor near the *Dt1* on GSD occurrence.

In Chapter 4, the effect of the genetic factor near the *Dt1* locus on plant phenotypes was studied in reference to the source and sink balance, which has been considered a factor to cause delay of senescence in vegetative organs. Near isogenic lines (NILs) segregating the stem determination types were used, and the D-types showed sever symptom of GSD compared with

the corresponding I-types. Generally, D-type plant tended to obtain greater assimilate supply (source) in node-basis than did I-type due to difference of leaf area index. Inferior production of flowers per node in the D-types was found and this was considered to cause lower number of pods per node and hence the excess source relative to the sink. There was a close positive correlation between the flower number per node and DSS.

The relation between the stem determination and the source and sink balance was further studied in Chapter 5 using ST and OA populations. In ST RILs, D-types produced less flowers per node than I-types, but it was not true in OA RILs, in which even D-types produced enough flowers to maintain the balance. This might be the reason why any QTLs were not detected near the *Dt1* locus for the OA RILs.

The stem determination gene of soybean has been identified to be an ortholog gene of arabidopsis *TERMINAL FLOWER1* (Liu et al. 2010), which is involved in inflorescence morphology through maintenance of meristem activity. Considering greater production of flower of the primary raceme in the I-types, it is suggested that the stem determination gene affects flower production in the racemes as well as node production.

In the D types, the flower number per raceme was greater as days to flowering was greater, and thus the genetic factor for flowering time also is thought to be involved in flower production in the racemes, and this is the reason why earlier genotypes tend to exhibit severer symptom of GSD in the D-types.

In conclusion, the genetic factor near the *Dt1* locus and its interaction with flowering time genes are considered as major determinant factors to cause genetic variation in DSS in soybean. These genetic factors govern flower production per node. In the genotypes that exhibit GSD symptom, superior flower production helps to avoid excess source relative to sink, which would lead to the improvement of GSD characteristic.

References

Abdel-Haleem, H, Carter, T.E., Purcell, L.C., King, C.A., Ries, L.L., Chen, P.Y., Schapaugh, W., Sinclair, T.R. and Boerma, H.R. 2012. Mapping of quantitative trait loci for canopy-wilting trait in soybean (*Glycine max* L. Merr). *Theor. Appl. Genet.* 125: 837-846.

Abe, J., Xu, D.H., Suzuki, Y., Kanazawa, A. and Shimamoto, Y. 2003. Soybean germplasm pools in Asia revealed by unclear SSRs. *Theor Appl Genet*. 106: 445-453.

Abe, J., Xu, D., Miyano, A., Komatsu, K., Kanazawa, A. and Shimamoto, Y. 2003. Photoperiod-insensitive Japanese soybean landraces differ at two maturity loci. *Crop Sci*. 43: 1300-1304.

Alvarez, J., Guli, C.L., Yu, XH. And Smyth, D.R. 1992. *terminal flower*: a gene affecting inflorescence development in *Arabidopsis thaliana*. *Plant J*. 2: 103-116.

Bernard, R.L. 1971. Two major genes for time of flowering and maturity in soybeans. *Crop Sci.* 11: 242-244.

Bernard, R.L. 1972. Two genes affecting stem termination in soybeans. *Crop Sci.* 12: 235–239.

Bernard, R.L., Nelson, R.L., Cremeens, C.R. 1991. USDA soybean genetic collection: isoline collection. *Soybean Genet. Newsl.* 18: 27-57.

Buzzell, R.I. 1971. Inheritance of a soybean flowering response to fluorescent-daylength conditions. *Can. J. Genet. Cytol.* 13: 703-707.

Chen, Q., Zhang, Z., Liu, C., Xin, D., Qiu, H., Shan, D., Shan, C. and Hu, G. 2007. QTL analysis of major agronomic traits in soybean. *Ag. Sci. in China*. 6: 399-405.

Cober, E.R., Tanner J.W. and Voldeng, H.D. 1996. Soybean photoperiod-sensitivity loci respond differentially to light quality. *Crop Sci.* 36: 606–610.

Cober, E.R. and Voldeng, H.D. 2001. A new soybean maturity and photoperiod-sensitivity locus linked to *E1* and *T*. *Crop Sci.* 41: 698–701.

Crafts-Brandner, S.J., Below, F.E., Harper, J.E. and Hageman, R.H. 1984. Effects of pod removal on metabolism and senescence of nodulating and nonnodulating soybean isolines. *Plant Physiol.* 75: 311-317.

Crafts-Brandner, S.J. and Egli, D.B. 1987. Sink removal and leaf senescence in soybean. *Plant*

Physiol. 85: 662-666.

Cregan, P.B., Jarvik, T., Bush, A.L., Shoemaker, R.C., Lark, K.G., Kahler, A.L., Kaya, N., VanToai, T.T., Lohnes, D.G., Chung, J. and Specht J. E. 1999. An integrated genetic linkage map of the soybean genome. *Crop Sci*. 39: 1464–1490.

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

Dorrance, A.E., McClure, S.A. and St. Martin, S.K. 2003. Effect of partial resistance on Phytophthora stem rot incidence and yield of soybean in Ohio. *Plant Dis.* 87:308-312.

Egli, D.B. and Zhen-wen, Y. 1991. Crop growth rate and seed number per unit area in soybean. *Crop Sci*. 31: 439-442.

Fehr, W.R., Caviness, C.E., Burmood, D.T. and Pennington, J.S. 1971. Stage of development descriptions for soybeans, Glycine Max (L.) Merrill. *Crop Sci.* 11: 929–931.

Foley, T.C., Orf, J.H. and Lambert, J.W. 1986. Performance of related determinate and indeterminate soybean lines. *Crop Sci.* 26: 5-8.

Furuya, Y. and Kato, K. 1963. Effect of drought stress at grain filling stage on seed yield and quality in rice and soybean. Kyushu Agric. Res. Rep. 8: 409-422*.

Furuya, T., Matsumoto, S., Shima, M. and Muraki, K. 1988. Maturation process of top organs in delayed stem maturation soybean plant. *Jpn. J. Crop Sci.* 57: 1-7*.

Furuya, T. and Umezaki, T. 1993. Simplified distinction method of degree of delayed stem maturation of soybean plants. *Jpn. J. Crop Sci.* 62: 126-127*.

Hardman, L.L. and Brun, W.A. 1971. Effects of atmospheric carbon dioxide enrichment at different development stages on growth and yield components of soybeans. *Crop Sci*. 11: 886-888.

Hill, C.B., Hartman, G.L., Esgar, R. and Hobbs, H.A. 2006. Field evaluation of green stem disorder in soybean cultivars. *Crop Sci.* 46: 879-885.

Hisano, H., Sato, S., Isobe, S., Sasamoto, S., Wada, T, Matsuno, A., Fujishiro, T.,Yamada, M., Nakayama, S., Nakamura, Y., Watanabe, S., Harada, K. and Tabata, S. 2007. Characterization of the soybean genome using EST derived microsatellite markers. *DNA Res*. 14: 271–281.

Hobbs, H.A., Hill, C.B., Gran, C.R., Koval, N.C., Wang, Y., Pedersen, W.L., Domier, L.L. and Hartman, G.L. 2006. Green stem disorder of soybean. *Plant Dis*. 90: 513-518.

Howell, R.W., Collins, F.I. and Sedgwick, V.E. 1959. Respiration of soybean seeds as related to weathering losses during ripening. *Agron. J*. 51: 677-679.

Isobe, K., Sekino, T., Nagura, R., Matsuura, R., Inoue, Y., Hashimoto, C., Takashima, T., Nonokawa, K., Maekawa, T. and Ishii, R. 2011. Effects of sowing time on the yield and the occurrence of delayed stem senescence in soybean in south kanto. *Jpn. J. Crop Sci*. 80: 408-419**.

Jiang, H. and Egli, D.B. 1995. Soybean seed number and crop growth rate during flowering. *Agron. J*. 87: 264-267.

Kabelka, E.A., Diers, B.W., Fehr, W.R., LeRoy, A.R., Baianu, I.C., You, T., Neece, D.J. and Nelson, R.L. 2004. Putative alleles for increased yield from soybean plant introductions. *Crop Sci*. 44:784–791.

Kaga, A., Shimizu, T., Watanabe, S., Tsubokura, Y., Katayose, Y., Harada, K., Vaughan, D.A. and Tomooka, N. 2012. Evaluation of soybean germplasm conserved in NIAS genebank and development of mini core collection. *Breeding Sci*. 61: 566-592.

Kilgore-Norquesta, L. and Sneller, C.H. 2000. Effect of stem termination on soybean traits in southern U.S. production systems. *Crop Sci*. 40: 83-90.

Konaka, N. and Takahashi, Y. 1965. Unusual maturation of soybean cultivar developed in Hokkaido prefecture in the warm district of Japan. *Jpn. J. Crop Sci*. 34: 492*.

Kosambi, D.D. 1943. The estimation of map distance from recombination values. *Ann. Eugenics* 12: 172-175.

Leopold, A.C., Niedergang-Kamien, E. and JANICK, J. 1959. Experimental modification of plant senescence. *Plant Physiol*. 34: 570-573.

Lincoln, S.E., Daly, M.J. and Lander, E.S. 1993. Constructing genetic linkage maps with MAPMAKER/EXP. Whitehead Institute for Biomedical Research, Cambridge, MA.

Liu, B., Watanabe, S., Uchiyama, T., Kong, F., Kanazawa, A., Xia, Z., Nagamatsu, A., Arai, M., Yamada, T., Kitamura, K., Masuta, C., Harada, K. and Abe, J. 2010. The soybean stem growth habit gene *Dt1* is an ortholog of arabidopsis *TERMINAL FLOWER1*. *Plant Physiol.*

153: 198-210.

Malvick, D. 2001. Green Stem of Soybean [Online]. Available by University of Illinois Extension http://www.ag.uiuc.edu/cespubs/pest/articles/200123d.html (verified 5 December 2005).

Mansur, L.M., Orf, J.H., Chase, K., Jarvik, T., Cregan, P.B. and Lark, K.G. 1996. Genetic mapping of agronomic traits using recombinant inbred lines of soybean. *Crop Sci*. 36: 1327-1336.

Matsumoto, S., Furuya, T. and Matsunaga, R. 1986. The occurrence of delayed stem maturation in early soybean varieties and a method for visual distinction. *Jpn. J. Crop Sci*. 55: 333-338***.

Mochizuki, A., Shiraiwa, T., Nakagawa, H. and Horie, T. 2005. The effect of temperature during the reproductive period on development of reproductive organs and the occurrence of delayed stem senescence in soybean. *Jpn. J. Crop Sci.* 74: 339-343**.

Molnar, S.J., Rai, S., Charette, M. and Cober, E.R. 2003. Simple sequence repeat (SSR) markers linked to *E1*, *E3*, *E4*, and *E7* maturity genes in soybean. *Genome* 46: 1024-1036.

Morita, K., Takahashi, W., Nabeshima, H., Nomura, M., Arai, S. and Iwai, S. 2006. Effect of green stem on soiled bean index at harvest of soybean by combine harvester. The Hokuriku Crop Sci. 41: 107-109*.

Morrison, M., Voldeng, H. and Cober, E. 2000. Agronomic changes from 58 years of genetic improvement of short-season soybean cultivars in Canada. Agron. J. 92: 780-784.

Nakamura, S. 1993. The present state and the future direction of soybean breeding for mechanical harvest. Tohoku Agric. Res. Extra Issue. 6: 47-56*.

Noodén, L.D. 1980. Senescence in the whole plant. *In* K.V. Thimann ed, Senescence in Plants. CRC Press, Boca Raton, FL. 219-258.

Noodén, L.D. 1984. Integration of soybean pod development and monocarpic senescence. *Physiol. Plant*. 62: 273-284.

Ogiwara, H. 2002. Chapter 3. Cultivation technique. Section 11. Delayed leaf senescence. *In* Agriculture, Forestry and Fisheries Research Council of Japan ed., Soybean-Technical development for improving national food self-sufficiency ratio. Annotated bibliography of

agriculture, forestry and fisheries research. No. 27: 291-294*.

Ojima, T., Takahashi, W., Nomura, M. and Asou, H. 2001. Relationship between number of pods per node, number of pods per stem weight and delayed stem maturation in soybean. *The Hokuriku Crop Sci*. 36: 81-83*.

Ookawa, T., Nishiyama, M., Takahiro, J., Ishihara, K. and Hirasawa, T. 1999. Differences in leaf senescence among reciprocally grafted plants of two soybean cultivars, Enrei and Tachinagaha. *Plant Prod. Sci.* 2: 51-52.

Phillips, D.A., Pierce, R.O., Edie, S.C., Foster, K.W. and Knowles, P.F. 1984. Delayed leaf senescence in soybean. *Crop Sci*. 24: 518-522.

Pierce, R.O., Knowles, P.F. and Phillips, D.A. 1984. Inheritance of delayed leaf senescence in soybean. *Crop Sci*. 24: 515-517.

Reinprecht, Y, Poysa, V.W., Yu, K., Rajcan, I., Ablett, G.R. and Pauls, K.P. 2006. Seed and agronomic QTL in low linolenic acid, lipoxygenase-free soybean (*Glycine max* (L.) Merrill) germplasm. *Genome*. 49: 1510-1527.

Saitoh, K., Isobe, S. and Kuroda, T. 1998. Significance of flower differentiation and development in the process of determining soybean yield: ―Relation between the number of pods and flowers―. *Jpn. J. Crop Sci.* 67: 70-78**.

Saitoh, K., Mahamood, T. and Kuroda, T. 2003. Difference in flower production and pod set performance among soybean cultivars with different stem-termination types and maturity groups. *Jpn. J. Crop Sci*. 72: 290-294**.

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

Schou, J.B., Jeffers, D.L. and Streeter, J.G. 1978. Effects of reflectors, black boards, or shades applied at different stages of plant development on yield of soybeans. *Crop Sci.* 18: 29-34.

Schwenk, F.W. and Nickell, C.D. 1980. Soybean green stem caused by bean pod mottle virus. *Plant Dis*. 64: 863-865.

Shibles, R., Anderson, I.C. and Gibson, A.H. 1975. Soybean. In Crop Physiology (Ed.) L.T.

Evans, Cambridge Univ. Press, Cambridge. 151-189.

Sinclair, T.R. and de Wit, C.T. 1975. Photosynthate and nitrogen requirements for seed production by various crops. *Science*. 189: 565-567.

Song, Q.J., Marek, L.F., Shoemaker, R.C., Lark, K.G., Concibido, V.C., Delannay, X., Specht, J.E. and Cregan P. B. 2004. A new integrated genetic linkage map of the soybean. *Theor. Appl. Genet*. 109: 122–128.

Specht, J.E., Chase, K., Macrander, M., Graef, G.L., Chung, J., Markwell, J.P., Germann, M., Orf, J.H. and Lark, K.G. 2001. Soybean response to water: A QTL analysis of drought tolerance. *Crop Sci*. 41: 493-509.

Takeda, H., Ohdaira, Y. and Takanashi, J. 2002. Degree of delayed leaf senescence among six soybean (*Glycine max* [L.] Merr.) cultivars, and the effect with depodding or without irrigation treatment. *Jpn. J. Crop Sci*. 71 (extra issue 1): 248-249.

Takeda, H., Ohdaira, Y. and Takanashi, J. 2003. Effect of delayed leaf senescence by periodical suppression of soil water after flowering stage in soybean (*Glycine max* [L.] Merr.). *Jpn. J. Crop Sci*. 72 (extra issue 1): 64-65.

Tanaka, Y. and Shiraiwa, T. 2009. Stem growth habit affects leaf morphology and gas exchange traits in soybean. *Ann. Bot.* 104: 1293-1299.

Tasma, I.M., Lorenzen, L.L., Green, D.E. and Shoemaker, R.C. 2001. Mapping genetic loci for flowering time, maturity, and photoperiod insensitivity in soybean. *Mol. Breed*. 8: 25-35.

TeKrony, D.M., Egli, D.B., Balles, J., Tomes, L. and Stuckey, R.E. 1984. Effect of date of harvest maturity on soybean seed quality and *Phomopsis* sp. seed information. *Crop Sci.* 24: 189-193.

Wang, S., Basten, C.J. and Zeng, Z.B. 2007. Windows QTL cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC. http://statgen.ncsu.edu/qtlcart/WQTLCart.htm.

Watanabe, S., Hideshima, R., Xia, Z., Tsubokura, Y., Sato, S., Nakamoto, Y., Yamanaka, N., Takahashi, R., Ishimoto, M., Anai, T., Tabata, S. and Harada, K. 2009. Map-based cloning of the gene associated with the soybean maturity locus E3. *Genetics.* 182: 1251–1262.

Watanabe, S., Xia, Z., Hideshima, R., Tsubokura, Y., Sato, S., Yamanaka, N., Takahashi, R.,

Anai, T., Tabata, S., Kitamura, K. and Harada, K. 2011. A map-based cloning strategy employing a residual heterozygous line reveals that the *GIGANTEA* gene is involved in soybean maturity and flowering. *Genetics* 188: 395–407.

Wilcox, J.R., Laviolette, F.A. and Athow, K.L. 1974. Deterioration of soybean seed quality associated with delayed harvest. *Plant. Dis. Rep*. 58: 130-133.

Wittenbach, V.A. 1982. Effect of pod removal on leaf senescence in soybean. *Plant Physiol.* 70: 1544-1548.

Xia, Z, Tsubokura, Y, Hoshi, M, Hanawa, M, Yano, C, Okamura, K, Ahmed, T.A., Anai, T, Watanabe, S, Hayashi, M, Kawai, T, Hossain, K.G., Masaki, H, Asai, K, Yamanaka, N, Kubo, N, Kadowaki, K, Nagamura, Y, Yano, M, Sasaki, T. and Harada, K. 2007. An integrated high-density linkage map of soybean with RFLP, SSR, STS, and AFLP markers using a single F_2 population. *DNA Res*. 14: 257–269.

Xia, Z., Watanabe, S., Yamada, T., Tsubokura, Y., Nakashima, H., Zhai, H., Anai, T., Sato, S., Yamazaki, T., Lu, S., Wu, H., Tabata, S. and Harada, K. 2012. Positional cloning and characterization reveal the molecular basis for soybean maturity locus E1 that regulates photoperiodic flowering. *Proc. Natl. Acad. Sci. USA.* 109: E2155-E2164.

Yamanaka, N., Nagamura, Y., Tsubokura, Y., Yamamoto, K., Takahashi, R., Kouchi, H., Yano, M., Sasaki, T. and Harada, K. 2000. Quantitative trait locus analysis of flowering time in soybean using a RFLP linkage map. *Breed. Sci*. 50: 109-115.

* In Japanese.

** In Japanese with English abstract.

*** In Japanese with English summary.

Publication list

(Chapter 3)

Fujii, K., Kato, S., Sayama, T., Tanaka, Y., Nakazaki, T., Ishimoto, M. and Shiraiwa, T. 2015. Stability verification of the effects of stem determination and earliness of flowering on green stem disorder of soybean against genetic background and environment. *Plant Prod Sci*. (in press)

[Relevant publications]

Tanaka, Y., Fujii, K. and Shiraiwa, T. 2010. Variability of Leaf Morphology and Stomatal Conductance in Soybean [*Glycine max.* (L.) Merr.] Cultivars. *Crop Science.* 50: 2525-2532.

Kato, S., Sayama, T., Fujii, K., Yumoto, S., Kono, Y., Hwang, TY., Kikuchi, A., Takeda, Y., Tanaka, Y., Shiraiwa, T. and Ishimoto, M. 2014. A major and stable QTL associated with seed weight in soybean across multiple environments and genetic backgrounds. *Theor. Appl. Genet*. 127: 1365–1374.

Kato, S., Fujii, K., Sayama, T., Ishimoto, M., Shiraiwa, T., Kikuchi, A. and Yumoto, S. 2015. Seed yield and its components of indeterminate and determinate lines in recombinant inbred lines of soybean. *Breeding Sci*. (in press)

Acknowledgement

This study was conducted over my pleasant and labored six years at the Laboratory of Crop Science, Graduate School of Agriculture, Kyoto University, and I am delighted with the accomplishment of this thesis. I appreciate unstinting support from a number of people. I would like to demonstrate my gratitude to Prof. Tatsuhiko Shiraiwa for his instruction throughout this study. I would like to express my appreciation to Dr. Tomoyuki Katsube-Tanaka, Dr. Koki Homma and Dr. Yu Tanaka for their advice and assistance to this thesis.

My appreciation also should be taken to Dr. Tetsuya Nakazaki and Dr. Keisuke Katsura (the Experimental Farm, Kyoto University) for their advice and generous assistance during the cultivation at the Experimental Farm of the Graduate School of Agriculture, Kyoto University.

I appreciate the technical support for the genetic analysis in Chapter 3 from Dr. Masao Ishimoto and Dr. Takashi Sayama (National Institute of Agrobiological Sciences), and also express my gratitude to Mr. Shin Kato (Tohoku Agricultural Research Center) for the collaborative research. The analysis was also supported by grants from the Ministry of Agriculture, Forestry, and Fisheries of Japan [Genomics for Agricultural Innovation (SOY2002)].

I would like to express my appreciation to Mr. Ryoichi Wada for the continuous support for the field experiments at the Kyoto Experimental Farm, Kyoto University. I would like to extend my gratitude to Dr. Custod'io R. P. Tacarindua, Dr. Yasuko Fukuda, Dr. Sonia Hossain, Mr. Yuichiro Yokomine, Ms. Shiho Tsujioka, Mr. Yohei Kawasaki, Mr. Rajen Bajgain, Mr. Yusaku Akamatsu, Mr. Keiichiro Nakashima, Mr. Akinori Yoshifuji, Ms. Yuko Nomoto, Mr. Naoto Mukainishi and the other members of the Laboratory of Crop Science, Graduate School of Agriculture, Kyoto University, for the cooperative work at the experimental field and for the delightful time at the laboratory.

Finally, I would like to deriver powerful statement of appreciation for my parents, relatives and all the friends for their support and encouragement.