A study on physiological mechanism of green stem disorder

in soybean (*Glycine max* (L.) Merr.)

- Analysis of inducing factors and evaluation of cultivar differences

with the light availability manipulation method -

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Abstract

Chapter 1 Introduction

Green stem disorder (GSD) in soybean is a symptom with which plants retain green stems and leaves as the pods mature. This causes tremendous difficulty of machine harvesting reducing time and energy efficiency by resistance against threshing and deteriorating appearance quality of seeds due to stem moisture. In spite of severity, the physiological mechanism of GSD occurrence is not understood and thus agronomical measures to avoid GSD has not been established. In order to elucidate the mechanism of GSD, the experimental treatment that promotes GSD needs to be detected, with which physiological processes can be compared between plants with and without GSD occurrence. The objective of this study was to develop new methods to promote GSD occurrences for studies of GSD and find a new insight on the physiological mechanism of GSD by using these methods.

Chapter 2 Effect of thinning and shade removal on green stem disorder in soybean

As new methods to promote GSD occurrences, the effect of thinning and shade removal on green stem disorder was analyzed. A cultivar ‘Sachiyutaka’ was grown at the experimental fields of NARO, Western Region Agricultural Research Center, Hiroshima, Japan, in the conventional crop management practices. I adjusted plant density at the developmental growth stage R1 (the beginning of flowering) or at R5 (the beginning of seed filling), from dense (22.2 plants m^{-2}) to sparse (5.56 plants m^{-2}) by thinning. We found that GSD occurrence was increased when plant density was changed, compared to the treatments that were maintained under either dense or sparse conditions. GSD was promoted more strongly when thinning was conducted at R5 than at R1 stage. And then a shading technique was employed at the field experiment to manipulate light-availability for the plants. Shading equipment surrounding plants, except for their upper-most leaves, was implemented to determine the association of shading and GSD. The results of the shade experiment revealed that GSD occurrence generally increased in treatments subjected to shade removal, compared to those that were shaded until R8 stage (full maturity) or never shaded since the time of sowing. GSD was strongly promoted by shade removal at R5 than at R1 stage. The shading results coincide with the results of the plant density experiment, indicating that an increase in light availability enhances source activity relative to sink at R5 stage, thereby promoting GSD occurrence in soybean.

Chapter 3 Effect of improved light environment on green stem disorder in soybean corresponded to enhanced source ability

The hypothesis that enhanced source ability by thinning or shade removal promoted GSD occurrences, which was indicated in chapter 2, was verified in order to find new insights on GSD mechanism. ‘Sachiyutaka’ soybean was grown in the field with the

treatments of varied duration and timing of shade removal suggested that GSD severity became higher as duration of shade removal at R5 became longer and late timing of shade removal (28 days after R5) had no significant effect on neither GSD severity nor growth parameters related to source ability. The transcriptome analysis was conducted on the harvested plants. The result of transcriptome analysis of main stems at 14 days or 28 days after R5 using RNA-seq indicated that the thinned plants showed overrepresented upregulation of photosynthetic genes and upregulation of genes of vegetative storage proteins. The dry matter dynamics also was measured. The thinned plants showed increase in weight of vegetative parts. The increased vegetative biomass included dry weight of leaves per unit leaf area, which is considered associating increased physiological capacity of the leaves. These results suggested that GSD promoted by thinning and shade removal corresponded to excess source ability caused by increase in photosynthesis.

Chapter 4 Effect of thinning on cultivar differences of green stem disorder in soybean

As well as elucidating the physiological mechanism of GSD, the thinning treatment was applied to the breeding GSD insensitive cultivars. GSD insensitive cultivars is expected to be an effective countermeasure to GSD. However, it is difficult to stably detect cultivar differences in GSD under conventional field conditions, because the occurrences of GSD largely vary by location and year. The thinning treatment may help accurate phenotyping for occurrences of GSD in breeding. To verify this possibility, the thinning treatment was applied to the four cultivars, for which the GSD severity values had been evaluated in an independent study. As a result, the cultivar differences in GSD severity were generally

comparable between the present and previous studies. However, the difference was more evident when the thinning treatment was conducted, exhibiting the GSD score of 2.8 of 'Hatsusayaka' compared with the GSD score of 3.6 of 'Sachiyutaka'. On the other hand, the scores of those cultivars were similar without the thinning treatment. A positive correlation between GSD severity and N concentration in the main stem could be seen but the increment of GSD score with unit increase of N concentration in the main stem differed between cultivars. Thus, although more cultivars need to be tested to prove, the thinning treatment could be useful as a phenotyping technique in the breeding of GSD-insensitive cultivars.

Chapter 5 General discussion

In these studies, light-availability manipulation methods were indicated to be the effective methods to easily and stably generate GSD for study of GSD in both physiological mechanism and breeding. And by using these methods, it was suggested that the enhanced source without reducing sink size could promote GSD occurrences. These results supported the hypothesis on the GSD physiological mechanism that sink-source balance affects GSD occurrences.