Varietal Difference in the Occurrence of Delayed Stem Senescence and Cytokinin Level in the Xylem Exudate in Soybeans

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Abstract: The objectives of this study were to evaluate the varietal difference in the occurrence of delayed stem senescence (DSS) and cytokinin level in the xylem exudate in soybeans and the relationship between the occurrence of DSS and cytokinin level in the xylem exudate. Pot experiments were carried out in the experiment field at Nihon University in 2010, 2012 and 2013. In this experiment, we used 11 soybean varieties, which were cultivated in the Kanto region, Japan. The degree of DSS (DSS score) was positively correlated with the days from sowing to flowering stage (S-R2), and was higher in the varieties with a longer length of S-R2, though the correlation was not significant. Under some conditions, the DSS score was negatively correlated with S-R2. Moreover, the DSS score was positively or negatively correlated with the main stem diameter, total node number, stem dry weight and seed weight depending on the growth parameter or sowing date. Thus, we concluded that the DSS score was not correlated with the growth parameters. On the other hand, the level of cytokinins such as t-ZR and iPA shown by their amount in xylem exudate from a plant at the seed filling stage was negatively correlated with the DSS score. Thus, we consider that one of the reasons for varietal difference in DSS occurrence may be the difference in cytokinin content of stem and leaves after the seed filling stage.

Key words: Cytokinin, Delayed stem senescence, Soybean (Glycine max (L.) Merr.), Varietal difference.

Delayed stem senescence (DSS) in soybean (Glycine max (L.) Merr.) is a phenomenon of retarded leaf and stem maturity. When DSS occurred in soybean plants, much chlorophyll and water remained in the stem at the maturity stage. In Japan, DSS is one of the most important problems for combine harvesting of soybean. Previous studies indicated the varietal difference in the occurrence of DSS (Matsumoto et al., 1986; Mochizuki et al., 2005; Isobe et al., 2011, 2014). For example, in Enrei and Tachinagaha, DSS occurred frequently in Kanto region, Japan (Hajika, 2005; Isobe et al., 2011, 2014). DSS was marked in the varieties with a shorter growing duration from sowing to flowering time (Isobe et al., 2014). Moreover, DSS did not occur in the later maturing varieties or varieties with smaller seed (Hajika, 2005). However, Inoue (2004) reported that DSS was marked in the later maturing varieties. These facts indicated that the occurrence of DSS is related to the length of the growing period in soybean.

On the other hand, the cytokinins are known as senescence-inhibiting plant hormones. In soybean, the flux of cytokinins in xylem drastically decreased from the flowering stage to pod-setting stage (Heindl et al., 1982; Nooden et al., 1990). Isobe et al. reported (2011) that the flux of cytokinins in xylem decreased from the flowering stage (R2) to seed filling stage (R5) in soybean with severe DSS, but it did not decrease in soybean with normal maturity. Sato et al. (2007) reported that the concentration of a kind of cytokinin, trans-zeatin riboside (t-ZR) in xylem exudate tended to be higher in soybeans with severer DSS than in those with normal maturity. Thus, cytokinins in xylem exudate after the pod-setting stage may be related to the occurrence of DSS. However, the varietal difference in the occurrence of DSS and cytokinin level in the xylem exudate is not clear in soybeans.

The objectives of this study were to evaluate the varietal difference in the occurrence of DSS and cytokinin level in the xylem exudate in soybean, and the correlation of the occurrence of DSS with the growth parameters and cytokinin level in the xylem exudate.

Material and Method

1. Plots and cultivation system

Pot experiments were carried out in the experimental field at Nihon University (Fujisawa-city, Kanagawa, Japan) in 2010, 2012 and 2013 to clarify the varietal difference in the degree of delayed stem senescence (DSS score) and cytokinin level in the xylem exudate. In this experiment, the soybean variety used were Enrei (Ecological type...
(Fukui 1965) (IIc), Tachinagaha (IIc), Tamadaikoku (IIc), Suzuroman (IIIc), Tsukuzairai, Otsuru (IIc), Nakasennari (IIc), Natto-syoryu (IIc), Tamahomare (IIIc) and Fukuyutaka (IVc) in 2010, and Enrei, Tachinagaha, Suzuroman, Tsukuzairai, Ginrei (IIc), Otsuru, Nakasennari, Natto-syoryu, Tamahomare and Fukuyutaka in 2012 and 2013. Four seeds per pot were sown on 14 May 2010, 8 or 24 (Otsuru) May 2012 and 7 May 2013 (May sowing plot) or 21 June 2010, 27 June 2012 and 29 June 2013 (June sowing plot) in 1/5000a pots containing 3 kg of field soil (Andosol), 2.4 g of ammonium sulfate, 5.6 g of superphosphate and 1.4 g of potassium chloride. The seedlings were thinned to 1 plant per pot at the expansion stage of the primary leaf. Fifteen pots (twenty pots in 2013) of all varieties were prepared in both plots. All pots were placed randomly in outside of experiment field from sowing to maturity, and weeds, diseases and insects were controlled as necessary during the cultivation period.

2. Measurement of growth and seed weight at maturity

Dates of the flowering stage (R2) and maturity stage (R8) were recorded. The main stem diameter, total node number, stem dry weight and seed weight of ten plants were measured at R8 in this experiment. The plants were cut at the cotyledonary node, and the main stem diameter was measured at the intermediate point between cotyledon node and primary leafnode with a vernier. Total node number and branch node number were counted on all main stems. Soybean seeds with a diameter exceeding 5 mm (3 mm in; Natto-syoryu and Suzuroman) were used for the seed weight survey. Stem dry weight was determined after oven-drying at 80°C for 48 h.

3. Sampling xylem exudate and analyzing cytokinin flux

Xylem exudate from five plants was sampled at the beginning of the seed filling stage (R5) in 2013. Each plant was cut at primary leaf node at 9 a.m. The xylem exudate from the decapitated stem was collected with silicone tube for 1 hr. Then, for labeled standard, 50 μL of 20 nM t-ZR or iP (isopentenyl adenosine) stable isotope solution was added to 1 mL of exudate. The mixed solution was loaded onto an MCX column (Oasis MCX6cc LP Extraction Cartridges) and eluted successively with 5 mL of 0.35 M acetic acid solution and methanol / 0.5% (v/v) acetic acid solution were used as a solvent. The molar amount of cytokinin in the xylem exudate corrected for 1 hr per plant at the R5 stage was considered to represent the cytokinin flux.

4. Assessment of delayed stem senescence (DSS) score

At the maturity stage (R8), 10 plants of each variety were sampled and used to assess the DSS score by the method proposed by Furuya and Umezaki (1993). The five DSS scores are shown by DSS1~DSS 5. DSS1 shows that the stem is green and yellow-green leaves or green leaves remained at more than one-third of the nodes of the plant; and DSS5, the stem is brown and dry, and all leaves have abscised (Sato et al., 2007).

5. Statistical analysis

All values were expressed as mean values. The data were analyzed statistically and significant differences between the May sowing plot and June sowing plot for each variety were determined by t-test and, between varieties were determined by Tukey’s multiple means test. The correlation coefficient between growth parameters or cytokinin flux and degree of delayed stem senescence (DSS score) were also calculated. Correlation analysis was done using Kaleida Graph ver.4.0 software.

Results

1. Date of flowering and maturity stage

Table 1 shows the days from sowing (S) to flowering stage (R2), from R2 to maturity stage (R8), and from S to R8 in May and June sowing plots. The days from S to R2, from R2 to R8 and from S to R8 in June plots were shorter than those in May plots in every year.

2. Growth and seed weight at maturity stage (R8)

The main stem diameter, total node number, stem dry weight and seed weight at maturity (R8) in 2010, 2012 and 2013, are shown in Tables 2, 3 and 4, respectively. The values in many varieties were significantly higher in the May sowing plot than in the June sowing plot. In 2010 (Table 4), the main stem diameter was largest in Nakasennari in both May and June sowing plots. The total node number was largest in Natto-syoryu in May sowing plot, and in Fukuyutaka in the June sowing plot. The stem dry weight was heaviest in Tamahomare in both sowing plots. The seed weight was heaviest in Natto-syoryu in May sowing plot, but there was no significant difference among the varieties in the June sowing plot. In 2012, the main stem diameter was largest in Ginrei in both plots, and the total node number was largest in Natto-syoryu and Fukuyutaka in June sowing plots. The stem dry weight was heaviest in Tamahomare in both sowing plots, and the seed weight was heaviest in Tsukuzairai in both sowing plots. In 2013, the main stem...
Table 1. Days from sowing (S) to flowering (R2) and maturity stage (R8).

| Plots | Variety          | 2010     | 2012     | 2013     |
|-------|------------------|----------|----------|----------|
|       | S to R2 | R2 to R8 | S to R8 | S to R2 | R2 to R8 | S to R8 |
| May sowing |
| Enrei  | 54      | 81      | 135     | 57      | 93      | 150     |
| Tchinagaha | 54     | 92      | 146     | 59      | 95      | 154     |
| Tamadaiikoku | 55   | 78      | 133     | –       | –       | –       |
| Suzuraman | 62     | 85      | 147     | 68      | 83      | 154     |
| Tsukaizairai | 63    | 92      | 155     | 70      | 100     | 170     |
| Ginrei  | –       | –       | –       | 62      | 92      | 154     |
| Otsuru  | 59      | 96      | 155     | 52      | 86      | 138     |
| Nakasennari | 59   | 98      | 157     | 63      | 91      | 154     |
| Natto-syoryu | 63  | 84      | 147     | 69      | 82      | 151     |
| Tamahomare | 63    | 95      | 158     | 69      | 92      | 161     |
| Fukuyutaka | 73    | 92      | 165     | 74      | 94      | 168     |
| June sowing |
| Enrei  | 42      | 63      | 105     | 39      | 58      | 97      |
| Tchinagaha | 39     | 70      | 109     | 37      | 72      | 109     |
| Tamadaiikoku | 42   | 76      | 118     | –       | –       | –       |
| Suzuraman | 45     | 74      | 119     | 45      | 62      | 107     |
| Tsukaizairai | 49   | 80      | 129     | 45      | 70      | 115     |
| Ginrei  | –       | –       | –       | 40      | 71      | 111     |
| Otsuru  | 45      | 74      | 119     | 44      | 63      | 107     |
| Nakasennari | 45   | 70      | 119     | 43      | 61      | 104     |
| Natto-syoryu | 49  | 73      | 122     | 45      | 68      | 113     |
| Tamahomare | 49    | 73      | 122     | 45      | 68      | 117     |
| Fukuyutaka | 57    | 83      | 140     | 49      | 68      | 117     |

–: not used in the experiment.

Table 2. Main stem diameter, total node number, stem dry weight and seed weight at maturity stage in 2010.

| Plots | Variety | Main stem diameter (mm) | Total node number (pl.) | Stem dry weight (g pl.) | Seed weight (g pl.) |
|-------|---------|-------------------------|-------------------------|-------------------------|---------------------|
| May sowing |
| Enrei  | 9.9 cde | 48.5 c                  | 9.1 c                   | 11.4 ab                 |
| Tchinagaha | 11.0 abc | 43.0 e                | 9.4 c                   | 10.7 b                  |
| Tamadaiikoku | 8.6 f | 52.1 e               | 8.6 c                   | 11.7 ab                 |
| Suzuraman | 9.3 def | 76.5 d                 | 8.9 c                   | 13.0 ab                 |
| Tsukaizairai | 9.1 cf | 90.9 bc                | 9.4 c                   | 12.9 ab                 |
| Otsuru  | 10.5 abcd | 53.7 c                | 10.1 bc                 | 10.7 b                  |
| Nakasennari | 11.7 a | 75.2 d                 | 12.4 b                  | 13.0 ab                 |
| Natto-syoryu | 10.1 bcde | 105.8 a               | 10.6 bc                 | 14.1 a                  |
| Tamahomare | 10.2 bcde | 87.7 cd                | 14.9 a                  | 12.9 ab                 |
| Fukuyutaka | 11.3 ab | 101.9 ab               | 12.6 ab                 | 10.3 b                  |
| June sowing |
| Enrei  | 7.5 BCD ** | 37.0 EF *** | 4.6 DE *** | 7.9 A *** |
| Tchinagaha | 7.9 ABC *** | 28.1 F **** | 3.8 E *** | 8.6 A * |
| Tamadaiikoku | 7.4 BCD ns | 36.4 EF *** | 5.8 BCD ** | 7.9 A ** |
| Suzuraman | 6.7 D ** | 43.3 DE *** | 4.2 E ** | 8.6 A ** |
| Tsukaizairai | 6.9 CD * | 51.4 CD *** | 4.5 E *** | 8.6 A *** |
| Otsuru  | 8.3 AB * | 48.0 CD * | 5.3 CDE *** | 8.5 A * |
| Nakasennari | 8.8 A ** | 55.9 BC *** | 6.2 BC ** | 9.8 A * |
| Natto-syoryu | 7.0 CD ** | 61.1 AB *** | 4.4 E *** | 11.2 A ** |
| Tamahomare | 8.2 AB ** | 54.4 BC *** | 7.6 A *** | 11.0 A ns |
| Fukuyutaka | 8.9 A ** | 66.0 A *** | 6.7 AB *** | 8.8 A ns |

Values followed by different letters within each sowing plot are significantly different at P = 0.05 by the Tukey test. ns, *, **, ***: no significant difference, 5%, 1% and 0.1% level of significant difference between May sowing and June sowing plots by t-test, respectively.
Table 3. Main stem diameter, total node number, stem dry weight and seed weight at maturity stage in 2012.

| Plots         | Variety    | Main stem diameter (mm) | Total node number (pl.⁻¹) | Stem dry weight (g pl.⁻¹) | Seed weight (g pl.⁻¹) |
|---------------|------------|-------------------------|---------------------------|--------------------------|-----------------------|
| May sowing    | Enrei      | 11.9 f                  | 51.8 d                    | 18.7 bc                  | 23.0 cd               |
|               | Tachinagaha| 12.4 cdef               | 49.8 d                    | 16.3 c                   | 23.1 cd               |
|               | Suzuroman  | 12.4 def                | 120.6 a                   | 22.0 b                   | 29.3 b                |
|               | Tsukuiizairai | 11.8 f              | 100.9 b                   | 20.8 bc                  | 35.1 a                |
|               | Ginrei     | 14.6 a                  | 73.2 c                    | 24.4 ab                  | 21.2 d                |
|               | Otsuru     | 13.2 bcd                | 70.1 c                    | 21.6 b                   | 25.7 bcd              |
|               | Nakasennari | 13.4 abcd              | 62.8 cd                   | 20.4 bc                  | 27.5 bc               |
|               | Natto-syoryu | 12.0 ef                | 134.5 a                   | 21.7 b                   | 29.9 ab               |
|               | Tamahomare  | 13.4 abc                | 78.6 c                    | 28.6 a                   | 31.4 ab               |
|               | Fukuyutaka | 14.0 ab                 | 127.1 a                   | 28.0 a                   | 27.4 bc               |
| June sowing   | Enrei      | 8.6 D ***               | 40.6 E ***                | 6.2 D ***                | 22.5 D ns             |
|               | Tachinagaha| 9.2 CD ***              | 35.8 F ***                | 7.3 D ***                | 25.0 CD ns            |
|               | Suzuroman  | 9.5 BCD ***             | 59.5 ABC ***              | 8.2 CD ***               | 28.4 BC ns            |
|               | Tsukuiizairai | 8.9 D ***             | 64.1 AB ***               | 7.9 D ***                | 36.8 A ns             |
|               | Ginrei     | 11.9 A ***              | 50.8 CD ***               | 11.0 AB ***              | 24.8 CD **            |
|               | Otsuru     | 10.1 BCD ***            | 48.5 DE ***               | 6.6 D ***                | 28.1 BC ns            |
|               | Nakasennari | 10.6 ABC ***           | 58.6 ABCD ns              | 10.2 BC ***              | 30.0 B *              |
|               | Natto-syoryu | 8.6 D ***              | 55.7 BCD ***              | 6.5 D ***                | 26.2 BCD *            |
|               | Tamahomare  | 10.4 ABC ***            | 63.9 AB ***               | 12.7 A *                 | 35.8 A *              |
|               | Fukuyutaka | 10.9 AB ***             | 65.6 A ***                | 10.1 BC ***              | 27.7 BC ns            |

Values followed by different letters within each sowing plot are significantly different at P = 0.05 by the Tukey test.

ns, *, **, ***; no significant difference, 5%, 1% and 0.1% level of significant difference between May sowing and June sowing plots by t-test, respectively.

Table 4. Main stem diameter, total node number, stem dry weight and seed weight at maturity stage in 2013.

| Plots         | Variety    | Main stem diameter (mm) | Total node number (pl.⁻¹) | Stem dry weight (g pl.⁻¹) | Seed weight (g pl.⁻¹) |
|---------------|------------|-------------------------|---------------------------|--------------------------|-----------------------|
| May sowing    | Enrei      | 14.0 c                  | 57.7 fg                   | 18.9 d                   | 15.1 cde              |
|               | Tachinagaha| 13.4 c                  | 47.1 g                    | 17.1 d                   | 9.2 c                 |
|               | Suzuroman  | 14.2 bc                 | 113.2 abc                 | 23.8 cd                  | 28.5 a                |
|               | Tsukuiizairai | 12.9 c               | 101.6 bcd                 | 24.7 abcd                | 12.4 de               |
|               | Ginrei     | 15.5 ab                 | 69.5 ef                   | 22.3 cd                  | 12.5 de               |
|               | Otsuru     | 16.1 a                  | 60.6 fg                   | 21.7 cd                  | 15.5 cde              |
|               | Nakasennari | 15.5 ab                 | 85.2 de                   | 29.1 ab                  | 11.8 de               |
|               | Natto-syoryu | 13.5 c                | 135.8 a                   | 21.5 d                   | 27.9 ab               |
|               | Tamahomare  | 13.8 c                  | 87.8 cde                  | 28.5 abc                 | 20.7 bc               |
|               | Fukuyutaka | 14.8 ab                 | 125.1 ab                  | 31.6 a                   | 18.5 cd               |
| June sowing   | Enrei      | 8.0 ABC ***             | 45.4 D *                  | 8.8 A ***                | 7.0 B ***             |
|               | Tachinagaha| 8.4 AB ***              | 32.4 E ***                | 6.7 ABC ***              | 6.3 B s               |
|               | Suzuroman  | 7.4 C ***               | 59.3 AB ***               | 4.9 CD ***               | 12.4 A ***            |
|               | Tsukuiizairai | 7.8 ABC ***         | 53.3 BC ***               | 7.4 AB ***               | 13.0 A ns             |
|               | Ginrei     | 8.5 A ***               | 49.7 CD ***               | 7.6 AB ***               | 12.4 A ns             |
|               | Otsuru     | 8.0 ABC ***             | 47.5 D ***                | 7.5 AB ***               | 8.6 B ***             |
|               | Nakasennari | 8.1 ABC ***            | 52.6 BCD ***              | 8.1 AB ***               | 12.5 A ns             |
|               | Natto-syoryu | 7.7 BC ***            | 56.4 ABC ***              | 6.2 BC ***               | 14.8 A ***            |
|               | Tamahomare  | 8.4 AB ***              | 59.5 AB ***               | 8.1 AB ***               | 13.3 A ***            |
|               | Fukuyutaka | 8.4 AB ***              | 63.7 A ***                | 7.9 AB ***               | 15.4 A ns             |

Values followed by different letters within each sowing plot are significantly different at P = 0.05 by the Tukey test.

ns, *, ***, ***; no significant difference, 5%, 1% and 0.1% level of significant difference between May sowing and June sowing plots by t-test, respectively.
3. DSS score at maturity stage

In 2010, 2012 and 2013, there was significant varietal difference at the 5% level in the DSS score at maturity stage (Fig. 1, 2 and 3). In 2010 and 2013, Tamahomare showed the highest DSS score in both sowing plots. In 2012, however, Fukuyutaka showed the highest DSS scores in both sowing plots. The DSS score in Tachinagahara was significantly lower than that in other varieties in all years. The DSS scores of many varieties were lower in the May sowing plot than in the June plot. However, the difference between the two sowing plots significantly varied with the variety (Fig. 1 – 4).

4. Cytokinin flux in xylem at the beginning of seed filling stage (R5) in 2013

There was a significant varietal difference at the 5% level in the t-ZR flux shown by molar amount of cytokinin in the xylem exudate corrected for 1 h from a plant at the R5 stage (Fig. 5). In May sowing plot, the t-ZR flux was highest in Enrei and Tamahomare and lowest in Fukuyutaka. In June sowing plot, the t-ZR flux was highest in Enrei, and
lowest in Tamahomare and Fukuyutaka. In all cultivars expect for Ginrei and Nakasennari, there were no significant differences in the \( t \)-ZR flux between the May and June sowing plots. In Ginrei and Nakasennari, the \( t \)-ZR flux was lower in the June sowing plot than in the May sowing plot. The iPA flux also varied significantly with the variety (Fig. 6). In the May sowing plot, it was highest in Enrei and Tamahomare and lowest in Ginrei and Fukuyutaka. In the June sowing plot, it was highest in Enrei and lowest in Fukuyutaka. The differences between the May and June sowing plots were not significant (Fig. 6).
5. Relationship between growth parameter and DSS score

DDS score was positively correlated with the lengths from sowing to flowering stage (S-R2), flowering to maturity stage (R2-R8) and from sowing to maturity (S-R8) in both May and June sowing plots in all years except for R2-R8 in June sowing plot in 2012 (Table 5). However, in the sum of the values for the plants in the two sowing plots, the correlation was negative except for S-R8 in 2013 (Table 5). The coefficients of the correlation of the DSS scores with main stem diameter, total node number, stem dry weight and seed weight are shown in Table 6. The coefficients of correlation were positive or negative depending on the growth parameter or sowing plot (Table 6).

6. Relationship between cytokinin flux and delayed stem senescence score

The coefficients of the correlation of DSS score with the t-ZR, iPA and t-ZR + iPA flux shown in Figs 5 and 6 are shown in Table 7. The DSS score was negatively correlated with the flux of the cytokinins in all sowing plots (Fig. 7 and 8).

**Discussion**

The degree of DSS occurrence (DSS score) varied significantly with the variety (Matsumoto et al., 1986). There have been several reports showing that DSS is associated with the growth length, main stem diameter and stem growth type (Inoue, 2004; Isobe et al., 2014; Shimada et al., 2007). Many previous researchers reported that the

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**Table 5.** Correlation coefficient between growth length and degree of DSS score each year.

| Year | Growth length | May sowing plot (n = 10) | June sowing plot (n = 10) | Both plots (n = 20) |
|------|---------------|--------------------------|--------------------------|---------------------|
| 2010 | Sowing — Flowering (R2) | 0.701* | 0.340 | -0.135 |
|      | Flowering (R2) — Maturity (R8) | 0.535 | 0.392 | -0.129 |
|      | Sowing — Maturity (R8) | 0.751* | 0.422 | -0.140 |
| 2012 | Sowing — Flowering (R2) | 0.908*** | 0.822*** | -0.313 |
|      | Flowering (R2) — Maturity (R8) | 0.076 | -0.117 | -0.616** |
|      | Sowing — Maturity (R8) | 0.716* | 0.407 | -0.493 |
| 2013 | Sowing — Flowering (R2) | 0.322 | 0.602 | -0.139 |
|      | Flowering (R2) — Maturity (R8) | 0.125 | 0.218 | -0.086 |
|      | Sowing — Maturity (R8) | 0.504 | 0.326 | 0.127 |

* and **: 5% and 1% level of significant difference, respectively.

**Table 6.** Correlation coefficient between growth parameter at maturity and DSS score each year.

| Year | Growth length | May sowing plot (n = 10) | June sowing plot (n = 10) | Both plots (n = 20) |
|------|---------------|--------------------------|--------------------------|---------------------|
| 2010 | Main stem diameter | 0.131 | 0.190 | -0.361 |
|      | Total node number | 0.560 | 0.521 | 0.044 |
|      | Stem dry weight | 0.554 | 0.519 | 0.199 |
|      | Seed weight | 0.075 | -0.155 | -0.458 |
| 2012 | Main stem diameter | -0.036 | -0.243 | -0.504* |
|      | Total node number | 0.719* | 0.784** | 0.023 |
|      | Stem dry weight | 0.691* | 0.457 | 0.423 |
|      | Seed weight | 0.773* | 0.538 | 0.579** |
| 2013 | Main stem diameter | 0.090 | 0.000 | -0.272 |
|      | Total node number | -0.024 | 0.625 | -0.131 |
|      | Stem dry weight | 0.413 | -0.104 | -0.169 |
|      | Seed weight | -0.225 | 0.719* | -0.133 |

* and **: 5% and 1% level of significant difference, respectively.

**Table 7.** Correlation coefficient between cytokinin flux (amount of cytokinin in xylem exudate) and degree of DSS score in 2013.

| Cytokinin | May sowing plot (n = 10) | June sowing plot (n = 10) | Both plots (n = 20) |
|-----------|--------------------------|--------------------------|---------------------|
| t-ZR      | -0.699* | -0.744* | -0.647** |
| iPA       | -0.686* | -0.622 | -0.683*** |
| t-ZR + iPA | -0.699* | -0.744* | -0.649** |

* and **: 5%, 1% and 0.1% level of significant difference, respectively.
DSS score was related to the growth length in soybean. For example, DSS often occurred in the varieties with longer length from seed filling to maturity stage (Inoue, 2004). In contrast, Isobe et al. (2014) reported that DSS often occurred in the varieties with shorter length from sowing to flowering stage. Thus, the results on the correlation of the DSS score with growth length were not consistent. In this study, we examined the correlation of the DSS score with several growth parameters for three years. The DSS score was positively correlated with the length from sowing to flowering stage (S-R2) (Table 1 and 5). However, the correlation was not significant in many cases as shown in Table 7. Moreover, the DSS score was negatively correlated with the length of S-R2 in the plants in both plots. Hence, we concluded that the differences in the DSS score among varieties cannot be explained by the differences in soybean growth length.

Inoue (2004) reported that DSS often occurred in the varieties with a thicker main stem, and Mochizuki et al. (2005) reported that the dry weight of aboveground part and seed yield were greater in the plants with a high DSS score than in the plants with a normal score. However, this is not consistent with our results. In this study, the DSS score was positively correlated with the main stem diameter in 2010, but negatively in 2012 (Table 2, 3, 4 and 6). Moreover, in this study, the DSS score was positively or negatively correlated with the total node number, stem dry weight and seed weight depending on the growth conditions. Thus, we concluded that the DSS occurrence was not related to the growth parameters such as total node number, stem dry weight and seed weight at maturity.

Many researchers reported that the occurrence of DSS in soybean significantly varied with the variety (Matsumoto et al., 1986; Mochizuki et al., 2005; Isobe et al., 2011, 2014). Sato et al. (2007) reported that the \( t \)-ZR flux during the early seed filling stage tended to be higher in the plants with DSS than in the normally matured plants. Cytokinins are produced in the root tip, and are known as senescence-inhibiting plant hormones (Sitton et al., 1967). Cytokinins produced in the root are transported carried through the xylem into the leaves and stem (Nooden and Letham, 1993). Thus, we considered that the cytokinin flux to stem and leaves after seed filling stage delayed the senescence of stem and leaves, and increased the DSS occurrence. In this study, \( t \)-ZR and iPA flux at the seed filling stage significantly varied with the variety (Fig. 5 and 6). The \( t \)-ZR and iPA flux was higher in Emrei, Tachinagaha and Otsuru than in the other varieties, and definitely lower in Ginrei, Tamahomare and Fukuyutaka. These results are consistent with those reported by Isobe et al. (2011). In the present study, a negative correlation was observed between the DSS score and the \( t \)-ZR or iPA flux at the R5 stage (Fig. 7 and 8). Moreover, except for iPA flux in June sowing plot, these correlations were statistically significant (Table 7, Fig. 7 and 8). Thus, one of the reasons for the difference in DSS occurrence among varieties may be the difference in the amount of cytokinin supplied to stem and leaves after seed filling stage. The growth condition can directly affect the occurrence of DSS (Takeda et al., 2002; Inoue, 2003; Sato et al., 2007). For example, the number of pods was decreased under drought or excess-wet soil conditions associated with an increased occurrence of DSS (Takeda et al., 2002; Sato et al., 2007). Moreover, Sato et al. (2007) reported that the \( t \)-ZR flux at the R5 stage tended to be higher in the soybean plants with DSS than in the normally matured plant. This study suggested that we should
examine the correlation between the amount of cytokinin in xylem exudate and DSS score directly under various culture conditions.

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* In Japanese.
** In Japanese with English abstract.
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