Effect of Waterlogging during Vegetative Stage on Growth and Yield in Supernodulating Soybean Cultivar Sakukei 4

Toshinori Matsunami, Gun-Ho Jung, Yukihiko Oki and Makie Kokubun

(Graduate School of Agricultural Science, Tohoku University, Aoba-ku, Sendai 981-8555, Japan)

Abstract: The supernodulating soybean cultivar Sakukei 4 was previously characterized by its superior capabilities of nitrogen (N) fixation and photosynthesis, and was expected to be high yielding. Since the N absorption by Sakukei 4 is largely dependent on N fixation, it may be strongly affected by waterlogging during the vegetative stage, which occurs frequently in major soybean producing areas. In this study, we investigated the reduced growth and yield resulting from waterlogging during vegetative stage and the subsequent recovery in Sakukei 4, in comparison with those in the normally-nodulating cultivar Enrei and non-nodulating genotype En1282. Under the field conditions, the reduction of growth and yield by waterlogging was greatest in En1282 among the three genotypes, indicating that capability of N fixation is essential for the recovery from waterlogging-induced injury. The waterlogging-induced yield reduction in Sakukei 4 resembled that in Enrei, although growth reduction was greater in Sakukei 4 than in Enrei. Irrespective of cultivar, the yield was associated more closely with the crop growth rate (CGR) during the post-treatment stage than with that during the waterlogging treatment. In the pot experiments, yields positively correlated with both above- and underground plant parts irrespective of cultivar, not significantly, but significantly with root dry weights in Enrei and with nodule dry weights in Sakukei 4. These results indicate that Sakukei 4 exhibits a marked decrease in dry matter production by waterlogging, but yield decrease is compensated to a level similar to that of Enrei because of its enhanced nodule growth during the recovery stage.

Keywords: Growth rate, Growth recovery, Nodule, Soybean, Supernodulation, Waterlogging.

Soybean characteristically requires more N for growth than other major crops (Sinclair and de Wit, 1975). Soybean seeds accumulate N in large quantities during the seed filling stage. Therefore, a large amount of N accumulated in the vegetative tissues is translocated to seeds; thereby, a rapid decline of leaf N contents occurs, accompanying the decline of photosynthetic capability (Sinclair and de Wit, 1975). This self-destructive N translocation is one factor defining the seed yield of soybean. In addition, lack of redistributed N from vegetative plant parts might limit yield by restricting the seed-filling duration (Sinclair and de Wit, 1975; Boote, 1981; Egli et al., 1984).

One effective option for enhancing the N supply to plants is increasing application of N fertilizers, either as basal application or as top-dressing during growth (Yoshida, 1979; Watanabe et al., 1983). However, a high input of N fertilizer can engender environmental problems and soybean productivity should be improved increasing the capacity of biological N fixation. Genetic improvement of symbiotic N fixation is one option for enhancement of the capability of N absorption. Several supernodulating soybean lines that form many more nodules than normally-nodulating cultivars have been isolated through attempts to induce mutations in nodulation capabilities (Carroll et al., 1985a, b; Day et al., 1986; Gremaud and Harper, 1989; Akao and Kouchi, 1992). Most supernodulating lines bred so far, however, showed inferior growth and yield performance compared to their related normally-nodulating parental cultivars. The supernodulating lines failed to produce practical benefits (Herridge and Rose, 2000; Sinclair, 2004).

Recently, a supernodulating cultivar "Sakukei 4" was released. This cultivar was derived from a high-yielding Enrei cultivar (Takahashi et al., 2003a). This genotype’s yield and agronomic performance were equal or superior to those of its parental cultivar under certain conditions (Takahashi et al., 2003b). Sakukei 4 is the first soybean cultivar ever bred that has the potential to be grown practically by farmers. Our previous study characterized Sakukei 4 as having a superior ability to maintain high leaf N and photosynthesis, irrespective of the rate and type of N fertilizer applied (Maekawa et al., 2003). This high photosynthetic capability of Sakukei 4 depends on high Rubisco and chlorophyll content of leaves (Maekawa and Kokubun, 2005). However, the growth performance of Sakukei 4 was inferior to that of its parental normally-nodulating Enrei cultivar (Maekawa...
Materials and Methods

1. Plant materials

The supernodulating cultivar Sakukei 4, its parental cultivar Enrei and the related non-nodulating line En1282 were used in the present experiments. Sakukei 4 is an improved genotype which was selected from the progeny of Enrei × En6500 (Takahashi et al., 2003b); En6500 is an EMS (ethyl-methyl-sulfonate)-induced mutant of Enrei (Akao and Kouchi, 1992). A recent genetic analysis indicated that this progeny is likely to be crossed naturally during a period of selection, with Tamahomare (Yamamoto et al., 2004). A non-nodulating mutant derived from Enrei, En1282, was provided by Dr. Akao, National Institute of Agrobiological Sciences, Japan.

2. Field experiment (Exp. 1)

The three genotypes were sown in the experimental fields of Tohoku University (38°16’ N, 140°50’ E) on 26 May 2003 and 28 May 2004 with 70-cm spacing between rows and 15-cm spacing between hills. The soil was a fine-textured clayey Terrace Yellow soil (Classification Committee of Cultivated Soils, 1996). Two seeds per hill were sown, and seedlings were thinned after establishment to one plant per hill. The experimental design was a randomized complete block with the main plots of waterlogging treatment and subplots of cultivar. Each plot was 12.6 m² (4.2 × 3.0 m), with three replications. Prior to sowing, N, P₂O₅ and K₂O fertilizers were applied at 2, 10 and 6 g m⁻², respectively. After seedling establishment, the basal parts of seedlings were ridged by intra-row soil; thereby, the soil surface of the intra-row space was kept lower by ca. 10 cm than that of the intra-plant space. Waterlogging treatment was imposed by sprinkler irrigation during a period from stage V5 to V9 in 2003 or from V6 to R1 in 2004 (Fehr et al., 1971). The respective durations of treatment were from 30 June to 17 July in 2003 and from 3 July to 26 July in 2004. By this treatment, the intra-row space was kept under a submerged condition during the period, whereas the intra-plant space was maintained above the water surface.

Three plants showing medium growth were sampled from each replication at the beginning and end of

| Year | June | July | Aug. | Sept. | June | July | Aug. | Sept. | June | July | Aug. | Sept. |
|------|------|------|------|-------|------|------|------|-------|------|------|------|-------|
| 2003 | 19.1 | 18.4 | 22.2 | 20.2  | 121  | 34   | 77   | 104   | 107  | 287  | 211  | 98    |
| 2004 | 19.9 | 23.8 | 23.6 | 21.2  | 189  | 196  | 186  | 109   | 137  | 156  | 72   | 85    |
the treatment, and at the seed-filling stage (R5; 91 days after sowing (DAS) in 2003, 104 DAS in 2004). The samples were separated into leaf, petiole, stem and pod, dried at 80°C for more than three days in a ventilated oven, and weighed. The leaf area of a plant of medium size in each plot was measured using a leaf-area meter (automatic area meter model AAM-7; Hayashi Denko Co, Ltd., Tokyo, Japan). After measurement, the leaves were dried and weighed. In the other samples, the leaf area was estimated from the ratios of leaf dry weight to leaf area. Crop growth rates (CGR) and average leaf area index (LAI) were calculated using data of the first and last days of the investigation period. At maturity, 10 plants for each replication were harvested; the yield and yield components were measured in both years.

3. **Pot experiment (Exp. 2)**

The supernodulating genotype Sakukei 4 and its parent cultivar, Enrei, were used in this experiment. The same soil as used in Exp. 1 was used. Four seeds per pot (16 cm diameter, 19 cm tall) were sown on 30 May 2003 and 31 May 2004; they were thinned to one plant per pot after emergence. Prior to sowing, fertilizer was applied at fixed rates: 0.3 g of N, 1.0 g of P₂O₅, 1.0 g of K₂O and 5 g of slaked lime per pot. The waterlogging treatment was imposed by submerging pots up to the soil surface in a water pool that was 19 cm deep, 3 m wide and 6 m long. The treatment was imposed for ten days starting on 10 DAS (V1), 24 DAS (V3) and 38 DAS (V8) in 2003, and 21 DAS (V3), 31 DAS (V6) and 41 DAS (V8) in 2004. In this experiment, the first, second and third periods in each year are respectively designated as W1, W2 and
Table 2. Effects of waterlogging at the vegetative growth stage on yield and yield components of three genotypes (Exp. 1).

| Year | Yield components | Enrei | | En1282 | | Sakukei 4 |
|------|------------------|-------|------|-------|------|-------|
|      | Cont. | Waterl. | Cont. | Waterl. | Cont. | Waterl. |
| 2003 | Yield (g m\(^{-2}\)) | 228 | 207 | 91 | 43 | 18* | (41) | 208 | 158 | (76) |
|      | No. of pods (m\(^{-2}\)) | 380 | 346 | 91 | 128 | 76* | (59) | 320 | 271 | (85) |
|      | No. of seeds per pod | 1.74 | 1.74 | (100) | 1.43 | 1.39 | (97) | 1.89 | 1.80 | (95) |
|      | No. of seeds (m\(^{-2}\)) | 663 | 602 | 91 | 183 | 104* | (57) | 600 | 491 | (82) |
|      | 100-seed weight (g) | 34.5 | 34.3 | (99) | 23.1 | 17.0 | (74) | 34.4 | 32.2 | (94) |
| 2004 | Yield (g m\(^{-2}\)) | 521 | 199** | (38) | 358 | 54** | (15) | 313 | 113** | (36) |
|      | No. of pods (m\(^{-2}\)) | 898 | 387** | (43) | 784 | 181** | (23) | 631 | 247** | (39) |
|      | No. of seeds per pod | 1.35 | 1.30 | (96) | 1.43 | 1.29 | (90) | 1.43 | 1.38 | (96) |
|      | No. of seeds (m\(^{-2}\)) | 1207 | 500** | (41) | 1123 | 233** | (21) | 901 | 345** | (38) |
|      | 100-seed weight (g) | 43.2 | 39.8 | (92) | 31.9 | 23.5 | (74) | 34.6 | 33.4 | (97) |

*, ** mean significant differences between control plots (Cont.) and waterlogged plots (Waterl.) at the 5% and 1% levels, respectively. Figures in parentheses show relative values of waterlogged plots (%) to the control plots.

Fig. 2. Correlation of yield with CGR determined during waterlogging treatment (A) and during post-treatment (B) for two years (Exp. 1).

*, ** mean significant correlations at the 5% and 1% levels, respectively.
W3 in each year. Four to five plants showing medium growth were sampled from each treatment period at the beginning and end of the treatment, and at seed-filling stage (R₅; 87 DAS in 2003, 94 DAS in 2004). The samples were separated into various parts and processed as described for Exp. 1.

In both experiments, the seeds were inoculated with a strain of Bradyrhizobium japonicum (Tokachi-Nokyoren, Obihiro, Japan). Weather data were obtained from the Sendai District Meteorological Observatory website.

Results

1. Climatic conditions

Climatic conditions in the two years differed: temperature and sunshine hours were substantially fewer in 2003 than in 2004, but precipitation had an opposite tendency (Table 1). Particularly, substantially lower temperatures and fewer sunshine hours occurred in July and August 2003, which markedly depressed growth and engendered substantially lower yields in 2003 than in 2004.

2. Field experiment (Exp. 1)

(1) Growth responses

Main stem length, top dry weight, and LAI were generally depressed by waterlogging in all genotypes, except that these traits of waterlogged plot exceeded those of control plot immediately after the waterlogging treatment in Enrei, 2004 (Fig. 1). Among the three traits, main stem length was less affected by the waterlogging treatment than the other two traits. The difference in growth performance between control and waterlogged plants was magnified with time after treatment. It was obvious that waterlogging-induced growth depression was more pronounced in En1282 and Sakukei 4 than in Enrei. Generally,
growth performance of Enrei was superior to the other two genotypes, but top dry weight and LAI of En1282 tended to be greater than those of Enrei and Sakukei 4 around the growth stage of 50 DAS in 2003 or 60 DAS in 2004. This superior growth of En1282 remained until the seed-filling stage in 2004, but not in 2003.

(2) Yield and yield components

In 2003, the yield in waterlogged plots of Enrei, En1282 and Sakukei 4 was 91, 41 and 76% of the control plots, respectively, but a significant yield reduction by waterlogging was observed only in En1282 (P<0.05) (Table 2). In 2004, however, a significant yield decrease was observed in all genotypes (P<0.01). The yield of the waterlogged plots of Enrei, En1282 and Sakukei 4 was 38, 15 and 36% of the control plots, respectively. No clear cultivar difference in the degree of yield decrease was observed between Sakukei 4 and Enrei. The significant yield decrease by waterlogging was attributable to the decrease of pod and seed numbers in all genotypes.

(3) Relationship between CGR and yield

Figure 2 shows the correlations between yield and CGR during waterlogging treatment and during post-treatment periods in both years. In Enrei and Sakukei 4, yields were closely correlated with CGR determined during post-treatment, but not with CGR during treatment. In En1282, yield was correlated with CGR during both periods.

3. Pot experiment (Exp. 2)

(1) Growth response to waterlogging

No marked cultivar difference existed in the effect of waterlogging on top dry weight (Fig. 3). The difference in top dry weight between control and waterlogged plants was more markedly greater at the post-treatment stage than at the stage immediately

![Graph showing correlations between yield and CGR during waterlogging treatment and during post-treatment periods in both years.](image-url)
Dry weights of roots and nodules were reduced substantially by waterlogging, except for the plots of Enrei in 2003 (Figs. 4, 5). Comparing the two cultivars, the magnitude of waterlogging-induced reduction of the nodule dry weight was more pronounced in Sakukei 4, particularly at the stage immediately after treatment (Fig. 5). Nonetheless, the nodule dry weights tended to be heavier in Sakukei 4 than in Enrei, irrespective of the treatment. Generally, the waterlogging-induced reduction of roots and nodules was more pronounced in 2004 than in 2003. Comparing the effects of different periods of treatment (W1, W2, W3), the dry weights of the three plant parts were more markedly reduced by the treatment at later stages (Figs. 3, 4, 5).

(2) Relationship between growth parameters during post-treatment period and yields

In Exp. 1, the yields of Enrei and Sakukei 4 were correlated closely with CGR during the post-treatment period, but not with that during the treatment (Fig. 2). Therefore, in this pot experiment, the relative growth rates (RGRs) of various plant parts during the post-treatment period were compared. No cultivar difference was apparent in RGRs of each plant part. However, the RGR of underground parts was higher than that of top parts; the RGR of nodules was highest among plant parts (Table 3).

The variation of top dry weight and root dry weight among treatments and years was more pronounced in Enrei whereas that of nodule dry weight was more pronounced in Sakukei 4 (Fig. 6). In Sakukei 4, the variation of yield among treatments and years was less than that of Enrei. Yields positively correlated with...
both above- and underground plant parts irrespective of cultivar, not significantly, but significantly with root dry weights in Enrei and with nodule dry weights in Sakukei 4 (P < 0.05) (Fig. 6).

**Discussion**

Numerous previous studies on excess moisture injury in soybean specifically examined the stress-induced decrease of growth and related eco-physiological parameters such as leaf color, photosynthetic activity, nodule activity and mineral absorption during the waterlogged period (Bennett and Albrecht, 1984; Sojka, 1985; Buttery, 1987; Sallam and Scott, 1987; Griffin and Saxton, 1988; Scott et al., 1989; Oostehuis et al., 1990; Sung, 1993; Sugimoto, 1994; Bacanamwo and Purcell, 1999). Few reports have described growth recovery after the removal of stress and its relation to final yield. Sugimoto and Satou (1990, 1993) demonstrated that depression of growth and yield caused by excess soil moisture was attributable to the leaf N deficit that was induced by the disabled N fixation activity by nodule bacteria and N absorption by roots. For this reason, the present study investigated the growth recovery capability and its relation to the yield of waterlogged Sakukei 4, which was previously characterized by having a higher capability of N fixation and photosynthesis than its parental cultivar.

Irrespective of cultivar, the effects of waterlogging

### Table 3. RGRs (g g⁻¹ d⁻¹) of various plant parts measured during the post-treatment period of two cultivars grown in two years (Exp. 2).

| Year | Enrei Top | Enrei Root | Enrei Nodule | Sakukei 4 Top | Sakukei 4 Root | Sakukei 4 Nodule |
|------|-----------|------------|-------------|--------------|---------------|-----------------|
| 2003 |           |            |             |              |               |                 |
| W1   | 0.06      | 0.06       | 0.10        | 0.06         | 0.06          | 0.12            |
| W2   | 0.04      | 0.05       | 0.13        | 0.04         | 0.05          | 0.10            |
| W3   | 0.04      | 0.04       | 0.06        | 0.04         | 0.05          | 0.07            |
| 2004 |           |            |             |              |               |                 |
| W1   | 0.06      | 0.05       | 0.10        | 0.06         | 0.04          | 0.08            |
| W2   | 0.01      | 0.03       | 0.08        | 0.02         | 0.03          | 0.07            |
| W3   | 0.01      | 0.02       | 0.07        | 0.00         | 0.02          | 0.04            |

W1, W2 and W3 indicate waterlogging treatment periods.

**Fig. 6.** Correlation of yield with top dry weight, root dry weight and nodule dry weight determined at the post-treatment time (87 DAS in 2003, 94 DAS in 2004) (Exp. 2).

* indicates a significant correlation at the 5% level.
treatment on growth were more markedly expressed during the post-treatment period than immediately after treatment in both years. In comparison with control plots, the top dry weights of waterlogged plots at the pod-filling stage were 86% and 36% in Enrei and Sakukei 4, respectively, in 2003, and 69% and 43% in Enrei and Sakukei 4 in 2004, respectively (Fig.1). Yield reduction by waterlogging was more pronounced in 2004 than in 2003, and the percentage of waterlogged plots was 58% and 36% in Enrei and Sakukei 4, respectively (Table 2). The marked yield reduction caused by waterlogging treatment in 2004 reflects the increased growth and yield of control plots under favorable climatic conditions irrespective of cultivar (Fig. 1, Table 1). The reduction of growth and yield by waterlogging was greatest in En1282 among the three genotypes (Fig. 1, Table 2), indicating that capability of N fixation is essential for the recovery from waterlogging-induced damage. It is noteworthy that the yield reduction of Sakukei 4 from waterlogging resembled that of Enrei, despite its more marked growth reduction than Enrei, suggesting that there might be a mechanism to compensate for growth reduction in Sakukei 4.

In 2004, when a marked yield reduction by waterlogging was observed, that reduction was primarily attributable to the fewer pods and seeds in all genotypes. In En1282, a marked decrease of 100-seed weight was also observed, suggesting that waterlogging treatment reduced the photosynthate supply during the seed filling period in this genotype. Sugimoto and Satou (1990) indicated that the non-nodulating line was more sensitive to waterlogging than the normally-nodulating genotype in terms of yield. They ascribed the decrease of 100-seed weight to the reduced dry matter production that might result from the lack of nitrogen supply during the seed-filling period. The results of this study support their inference.

In 2003, Enrei and Sakukei 4 exhibited the markedly reduced growth immediately after the waterlogging treatment, exhibiting comparable yields to those of the control plants. In 2004, the two cultivars exhibited no marked reduction of growth immediately after the waterlogging treatment, but the yield reduction was substantial (Fig. 1, Table 2). These results suggest that the yield performance under waterlogging conditions might be affected by the recovery capability rather than the degree of growth reduction measured immediately after waterlogging treatment. The correlations of yield with CGR measured during the waterlogging treatment and the post-treatment period revealed that the yields of the two cultivars were closely associated with CGR during the post-treatment period, but not with CGR during treatment (Fig. 2), which confirmed our presumption.

There have been inconsistent views on the most critical growth stage that determines soybean yield. In a study in which assimilate supply was modified by shading or plant shape alteration, Kokubun (1988) showed that yield was defined by CGR during a period from flowering to pod growth (R1 to R4). In the present study, CGR during post-treatment period (R1 to R5) was closely correlated with yield (Fig. 2). This result confirmed the view that the critical stage that limits yield is a period from flowering to pod growth or early seed growth. In a study using various soybean genotypes, Shiraiwa et al. (2004) found that yield significantly correlated with CGR during a period after the beginning of seed filling (R5). Since we did not measure the change of dry weights after R5 in the present study, it is not clear how the dry matter production after R5 contributed to an increase in yield.

A comparison of RGR of various plant parts during the post-treatment period revealed no clear difference between Enrei and Sakukei 4 (Table 3). Noticeably, however, the RGR of underground parts was higher than that of top parts. The RGR of nodules was the highest among those of all plant parts. That is, nodule growth is the most vigorous in all plant parts during the period of recovery from waterlogging. The yield correlated with dry weight of above- and underground plant parts irrespective of cultivar though not significantly, but significantly with nodule dry weight in Sakukei 4, and with root dry weight in Enrei (Fig. 6). These results suggest that the alleviation of waterlogging-induced yield reduction of Sakukei 4 appears to depend on its enhanced nodule growth capability during the recovery stage.

The physiological functions harmed by waterlogging were identified as the N-fixation activity of nodules and the nutrient absorption of roots associated with the depressed respiration of roots and nodule bacteria caused by oxygen deficiency in waterlogged soils (Sugimoto and Satou, 1990, 1993). The results of the present study suggest that Sakukei 4, with its superior nodule-formation capability, has a higher capability for recovering from waterlogging-induced growth depression, probably because of the recovery of N fixation and photosynthesis. Further studies are necessary to clarify the physiological mechanism that determines how nodule-growth recovery mitigates yield reduction.

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