Effects of Short-Term Waterlogging on Soybean Nodule Nitrogen Fixation at Different Soil Reductions and Temperatures

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Abstract: Short-term waterlogging often occurs in upland fields converted from paddies in Japan, since paddy fields have poor surface drainage and a high water table. The objective of this study was to clarify the effects of different durations of waterlogging (W) and waterlogging with soil reduction (WR) on soybean nitrogen fixation at different soil temperatures. Acetylene reduction activity (ARA) was reduced by W treatment for 3, 5 and 7 days (d), ARA was restored to the same level as in the control (C) at 3 days after the end of the treatment (DAT). However, ARA reduced by the WR treatment for 3, 5 and 7 d in a greenhouse was not restored. In the experiments under three soil temperature conditions (lower than 30ºC, around 34ºC and higher than 36ºC) and with two waterlogging durations (3 and 5d) in the growth chamber, ARA reduced by the W treatment was restored after the treatment irrespective of soil temperature and waterlogging duration whereas ARA reduced by WR was restored only after the short-term waterlogging at a low soil temperature. The nodule dry weight and respiration rate of the root system significantly decreased when ARA was greatly reduced. In addition, ARA at 3 DAT was positively correlated with stomatal conductance (SC) at 4 DAT. We concluded that the recovery of nodule nitrogen fixation from the reduction by waterlogging is strongly affected by the oxidation-reduction potential and temperature of the soil, and the nodule damage could be evaluated by SC.

Key words: Acetylene reduction activity, Oxidation reduction potential, Soil reduction, Soil temperature, Soybean (Glycine max), Waterlogging.

Received 28 December 2010. Accepted 20 April 2011. Corresponding author: S. Shimada (shinji@affrc.go.jp, fax +81-29-838-8532).

This research was supported by Scientific Research (B) (General) grant no. 22380014, a Grant-in-Aid for Scientific Research from Japan Society for the Promotion of Science.

Abbreviations: ARA, acetylene reduction activity; DAT, days after treatment; SC, stomatal conductance; ORP, oxidation reduction potential; W, Waterlogging treatment; WR, Waterlogging with soil reduction treatment.
lead to the development of adventitious roots or the formation of aerenchyma. Consequently, nodule nitrogen fixation is almost stopped due to the severe restriction of gas exchange between the soil and atmosphere (Huang et al., 1975; Shimamura et al., 2002; Thomas et al., 2005). Therefore, the recovery of nitrogen fixation from the reduction in waterlogging after the removal of excess water is important for the acquisition of nitrogen in soybean under short-term waterlogging stress.

Waterlogging occurring for less than a week has been shown to reduce the yield in soybean (Griffin and Saxton, 1988; Sullivan et al., 2001). Even four days of waterlogging reduced the yield significantly (Scott et al., 1989), and the decrease was more marked in clayey soil (Rhine et al., 2010; Scott et al., 1989).

Concerning the effect of waterlogging lasting less than a week, there have been reports on stomatal conductance (SC) (Kozlovski, 1984; Ahnold et al., 2002), photosynthesis (Huang et al., 1975; Oosterhuis et al., 1980; Sugimoto, 1994) and nitrogen fixation of the nodule (Andereva et al., 1987; Huang et al., 1975; Huang and LaRue, 1985; Sugimoto, 1994; Sung, 1993). Nitrogen fixation in the nodule is often evaluated using acetylene reduction activity (ARA). After short-term waterlogging, ARA was restored to the same value as that in the control plant after the waterlogging in some studies (Huang et al., 1975; Huang and LaRue, 1985), but not in other studies (Andereva et al., 1987; Sung, 1993). Thus, it is uncertain whether ARA is restored after waterlogging.

Direct damage to the root tissues and cells is caused by hypoxia or anoxia in the root zone due to waterlogging (Russel et al., 1990; Vartapetian and Jackson, 1997). Also, the effect of waterlogging has been attributed to chemical changes in the soil, where the absence of aeration lowers the oxidation reduction potential (ORP) of the soil and harmful substances such as ferrous ions and hydrogen sulfide are produced (Marschner, 1995). At a higher soil temperature, the oxygen requirement for respiration increases, and the ORP is decreased causing more serious damage (Trought and Drew, 1982). Therefore, we considered differences in ORP and soil temperature as possible factors leading to these conflicting results. However, there are few studies on the effect of short-term waterlogging on the restoration of nitrogen fixation in the nodule taking soil ORP and temperature into consideration.

Here, we examined the degree of recovery of ARA from the suppression by waterlogging for less than a week under different ORP and soil temperature conditions. We investigated the response of the soybean plant (SC, SPAD value, nodule dry weight and respiration rate of root system) to waterlogging and analyzed the association between the recovery of ARA and these parameters.

Materials and Methods

1. Culture conditions and treatments

Three experiments were conducted. The effects on dry weight of plants and nodules and on acetylene reduction activity (ARA) of nodules were examined in experiment 1; the effects on chlorophyll content (SPAD value) and SC of leaves, ARA of nodules and oxygen concentration (OC) and oxidation reduction potential (ORP) in the rhizosphere in experiment 2, and the effects on respiration in rhizosphere in experiment 3. The seeds of soybean (Glycine max (L.) Merr.) cultivar ‘Enrei’ were sown in a 1/5000 Wagner pot (159 mm diameter, 190 mm tall) containing vermiculite and silica sand (volume rate, 1:1). Four or five seeds were sown per pot, and were inoculated with a suspension of Bradyrhizobium japonicum strain USDA 110 (107 cells mol−1 seed−1). In Exp.1-1, 1-2, and Exp.2-1 seeds were sown on 18 June, 8 July and 23 July 2009, respectively, in a greenhouse (GH) under natural light in Tsukuba, Japan. GH was controlled so that day/night temperatures were 25–35/20–25°C, but the day length was not regulated. The emerged seedlings were thinned to one plant per pot. The nutrient solution was prepared according to Akao and Kouchi (1989) (1.11 mmol L−1 MgSO4·7H2O, 1.35 mmol L−1 K2SO4, 0.35 mmol L−1 KH2PO4, 0.19 mmol L−1 CaCl2·2H2O, 2.70 mmol L−1, 53 μmol L−1 Fe-EDTA, 50.9 μmol L−1 H3BO3, 10.0 μmol L−1 MnCl2·4H2O, 0.8 μmol L−1 ZnSO4·7H2O, 0.40 μmol L−1 CuSO4·5H2O, and 1.41 μmol L−1 NaMoO4·2H2O) and adjusted to pH 6.3. The plants were watered (100 ml) three times per week using a nutrient solution (0.75 mmol L−1 KNO3) in the first week and then with N-free nutrient solution. Plants were grown in Wagner pots without drain stoppers and were irrigated with water at intervals to avoid stress due to a deficit or excess of water during cultivation. The plants were treated with pesticides as necessary.

In other experiments, the plants were grown in a growth chamber (GC; NK system, Osaka, Japan). GC was controlled so that day/night temperatures were 35–25°C, the photosperiod was 14 h, and the photosynthetic photon flux density (PPFD) just above the plant was higher than 250 μmol m−2 s−1. Soybean plants grown for 36–57 days in GH and 35–43 days in GC were treated as described below. The soil temperature was adjusted to above 36°C during the treatment (high), around 35°C (medium-high) and lower than 30°C (low).

The plants were exposed to waterlogging (W) and waterlogging + reduction by adding soluble starch (WR). W was defined as waterlogging with 2 cm or more above the soil surface and WR was defined as waterlogging as in W with the addition of a starch solution (2 g L−1) to reduce the soil. The duration of these treatments was 3, 5 and 7 days. The plants without treatment were used as a control (C). In some experiments, the soil temperature was...
adjusted to about 36°C, by setting the pot on a water bath at 38°C, alternatively, it was about 30°C in the GC. The replicate was 3–4 pots per treatment in all experiments.

2. Measurements

The oxygen concentration (OC) in the pot was measured using an oxy-meter with a needle sensor (OXY-4 mini, Presens, Regensburg, Germany). The oxygen reduction potential (ORP) in the pot was measured using an ORP meter (RM-39P, TOA TDK, Tokyo, Japan). The ORP meter logged the results every half hour throughout the experiment. Moreover, soil temperature and air temperature were measured using a Thermo Recorder (TR-52, T&D Corporation, Nagano, Japan) during the experimental period. The oxygen, ORP and thermo sensors were set at 5 cm below the soil surface.

The nitrogenase activity of the root system was measured as in situ flow-through acetylene reduction activity (ARA). ARA was measured before treatment, immediately after treatment (0 DAT: 2, 3 hours after free water was drained from the waterlogged area), and at 1 and 3 days after the end of the treatment (DAT). The 1/5000 Wagner pot was sealed with a plastic lid with holes and vinyl tape. Plants were grown through a 3-cm hole in the lid, and this hole was sealed with a silicon plug and pate (PROVIL novo Light, Heraeus Kulzer, Hanau, Germany). The gas mixture (10 kPa pC₂H₂, 21 kPa pO₂ and 69 kPa pN₂) flowed through the drainage port located at the bottom of the Wagner pot. Gas flowed out through the exit port located on the lid, and a tube connected the exit port to the flow. The gas flow rate to the pot was 2 L min⁻¹, and the air temperature during measurements of ARA was set at 26°C. Gas was sampled at the tube with a syringe and injected into a GC-8A gas chromatograph (Shimadzu, Kyoto, Japan) to determine the ethylene (C₂H₄) concentration. ARA was calculated as the product of the ethylene concentration in the gas sample (μmol L⁻¹), and the specific ARA per unit nodule dry weight.

SC of the leaf and SPAD value of the terminal leaflet were measured using a leaf porometer Model SC-1 (Decagon Devices Inc., Pullman, WA) and SPAD-502 (Konica Minolta Sensing Inc., Osaka, Japan), respectively, at the abaxial side of the terminal leaflet just below the recently fully expanded leaf on the main stem. The values of SC and SPAD were measured at least 3 and 6 times on the terminal leaflet, respectively, and averaged. In addition, in some of these experiments, we measured the respiration rates of the rhizosphere using the LI-840 (Li-COR, Lincoln, USA) before treatment and at 3 DAT, and the nodules measured were sampled.

After measurements, the plants were sampled and dried for more than 2 days at 85°C. We then measured the dry weight (DW) of each part of the plant.

3. Statistical analysis

Data was analyzed using statistical software (SAS Add-In for Microsoft Office, SAS institute Inc. NC, USA). Differences among the means of the values were tested by analysis of variance (ANOVA). When the F-test indicated significance of differences (P < 5%), the data were analyzed by Tukey’s multiple range test at a probability level of 5%.

Results

1. Oxygen concentration (OC) and oxidation-reduction potential (ORP) in soil

Changes in the ORP and OC in the soil under W and WR treatments were similar in all experiments. Figure 1 shows typical changes in the ORP and OC of the soil at the medium-high temperature (Exp. 2-4). In this experiment, the OC in WR declined to 0 hPa within 6 hours after the start of the treatment. In contrast, it took about a day (d) to reach an OC of approximately 0 kPa in W. The value of ORP in WR decreased to the minimum value (approximately −300 mV) 1–2 d later, and then gradually
increased. In contrast, the ORP in W slowly decreased, and was between 0–200 mV at 5d (data not shown), which was higher than that in WR treatment.

2. Acetylene reduction activity (ARA) under different conditions

As shown in Fig. 2A, in all treatments for 3 d (W3), 5 d (W5) and 7 d (W7), ARA was detected only slightly at the end of the treatment (0 DAT). ARA in all treatments was restored to half the level in C at 1 DAT, and the same level as in C at 3 DAT. As shown in Fig. 2B, in all treatments for 3 d (WR3), 5 d (WR5) and 7 d (WR7), ARA could not be detected at 0 DAT. However, in the WR3 and WR5 treatments ARA was detected at 1 DAT and was restored to about 1/3 of the value in C at 3 DAT (Fig. 2B). ARA in the WR7 treatment was not restored at 1 and 3 DAT.

As shown in Fig. 3, ARA in response to W and WR treatments greatly varied with the duration of treatment and soil temperature. In W, the ARA in W3 and W5 at the low temperature (Figs. 3A and B) and W3 at medium-high temperature (Fig. 3C) was restored to the same level as that in C at 3 DAT. However, the restored ARA after W5 at the medium-high temperature (Fig. 3D) was significantly lower than the value in C, although the diminution was not large. By contrast, ARA in WR3 at the low and medium-high temperatures (Figs. 3A and C) was restored to about the same level as that in C at 3 DAT. ARA in WR5 at the low temperature was restored slightly at 3 DAT (Fig. 3B), but not in WR5 at the medium-high temperature and in WR3 at the high temperature (Figs. 3D and E). The ARA levels in these treatments gradually increased with time until 3 DAT.
The effects of treatment on SPAD value were similar to those on SC. In particular, WR in Exps. 2-2, 2-4 and 2-5, in which SC was much lower in WR than in W, showed a lower SPAD value of 21 or less, and the leaf was yellow (Table 1).

4. Correlation between ARA and SC
As Table 2 shows, SC at 4 DAT was significantly positively correlated with ARA at 3 DAT.
Effect of W and WR treatments for 3 and 5 days under different soil temperature conditions on SC of the terminal leaflet. Vertical bars represent SE (n = 3, 4). The gray squares in figures indicate the period of treatment. Within each experiment and each day of measurement, means with the same letter within a set are not significantly different at the 5% level, based on Tukey’s multiple range test.

†† Same as in Fig. 3.

Table 1. Effect of W and WR treatments lasting 3 and 5 days under different soil temperature conditions on SPAD value of the terminal leaflet.

| Measurement time | Temp. | Exp.2-2 Low† | Exp.2-3 Medium-high | Exp.2-4 Medium-high | Exp.2-5 High |
|------------------|-------|--------------|---------------------|---------------------|-------------|
|                  |       | Low          |                     |                     |             |
|                  |       | Period 5     | 3                   | 3                   | 5           |
| Pre-Treatment    | C     | 37.9 a        | 53.5 a              | 37.8 a              | 39.6 ab     |
|                  | W     | 36.2 a        | 36.3 a              | 40.7 a              | 40.7 a      |
|                  | WR    | 36.5 a        | 37.0 a              | 39.6 a              | 39.7 ab     |
| Post-Treatment   | C     | 34.9 a        | 34.2 a              | 37.6 a              | 37.9 ab     |
|                  | W     | 33.7 a        | 34.2 a              | 29.8 b              | 35.4 b      |
|                  | WR    | 21.0 a        | 33.2 a              | 18.3 c              | 21.1 c      |

Measurement of SPAD value was conducted at 4 DAT. Growth chamber conditions; day/night temperatures of 35/25ºC in Exps. 2-3, 2-4 and 2-5 and 30/25ºC in Exp. 2-2, a 14-h photoperiod, and a PPFD of more than 250 μmol m⁻² s⁻¹ in Exps. 2-2, 2-3, 2-4 and 2-5. Data with the same letter in each column are not significantly different at the 5% level based on Tukey’s multiple range test.

† W indicates the waterlogging treatment; WR indicates the waterlogging + reduction treatment; C indicates the Control. †† Same as in Fig. 3.
5. Dry weight (DW) and respiration rate of root system

As Table 3 shows, DW in all organs was suppressed by the waterlogging and waterlogging plus reduction. In particular, the nodule DW at the high soil temperature was markedly reduced. The nodule DW was not significantly reduced in Exps. 1-1, 2-1 and 2-3, in which the ARA in WR was restored to the level of the control at 3 DAT. However, the nodule DW significantly decreased in Exps. 1-2, 2-2 and 2-5, in which ARA in WR (WR3, WR5, WR7) was not restored at 3 DAT (Table 3, Figs. 2, 3). The root and shoot DW in WR in which ARA was not restored at 3 DAT (Exps. 1-2, 2-2, 2-4 and 2-5) were significantly decreased except for shoot DW in Exp. 1-2 (only WR7).

Fig. 5 shows the effect of W5 and WR5 over 5 d on the respiration rate in the rhizosphere of soybeans in Exp. 3 (medium-high). Before treatment, there was no difference in the respiration rate among the treatments (C, W5 and WR5). At 3 DAT, however, the respiration rate in WR5 was significantly lower than that in the control (C) (Fig. 5)

Table 3. Effect of W and WR treatments lasting 3, 5, or 7 days under different soil temperature conditions on nodule, root and shoot DW.

| Temp.       | Exp. 1-1 High | Exp. 1-2 High | Exp. 2-1 Low | Exp. 2-2 Low | Exp. 2-3 Medium-high | Exp. 2-4 Medium-high | Exp. 2-5 High |
|-------------|---------------|---------------|--------------|--------------|----------------------|----------------------|--------------|
| Pre-treat   | —             | 0.19 ab       | 0.38 a       | 0.29 b       | 0.19 a               | 0.18 a               | 0.16 a       |
| C           | 0.32 ab       | 0.44 c        | 0.48 ab      | 0.49 c       | 0.27 bc              | 0.41 b              | 0.37 b       |
| W3          | 0.57 b        | —             | 0.48 ab      | —            | 0.30 c               | —                    | —            |
| W5          | 0.28 a        | —             | —            | 0.49 c       | —                    | 0.42 b              | —            |
| nodule DW   | W7            | 0.28 ab       | —            | —            | —                    | —                    | —            |
| (g plant⁻¹) | WR3           | —             | 0.27 b       | 0.52 b       | —                    | 0.24 ab              | —            |
| WR5         | —             | 0.22 ab       | —            | 0.17 a       | —                    | 0.13 a               | —            |
| WR7         | —             | 0.11 a        | —            | —            | —                    | —                    | —            |
| Root DW     | W7            | 1.92 a        | —            | —            | —                    | —                    | —            |
| (g plant⁻¹) | WR3           | —             | 1.17 a       | 1.55 a       | —                    | 0.93 a               | —            |
| WR5         | —             | 1.13 a        | —            | 1.48 a       | —                    | 0.83 a               | —            |
| WR7         | —             | 1.15 a        | —            | —            | —                    | —                    | —            |
| Shoot DW    | W7            | 6.61 a        | —            | —            | —                    | —                    | —            |
| (g plant⁻¹) | WR3           | —             | 6.83 b       | 11.12 a      | —                    | 5.55 b               | —            |
| WR5         | —             | 6.97 b        | —            | 8.61 b       | —                    | 5.39 a               | —            |
| WR7         | —             | 7.44 bc       | —            | —            | —                    | —                    | —            |

Greenhouse conditions included day/night temperatures of 25–35/20–25°C, no supplemental light, (natural day length) in Exp. 1 and 2-1. Growth chamber conditions included day/night temperatures of 35/25°C in Exps. 2-2, 2-4 and 2-5 and day/night temperatures of 30/25°C in Exp. 2-2, a 14 h photoperiod, and a PPFD of more than 250 μmol m⁻² s⁻¹ in Exps. 2-2, 2-3, 2-4 and 2-5. Data with the same letter in each column are not significantly different at the 5% level. Based on Tukey’s multiple range test.

W indicates the waterlogging treatment; WR indicates the waterlogging + reduction treatment; C indicates the Control. Numbers indicate the period of treatment. \(^{11}\) Same as in Fig. 3.
significant lower than that in C, while that in W5 was not.

**Discussion**

Long-term waterlogging has been shown to promote acclimatization to the waterlogging conditions in soybean plants and result in plant adaptation (development of lateral roots and adventitious roots, thickening of stems, and formation of aerenchyma in roots and nodules) to relieve the damage (Bacanamwo and Purcell, 1999b; Shimamura et al., 2002). However, in the present experiment, the development of lateral roots and thickening of stems were slight in the W and WR treatments and obvious plant adaptation was not observed because the waterlogging treatment period was less than one week.

In the W condition, the OC at 5 cm below the soil surface declined to about 0 kPa within 1 or 2 d from the start of the W treatment, and the ORP also dropped to nearly 0 mV at the lowest (Fig. 1). The ARA in W7 recovered to the same level as in C at 3 DAT although the recorded maximum soil temperature was nearly 39°C in the greenhouse experiment (Fig. 2A). In the growth chamber experiment (Fig. 3, Exps. 2-2, 2-3, 2-4 and 2-5), W did not cause serious reduction of ARA. These results indicate that the soybean nodule is very tolerant to waterlogging (excess water) and anaerobic conditions without severe soil reduction. By contrast, the ORP in the WR treatment showed a very low value (around -300 mV) at 2 d after the start of treatment (Fig. 1). ARA was not restored at 3DAT after WR3 with a high soil temperature (Fig. 3E) and WR5 at low and medium-high temperatures (Figs. 3B and D).

The change in the SC of the leaf due to W and WR was similar to that in ARA (Figs. 3 and 4). In Exps. 2-2, 2-4 and 2-5, in which ARA was not restored after the treatment, both the SC and SPAD value of WR were greatly decreased (Figs. 4B, D and E, Table 1). Furthermore, there was a significantly positive correlation between the SC measured at 4 DAT and ARA measured at 3 DAT (Table 2). These positive correlations indicate that the soil reduction caused damage to both root function related to stomatal opening and the ARA of the nodule to similar degrees.

Although SC decreased during the W treatment, it was restored to the same level as in C at 3 DAT (Fig. 4). Waterlogging has been reported to decrease the SC of the leaf (Kozlowski, 1984; Ahmed et al., 2002). Unknown materials derived from the root have been suggested to be involved (Else et al., 1996; Else et al., 2009) and ABA was a candidate (Jackson and Hall, 1987; Jackson, 2002). Waterlogging was also reported to result in the decline of SC without a decrease of leaf water potential in the soybean (Oosterhuis et al., 1990). Therefore, it was considered that the temporary decline of SC in W was due to the temporary stomatal closure caused by chemical materials from the root as described in those papers.

By contrast, the SC of WR was not restored to the same level as in C, not only during the treatment but also after the treatment (Fig. 4). In addition, the respiration rate under WR greatly decreased compared to C and W (Fig. 5), and the nodule dry weight significantly decreased in Exps. 2-2, 2-4 and 2-5, in which ARA was not restored. These results indicate that the marked reduction of soil directly damaged the cells and tissues of the root system and nodules. As a result, it is considered that a decline of root function relating to stomatal opening and of ARA occurred. The generation of harmful substances (e.g., ferrous ion, hydrogen sulfide) begins under severe reduction of the soil, and it is known that hydrogen sulfide is generated below about −50 mV (Marschner, 1995). In WR, the ORP temporarily dropped to −300 mV; therefore, harmful substances may be generated in the soil and damage the roots and nodules in WR.

Four days of waterlogging has been reported to significantly suppress nitrogen fixation (Sung, 1993) and make irreversible changes in the ultrastructure of cells in the nodules (Andreeva et al., 1987). In contrast, Huang and LaRue (1985) reported that ARA reduced by waterlogging for 8 days was restored to the same degree as in the control at 2 days after waterlogging. They cultivated soybean in pots filled with Turface (a porous ceramic soil conditioner) and grew the plants in a controlled-environment greenhouse. We believe that this condition did not cause severe reduction in the soil although they did not measure ORP in the soil. On the other hand, Andreeva et al. (1987) cultivated soybean in pots filled with quartzite sand (5 plants per pot), and in their experiment...
the soybean nodules at the middle and lower layers of the pot died after 4–5 days of waterlogging. In addition, the experiment of Sung (1993) was conducted in an experimental field. The results of these papers and our experiments indicate that ARA was not restored much after treatment in conditions under which severe soil reduction occurred.

The chlorophyll content (SPAD value) of the leaf was not obviously affected by 3 d of W at a low soil temperature, but the SPAD value declined at higher soil temperatures or with longer durations of waterlogging treatments. In particular, SPAD value in WR markedly decreased. Waterlogging causes inhibition of not only the nitrogen fixation of the nodule (Huang et al., 1975; Shimamura et al., 2002) but also the nitrogen absorption by the root system (Sugimoto, 1994), as shown by the decreased SPAD value in the leaf (Bacanamwo and Purcell, 1999a) or the nitrogen contents in the leaf (Rhine et al., 2010). This indicates that the adverse effect of waterlogging was promoted by a lower ORP in the soil, a higher temperature of the soil and a longer duration of waterlogging.

A high temperature has been reported to promote the oxygen requirement for respiration of roots, and the growth rate and senescence rate of the plants (Trought and Drew, 1982). In addition, the nitrogen fixation (ARA) was closely correlated with the soil temperature (Denison and Sinclair, 1985). The nitrogen fixation decreased at above 34°C soil temperature and did not recover after high-temperature-induced declines (Sinclair and Weisz, 1985). Some papers reported that waterlogging damage to alfalfa would be more severe at a higher soil temperature than lower soil temperature (Heinrichs, 1972; Thompson and Fick, 1981). These findings were consistent with our results that the adverse effect of waterlogging was severer at a higher soil temperature.

These experiments clearly showed that the reduction in ARA due to waterlogging for several days is small in soybeans if the ORP value of the soil is not below around 0 mV. However, if the waterlogging caused severe soil reduction, for example if ORP reached approximately −300 mV, the nodules were irreversibly damaged, and this damage became more severe with higher soil temperatures or longer duration. In addition, there was a significantly positive correlation between SC in the leaf and ARA at or longer duration. In addition, there was a significantly positive correlation between SC in the leaf and ARA at

The effects of soil temperature and soil reduction on nodule nitrogen fixation should be considered when developing criteria for drainage measures.

Acknowledgements

We thank Prof. Minamisawa K and Dr. Itakura M of Tohoku University for providing rhizobia (Bradyrhizobium japonicum USDA110) and Mrs. Inoue and Mrs. Horikoshi for their laboratory help. We also thank Dr. Mitsunaga T for advice on statistics analysis.

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* In Japanese with English summary.
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