Secondary Aerenchyma Formation and its Relation to Nitrogen Fixation in Root Nodules of Soybean Plants (*Glycine max*) Grown under Flooded Conditions

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Abstract: Soybean (*Glycine max* (L.) Merr.) is considered to be susceptible to flooding, a major agronomic problem in the world, and nitrogenase activity rapidly declines due to oxygen deficiency in root nodules. We investigated nodule acclimation to flooding at the morphological level using a soybean cultivar possessing the ability to form secondary aerenchyma. After 1 week of treatment, lenticles were formed on the surface of the root nodules, and secondary aerenchyma were observed through the lenticles under both irrigated and flooded conditions. As the plant grew, the nodule epidermis came off, and well-developed secondary aerenchyma covered the nodule surface. The secondary aerenchyma originated from the secondary meristem (phellogen) girdling the sclerenchyma, and the degree of development was greater in flooded nodules than in irrigated ones. Although root nodulation and total nitrogenase activity (TNA) decreased under flooded conditions, there were no differences in shoot N concentration, specific nitrogenase activity (SNA) and relative ureide-N in the xylem bleeding sap between plants in the irrigated and flooded conditions. Under flooded conditions, however, when the entry of oxygen into the secondary aerenchyma formed in the hypocotyl was inhibited by vaseline treatment (pasting on the surface of the hypocotyl), the shoot N concentration, TNA, SNA, the ureide-N concentration and the relative ureide-N in the sap declined remarkably. These results suggested that secondary aerenchyma formation in soybean plants is a morphological acclimation response to flooding stress, and that one of the functions is to supply atmospheric oxygen to root nodules, which consequently enables nodule activity to be maintained.

Key words: Flooding, *Glycine max*, Nodule activity, Oxygen transport system, Root nodule, Secondary aerenchyma, Soybean.

In flooded soils, the oxygen respiration of plant roots is inhibited, and the roots develop several physiological disorders. Therefore, it is difficult for many upland plants without tolerance to flooding to survive for long under these conditions. In contrast, wetland and some upland plants can survive under flooded conditions, because aerenchyma form in their roots (Smirnoff and Crawford, 1983; He et al., 1999; Longstreth and Borkhuisenius, 2000). Aerenchyma have the function of transporting oxygen for respiration to roots, and it has been clearly established that the partial oxygen pressure in gas in roots is over 14 kPa (Armstrong et al., 2000; Arikado, 1961), and that oxygen is actively released from the apex of the root possessing aerenchyma to the outside (Armstrong, 1971). These results suggest that oxygen respiration is maintained in these roots, and that the root surface is protected from toxic reduced substances by this oxidation barrier.

Leguminous plants have a symbiotic relationship with root nodule bacteria, and, therefore, oxygen is required for respiration of both roots and nodules. When there is an oxygen deficiency in flooded soils, the nitrogenase activity (acetylene reduction rate) of the nodules is rapidly inhibited (Bisseling et al., 1980; Hong et al., 1977), and root nodulation is decreased (Minchin and Pate, 1975; Sugimoto, 1994). Therefore, an internal aeration system to supply atmospheric oxygen to root nodules is required to prevent the decline of nitrogen fixation and the decrease of nodulation under these conditions.

In some leguminous plants with flooding tolerance, such as *Glycine soja* (Arikado, 1954), *Viminaria juncea* (Walker et al., 1983) and *Lotus uliginosus* (James and Crawford, 1998; James and Sprent, 1999), a white spongy tissue having much gas space covers the stem base, roots and nodules and it connects tissues at the junctions under flooded conditions. Therefore, this tissue is regarded as a continuous aerenchymatous pathway, and is called secondary aerenchyma (Williams and Barber, 1961; Jackson and Armstrong, 1999). It consists of thin walled, non-suberized and living parenchymatous cells with abundant intercellular spaces filled with air between their cells (Scott and Wager, 1888; Batten, 1918; Lempe et al., 2001; Stevens et al., 1997; Walker et al., 1983; Angeles, 1992; Arber, 1920; Metcalfe, 1931; Sifton, 1945). The aerenchyma are connected to the ambient atmosphere through hypertrophied lenticles in the epidermis of stem, roots and nod-
ules, with lenticels in the stem segments situated above
the water level being especially crucial as entry points for
oxygen diffusion to flooded tissues (Walker et al., 1983;
The infected tissue of the nodules in these leguminous
plants is pink, and the nitrogenase activity is highly
maintained, even under flooded conditions (Walker et al.,
1983; James and Crawford, 1998; James and Sprent,
1999). Some research has demonstrated using 15N as a
tracer that atmospheric nitrogen entered into the aerenchyn-
ma developing in the stem above the water level, and
was transported to the flooded nodules via aerenchyma
under nitrogen gas deficient conditions in flooded soils
(Walker et al., 1983; Saraswati et al., 1992).

Soybean, which is one of the important leguminous
crops, is considered to be susceptible to flooding stress
because the plant has a limited ability to form secondary
aerenchyma (Arikado, 1954). In fact, nitrogenase activ-
ity rapidly declines due to oxygen deficiency in root
nodules after flooding treatment (Huang and LaRue,
1985; Sugimoto, 1994). However, recent research
revealed a soybean cultivar that forms profuse secondary
aerenchyma in the hypocotyl, roots, and nodules under
flooded conditions (Mochizuki et al., 2000; Shimamura
et al., 2001a). In this cultivar, root growth is extremely
inhibited when the entry of atmospheric oxygen into the
secondary aerenchyma developing in the hypocotyl
above the water level is prevented (Shimamura et al.,
2000).

In this study, the process of secondary aerenchyma
formation and the contribution of the aerenchyma to the
nitrogen fixing activity of the root nodules in soybean
plants grown under flooded conditions were investigated.

Materials and Methods

The soybean (Glycine max (L.) Merr.) cultivar ‘Aso
aogari’ which has an excellent ability to develop aeren-
chyma under flooded conditions (Shimamura et al.,
2001b), was used. The experiments were carried out in a
greenhouse under natural light at the Kyushu University
Farm in Fukuoka, Japan, from June to August in
2001. Three seeds inoculated with Bradyrhizobium japonicum
strain USDA110 were sown in paddy soil (Clay
loam; Coarse sand 13.9%, Fine sand 27.2%, Silt 34.3%
and Clay 24.6%) in a plastic pot (11.3 cm diameter×14
cm height) in Experiment 1, and in a plastic pot (15.9
cm diameter×19 cm height) in Experiments 2 and 3.
Fertilizer was not applied. The seedlings were thinned
to one plant per pot after germination. The averages of
the greenhouse and flood water temperatures for 4 weeks
after the initiation of soil water treatment were 28.8 and
28.5°C, respectively.

1. Formation of secondary aerenchyma in the root
nodules (Experiment 1)

The pots were divided into two groups at the V1
growth stage (Fehr and Caviness, 1977); one group was
continuously flooded at 3 cm above the soil surface by
putting 15 pots per container (48×77×17 cm), and the
other was normally irrigated. Five seedlings for each
treatment were harvested at 1, 2 and 4 weeks. Three
nodules (over 2, 3 and 4 mm diameter at 1, 2 and 4
weeks, respectively) per seedling were selected. The
outside structures of the nodules were observed with a
stereoscopic microscope (SMZ-10, Nikon Co. Ltd.,
Tokyo Japan). Then, transverse cross-sections 100-200
μm in thickness were cut on a plant microtome (MTH
-1, Nippon Medical & Chemical Instruments Co. Ltd.,
Osaka Japan), and viewed with a light microscope
(OPTIPHOT-2, Nikon Co. Ltd., Tokyo Japan).

2. Assay of nodule activity (nitrogenase activity)
by the reduction of acetylene to ethylene (Experiment 2)

The seedlings were subjected to irrigated or flooded
treatment at the V1 growth stage. Flooding pots were
put in containers (69×37×27 cm) (six pots per con-
tainer) and water table was kept at 3 cm above the soil
surface. After 3 weeks of soil water treatment, vaseline
was applied to the surface of hypocotyls above the soil
in some plants (Table 1). The hypocotyl was sealed with
vaseline for preventing the entry of atmospheric oxygen
into the aerenchyma exposed to air through lenticels of
the hypocotyl (Armstrong, 1968; Jackson and Attwood,
1996). The seedlings at 4 weeks of soil water treatment
were harvested (seven seedlings in IN, FN and FV, and
six seedlings in IV). The root systems with shoots were
washed to remove the soil and the systems were kept in
the polyethylene bags (one seedling per bag) at room
temperature (30°C) for 1 hour (personal communica-
tion; T. Yamakawa). Acetylene reduction activity was
determined using a previously reported method, and
irrigated nodules were compared with flooded ones
(Bisseling et al., 1980; Sugimoto, 1994). That is, the root
systems detached from the shoots were placed in gas-
tight 500 ml Erlemeyer flasks, and 50 ml of air in the
flasks were replaced with acetylene. Gas samples (1 ml)
were extracted at 5 and 35 min, and analyzed on a gas
chromatograph (GC-8A, Shimadzu Co. Ltd., Kyoto
Japan) (0.3 cm (i.d.)×100 cm column of 80–100 mesh
Porapak N and a column temperature of 80°C with
nitrogen as a carrier gas). The number of nodules and

<table>
<thead>
<tr>
<th>Soil water treatment</th>
<th>Vaseline treatment*</th>
<th>IN</th>
<th>Irrigated</th>
<th>Non-treated</th>
<th>IV</th>
<th>Irrigated</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>FN  flooded</td>
<td></td>
<td>F</td>
<td>Flooded</td>
<td>Treated</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* : Water level was maintained at 3 cm above the soil surface.
** : The hypocotyl was sealed with vaseline after 3 weeks of soil water treatment.

Table 1. Components of treatment conditions in Experiments 2 and 3.
the dry weight of the plants were measured after drying in an oven at 80°C for 48 hours. Shoot N concentration was determined on 300 mg of finely ground samples using the method of Kjeldahl.

3. Collection and analysis of xylem bleeding sap (Experiment 3)

The experimental design was the same as in Experiment 2. After 3 weeks of soil water treatment, vaseline was applied to the hypocotyl above the soil surface in some plants (Table 1). After 4 weeks of soil water treatment, the hypocotyls were cut and covered with a silicon tube. The first drop of xylem sap at the cut surface was discarded to avoid contamination from damaged cells. Subsequent drops were collected onto ice at 3-min intervals for 1 hour. The xylem sap in each plant was weighed, and the bleeding sap rate was calculated. Seven samples per treatment were stored at -30°C in a freezer until analysis. Concentrations of amino-N, nitrate-N, and ureide-N (allantoin and allantonic acid) in the xylem sap were determined using the ninhydrin technique (Herridge, 1984), using a 1:1 (mol) asparagine:glutamine standard, the salicylic acid technique (Cataldo et al., 1975), and the method of Young and Conway (1942), respectively. The absorbance was analyzed on a spectrophotometer (UV-1200 UV-VIS, Shimadzu Co. Ltd., Kyoto Japan).

Results

1. Morphological changes of root nodules by flooding treatment

After 1 week of treatment, lenticels were formed on the surface of the root nodules, and secondary aerenchyma, which consisted of white and spongy tissue, was observed through the lenticels under both the irrigated and flooded conditions. However, both the lenticels and the secondary aerenchyma developed well in the flooded nodules (Fig. 1A and D). After 2 weeks of treatment, almost all of the epidermis in the flooded nodules came off, and the secondary aerenchyma covered the nodule surface in place of the epidermis. At this time, flooded plants had a continuous aerenchymatous pathway from the hypocotyl above the water level to the flooded roots and nodules (data not shown) as found in previous studies for other species (Walker et al., 1983; James and Crawford, 1998; James and Sprent, 1999). By contrast, in the irrigated nodules, the lenticels developed extensively, and barely any epidermis came off (Fig. 1B and E). Irrigated plants did not have the aerenchymatous pathway (data not shown). After 4 weeks, in both the irrigated and flooded nodules, the epidermis came off, and the secondary aerenchyma covered the surface (Fig. 1C and F). In the transverse sections of these plants, however, the layer of white secondary aerenchyma was much thicker in the flooded nodules than in the irrigated ones (Fig. 1G and H). The secondary aerenchyma originating from the secondary meristem appeared on the outer side of the sclerenchyma under both the irrigated and flooded conditions (Fig. 1I).

2. Effect of flooding and hypocotyl vaseline treatments on plant growth, nitrogenase activity of root nodules and xylem bleeding sap components

In the plants without vaseline treatment, the growth was clearly decreased by flooding. The shoot, root and total dry weight of the flooded plants were 40%, 44%, and 42% of the irrigated ones with significant differences at the 1% level, respectively. In addition, the number and dry weight of the root nodules was 42% and 66%, respectively (Table 2). However, there was no significant difference in shoot N concentration between the irrigated and flooded plants. The total nitrogenase activity (TNA) (μmol C₃H₄ plant⁻¹ h⁻¹) of the root nodules was reduced by the flooding treatment, while there was no significant difference in specific nitrogenase activity (SNA) (μmol C₃H₄ g⁻¹DW h⁻¹) between the irrigated and flooded nodules (Table 3). The bleeding sap rate was much lower in the flooded plants than in the irrigated ones (Table 4). Although the ureide-N concentration of the xylem sap was higher in the flooded plants than in the irrigated ones (Table 3), there was no significant difference in the relative ureide-N, α-amino-N and nitrate-N to total N concentration between the irrigated and flooded plants (Table 6).

The effects of the vaseline treatment on plant growth, root nodulation, TNA, SNA, bleeding sap rate and the xylem bleeding sap components were different from the effects in the irrigated ones. Under irrigated conditions, vaseline treatment did not restrict these characteristics in contrast to non-vaseline treatment (Tables 2, 3, 4, 5 and 6). Therefore, it seemed that the treatment had no direct effect on the growth of the plants. Under the flooded conditions, although the plant growth and the root nodulation were not restricted statistically by vaseline treatment, the shoot N concentration of the vaseline-treated plants was 50% of that in the plants without vaseline treatment (Table 2). Moreover, we observed more black roots and less green leaves in the plants with vaseline treatment than in those without vaseline treatment (data not shown). Nodule activity was greatly reduced by vaseline treatment, i.e., the TNA and SNA in the plants treated with vaseline was only 0.27%, and 1.02% in the plants without vaseline treatment (Table 3). Although the effect of the vaseline treatment on the bleeding sap rate was not detected statistically, the rate was 43% of the plants without vaseline treatment (Table 4). Furthermore, the effect of the vaseline treatment on the N concentration and the relative N in the xylem sap was detected in the flooded plants. The ureide-N concentration declined to only 0.86% of the plants without vaseline treatment (Table 5), consequently, the relative ureide-N was 1.44% (Table 6).
Discussion

1. Process of secondary aerenchyma formation in the root nodules

In soybean plants, lenticels were formed on the surface of the root nodules, and secondary aerenchyma developed in the lenticels (Fig. 1). As the soybean plants grew, a secondary meristem girdled the sclerenchyma.
Table 2. Effect of flooding and vaselinel treatments on nodulation and plant growth of soybean.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Nodule No. (no. plant&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Nodule dry weight (g plant&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Root dry weight (g plant&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Shoot dry weight (g plant&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Total dry weight (g plant&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Shoot N concentration (mg g&lt;sup&gt;-1&lt;/sup&gt;DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IN</td>
<td>188.6 ± 33.2 a*</td>
<td>0.87 ± 0.19 a</td>
<td>3.25 ± 0.41 a</td>
<td>13.04 ± 1.44 a</td>
<td>17.17 ± 1.87 a</td>
<td>26.11 ± 1.46 a</td>
</tr>
<tr>
<td>IV</td>
<td>222.5 ± 43.9 a</td>
<td>0.73 ± 0.12 a</td>
<td>2.72 ± 0.31 a</td>
<td>11.42 ± 0.51 a</td>
<td>14.87 ± 0.58 a</td>
<td>24.27 ± 1.27 a</td>
</tr>
<tr>
<td>FN</td>
<td>80.0 ± 15.1 b</td>
<td>0.57 ± 0.05 ab</td>
<td>1.42 ± 0.17 b</td>
<td>5.17 ± 0.74 b</td>
<td>7.16 ± 0.90 b</td>
<td>25.15 ± 2.20 a</td>
</tr>
<tr>
<td>FV</td>
<td>54.3 ± 13.4 b</td>
<td>0.28 ± 0.30 b</td>
<td>1.04 ± 0.09 b</td>
<td>4.82 ± 0.56 b</td>
<td>6.14 ± 0.45 b</td>
<td>12.56 ± 0.38 b</td>
</tr>
</tbody>
</table>

* : Means followed by the same letter within a column are not significantly different, as determined by Tukey-Kramer (P<0.01). Means±SD, n=7 (IN, FN, FV) and 6 (IV).

Table 3. Effect of flooding and vaselinel treatments on nodule nitrogenase activity of soybean.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total nitrogenase activity (TNA) (µmol C&lt;sub&gt;2&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt; plant&lt;sup&gt;-1&lt;/sup&gt; h&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Specific nitrogenase activity (SNA) (µmol C&lt;sub&gt;2&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt; g&lt;sup&gt;-1&lt;/sup&gt;DW h&lt;sup&gt;-1&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IN</td>
<td>81.03 ± 13.69 a*</td>
<td>71.81 ± 16.86 a</td>
</tr>
<tr>
<td>IV</td>
<td>60.83 ± 11.83 a</td>
<td>83.54 ± 12.16 a</td>
</tr>
<tr>
<td>FN</td>
<td>37.38 ± 8.83 b</td>
<td>65.44 ± 13.78 a</td>
</tr>
<tr>
<td>FV</td>
<td>0.10 ± 0.03 c</td>
<td>0.67 ± 0.42 b</td>
</tr>
</tbody>
</table>

* : Means followed by the same letter within a column are not significantly different, as determined by Tukey-Kramer (P<0.01). Means±SD, n=7 (IN, FN, FV) and 6 (IV).

Table 4. The relative ureide-N, α-amino-N and nitrate-N to total N concentration in the xylem bleeding sap collected from soybean plants.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Relative-N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IN</td>
<td>70.09 ± 4.21 a*</td>
</tr>
<tr>
<td>IV</td>
<td>67.74 ± 3.62 a</td>
</tr>
<tr>
<td>FN</td>
<td>74.34 ± 5.32 a</td>
</tr>
<tr>
<td>FV</td>
<td>1.44 ± 0.23 b</td>
</tr>
</tbody>
</table>

* : Means followed by the same letter within a column are not significantly different, as determined by Tukey (P<0.01). Means±SD, n=7.

Table 5. Concentrations of uride-N, α-amino-N, and nitrate-N in the xylem bleeding sap collected from soybean plants.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ureide-N (µ mol ml&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>α-amino-N (µ mol ml&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Nitrate-N (µ mol ml&lt;sup&gt;-1&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IN</td>
<td>8.43 ± 1.91 b*</td>
<td>3.40 ± 0.93 b</td>
<td>0.20 ± 0.05 b</td>
</tr>
<tr>
<td>IV</td>
<td>9.99 ± 4.63 b</td>
<td>4.29 ± 1.83 b</td>
<td>0.37 ± 0.08 ab</td>
</tr>
<tr>
<td>FN</td>
<td>20.86 ± 5.18 a</td>
<td>6.96 ± 2.23 b</td>
<td>0.24 ± 0.05 ab</td>
</tr>
<tr>
<td>FV</td>
<td>0.18 ± 0.14 c</td>
<td>11.74 ± 2.13 a</td>
<td>0.49 ± 0.23 a</td>
</tr>
</tbody>
</table>

* : Means followed by the same letter within a column are not significantly different, as determined by Tukey (P<0.01). Means±SD, n=7.

and a secondary aerenchyma layer covered the whole nodule; consequently, the epidermis came off in both the irrigated and flooded nodules. From the process of lentil formation in the woody plant stems (Eames and MacDaniels, 1947), we propose that the morphological changes in the root nodules of soybean plants are as follows. First, the secondary meristem, which is partly active, differentiates into secondary aerenchyma. Second, the lentilicel is formed when the development of the aerenchyma breaks the epidermis. Third, the secondary meristem is active in a whole nodule, and its surface is covered with the aerenchyma. Last, the epidermis comes off.

Secondary aerenchyma closely resembled the structures of lenticels and the complementary cells developing in the stems of woody plants (Eames and MacDaniels, 1947). Therefore, we suggest that the function of the lenticels in the nodules is to enhance the gaseous diffusion between nodules and soils under the irrigated conditions, as lenticels in woody plant stems have the function of permitting the free movement of gas into and out of the stems. Especially under the flooded conditions, the nodules showed an earlier and greater development of secondary aerenchyma, and the plants had a continuous aerenchymatous pathway from the hypocotyl above the water level to the flooded roots and nodules. These responses seem to enhance the gas exchange between the nodules and the atmosphere immediately above the water surface.

2. Effect of flooding and vaselinel treatments on nodule activity

In plants without vaselinel treatment, the root nodulation and TNA were decreased by flooding stress (Tables 2 and 3). However, the bacteroid zone in the transverse sections of these root nodules was pink in color, due to the presence of leghemoglobin (Fig. 1), and SNA was at the same level in the flooded plants as in the irrigated ones (Table 3). Although the potential to reduce acetate at ambient oxygen concentrations in Erlenmeyer flasks was not correctly indicative of the ability to fix nitrogen under hypoxic conditions in flooded soils, it was
clear that nodule activity was highly maintained under flooded conditions, because there was no difference in the relative ureide-N in the xylem bleeding sap between the irrigated and flooded plants (Table 6). Furthermore, the shoot N concentration was at the same level in the flooded plants as in the irrigated ones (Table 2), indicating that the flooded nodules supplied the shoot with the fixed nitrogen. The ureide-N concentration of the xylem sap was much higher in the flooded plants than in the irrigated ones (Table 5). This cause may not be that root nodule activity was enhanced by flooding, but that the bleeding sap rate was much lower in the flooded plants than in the irrigated ones (Table 4). Under flooded conditions, however, TNA, SNA, the ureide-N concentration and the relative ureide-N sharply declined, and nodule activity was barely present when the vaseine treatment was applied to inhibit the entry of oxygen into the secondary aerenchyma (Tables 3, 5 and 6). There was a decrease in shoot N concentration in the flooded plants with vaseine treatment in contrast to the flooded plants without vaseine treatment (Table 2). This result suggests that there were large differences in nitrogen fixation activity between the flooded plants with and without vaseine treatment, as indicated by the acetylene reduction data and ureide data (Tables 3 and 5).

In flooding-tolerant leguminous plants *Lotus uliginosus* (James and Sprent, 1999) and *Viminaria juncea* (Walker et al., 1983), secondary aerenchyma developed well in the stem, roots, and nodules, and nitrogenase activity was highly maintained under flooded conditions, so that it was suggested that atmospheric oxygen entering through lenticels was supplied to the nodules via the secondary aerenchyma. Lenticles have the function as the primary entry points for oxygen diffusion to oxygen-stressed roots in several plants (Hook and Scholten, 1978). It was reported that putting lanolin on the stem lenticels above the water level restricted the root extension rate, root fresh mass and internal root aeration in woody species under hypoxic conditions (Armstrong, 1968; Jackson and Attwood, 1996). In aquatic plants, oxygen transport from aerial parts to flooded ones is enhanced by mass-flow system (Dacey, 1980; Dacey and Klug, 1982a; Dacey and Klug, 1982b; Dacey, 1987). We also believe that secondary aerenchyma and hypertrophied lenticels in soybean plants have such functions, because the aerenchyma developed in the hypocotyl, roots, and nodules, and because nodule activity was remarkably restricted by blocking of the oxygen transport system.

Flooding-induced or nitrogen-acclerated oxygen deficiency in the rhizosphere generally restricts root nodule activity and nodulation in soybean plants (Bacanamwo and Purcell, 1999; Sung, 1993; Huang and LaRue, 1985; Sugimoto, 1994). Our results were similar to those reported previously in relation to the reduction of root nodulation, while there was an obvious difference between them with respect to nodule activity. When low nodule activity was measured within a short term (about 1 week) after oxygen deficiency treatment in the previous studies, the stem, root, and nodules of the soybean plants might not yet possess well-developed secondary aerenchyma. Even in nodules, it took 2 weeks to possess well-developed aerenchyma. In cases following long-term (over 1 week) oxygen deficiency treatment, the cultivars might show a low ability to form secondary aerenchyma. Indeed, a varietal difference in the degree of secondary aerenchyma development was detected in soybean plants (Shimamura et al., 2001b). Therefore, not enough oxygen to maintain nodule activity seemed to be transported in previous studies.

In this study, although nodule activity was maintained even under flooded conditions, the growth of flooded plants was lower than that of the irrigated plants. Secondary aerenchyma in soybean plants seems to be inferior to that of the flood-tolerant leguminous plant *Lotus uliginosus* (James and Crawford, 1998; James and Sprent, 1999) in oxygen transport potential. Additionally, conductive resistance to water absorption may increase, and nutrient uptake may be restricted, because the bleeding sap rate greatly declined due to the flooding treatment (Table 4). However, there is little information available on secondary aerenchyma functions (Jackson and Armstrong, 1999), so further investigation of the aerenchyma is necessary to improve the flooding tolerance in soybean plants.

**Acknowledgements**

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**References**


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