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# Secondary Aerenchyma Formation and Root Growth Response of Soybean (*Glycine max*) Seedlings under Flooded Conditions

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## Abstract

Most of wetland species can develop their roots into flooded soils because of the presence of longitudinal aerenchyma channels that facilitates oxygen diffusion from shoot to root tips. This tissue is called as primary aerenchyma because it is formed in fundamental tissues. It is also formed in rice root, consequently rice plants can grow well in paddy field. On the other hand, it is considered that most of mesophytes such as field crops cannot grow under flooded and excess moisture conditions because of their low ability to develop aerenchyma. However, we found that soybean plants could develop aerenchyma and grow well in flooding compared with other leguminous crops such as *Vigna* and *Phaseolus* species. This type of aerenchyma, which is consisted of white spongy tissue filled with gas space and is differentiated from secondary meristem (phellogen), is called as secondary aerenchyma. It plays a role in supplying oxygen from the aerial parts to the flooded roots and nodules. In our recent study, it was observed that there was a wide range of varietal differences on secondary aerenchyma formation and adventitious root development in soybean seedlings under flooding. Although the research for secondary aerenchyma in soybean plants is on the way, it may be able to breed soybean varieties with flooding tolerance.

**Keywords:** anaerobiosis, flooding, secondary aerenchyma, soybean, water logging

## Introduction

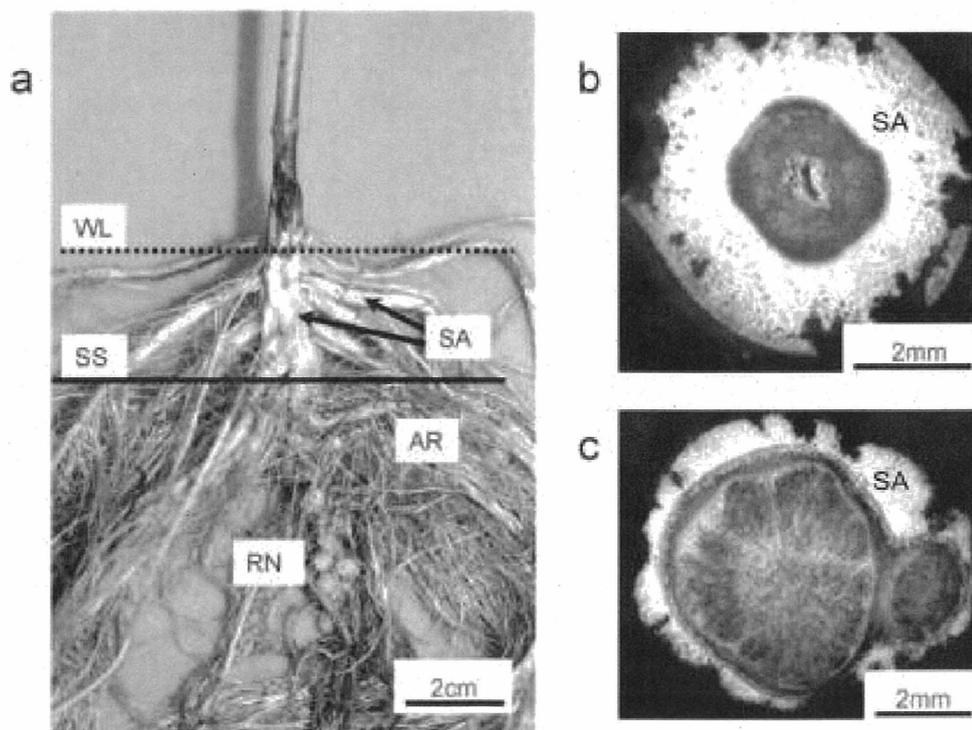
Flooding is a major problem in many areas of the world and most of mesophytes such as field crops are susceptible to the stress. Therefore, an understanding of the morphological mechanisms of flooding tolerance is important for developing flood-tolerant genotypes. Aerenchyma formation is one of the morphological changes that occurs in plants grown under flooded and hypoxic conditions and it is thought to enhance the internal diffusion of atmospheric and photosynthetic oxygen from the aerial parts to the flooded roots, allowing the roots to maintain aerobic respiration (Armstrong, 1979). The two types of aerenchyma are classified. One is cortical (lysigenous or schizogenous) aerenchyma formed in the roots of rice, maize, barley, wheat and some *Rumex* species. The other is white spongy tissue filled with gas spaces and is formed in the stem, hypocotyl, tap root, adventitious roots and root nodules of *Glycine soja*, *Sesbania rostrata*, *Lotus uliginosus* and *Viminaria juncea* grown under flooded conditions. It is differentiated from secondary meristem (phellogen) and is called secondary aerenchyma (Jackson and Armstrong, 1999). Secondary aerenchyma seems to play a role in supplying oxygen to roots and nodules. In

contrast to cortical aerenchyma, however, there is little information about the morphological and anatomical processes of secondary aerenchyma formation and its function.

Soybean is generally susceptible to flooding, and its growth and yield are negatively affected by the stress. Arikado (1954) suggested that soybean had low flooding tolerance because of its low ability to develop aerenchyma in the shoot and roots. However, some soybean genotypes can form lysigenous aerenchyma in the root cortex (Bacanamwo and Purcell, 1999) and secondary aerenchyma in the hypocotyl and tap root of seedlings grown under flooded conditions (Mochizuki *et al.*, 2000). In this paper, we introduced our recent studies of the morphological and anatomical processes of secondary aerenchyma formation and its function as an oxygen pathway from the aerial parts to flooded roots, and we described here the relationship between aerenchyma formation and the flooding tolerance of soybean.

### Secondary aerenchyma formation in flooded soybean

We investigated secondary aerenchyma formation in soybean seedlings grown under flooded conditions (Shimamura *et al.*, 2003). As a result, within 3 weeks of flooding, large volumes of secondary aerenchyma developed in flooded hypocotyl, tap root, adventitious roots and nodules (Fig. 1). This tissue has large intercellular spaces and consists of living cells whose walls do not become suberized, whereas phellem has no intercellular spaces and consists of dead cells whose walls become suberized. It is differentiated from secondary meristem (phellogen) and is homologous with cork tissue (Fig. 2). Cells were exposed to the outside through lenticels in hypocotyl and roots where epidermis and cortex layers were collapsing. The outer part of the nodules was covered with a layer of secondary aerenchyma that was relatively thinner in the nodules than in the hypocotyl and roots. Nodules attached to the face of roots had pink infected tissues, which suggested the presence of leghemoglobin and the survival of nodules under flooded conditions.



a, root system; b, transverse section of an adventitious root; c, transverse section of a nodule. WL, water level; SS, soil surface; AR, adventitious roots; RN, root nodules; SA, secondary aerenchyma.

Figure 1. Root system and secondary aerenchyma development in soybean seedlings after 3 weeks of flooding.

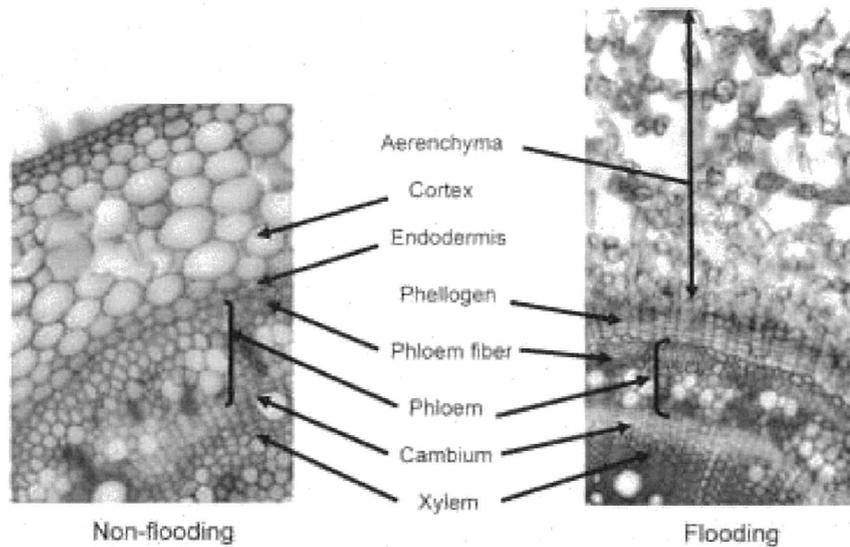
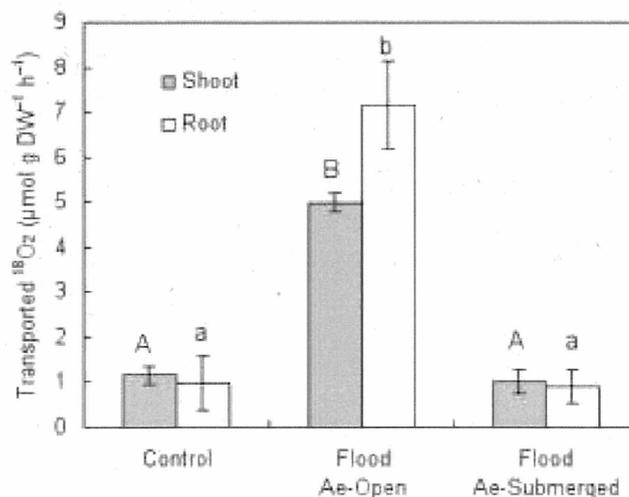


Figure 2. Cross sections of young hypocotyl in soybean.

### Function of secondary aerenchyma in flooded soybean

Oxygen dynamics in aerenchymatous stems were investigated using Clark-type  $O_2$  microelectrodes, and  $O_2$  transport to roots was evaluated using stable-isotope  $^{18}O_2$  as a tracer, for plants with shoots in air and roots in flooded sand or soil (Shimamura et al., 2010).

After introducing  $^{18}O_2$  gas via the stem lenticels, significant  $^{18}O_2$  enrichment in water extracted from roots after 3 h was confirmed, suggesting that transported  $O_2$  sustained root respiration. In contrast, slight  $^{18}O_2$  enrichment was detected 3 h after treatment of stems that lacked aerenchyma and lenticels. The results showed that hypertrophic lenticels in the lower stem of soybean, just above the water surface, are entry points for  $O_2$ , and these connect to aerenchyma and enable  $O_2$  transport into roots in flooded soil.



Flood Ae-Open, aerenchyma and lenticels above the water; Flood Ae-Submerged, submerged aerenchyma and lenticels. Values are the mean  $\pm$  s.e. Means in the shoot followed by the same upper-case letter and means in the root followed by the same lower-case letter do not differ significantly ( $P < 0.01$ , Tukey-Kramer's test).

Figure 3. Volume of  $^{18}O_2$  transported from the stem to the roots with or without exposure of the aerenchyma to the  $^{18}O_2$  gas in each treatment.

## Interspecific difference in secondary aerenchyma formation of flooded leguminous crops

We investigated the secondary aerenchyma formation in hypocotyls just below the soil surface of young seedlings in wild soybean and six summer leguminous crops grown under upland and flooded conditions for 14 days (Mochizuki *et al.*, 2000).

Under the upland conditions, secondary aerenchyma was scarcely observed in any species. Under the flooded conditions, however, there was an interspecific difference in the secondary aerenchyma formation. Secondary aerenchyma area per transverse section of hypocotyls under the flooded conditions was largest in soybean 'Asoaogari'; followed by soybean 'Akisengoku', wild soybean 'D5', cowpea 'Sanjakusasage', mung bean 'Bundomame', and mung bean 'Acc. 7703', and those in the other crops were less than 1 mm<sup>2</sup> (Table 1). Since a significant positive correlation was found between dry weight ratio (the ratio of dry weight of the aerial part under the flooded conditions to that under the upland conditions) and secondary aerenchyma area ( $r=0.738^*$ ), it is suggested that the ability of secondary aerenchyma formation is related to the flooding tolerance in leguminous crops.

Table 1. Secondary aerenchyma area of hypocotyl and dry weight ratio in summer leguminous crops

Scientific name	Variety	Secondary aerenchyma area (mm <sup>2</sup> )	Secondary aerenchyma ratio (%)	Dry weight ratio (%)
<i>Glycine soja</i> Sieb. et Zucc.	D5	4.37±0.26b	173.01±15.88a	93±18.2a
<i>G. max</i> Merr.	Asoaogari	9.77±1.84a	146.19±35.76a	84±18.2ab
	Akisengoku	7.80±1.92a	71.14±16.40b	97±18.5a
<i>Vigna angularis</i> Ohwi et Ohashi	Acc.7703	1.52±0.92cd	59.45±40.50bc	52± 7.8bc
	Bundomame	1.97±0.45cd	58.03± 2.16bc	51± 9.3bc
<i>V. mungo</i> Hepper	Sanjakusasage	2.63±1.09bc	27.06± 7.96cd	69±25.4abc
	Acc.3061	0.49±0.05cd	12.50± 0.99d	66± 6.1abc
<i>V. radiata</i> R. Wilczek	Acc.3083	0.31±0.21d	9.89± 7.50d	70± 6.5ab
	Hayateshouzu	0.48±0.18cd	10.73± 3.20d	53±12.6bc
<i>V. sinensis</i> Endle.	Erimoshouzu	0.49±0.27cd	8.50± 0.31d	49±17.0bc
<i>Phaseolus vulgaris</i> L.	Dover	0.85±0.14cd	7.36± 1.67d	35± 3.7c

Secondary aerenchyma ratio is the percentage of secondary aerenchyma area to total area. Dry weight ratio is the percentage of dry weight of the aerial part under flooded conditions to that under upland conditions. Values followed by the same letter in each column are not significantly different at  $P<0.05$  by Duncan's multiple test.

## Effects of hypoxia on dry matter production and root development in soybean and wild soybean cultivars

Using 91 soybean and wild soybean cultivars, effects of hypoxia on dry matter production and root development were examined (Sakazono *et al.*, 2011). Seedlings of eight days after sowing were grown in solution culture with (control) and without (hypoxia) O<sub>2</sub>. Seven days after treatment, dry weight, root characters and hypocotyl diameter were measured. Root characters were measured with Win RHZO (Regent Instruments Inc., Quebec, Canada). In this experiment, we use the ratio of hypocotyl diameter (ratio of hypocotyl diameter in hypoxia to that of control) as an indicator of secondary aerenchyma development in hypoxia.

Effect of hypoxia was higher in root growth than shoot growth. Total root length, root surface area and root volume were decreased by hypoxia, whereas average root diameter and

hypocotyl diameter were increased. However, in every trait including hypocotyl diameter, there was wide variation among cultivars.

Table 2. Effects of hypoxia on dry matter production and root development in soybean and wild soybean cultivars

Trait	Treatment	Average ( Max. - Min. )
Total dry weight (mg)	Hypoxia	164.6 ( 262.8 - 19.5 )
	Cont.	188.9 ( 359.3 - 19.8 )
	Ratio (hypoxia / cont.)	0.89 ( 1.08 - 0.65 )
Root dry weight (mg)	Hypoxia	65.6 ( 101.1 - 8.5 )
	Cont.	93.3 ( 162.7 - 9.8 )
	Ratio (hypoxia / cont.)	0.73 ( 1.02 - 0.55 )
Shoot dry weight (mg)	Hypoxia	99.1 ( 185.7 - 11.0 )
	Cont.	95.8 ( 196.6 - 10.0 )
	Ratio (hypoxia / cont.)	1.05 ( 1.29 - 0.81 )
Total root length (cm)	Hypoxia	159.2 ( 405.6 - 58.2 )
	Cont.	477.7 ( 916.1 - 71.5 )
	Ratio (hypoxia / cont.)	0.37 ( 0.87 - 0.16 )
Root surface area (cm <sup>2</sup> )	Hypoxia	31.0 ( 56.9 - 7.3 )
	Cont.	79.7 ( 136.8 - 9.2 )
	Ratio (hypoxia / cont.)	0.43 ( 0.68 - 0.23 )
Root volume (cm <sup>3</sup> )	Hypoxia	0.50 ( 0.87 - 0.07 )
	Cont.	1.08 ( 1.71 - 0.10 )
	Ratio (hypoxia / cont.)	0.46 ( 0.93 - 0.30 )
Average root diameter (mm)	Hypoxia	0.66 ( 1.62 - 0.39 )
	Cont.	0.53 ( 0.69 - 0.38 )
	Ratio (hypoxia / cont.)	1.21 ( 1.69 - 0.96 )
Hypocotyle diameter (mm)	Hypoxia	3.69 ( 4.85 - 1.30 )
	Cont.	2.45 ( 3.35 - 1.06 )
	Ratio (hypoxia / cont.)	1.51 ( 2.01 - 1.13 )

## Conclusions

It is clearly that the secondary aerenchyma formed in soybean plays a role in supplying oxygen from aerial parts to roots and nodules, and there are interspecific and intraspecific differences in the secondary aerenchyma formation. Although the research for secondary aerenchyma in soybean plants is on the way, it may be able to breed soybean varieties with flooding tolerance.

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