



Aerenchyma formation in crop species: A review

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ABSTRACT

Flooding is a major problem in many crop areas around the world. However, many wetland plant species can expand their roots into flooded soils because of the presence of longitudinal aerenchyma channels that facilitate oxygen diffusion from the shoots to the root tips. Aerenchyma also forms in rice roots, allowing rice plants to grow well in flooded paddy fields. Aerenchyma formation therefore helps plants to survive flooding. “Primary aerenchyma” forms in the roots of some cereal crops such as rice, maize, barley and wheat. “Secondary aerenchyma” forms in the stem, hypocotyl, tap root, adventitious roots, and root nodules of some legume crops such as soybean. This paper reviews the recent progress in the study of aerenchyma formation, and highlights the role that primary aerenchyma in cereal crops and secondary aerenchyma in soybean can play in improving their tolerance to flooding.

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1. Introduction

Flooding is a major problem in many areas of the world, and most crop species are susceptible to flooding stress. Dudal (1976) estimated that 12% of the world's soils are likely to suffer from excess water at some time. Considerable transient and persistent flooding of the soil and deeper submergence of crops occurs in many rainfed farmlands (Jackson, 2004). Increasing the flooding tolerance of crop species would therefore help to increase and stabilize crop production around the world. To this end, utilizing diverse genetic resources for breeding is a potentially important strategy (Setter and Waters, 2003).

The principal cause of damage to plants grown in flooded soil is an inadequate supply of oxygen to the submerged tissues. In addition, the depletion of oxygen in the soil results in a shift from aerobic to anaerobic microbial processes, which leads to the reduction of oxidized compounds and the production of phytotoxic ions such as Mn^{2+} , Fe^{2+} , and S^{2-} (Blom, 1999; Ponnamperna, 1984). Thus, internal O_2 transport from shoots to roots is essential to the survival and efficient functioning of roots. Consequently, any mechanism that can supply oxygen to the flooded tissues is beneficial under long-term flooding, such as the development of aerenchyma to facilitate gas diffusion to the roots (Armstrong, 1979).

Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; MT, metallothionein; RBOH, respiratory burst oxidase homolog; ROL, radial O_2 loss; ROS, reactive oxygen species.

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Aerenchyma formation is a morphological change that occurs in plants grown under flooded or hypoxic conditions, and it is known 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). Two types of aerenchyma have been identified: One is cortical aerenchyma (i.e., primary aerenchyma), which forms in the roots of rice (*Oryza sativa*), maize (*Zea mays*), barley (*Hordeum vulgare*), and wheat (*Triticum aestivum*) (Armstrong, 1979; Evans, 2003; Jackson and Armstrong, 1999; Nishiuchi et al., 2012). The other is a white, spongy tissue filled with gas spaces that forms in the stem, hypocotyl, tap root, adventitious roots, and root nodules of legumes such as soybean (*Glycine max*), wild soybean (*Glycine soja*), *Sesbania rostrata*, and *Viminaria juncea* grown under flooded conditions (Arikado, 1954; Mochizuki et al., 2000; Saraswati et al., 1992; Walker et al., 1983). Because it differentiates centrifugally from phellogen and is secondary tissue, it is called “secondary” aerenchyma (Arber, 1920; Fraser, 1931; Jackson and Armstrong, 1999; Williams and Barber, 1961).

In the present review, we summarize the recent progress in understanding both primary aerenchyma in cereal crops and secondary aerenchyma in soybean plants. Aerenchyma formation may contribute to long-term avoidance of anoxia under flooded conditions, and improve crop growth and yield.

2. Aerenchyma formation in cereal crops

Two main types of primary aerenchyma have been recognized: schizogenous and lysigenous aerenchyma (Evans, 2003; Jackson and Armstrong, 1999). Schizogenous aerenchyma develops gas

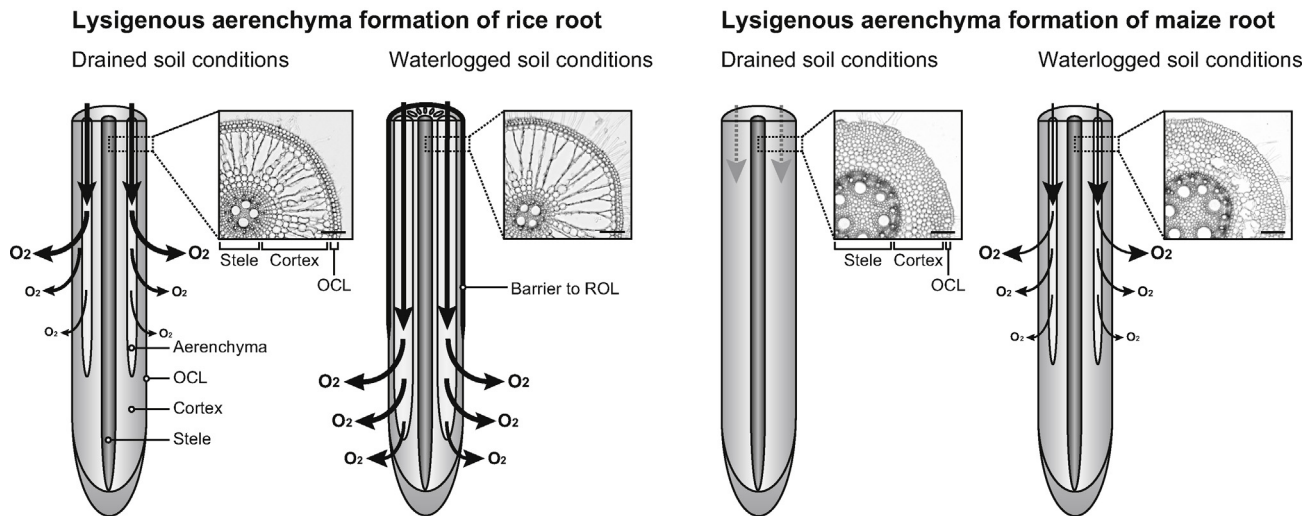


Fig. 1. Lysigenous aerenchyma formation in cereal crop species. Lysigenous aerenchyma forms in roots as a result of the death and subsequent lysis of cortical cells, thereby creating gas spaces. In rice roots, lysigenous aerenchyma is constitutively formed under drained soil conditions, and its formation is enhanced under flooded soil conditions. Longitudinal diffusion of O_2 toward the root apex can be further enhanced by the induction of a barrier to radial O_2 loss (ROL) in the outer cell layers (OCL) of the roots. In maize and other dryland cereal crops, lysigenous aerenchyma does not form under drained soil conditions, but is induced by soil waterlogging. Scale bars: 100 μm .

spaces through cell separation and differential cell expansion without cell death. By contrast, lysigenous aerenchyma is formed by the death and subsequent lysis of some cells in cereal crops such as rice (Jackson et al., 1985b), maize (Drew et al., 1981), wheat (Trought and Drew, 1980), and barley (Arikado and Adachi, 1955). In the roots, lysigenous aerenchyma forms in the cortex (Fig. 1), whereas in the stems, it can form in the cortex and the pith cavity (Armstrong, 1979). The lysigenous aerenchyma forms specifically in the root cortex, but not in other root tissues such as the epidermis, hypodermis, endodermis, or stele (Evans, 2003; Jackson and Armstrong, 1999). The contents of the cells that form the lysigenous aerenchyma are completely digested, leaving just the cell wall surrounding the gas space (Fig. 1; Evans, 2003). Yet even in the cortex, some cells remain intact and form radial bridges immediately adjacent to the lysing cells to help the root retain structural rigidity (Fig. 1; Armstrong and Armstrong, 1994).

In some wetland plant species, root lysigenous aerenchyma forms constitutively in drained soil (Jackson et al., 1985b; Visser and Bögemann, 2006), and its formation is further enhanced by flooding of the soil (Fig. 1; Abiko et al., 2012; Colmer et al., 2006; Shiono et al., 2011). In rice, aerenchyma formation under aerobic conditions begins at the apical parts of the roots and gradually develops during root elongation, while under stagnant deoxygenated conditions is enhanced in all parts of the roots (Shiono et al., 2011). Moreover, longitudinal diffusion of O_2 towards the root apex can be further enhanced by the induction of a barrier to radial O_2 loss (ROL) that minimizes the loss of O_2 into the surrounding environment (Fig. 1; Nishiuchi et al., 2012). On the other hand, in dryland species such as maize, wheat, and barley, lysigenous aerenchyma does not form under well-drained soil conditions, but is induced by poor aeration (McDonald et al., 2001; McPherson, 1939; Trought and Drew, 1980).

2.1. Physiology of lysigenous aerenchyma in cereal crops

Ethylene is involved in the formation of lysigenous aerenchyma (Drew et al., 2000; Jackson and Armstrong, 1999). In maize roots, stimulation of ethylene biosynthesis by hypoxia resulted from increased activities of two enzymes involved in the ethylene biosynthesis pathway: 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and ACC oxidase (Fig. 2; He et al., 1996a). The

treatment of maize roots with inhibitors of ethylene action (e.g., silver ions) or of ethylene biosynthesis (e.g., aminoethoxyvinylglycine, aminoxyacetic acid, cobalt chloride) effectively blocks aerenchyma formation under hypoxic conditions (Drew et al., 1981; Jackson et al., 1985a; Konings, 1982). Moreover, the production of lysigenous aerenchyma can be induced by treatment with

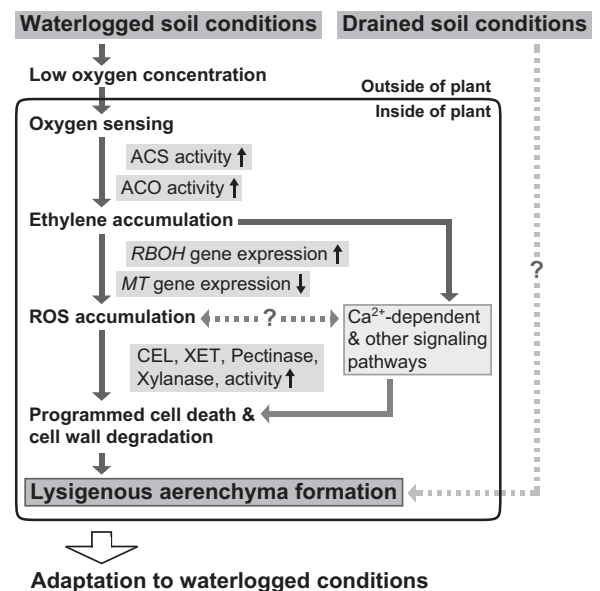


Fig. 2. Model of lysigenous aerenchyma formation in cereal crop species. Waterlogging promotes biosynthesis and accumulation of ethylene by enhancing the activities of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS) and ACC oxidase (ACO), followed by induction of expression of *respiratory burst oxidase homolog* (*RBOH*) gene. The *RBOH* activity leads to the production and accumulation of O_2^- in the apoplast, which is spontaneously or enzymatically converted to H_2O_2 . In the cortical cells, decreased expression of *metallothionein* (*MT*) gene prevents scavenging of reactive oxygen species (ROS), thereby leading to higher accumulation of ROS, which activates programmed cell death and lysis of the cortical cells. Ca^{2+} -dependent and other signaling pathways are also activated by ethylene accumulation, and this leads to the activation of some cell wall degradation enzymes, including cellulase (CEL), xyloglucan endotransglycosylase (XET), pectinase, and xylanase. The relationship between ROS and these signaling pathways is unclear. Thus far, no information about the molecular basis of constitutive aerenchyma formation is available.

ethylene, even under aerobic conditions (Drew et al., 1981; Jackson et al., 1985a). These observations indicate that ethylene triggers inducible aerenchyma formation.

Ethylene-responsive aerenchyma formation is affected by chemical inhibitors or stimulators of programmed cell death and other signaling pathways. Signaling pathways that depend on the heterotrimeric G-protein, phospholipase C, inositol 1,4,5-trisphosphate, and Ca^{2+} are involved in the formation of lysigenous aerenchyma in maize roots (He et al., 1996b). Subbaiah et al. (1994) proposed that under oxygen deprivation, Ca^{2+} is released from mitochondria into the cytoplasm. The elevated cytosolic Ca^{2+} provokes subsequent activation of kinases and phosphatases during aerenchyma formation (Subbaiah and Sachs, 2003). These Ca^{2+} -dependent signaling pathways may induce the expression of the genes responsible for aerenchyma formation (Fig. 2; Drew et al., 2000; Subbaiah and Sachs, 2003).

In the final stage of lysigenous aerenchyma formation, the cell wall is degraded enzymatically (Fig. 2). Changes in esterified and de-esterified pectins in the cell wall of cells in the maize cortex are observed during cell death (Gunawardena et al., 2001). Subsequently, the cell wall is degraded by the combined action of pectolytic, xylanolytic, and cellulolytic enzymes (Evans, 2003; Jackson and Armstrong, 1999). The activity of cellulase is increased by treatment with ethylene, okadaic acid, or reagents that increase intracellular Ca^{2+} , and is decreased by treatment with K252a and inhibitors of Ca^{2+} increase (He et al., 1996b). The expression of a gene that encodes xyloglucan endotransglycosylase, which is a cell-wall-loosening enzyme, is induced in maize roots under flooding, and this induction is inhibited by treatment with an ethylene biosynthesis inhibitor (Saab and Sachs, 1996). Treatment with an ethylene biosynthesis inhibitor also prevents the formation of lysigenous aerenchyma, suggesting that the induction of xyloglucan endotransglycosylase production in response to ethylene is involved in aerenchyma formation through cell-wall modification (Saab and Sachs, 1996).

2.2. Gene expression during the formation of lysigenous aerenchyma in cereal crops

Reactive oxygen species (ROS) are key factors in signal transduction stimulated by abiotic stress in plants (Suzuki et al., 2011). Respiratory burst oxidase homolog (RBOH), a plant homolog of gp91phox in mammalian NADPH oxidase, has an important role in ROS-mediated signaling, such as in the defense response, programmed cell death, and development in plants (Torres and Dangel, 2005). In rice, ethylene-induced, H_2O_2 -mediated epidermal cell death during the emergence of adventitious roots is regulated by RBOH (Steffens and Sauter, 2009). Moreover, expression of the gene encoding metallothionein-2b (MT2b), a possible scavenger of ROS, is down-regulated in response to the presence of ethylene in epidermal cells, thereby amplifying the accumulation of H_2O_2 produced by RBOH (Steffens and Sauter, 2009). It has been also reported that down-regulation of *MT2b* or the application of H_2O_2 promotes aerenchyma formation in the internodes of rice stems (Steffens et al., 2011). Although the antioxidative capacity of MTs against H_2O_2 has not been directly evaluated, an animal MT has more than 100 times the antioxidant activity of reduced glutathione against hydroxyl radicals ($\cdot\text{OH}$) (Thornalley and Vasák, 1985). Rice *MT2b* and cotton (*Gossypium hirsutum*) *MT3a* also have high in vitro antioxidative capacity against $\cdot\text{OH}$ (Wong et al., 2004; Xue et al., 2009). Moreover, down-regulation of the rice *MT2b* gene causes high H_2O_2 accumulation during defense signaling (Wong et al., 2004).

Recently, Rajhi et al. (2011) identified genes associated with lysigenous aerenchyma formation in maize roots by means of microarray analysis combined with laser microdissection. A gene

encoding RBOH is strongly up-regulated in the cortical cells and slightly less strongly up-regulated in the stelar cells and the outer cell layers of maize roots under flooded conditions (Fig. 2; Yamauchi et al., 2011). By contrast, a gene encoding a *MT* is constitutively expressed in all of the cortical cells, the stelar cells, and the outer cell layers of maize roots under aerobic conditions. Under flooded conditions, *MT* is barely expressed in the cortical cells but is still highly expressed in the stelar cells and the outer cell layers (Fig. 2; Yamauchi et al., 2011). These results suggest that H_2O_2 and other ROS are scavenged by the constitutively expressed *MT* in stelar cells and the outer cell layers, whereas decreased *MT* expression in the cortical cells prevents ROS scavenging, thereby leading to greater ROS accumulation, which activates the subsequent processes involved in lysigenous aerenchyma formation in maize roots (Fig. 2). Some living cells in the cortex are important for the structural integrity of the root and for both apoplastic and symplastic transport of nutrients (Evans, 2003). Regulation of *MT* gene expression in these cells may have a role in controlling cell type-specific ROS accumulation and programmed cell death.

2.3. Lysigenous aerenchyma as an alternative trait for tolerance to flooding in cereal crops

Setter and Waters (2003) suggested that the rate of root aerenchyma formation was positively correlated with the tolerance to flooding of oats, triticale (*Triticosecale*), wheat, and barley; that is, aerenchyma forms more extensively in oats and triticale, which are more tolerant to flooding, than in wheat and barley, which are less tolerant. Furthermore, Thomson et al. (1992) reported that differences in aerenchyma formation among wheat and triticale cultivars were correlated with differences in their flooding tolerance. After 21–28 days' growth of plants in flooded soil, aerenchyma in sections of nodal roots taken at 50 mm from the root apex covered 22% of the root cross-sectional area in wheat (cv. Gamenya) and 31% in triticale (cv. Muir); and shoot fresh weights decreased by 21% in wheat but there was no effect in triticale. Aerenchyma formation of rice (cv. Calrose) covered 40–45% of the root cross-sectional area under the same conditions. Thus, Thomson et al. (1992) concluded that the poorer growth of wheat and triticale than of rice in flooded soil might be due to differences in the morphology and anatomy of the root systems, and specifically to differences in aerenchyma formation and ROL profiles.

Longitudinal diffusion of O_2 towards the root apex can be further enhanced by the induction of a barrier to ROL that minimizes the loss of O_2 into the surrounding environment before it can reach the root apex (Armstrong, 1979). This barrier may also impede the movement of soil-derived toxins and gases into the roots (Armstrong, 1979; Colmer, 2003; Greenway et al., 2006). Wetland plant species generally use these traits for adaptation to flooded environments (Colmer, 2003; Malik et al., 2011). Under field conditions, the extensiveness of aerenchyma formation in adventitious roots was positively correlated with the yield of 17 spring wheat cultivars grown under intermittent flooding, but not in barley (Setter et al., 1999). There was no significant suberization, which might be one of the components of the development of a barrier to ROL (Nishiuchi et al., 2012), at the hypodermis or the epidermis of either barley or wheat (Setter et al., 1999). These results suggest that an ability to form a continuous low-resistance pathway for O_2 diffusion from the shoot to the roots (i.e., functional aerenchyma with low ROL combined with the formation of a barrier to ROL) could be essential to improving the flooding tolerance of cereal crops.

Crossing dryland crop species with selected wild relatives from wetland habitats is a promising approach to improving flooding tolerance. For example, the flooding tolerance of *Hordeum marinum*, a wild relative of barley in the Triticeae, is associated with root

aeration traits that are superior to those of its dryland relatives (Garthwaite et al., 2003; McDonald et al., 2001). Recently, Malik et al. (2011) demonstrated that wide hybridization of flooding-tolerant *H. marinum* with flooding-intolerant wheat produced an amphiploid with a level of tolerance between those of its parents. They also showed that a barrier to ROL and higher adventitious root porosity were apparently transferred to the amphidiploid from *H. marinum*. *Zea nicaraguensis*, a flooding-tolerant wild relative of maize, was discovered in the coastal plains of Nicaragua (Iltis and Benz, 2000). Its high flooding tolerance, as well as its ability to cross with maize, makes *Z. nicaraguensis* a useful genetic resource for improving flooding tolerance in maize (Mano et al., 2007). Analysis of quantitative trait loci (QTLs) associated with constitutive aerenchyma formation using a backcross population of the maize inbred line Mi29 with *Z. nicaraguensis* suggested that this trait is highly heritable and controlled by QTLs (Mano and Omori, 2008, 2009). In addition to constitutive aerenchyma formation, *Z. nicaraguensis* can also induce the formation of a barrier to ROL in adventitious roots (Abiko et al., 2012). Several QTLs controlling flooding tolerance-related traits have been identified in *Z. nicaraguensis* (Mano et al., 2007, 2009; Mano and Omori, 2008, 2009). Thus, transfer of one or more of these QTLs into a common genetic background of maize through crossing and marker-assisted selection should help to produce a new flooding-tolerant maize line.

3. Aerenchyma formation in soybean

Six decades ago, Arikado (1954) reported that wild soybean, the ancestor of today's cultivated soybean, produced a conspicuous aerenchymatous phellem that differentiated from the phellogen in the stems, hypocotyls, adventitious roots, and nodules of this species when the plants were exposed to flooded soil 16 days after sowing. In addition, studies based on a single cultivar "Shiro-daizu" suggest that cultivated soybean has a low ability to develop aerenchyma in its stems, roots, and nodules under flooding. However, it has been reported that secondary aerenchyma covers the soybean nodule surface in flooded soils (Pankhurst and Sprent, 1975) and under hypoxic rhizosphere conditions (Parsons and Day, 1990), and is an obvious change compared with the morphology under non-flooded or non-hypoxic conditions.

Nodules, stems, hypocotyls, and adventitious roots of soybean produce high constitutive amounts of secondary aerenchyma under flooding or hypoxia (Fig. 3; Mochizuki et al., 2000; Pires et al., 2002; Rhine et al., 2010; Shimamura et al., 2002, 2003, 2010; Thomas et al., 2005; Youn et al., 2008). Therefore, many soybean cultivars possess a high ability to develop secondary aerenchyma. Development of secondary aerenchyma enhances formation of hypertrophic lenticels on the surface of stems, roots and nodules and the aerenchyma is exposed to the atmosphere through the lenticels, so white and spongy tissues are visible. This phenomenon

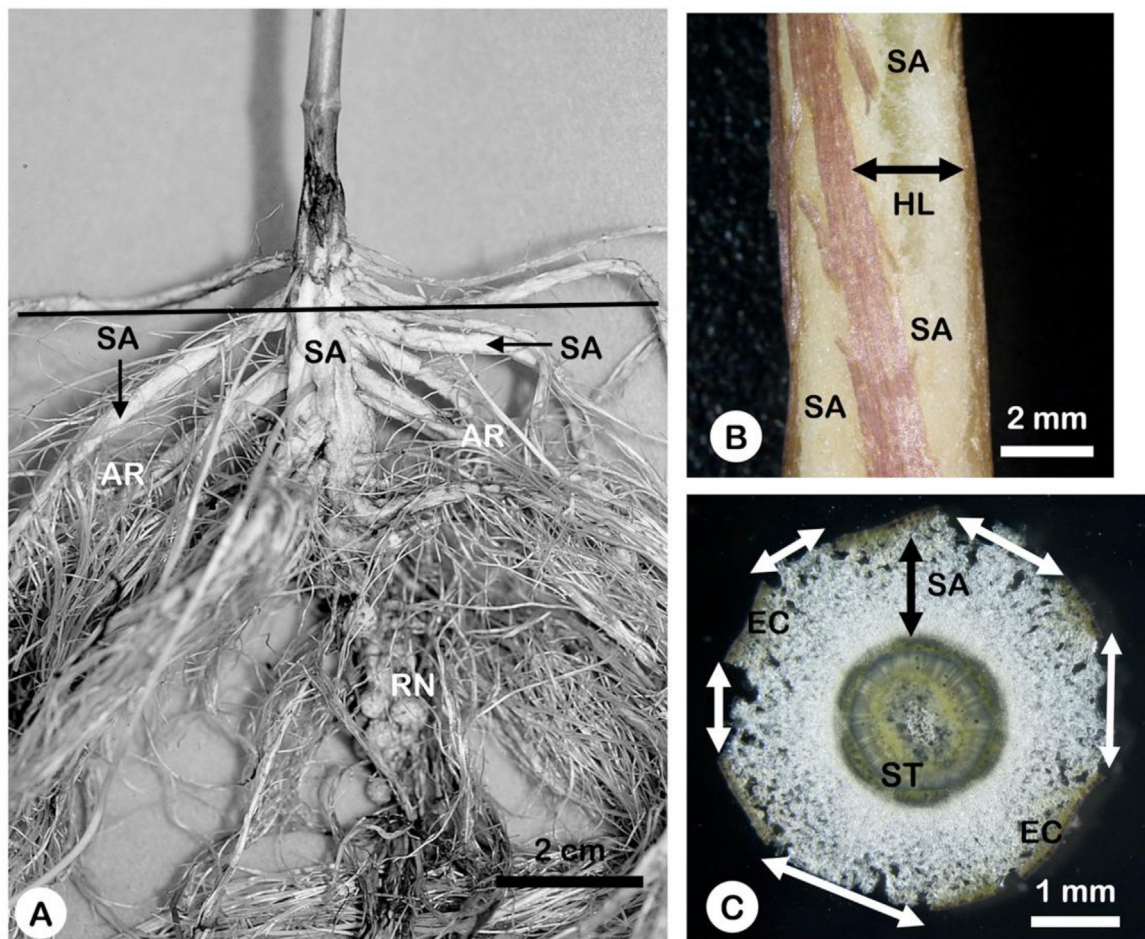


Fig. 3. Root system and secondary aerenchyma development in soybean seedlings under flooding. When the primary leaves had fully expanded, the plants were continuously grown under flooded conditions, with the water level maintained 3 cm above the soil surface. (A) Root system after 3 weeks of flooding. The black line indicates the water level. (B) Hypocotyl after 6 days of flooding. (C) Transverse section of hypocotyl after 6 days of flooding. White two-directional arrows indicate hypertrophic lenticels, and the secondary aerenchyma is exposed to the atmosphere through the lenticels. AR, adventitious roots; EC, epidermis and cortex; HL, hypertrophic lenticel; RN, root nodules; SA, secondary aerenchyma; ST, stele. Photograph (A) reproduced from Shimamura et al. (2003) with the permission of Springer.

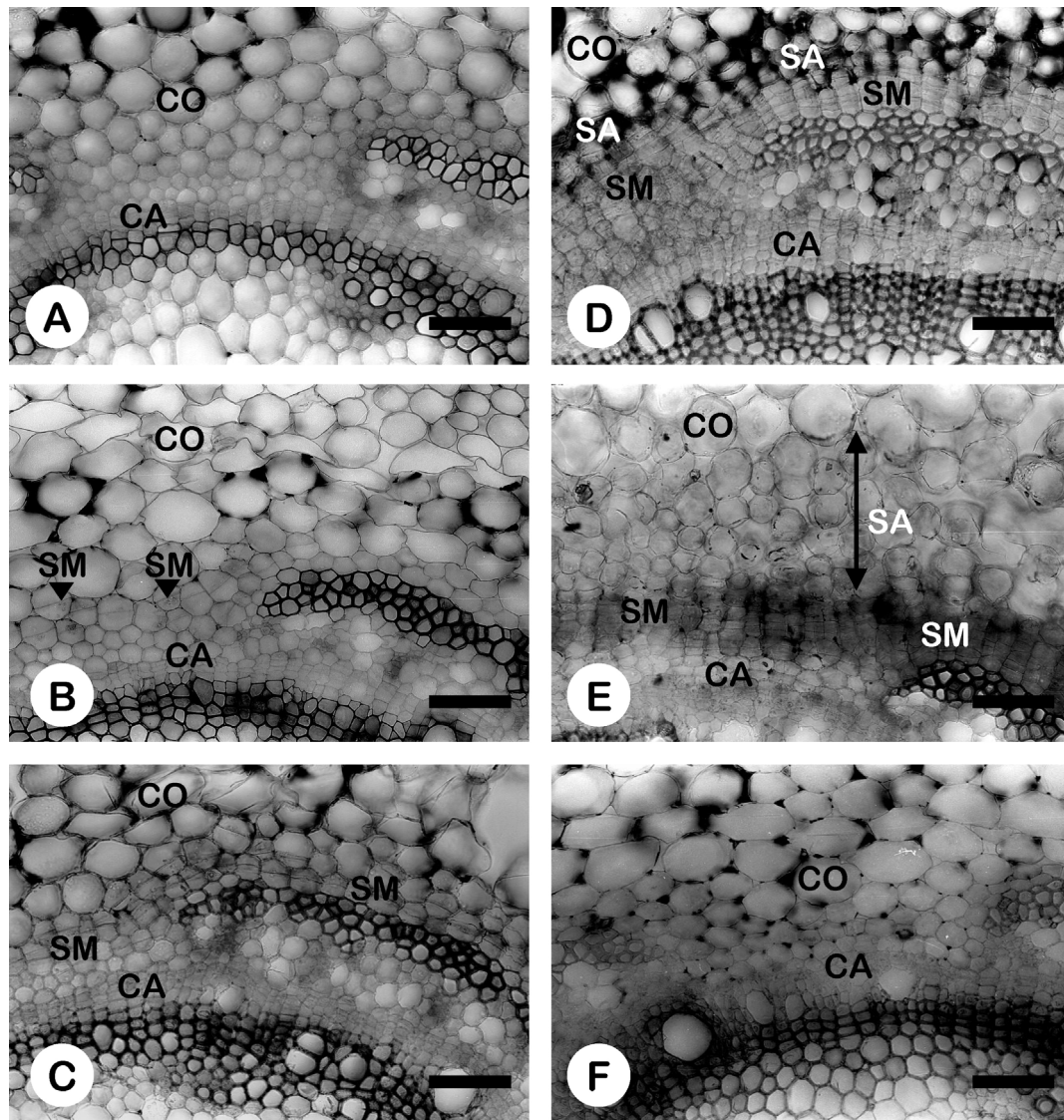


Fig. 4. Processes involved in secondary aerenchyma formation in the hypocotyl of soybean. When the primary leaves had fully expanded, the plants were continuously grown under flooded conditions with the water level maintained 3 cm above the soil surface or control (non-flooded) conditions. Transverse sections of the hypocotyl were obtained from 1 to 2 cm above the soil surface. (A) After 1 day of flooding, (B) after 1.5 days of flooding, (C) after 2 days of flooding, (D) after 3 days of flooding, (E) after 4 days of flooding, and (F) after 4 days of irrigation (but with aerobic soil conditions maintained). CA, cambium; CO, cortex; SA, secondary aerenchyma; SM, secondary meristem (i.e., phellogen). Scale bar = 0.1 mm. Photographs reproduced from Shimamura et al. (2003) with the permission of Springer.

has also been discovered in flood-tolerant legumes such as *Sesbania aculeata* (Scott and Wager, 1888), *S. rostrata* (Saraswati et al., 1992; Shiba and Daimon, 2003), *Neptunia oleracea* (Metcalf, 1931), *Melilotus siculus* (Teakle et al., 2011; Verboven et al., 2012), and *V. juncea* (Fraser, 1931; Walker et al., 1983).

3.1. Morphology and anatomy of secondary aerenchyma in soybean

In secondary growth in soybean roots, the epidermis, cortex, and endodermis are disrupted and sloughed off, so cork tissue is produced as a protective tissue between the phellogen and the epidermis under non-flooded conditions (Lersten and Carlson, 1987). In contrast, soil flooding or rhizosphere hypoxia triggers the formation of secondary aerenchyma by the phellogen in the plant parts affected by these stresses. Morphologically, the process of secondary aerenchyma formation consists of three phases: (1) phellogen development, (2) centrifugal differentiation of the cells that will become aerenchymatous phellem from the phellogen

cells, and (3) elongation of aerenchyma cells to develop a porous tissue. These stress responses appear within a few days after flooding in the hypocotyls of young soybean plants with fully expanded primary leaves (Shimamura et al., 2003). In flooded hypocotyls, morphological changes are not visible after 1 day of flooding (Fig. 4(A)), but the initiation of secondary meristem (phellogen) from the parenchyma tissue outside of the interfascicular cambium and an immature phellogen layer develops after 1.5 days of flooding (Fig. 4(B)). The phellogen layer then develops outside of the phloem fibers and connects with other phellogen areas to form a ring that girdles the stele after 2 days (Fig. 4(C)). The mature phellogen, which can comprise multiple layers, can differentiate new aerenchymatous cells toward the outside after 3 days (Fig. 4(D)), and loosely packed aerenchyma cells surround the stele after 4 days (Fig. 4(E)). In contrast, phellogen development is not drastically enhanced under control (non-flooded) conditions after 4 days of initiation (Fig. 4(F)).

In adventitious roots, lateral roots, and the tap root, phellogen that originated in the pericycle lying just below the endodermis also produces secondary aerenchyma from the center of the root

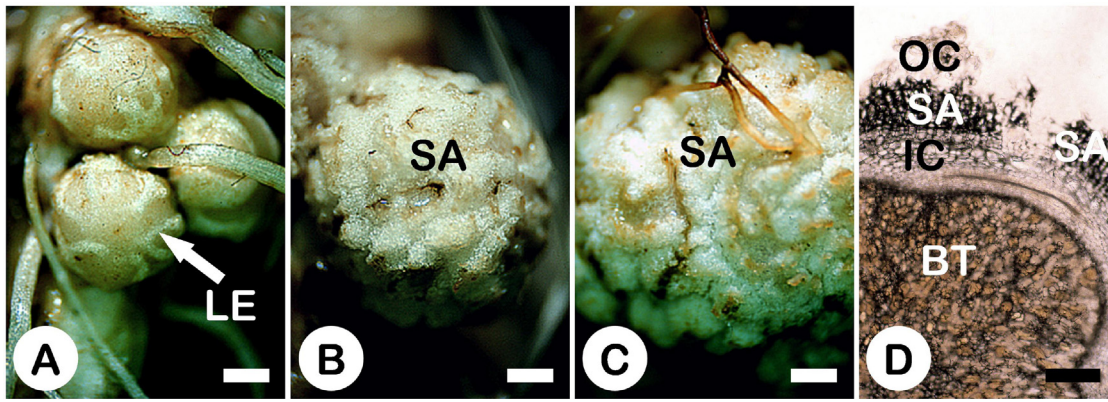


Fig. 5. External and internal structures of the root nodules of flooded soybean plants. When the primary leaves had fully expanded, the plants were grown continuously under flooded conditions, with the water level maintained 3 cm above the soil surface. Root nodules showing lenticels and secondary aerenchyma development after (A) 1, (B) 2, and (C) 4 weeks of flooding. The secondary aerenchyma is exposed to the atmosphere through the lenticels in nodule (A) as well as hypocotyl (see Fig. 3). The epidermis and cortex are sloughed off and the nodules in B and C are covered with secondary aerenchyma. (D) Transverse section of root nodule in soybean seedling after 1 week of flooding. Secondary aerenchyma tissues contain air, so the cell shape is not clearly visible in the photo. Scale bar = 1 mm (A–C) or 0.2 mm (D). BT, bacteroid tissue; IC, inner cortex; LE, lenticel; OC, outer cortex; SA, secondary aerenchyma. Photographs (A–C) reproduced from Shimamura et al. (2002) with the permission of the Crop Science Society of Japan.

towards the epidermis (Thomas et al., 2005). Similarly, the response of aerenchyma formation is observed in the main and lateral roots of *S. rostrata* and *Sesbania cannabina* (Shiba and Daimon, 2003) and of *M. sicutus* (Teakle et al., 2011). As a result of aerenchyma development, the epidermis and cortex of these species easily collapse, and secondary aerenchyma is soon exposed to the atmosphere through hypertrophic lenticels in the hypocotyls and roots. In *S. aculeata*, however, the phellogen originates from the first or second layer of the cortex on the outside of the endodermis in flooded roots (Scott and Wager, 1888). Thus, the origin of the phellogen from which the aerenchyma develops seems to differ among leguminous plants.

In the root nodules, secondary aerenchyma is also produced by the phellogen that arises from the cortical parenchyma outside of the sclerenchyma (Pankhurst and Sprent, 1975; Parsons and Day, 1990; Shimamura et al., 2002; Thomas et al., 2005). Lenticels develop extensively on the surface of root nodules under hypoxic or flooded conditions, exposing secondary aerenchyma at the nodule surface (Fig. 5(A and D)). Thereafter, the epidermis and outer cortex of the nodules collapse entirely and become detached from the nodules; thereby increasing the surface area of the lenticels (which is enhanced by development of the aerenchyma), and the entire nodule surface becomes covered by aerenchyma (Fig. 5(B and C)).

In members of the Lythraceae, Onagraceae, Melastomataceae, Euphorbiaceae, Myrtaceae, and Fabaceae, the development of secondary aerenchyma enhances the formation of hypertrophic lenticels on the surface of the stems and roots, and the aerenchyma is exposed to the atmosphere through the lenticels (see Section 3.2). In roots and stems with completely developed aerenchyma, the epidermis and cortex have collapsed and detached from the remaining tissues. Thus, the secondary aerenchyma directly contacts the soil water, but the cells do not let the water into the intercellular spaces.

Secondary aerenchyma consists of spongy and highly porous tissues with loosely packed parenchymatous cells, so the formation of secondary aerenchyma progressively increases the porosity (the volume of internal gas-filled spaces) in each organ. Non-leguminous *Lythrum salicaria* and leguminous *M. sicutus*, which can produce high amounts of aerenchyma under flooded or hypoxic stagnant conditions, possess high porosity in their roots and shoots. For example, the porosity of the basal stem of 17-week-old *L. salicaria* plants after 8 weeks of flooding reached 60–70% (Stevens et al., 1997), and the porosity of the hypocotyls and roots of 6-week-old *M. sicutus* plants grown in hypoxic stagnant agar solution for 3 weeks was 34–43% (Verboven et al., 2012). In addition, in the

flood-tolerant legume *V. juncea*, the root porosity of 12-week-old seedlings flooded for 7 and 14 days was 12% and 19%, respectively, compared with 5% under non-flooded conditions; and the porosity of the stem, roots, and nodules in flooded 21-week seedlings was 32%, 33%, and 12%, respectively, versus 2%, 7%, and 4% under non-flooded conditions (Walker et al., 1983). Similarly, the porosity of irrigated hypocotyls in soybean plants that lacked secondary aerenchyma was about 7%, whereas that of hypocotyls with a high proportion of aerenchyma was 24% after 7 days and 33% after 14 days of flooding (Shimamura et al., 2002).

3.2. Gas transport system of secondary aerenchyma in soybean

Structurally and functionally, secondary aerenchyma resembles lenticel tissue. Lenticels are also produced by a phellogen layer and are commonly found on the stems and roots of woody plants and herbaceous dicotyledons. Lenticel tissues consist of loosely arranged, thin-walled cells (complementary cells) with abundant intercellular spaces that enhance gas exchange between the plant's internal tissues and the atmosphere, similarly to how stomata function in the leaf. The diffusion rate of gases in air is approximately 10,000 times that in water (Armstrong, 1979), and the diffusion of atmospheric O_2 into roots is greatly decreased in flooded soils. Therefore, flooded roots suffer from insufficient O_2 . To avoid oxygen shortages in these tissues, hypertrophic lenticels that facilitate gas exchange develop quickly, promoted by increased phellogen activity as a result of flooding.

The hypertrophic lenticels provide a greater surface area in submerged portions of the stem base and appear to be important entry points for transporting oxygen into the oxygen-stressed roots of several plants (Hook and Scholtens, 1978). They also facilitate O_2 entry into the aerenchyma of nearby adventitious roots in various herbaceous dicots and woody species (Jackson and Ricard, 2003). Blocking of the hypertrophic lenticels at the base of the stem with lanolin inhibited O_2 transport into the roots and decreased the growth of *Salix viminalis* (Jackson and Attwood, 1996) and of various woody plants (Armstrong, 1968). Dittert et al. (2006) reported O_2 transport into the roots via hypertrophic lenticels just above the water surface in flooded *Alnus glutinosa*: the O_2 concentration in the rhizosphere decreased from $100 \mu\text{mol L}^{-1}$ to almost $0 \mu\text{mol L}^{-1}$ within a few minutes after N_2 gas was applied to the base of stems. These results indicate that hypertrophic lenticels just above the

water line are the first entry points for atmospheric oxygen into flooded parts of woody plants.

These responses have also been reported in secondary aerenchymatous plants, including soybean; and continuity of the secondary aerenchyma between the parts of the shoots above the water line and the flooded tissues (e.g., shoot bases, roots, and nodules) is necessary to provide a pathway for gas exchange that lets plants survive under flooded conditions. When the secondary aerenchyma that develops in submerged portions of the lower shoot of *L. salicaria* was peeled away from the shoot, this artificial disruption of continuity caused a significant reduction in root O₂ levels, indicating that the aerenchyma transports O₂ from the atmosphere into the submerged roots (Stevens et al., 2002). Shimamura et al. (2003) prevented the entry of atmospheric O₂ into the secondary aerenchyma of flooded soybean seedlings through hypertrophic lenticels by applying petroleum jelly and plastic paraffin film to the hypocotyl surface: the secondary aerenchyma and hypertrophic lenticels could not permit oxygen entry. After 14 days, the dry weight of flooded plants was 0.86 g in the treatment and 1.62 g in the control. The petroleum jelly treatment damaged the root growth more severely than the shoot growth, so the shoot-to-root ratio in this treatment was significantly higher than in the control. These symptoms were observed after only 7 days of treatment.

In addition, when secondary aerenchyma exposed to the atmosphere through lenticels on the upper stem just above the water surface was completely submerged, the partial pressure of oxygen (pO₂) within the aerenchyma gas spaces in the lower stem decreased from 14.7 kPa to 6.1 kPa during 120 min (Shimamura et al., 2010). After the water level decreased and the aerenchyma was exposed to the air for a few minutes, measurements confirmed quick movement of oxygen into the lower stem through the secondary aerenchyma, and the pO₂ in the aerenchyma of the submerged lower stems quickly increased to 14 kPa, which is near the original level. When the secondary aerenchyma just above the water surface in the stem of soybean plants after approximately 5 weeks of flooding was exposed to ¹⁸O₂ as a tracer gas for 3 h, the concentration of H₂¹⁸O in the roots increased significantly, whereas exposure of a stem with no secondary aerenchyma to ¹⁸O₂ had little effect on the H₂¹⁸O concentration in the roots, confirming that ¹⁸O₂ is transported to flooded roots through the aerenchyma and is consumed by root respiration in intact soybean plants (Shimamura et al., 2010).

In addition to its significant role in the movement of oxygen into flooded roots, the secondary aerenchyma plays a role in venting CO₂ from the roots in soybean (Shimamura et al., 2010). A high CO₂ level in the root zone is toxic for soybean plants under flooding and anoxia (Araki, 2006; Boru et al., 2003), so the ability to vent CO₂ into the atmosphere seems to be additionally important for the survival of flooded soybean plants. Thus, secondary aerenchyma might moderate CO₂ stress.

Legumes, including soybean, can form nodules on the roots and obtain organic nitrogen compounds from them. Although N₂ gas from the atmosphere is converted into organic nitrogen compounds by fixation in the nodules, a high amount of oxygen is required to meet the high-energy requirements of the symbiotic nitrogen-fixing microorganisms. Therefore, low-oxygen conditions such as flooding can suppress nitrogen fixation in many legumes. Thomas et al. (2005) reported that only 1 day of flooding was required to cause a sharp decline in the xylem sap concentrations of ureides and glutamine, which result from nitrogen fixation, in soybean plants. The reduction of nitrogen fixation can lead to a nitrogen shortage in the plant body, leading to symptoms such as leaf chlorosis. In addition, flooding greatly decreases the amount of N₂ gas in the rhizosphere, further limiting nitrogen fixation by the root nodules under flooding.

In contrast, the root nodules of wetland legumes are much less sensitive to flooding, and can sustain high nitrogen fixation rates by means of secondary aerenchyma. The relative contributions of secondary aerenchyma and the stem to the movement of N₂ down to root nodules have been demonstrated in *S. rostrata*. Using stems without nodules and with secondary aerenchyma exposed to a ¹⁵N₂ tracer, Saraswati et al. (1992) demonstrated that N₂ is transported to the root nodules for fixation; about 40% of the transport occurred through the secondary aerenchyma and 60% through the stem.

The gas exchange between flooded root nodules and the atmosphere via the aerenchyma has also been demonstrated using the acetylene (C₂H₂) reduction assay (i.e., the reduction of acetylene to ethylene by nitrogenase in root nodules). When *V. juncea* seedlings with extensive secondary aerenchyma were flooded for 7 days and then exposed to C₂H₂, the seedlings evolved substantially greater amounts of ethylene (C₂H₄) than those exposed to 0, 1, or 3 days of flooding and that lacked fully developed aerenchyma (Walker et al., 1983). In addition, when the secondary aerenchyma just above the water level in the lower stem of 21-week-old *V. juncea* plants was exposed to a ¹⁵N₂ tracer gas, significant ¹⁵N enrichment of the nodules and shoots was detected. However no significant ¹⁵N enrichment was detected in a non-flooded plant with poorly developed aerenchyma or in a continuously flooded plant whose basal aerenchyma had been blocked with petroleum jelly (Walker et al., 1983). Shimamura et al. (2002) similarly demonstrated that blocking of the secondary aerenchyma of soybean at the base of the stem with petroleum jelly for 1 week decreased root nodulation and nodule activity under flooded conditions.

3.3. Secondary aerenchyma as an alternative trait for tolerance of flooding in soybean

Mochizuki et al. (2000) reported a correlation between the flood tolerance of several legume crops, including soybean, and the development of secondary aerenchyma in their hypocotyls, and suggested that the most tolerant crops tended to produce a high amount of aerenchyma. Soybean is generally susceptible to flooding during its vegetative stage (Griffin and Saxton, 1988; Linkemer et al., 1998; Scott et al., 1989; Sojka, 1985), even though it can develop secondary aerenchyma in flooded stems, roots, and root nodules. Although aerenchyma forms rapidly in the hypocotyls in response to flooding stress (Shimamura et al., 2003), the development of a network of secondary aerenchyma in the flooded roots requires a few weeks of flooding (Shimamura et al., 2003; Thomas et al., 2005), during which period the soybean plants are injured by an oxygen shortage. Therefore, to improve the flooding tolerance of soybean, it will be necessary to investigate not only the potential for the transportation of oxygen via the aerenchyma, but also the rate of development of a network of aerenchyma in flood-tolerant legumes.

4. Conclusion

The research described in this literature review clearly demonstrates the importance of aerenchyma for increasing oxygen transport to flooded tissues. In addition, the improved gas exchange provided by the aerenchyma clearly improves nitrogen fixation by the nodules of soybean. Because most agricultural fields are at least occasionally flooded, the development of crops capable of tolerating flooding by rapidly developing efficient networks of aerenchyma would have important benefits for farmers. However, although the benefits of aerenchyma are clear, the mechanisms that underlie the formation of this tissue are not yet fully understood; in particular, the genetic bases for these mechanisms and the associated transcription factors and signaling pathways must be

elucidated by future research before modern breeding techniques can be used to improve crops that are at high risk of flood damage.

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