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主論文の要約

論文題目 Study on formation of barriers to radial oxygen loss in lateral and adventitious roots of rice
(イネの側根および不定根における酸素漏出バリア形成に関する研究)

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論文内容の要約

A global warming would increase the risk of flood. Flooding is the leading cause of weather-related disaster globally and has been estimated to cause 20% losses of crop productions worldwide. Thus, improvement of crops for higher tolerance to flooding is important for their stable productions. Waterlogged soils are typically anaerobic and chemically reduced, and accumulate toxic compounds, thereby impeding plants growth and causing yield loss in many crops. Oxygen diffusion is approximately 10,000 times slower in water than in air. Therefore, critical damages to plants often occur if oxygen is not adequately supplied to submerged tissues (i.e., roots). Internal aeration is crucial for root growth in waterlogged soil. Rice possesses several traits that enable to adapt in waterlogged soil or even in partial submergence. Important traits for survival during flooding in wetland species (including rice) are formations of aerenchyma and a barrier to ROL in roots and formation of gas film on the leaves. A barrier to ROL promotes the adaptation of plants to waterlogged soil conditions. A ROL barrier is formed in the basal parts of roots and contributes to the enhancement of longitudinal diffusion on oxygen (O₂)

via aerenchyma towards the root tips. So far, it has been unclear (1) whether the ROL barrier can be formed in lateral roots as well as the adventitious roots, (2) how the ROL barrier formation is controlled, and (3) what are the important components of the ROL barrier. To provide answers to these questions, I used rice as a material because it is well known that the adventitious roots of rice can tightly form a ROL barrier in waterlogged soil.

In Chapter 1, previous findings on the root ROL barrier are outlined as General Introduction.

In Chapter 2, I investigated whether a ROL barrier was formed in L- and S-type lateral roots (LRs) of rice under aerated or stagnant deoxygenated conditions, since it was unclear whether the ROL barrier can be formed in the LRs of rice. Rice possesses two types of LRs: L-type and S-type. L-type LRs are generally long, thick, and capable of branching, whereas S-type LRs are short, thin, and incapable of branching. In this study, I examined whether the L- and S-type LRs of rice can form ROL barriers. In L-type LRs, ROL barrier was formed under stagnant deoxygenated conditions and the aerenchyma, which is constitutively formed in aerated conditions, was further developed under stagnant deoxygenated conditions, suggesting that these traits promote the supply of oxygen to the tips of L-type LRs. However, neither a ROL barrier, nor aerenchyma was formed in S-type LRs, and thus ROL was observed mostly in basal parts. Patterns of suberin deposition, which is thought to be one of the components of the ROL barrier, were consistent with the patterns of ROL barrier-formation in L- and S-type LRs. These results suggest that L- and S-type LRs play distinct roles in growth of rice plants in waterlogged soil.

and in oxygenating the rhizosphere.

In Chapter 3, I evaluated whether longer L-type LRs, which were extended by the cutting of tips of the adventitious roots, were able to tightly form a ROL barrier, as like the case of longer adventitious roots. Although L-type LRs has an ability to form a ROL barrier, the ROL barrier was still permeable to O₂, that is, the ROL barrier was less tightly formed in L-type LRs under stagnant deoxygenated conditions. The less-tightly formed ROL barrier might be due to shorter root length (maximum length: ~60mm) of L-type LRs. Indeed, in the adventitious roots of rice, a ROL barrier is tightly formed in longer roots (>110 mm long), but weakly formed in shorter roots (<60mm long) under stagnant deoxygenated conditions. Thus, I examined whether L-type LRs by cutting of the adventitious root tips could become longer and a ROL barrier could be more tightly formed in the extended L-type LRs when grown under stagnant deoxygenated conditions. The cutting in the tip of the adventitious root showed the morphological and anatomical changes in L-type LRs. The numbers, lengths, and diameters of L-type LRs were enhanced by the cutting treatment. A ROL barrier was more tightly formed in the longer L-type LRs (90–110 mm in length) extended by the adventitious root tip cutting under stagnant deoxygenated conditions. In L-type LRs as well as the adventitious roots, I confirmed that the ROL barrier could be formed more tightly in longer roots than in shorter roots. The pattern of suberin accumulation in the exodermis and the sclerenchyma was consistent with the profile of ROL barrier formation. Aerenchyma formation was also promoted in the longer L-type LRs under stagnant deoxygenated conditions, suggesting that the larger aerenchyma areas and tightly formed ROL barrier enhance the internal diffusion oxygen to the root tips along L-

type LRs.

In Chapter 4, I evaluated that wax biosynthesis gene *LEAF GAS FILM-1 (LGF1)* was involved in ROL barrier formation using a rice mutant (*dripping wet leaf 7 (drp7)*), in which *LGF1* gene is deficient. In Arabidopsis, it was reported that some components of wax are associated with suberin in roots. I found that a wax biosynthesis gene, *Leaf Gas Film-1 (LGF1)*, was highly expressed in our parts of rice roots (including the epidermis, exodermis and sclerenchyma) and its expression was up-regulated in roots when rice plants were grown under stagnant deoxygenated conditions. These evidences raised the possibility that accumulation of wax produced by *LGF1* may be involved in the ROL barrier formation in rice roots. To explore this possibility, I used a rice mutant, *dripping wet leaf (drp7)*, which has a loss-of-function mutation of *LGF1* gene. Originally, it was reported that *drp7* mutant declined hydrophobicity on the leaf surface, thereby losing gas film formation on the leaves during submergence. I verified that a ROL barrier was weakly formed in the adventitious roots of *drp7* mutant, whereas its wild-type 'Kinmaze' tightly formed the ROL barrier was tightly formed in roots under stagnant deoxygenated conditions. The introduction of *Ubiquitin-promotor::LGF1 (pUb::LGF1)* to *drp7* mutant restored a tightly-formed ROL barrier. Histochemical GUS staining of the *pLGF1::GUS* transformant showed that *LGF1* gene was mainly expressed in the outer cell layers (*i.e.*, the exodermis, the sclerenchyma, and the most outer cortical cell) as well as the central cylinder under stagnant deoxygenated conditions. Moreover, suberin accumulation in the exodermis and the sclerenchyma was weaker in *drp7* mutant than in WT under stagnant deoxygenated conditions. These findings support the hypothesis that components of wax associated with

suberized tissue in rice roots and suberin-associated root wax is involved in a ROL barrier formation in rice roots under stagnant deoxygenated conditions.

In Chapter 5, I discussed a role of the ROL barrier formed in LR_s as well as the adventitious roots in growth of rice plants in waterlogged soils (*i.e.*, in paddy fields) and involvement of wax in the function of ROL barrier in rice roots as
General Discussion.