

Lysigenous aerenchyma formation in maize root is confined to cortical cells by regulation of genes related to generation and scavenging of reactive oxygen species

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To adapt to waterlogging, maize (*Zea mays*) forms lysigenous aerenchyma in root cortex as a result of ethylene-promoted programmed cell death (PCD). *Respiratory burst oxidase homolog (RBOH)* gene encodes a homolog of gp91^{phox} in NADPH oxidase, and has a role in the generation of reactive oxygen species (ROS). Recently, we found that during aerenchyma formation, *RBOH* was upregulated in all maize root tissues examined, whereas an ROS scavenging-related *metallothionein (MT)* gene was downregulated specifically in cortical cells. Together these changes should lead to high accumulations of ROS in root cortex, thereby inducing PCD for aerenchyma formation. As further evidence of the involvement of ROS in root aerenchyma formation, the PCD was inhibited by diphenyleneiodonium (DPI), an NADPH oxidase inhibitor. Based on these results, we propose a model of cortical cell-specific PCD for root aerenchyma formation.

In both wetland and non-wetland plants, lysigenous aerenchyma is formed in roots by creating gas spaces as a result of death and subsequent lysis of some cortical cells, and allows internal transport of oxygen from shoots to roots under waterlogged soil conditions.¹⁻³ In rice (*Oryza sativa*) and some other wetland plant species, lysigenous aerenchyma is constitutively formed under aerobic conditions, and is further enhanced under waterlogged conditions.⁴ On the other hand, in non-wetland plants, including maize (*Zea mays*), lysigenous

aerenchyma does not normally form under well-drained soil conditions, but is induced by waterlogging.⁵ Ethylene is involved in lysigenous aerenchyma formation,^{1-3,6,7} but the molecular mechanisms are unclear.

We recently identified two reactive oxygen species (ROS)-related genes that were specifically regulated in maize root cortex by waterlogged conditions, but not in the presence of an ethylene perception inhibitor 1-methylcyclopropene (1-MCP).⁵ One was *respiratory burst oxidase homolog (RBOH)*, which has a role in ROS generation and the other was *metallothionein (MT)*, which has a role in ROS scavenging. These results suggest that ROS has a role in ethylene signaling in the PCD that occurs during lysigenous aerenchyma formation.

Cell Type-Specific Expression of Genes Related to ROS Generation and Scavenging

In maize, lysigenous aerenchyma is formed in root cortical cells under waterlogged conditions, but not under aerobic conditions. However, ethylene can induce its formation even under aerobic conditions (Fig. 1A).

RBOH, a plant homolog of gp91^{phox} in plasma membrane-associated NADPH oxidase of mammals, converts O₂ to superoxide anion (O₂^{•-}), thereby leading to production of hydrogen peroxide (H₂O₂).^{8,9} In laser microdissection-isolated root tissues from waterlogged plants, expression of the *RBOH* gene is upregulated strongly in the cortical cells and slightly less

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strongly in the stelar cells and the outer cell layers (Fig. 1B).

MTs are low molecular weight, cysteine-rich metal-binding proteins that, in animals, seem to have roles in metal homeostasis, general stress responses and ROS scavenging.^{10,11} The *MT* gene was constitutively expressed in all of the laser microdissection-isolated root tissues under aerobic conditions, but interestingly, it was hardly expressed in the cortical cells under waterlogged conditions (Fig. 1B).

Involvement of ROS in Lysigenous Aerenchyma Formation in Maize Roots

To confirm the involvement of RBOH-produced ROS in lysigenous aerenchyma formation, we grew maize under waterlogged conditions with or without treatment of diphenyleneiodonium (DPI), an NADPH oxidase inhibitor. As shown in Figure 1C, aerenchyma formation of maize primary roots under waterlogged conditions was inhibited by the treatment with 1 μ M or 5 μ M DPI compared with no DPI treatment (-DPI), suggesting that the ROS generation mediated by NADPH oxidase (i.e., RBOH) under waterlogged conditions partly contributes to aerenchyma formation in maize roots.

ROS are key factors that transduce signals stimulated by environmental stresses or pathogen infections in plants. However, ROS, which are potentially toxic, can cause cellular damage, and thus their production must be tightly regulated.^{8,12} RBOHs are considered as the main producers of ROS for stress-stimulated signaling in plants.^{8,9,12} Several studies have shown that transcriptional activation of *RBOH* genes is accompanied by an oxidative burst,¹²⁻¹⁴ and the positive feedback regulation of the *RBOH* gene expression at transcriptional and post-transcriptional levels contributes to amplification of the ROS signals.^{12,15-17}

There is increasing evidence that plant MTs regulate the accumulation of ROS: downregulation of the rice *MT2b* gene contributed to high H_2O_2 accumulation during defense signaling¹⁸ and ethylene-induced epidermal cell death,¹⁹ and H_2O_2 treatment and knockdown of *MT2b* gene promoted lysigenous aerenchyma formation

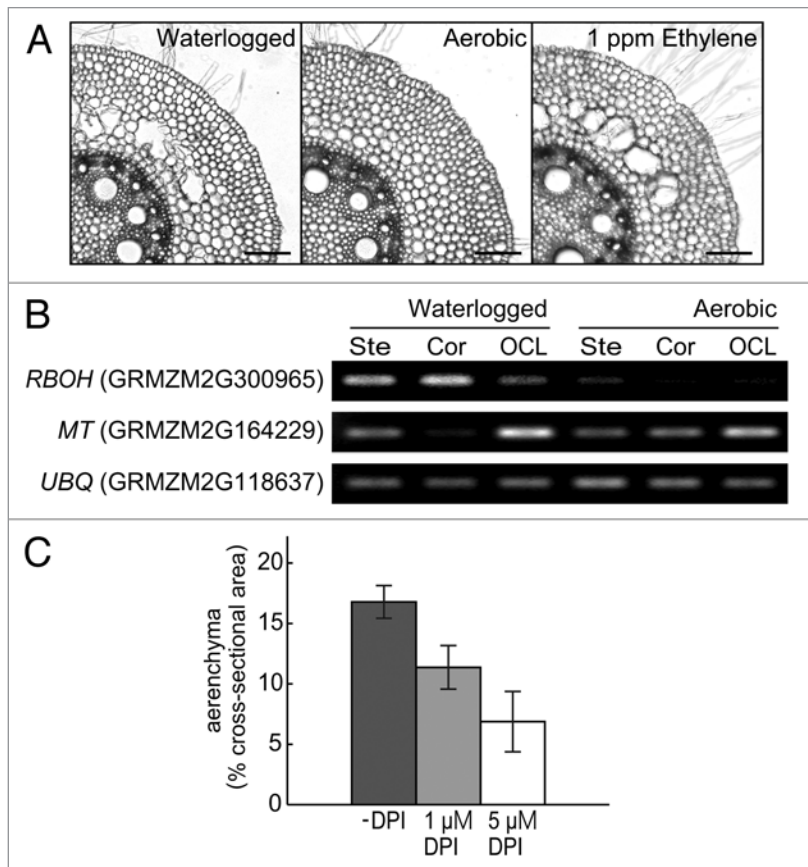


Figure 1. (A) Aerenchyma formation of maize primary roots under waterlogged conditions, aerobic conditions and aerobic conditions with 1 ppm ethylene. Tissue sections were prepared at 24 h after the start of treatments. Bars, 100 μ m. (B) Cell type-specific expression analysis of the *RBOH* and the *MT* genes. Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) was performed. The alphanumeric symbols in parentheses indicate the Gene IDs of the Maize sequence database. The Ubiquitin (UBQ) was used as a control. Ste, stelar cells; Cor, cortical cells; OCL, outer cell layers. (C) Aerenchyma formation in maize primary roots at 24 h grown under waterlogged conditions with or without DPI treatment. All values are means ($n = 4$) \pm SD.

in rice internodes.²⁰ Biochemical analysis revealed that an animal Zn or Cd binding MT has more than 100 times higher antioxidant activity against hydroxyl radicals (OH) than does reduced glutathione (GSH).^{10,21} Rice *MT2b* and cotton *MT3a* have higher antioxidative capacity against hydroxyl radicals than do the other antioxidants in vitro,^{18,22} and many cysteine residues in proteins are remarkably reactive to oxidizing agents.^{11,23} These results suggest that the MTs are responsible for reductions of H_2O_2 as well as other ROS in plant cells, although the antioxidative capacity of MTs against H_2O_2 has not been directly evaluated.

Sauter and colleagues^{19,20} proposed a model in which ethylene-promoted downregulation of *MT2b* gene expression enhances accumulation of H_2O_2

produced by NADPH oxidase, thereby inducing epidermal cell death in rice or aerenchyma formation in rice internodes. We propose a similar model for lysigenous aerenchyma formation in maize roots (Fig. 2). Waterlogging promotes biosynthesis and accumulation of ethylene, followed by induction of *RBOH* expression. The *RBOH* activity leads to the production and accumulation of $O_2^{\cdot -}$ at the apoplast, which is spontaneously or enzymatically converted to H_2O_2 . H_2O_2 can easily diffuse into the cytosol through the plasma membrane.²⁴ In the cytosol of stelar cells and cells in the outer cell layers, in which MT is constitutively expressed, H_2O_2 and other ROS are scavenged by MT. In contrast, in the cortical cells, the decreased *MT* expression prevents ROS scavenging, thereby leading to higher ROS accumulation, which activates

the subsequent processes of PCD and lysis of the cortical cells (i.e., lysigenous aerenchyma formation) in maize roots.

Conclusion and Perspectives

The preceding results suggest that both upregulation of *RBOH* gene expression and downregulation of *MT* gene expression in cortical cells contribute to cell type-dependent ROS accumulation in maize roots under waterlogged conditions. This results in accumulation of ROS in cortical cells, but not in the other root tissues, thereby inducing aerenchyma formation only in the root cortex. However, some cortical cells escape PCD and remain alive after completion of lysigenous aerenchyma formation. So far, it is unknown what factors determine which cells undergo PCD in the root cortex during aerenchyma formation in response to waterlogging stress. It would be of interest to examine whether cell fate is determined by differences in *MT* gene expression among the cortical cells.

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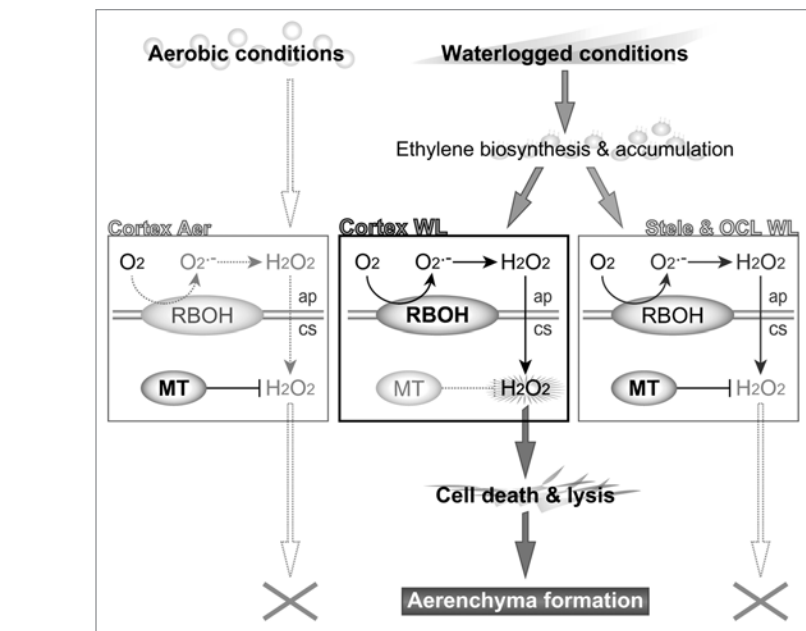


Figure 2. Model of lysigenous aerenchyma formation in maize root under waterlogged conditions. Waterlogging promotes biosynthesis and accumulation of ethylene. *RBOH* expression is upregulated in the cortical cells, the stele cells and the outer cell layers, whereas *MT* expression is downregulated specifically in the root cortex. This leads to high accumulation of ROS (including H₂O₂), which initiates PCD and lysis of the cortical cells (i.e., aerenchyma formation). Note that direct scavenging of H₂O₂ by MT proteins remains to be elucidated, but some genetic studies support MT-mediated H₂O₂ reduction. Aer, aerobic conditions; WL, waterlogged conditions; ap, apoplast; cs, cytosol.

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