論文の内容の要旨

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論文題目 **Study of aerenchyma formation in maize roots under waterlogged conditions**

湛水条件下におけるトウモロコシの根の通気組織形成に関する研究

To escape low oxygen conditions, numerous adaptations at the anatomical, morphological and metabolic level took place in plants suffering from waterlogged conditions. One of the structural adaptations is aerenchyma formation. Aerenchyma is a tissue consisting of longitudinal gas spaces separated by the strands of living cells, found in the root cortex of waterlogged plants. It enables the passage of gases in and out of tissues in plant roots. Internal transport of oxygen via the aerenchyma from shoots to roots is especially important for survival under waterlogged conditions. However, the molecular mechanism of lysigenous aerenchyma formation remains to be elucidated. The aim of this study was to identify aerenchyma formation-associated genes expressed in maize roots as a basis for understanding the molecular mechanism of aerenchyma formation.

1. Root growth and aerenchyma formation of maize under waterlogged or aerobic conditions

In this section, the growths of roots and shoots and aerenchyma formation were investigated. Three-day-old seedlings were treated under waterlogged conditions for 48 hours (h). The growth of the roots was retarded under waterlogged conditions compared with the roots grown under aerobic conditions. No effect was found of the waterlogged treatment on the elongation of the shoots. Three-day-old aerobically grown seedlings were then subjected to waterlogging treatment for 48 h and aerenchyma formation was investigated at different levels at the basal part of the root. I found that the gas spaces increased significantly during the treatment and the most inducible part was at 1.5-2 cm from the root-shoot junction.

2. Identification of genes involved in aerenchyma formation in maize roots using a laser microdissection and microarray analysis

Ethylene is known to be involved in aerenchyma formation in maize roots. It has been reported that aerenchyma is formed through programmed cell death (PCD). Three-day-old aerobically grown seedlings were then treated with ethylene (1 ppm) under aerobic conditions. The result showed that ethylene induced aerenchyma formation, which started between 6 h and 12 h after the ethylene treatment. On the other hand, aerenchyma formation was not observed at the basal region of roots of 4-day-old seedlings grown under aerobic conditions. The use of 1-methylcyclopropene (1-MCP; 1 ppm), an inhibitor of ethylene perception, completely blocked the formation of aerenchyma in the maize cortical cells when treated under waterlogged conditions for 24 h. Whereas the treatment of seedlings under waterlogged conditions without the 1-MCP pre-treatment induced aerenchyma formation starting between 18 h and 24 h after the treatment. Because aerenchyma is formed in the cortical cells of the root, cortical cells of roots treated under waterlogged conditions with or without pre-treatment with 1-MCP or aerobic conditions were isolated by laser microdissection and their mRNA levels were examined with a microarray. As a result, the signal intensities of 575 genes among 42,034 gene probes spotted on a microarray slide were significantly different between the two conditions for inducing and not inducing aerenchyma formation. Among them, it was likely that 239 genes were up-regulated and 336 genes were down-regulated under conditions for inducing aerenchyma formation (waterlogged conditions). The differentially expressed genes

included genes related to generation or scavenging of reactive oxygen species (ROS), cell wall loosening and degradation, and Ca^{2+} signaling.

3. Confirmation of microarray result using a semi quantitative RT-PCR and analysis of tissue specificity of the selected genes

Production of ROS has been implicated in diverse physiological processes including PCD in plants. One of major sources of ROS in plants is a reaction mediated by NADPH oxidase, which is responsible for the conversion of O_2 to superoxide anion (O_2), thereby leading to production of hydrogen peroxide (H_2O_2). Several ethylene-mediated waterlogging-responsive genes related to ROS-generation or ROS-scavenging were found. The up-regulated genes contain gene encoding respiratory burst oxidase homolog (RBOH) and the down-regulated genes contain gene encoding metallothionein (MT). The *RBOH* gene is involved in ROS generation and the *MT* gene is involved in ROS scavenging. To determine the tissue specificity of these genes, the stelar cells and the cortical cells of maize roots, treated or not under waterlogged conditions, were collected separately using the laser microdissection and the expression of these genes were analyzed using a semi quantitative RT-PCR. The up-regulation of *RBOH* expression was observed in both cortical cells and stelar cells, but the mRNA levels appeared to be slightly higher in cortical cells than in stelar cells under waterlogged conditions. Interestingly, the *MT* gene was constitutively expressed in both cortical cells and stelar cells under aerobic conditions, but the *MT* mRNA levels were decreased specifically in cortical cells under waterlogged conditions. In addition, the use of diphenylene iodonium (DPI), an inhibitor of RBOH activity, reduced the aerenchyma formation in the root cortical cells treated under waterlogged conditions.

The last step of aerenchyma formation involves cell wall loosening and degradation, in which many enzymes are involved. Genes related to cell wall degradation were also selected from the microarray experiment. Among the up-regulated genes, genes encoding xyloglucan endo-transglycosylase (XET), cellulose (CEL) and polygalacturonase (PG). These genes are related to cell wall loosening and degradation. The tissue specificity analysis showed that, *XET* and *CEL* genes were specifically expressed in the root cortical cells under waterlogged conditions. However, the *XET* was up-regulated in both cortical cells and stelar cells. A gene encoding plasma membrane (PM) *H^+-ATPase* as the cortical cell-specific up-regulated

gene. It is proposed that extrusion of an intracellular H^+ into the cell wall by PM H^+-ATPase results in a decrease of apoplastic pH, which induces cell wall loosening, possibly mediated by low-pH activated XETs.

Many studies have suggested that the cytosolic calcium ion (Ca^{2+}) functions as a second messenger for signaling pathways in response to oxygen deprivation. Ca^{2+} signaling may also be involved in aerenchyma formation in maize roots. In this study, several genes implicated in calcium signaling such as genes encoding calcineurin B-like protein (CBL), Ca^{2+}-binding domain containing proteins and calmodulin-like protein (CML), were selected. The expressions of *CBL* and *CML* were observed in both cortical cells and stelar cells under waterlogged conditions, but the changes in expression were more pronounced in cortical cells than in stelar cells. However, the expression of the cyclic nucleotide-gated ion channel 2 (*CNGC*) gene was repressed under waterlogged conditions.

In conclusion, in this study, genes related to many types of molecular function (e.g. ROS generation or scavenging, cell wall modification, and Ca^{2+} signaling) were found as up-regulated or down-regulated in root cortical cells under waterlogged conditions, and their expression was likely to be regulated by ethylene. The data should provide a basis for an understanding of the molecular mechanism of inducible lysigenous aerenchyma formation in plants.