# Identification of genes expressed during aerenchyma formation in maize roots using laser microdissection and a microarray

Mikio Nakazono[1], Imene Rajhi[1], Hirokazu Takahashi[1], Katsuhiro Shiono[1], Kazuhiro Ohtsu[2], Nobuhiro Tsutsumi[1], Tieming Ji[2], Daniel S. Nettleton[2], Patrick S. Schnable[2], and Naoko K. Nishizawa[1]

[1] Graduate School of Agricultural and Life Sciences, University of Tokyo, 1-1-1 Yayoi, Bunkyo, Tokyo 113-657, Japan

[2] Iowa State University, Ames, IA 50011-3650, USA

Contact: Mikio Nakazono, Phone: +81-3-5841-5074, Fax: +81-3-5841-5183,
E-mail: anakazo@mail.ecc.u-tokyo.ac.jp

## ABSTRACT

Laser microdissection (LM) is a new method that makes it possible to obtain large homogeneous populations of cells from tissue sections in one step. LM in combination with microarray analyses can monitor changes in transcript levels in specific cell types, in which morphological or physiological changes are observed. In this study, we used LM and a microarray to monitor genes expressed during aerenchyma formation in maize (*Zea mays*) roots. In maize, hypoxia stimulates ethylene biosynthesis, which induces cell death in the root cortex, thereby forming aerenchyma in the roots. Roots of 3-d-old maize (inbred line B73) seedlings were waterlogged for 6 h and then were fixed and embedded in paraffin. We isolated root cortex cells from the paraffin-embedded sections using LM, extracted RNA and carried out a maize cDNA microarray. Finally, we identified several genes that were expressed specifically in the root cortex during aerenchyma formation. Possible roles of these genes in aerenchyma formation are discussed.

KEYWORDS: Aerenchyma, Laser microdissection, Maize, Microarray, Root aeration, Waterlogging

## 1. INTRODUCTION

Waterlogging is an environmental stress that affects agricultural production in many regions. The main cause of damage to plants under waterlogged conditions is oxygen shortage, which affects nutrient and water uptake (Bailey-Serres and Voesenek, 2008). Some plants adapt to oxygen shortage by formation of aerenchyma (Jackson and Armstrong, 1999; Evans, 2003), which is an airy space found in the root cortex of waterlogged plants. In maize roots, aerenchyma is formed by programmed cell death in the mid cortex at basal regions of roots (Drew *et al.*, 2000). The large air spaces in the aerenchyma enable diffusion of gases between shoots and roots. Under waterlogging conditions, ethylene, a gaseous plant hormone, induces the expression of genes related to aerenchyma formation and promotes oxygen transport in roots (Shiono et al., 2008). Thus, ethylene is thought to be a trigger of aerenchyma formation. So far, it is unclear what genes are involved at the early stage during the aerenchyma formation although some genes (*e.g.*, *Xyloglucan endo-transglycosylase* (*XET*) gene; Saab and Sachs, 1996) expressed at the later stage of the aerenchyma formation were identified. The aim of this study is to identify aerenchyma formation-associated genes expressed at the early stage of the aerenchyma formation in maize root cortex isolated by laser microdissection (LM) and to understand the molecular mechanism of aerenchyma formation.

## 2. MATERIALS AND METHODS

### 2.1. Plant materials and treatments

Maize (*Zea mays*, inbred line B73) kernels were placed on germination paper, rolled-up in the paper, and placed in a flask as described by Nakazono et al. (2003). Water was added to the flask to keep the paper moist, but not enough to submerge the roots. Seedlings were germinated and grown at 28$^{o}$C under constant light in a growth chamber. Three-day-old seedlings were grown under several conditions. For the waterlogging treatments, the primary root (but not the shoots) was waterlogged. To examine the effect of ethylene on the aerenchyma formation, the seedlings were treated with ethylene gas (5 ppm) under aerobic conditions or pre-treated with an ethylene perception inhibitor 1-methylcyclopropene (1-MCP; 1 ppm) for 12 h before the waterlogging treatment. The ethylene or 1-MCP treatment of the seedlings was conducted in an airtight container.

### 2.2. Measurement of aerenchyma

Cross-sections were cut from fresh root samples with a hand-held razor blade and viewed with a microscope (DMIRBE M2FLIII, Leica) fitted with a CCD camera (VB-7000, Keyence) linked to a computer. Aerenchyma formation was measured along the basal and apical regions of the primary roots with Image J software (Ver. 1.39u, NIH, Bethesda, MD).

### 2.3. Treatments for gene analysis, laser microdissection (LM), microarray and quantitative RT-PCR

Three-day-old plants were treated for 6 h under four conditions and cortex cells were collected from 1) basal regions under waterlogged conditions, 2) apical regions under waterlogged conditions, 3) basal regions under aerobic conditions, and 4) basal regions pre-treated with 1-MCP under waterlogged conditions.

The basal and apical parts of roots were fixed in Farmer’s fixative (75% ethanol, 25% acetate) and embedded in paraffin. Cortex cells were isolated from paraffin-embedded root sections with a Veritas Laser Microdissection System (Molecular Devices) as shown in Figure 1. RNA was extracted from the LM-isolated cortex cells and amplified. The amplified RNA was analyzed with a 15K maize cDNA microarray. Each experiment was performed six times using independently isolated samples (six biological replicates). We selected genes whose transcript intensities differed by at least a factor of 2 from the control intensities in each experiment ($q<0.05$). Transcript levels of each gene were measured by real-time quantitative RT-PCR using a LightCycler (Roche Diagnostics) and the QuantiTect SYBR Green RT-PCR kit (Qiagen) according to the manufacturers’ protocols.

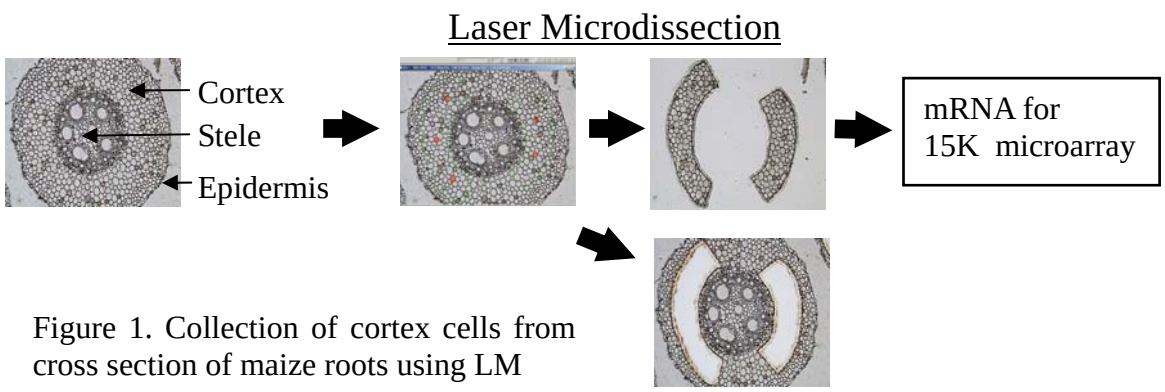


Figure 1. Collection of cortex cells from cross section of maize roots using LM

## 3. RESULTS AND DISCUSSION

### 3.1. Aerenchyma formation in maize primary roots

In 3-day-old seedlings that had been kept under waterlogged conditions for 24 h, an aerenchyma formed at the basal region (Fig. 2A) but not in the apical region (data not shown). Aerenchyma formation was inhibited by pre-treatment with 1-MCP (Fig. 2B), but was induced by ethylene treatment under aerobic conditions (Fig. 2C). Aerenchyma formation was not observed at the basal region of roots of 4-day-old seedlings grown under aerobic conditions (negative control; Fig. 2D)

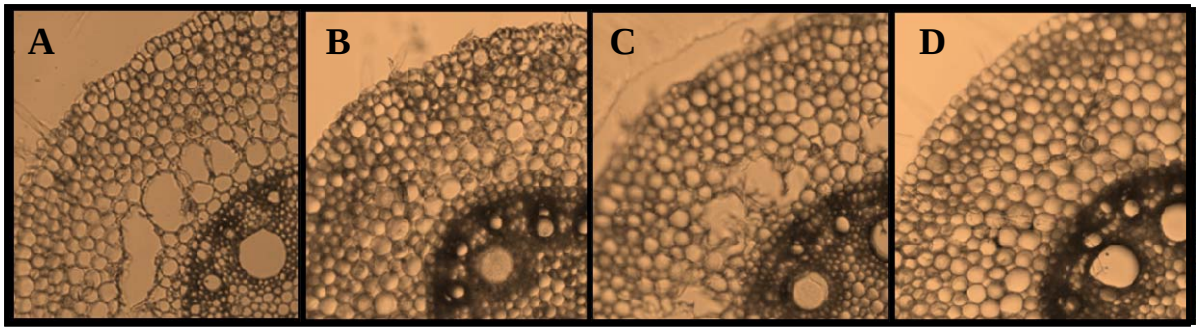


Figure 2. Cross sections of basal regions of a primary root under several growth conditions. (**A**, **B**) Waterlogged conditions without (**A**) or with (**B**) pre-treatment with 1 ppm 1-MCP. (**C, D**) Aerobic conditions with (**C**) or without (**D**) treatment with 5 ppm ethylene gas.

### 3.2. Changes in gene expression during aerenchyma formation

Analyses of cross sections of roots treated with the above conditions for 0, 6, 12, 18 and 24 h revealed that under aerobic conditions, aerenchyma formation started after 6 h of ethylene treatment, while it started after 18 h of waterlogging treatment. As a result, we examined gene expression in cortex cells at 6 h after the treatments.

The microarray data revealed 35 genes in the cortex cells, whose transcript levels were either increased of decreased by a factor of 2 or more. Of these genes, 19 were up-regulated and 16 were down-regulated genes during the aerenchyma formation.

We are currently using quantitative RT-PCR to check the expressions of some of these genes (*e.g.*, actin-depolymerizing factor (ADF; Fig. 3), pectin acetyltransferase, transcription factors and protein kinases). ADF and pectin acetyltransferase may be involved in changes of cell structure (*i.e.*, cell expansion) that occur before the PCD during the aerenchyma formation.

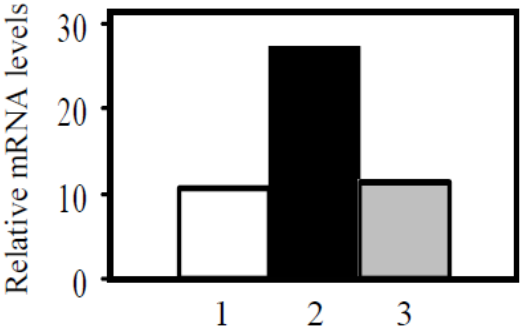


Figure 3. Quantitative RT-PCR of the gene encoding actin depolymerizing factor (ADF) using the LM-isolated cortex cells under aerobic conditions (1) or waterlogged conditions without (2) or with (3) the pre-treatment with 1-MCP.

## ACKNOWLEDGMENTS

We thank Drs. A. Oyanagi, K. Kawaguchi and F. Abe (National Institute of Crop Science, Japan), Dr. Y. Mano (National Institute of Livestock and Grassland Science, Japan) and Dr. T. Abiko and Ms. H. Kamakura (The University of Tokyo, Japan) for supporting our research. This work was supported by the Program for Promotion of Basic and Applied Researches for Innovations in Bio-oriented Industry (BRAIN), a grant from the Ministry of Agriculture, Forestry and Fisheries of Japan, and a grant from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

## REFERENCES

Bailey-Serres, J. and Voesenek, L.A.C.J. 2008. Flooding Stress: Acclimations and genetic diversity. *Annu. Rev. Plant Biol.* 59, 313–339.

Drew, M.C., He, C.-J. and Morgan, P.W. 2000. Programmed cell death and aerenchyma formation in roots. *Trends Plant Sci.* 5, 123-127.

Evans, D.E. 2003. Aerenchyma formation. *New Phytol.* 161, 35-49.

Jackson, M.B. and Armstrong, W. 1999. Formation of aerenchyma and the process of plant ventilation in relation to soil flooding and submergence. *Plant Biol.* 1, 274-287.

Nakazono, M., Qiu, F., Borsuk, L.A. and Schnable, P.S. 2003. Laser capture microdissection, a tool for the global analysis of gene expression in specific plant cell types: Identification of genes differentially expressed in epidermal cells or vascular tissues of maize. *Plant Cell* 15. 583-596.

Saab, I.N. and Sachs, M.M. 1996. A flooding-induced xyloglucan *endo*-transglycosylase homolog in maize is responsive to ethylene and associated with aerenchyma. *Plant Physiol.* 112, 385-391.

Shiono, K., Takahashi, H., Colmer, T.D. and Nakazono, M. 2008. Role of ethylene in acclimations to promote oxygen transport in roots of plants in waterlogged soils. *Plant Sci.* 175, 52-58.