

## Review

# Mechanisms of aerenchyma formation in maize roots

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**Respiration is very sensitive to waterlogged conditions. Under these conditions, plant roots suffer from lack of available oxygen. In fact, waterlogging reduces the exchange of gases between the plant and the atmosphere. When plants cannot receive sufficient oxygen level for respiration, they form aerenchyma in their roots which function as reservoirs of oxygen in the submerged plant. Aerenchyma is formed in maize (*Zea mays*) roots in response to different types of stress such as waterlogging, mechanical impedance, drought and nutrient deficiencies. Ethylene plays a crucial role in aerenchyma formation. Under waterlogged conditions, it can be cumulated in the submerged tissue and induces genes implicated in aerenchyma formation. These genes are related to calcium signaling, cell wall degradation and reactive oxygen species (ROS). In this review, the authors focused on the recent findings on aerenchyma in maize roots and explained the mechanisms of its formation under waterlogged conditions.**

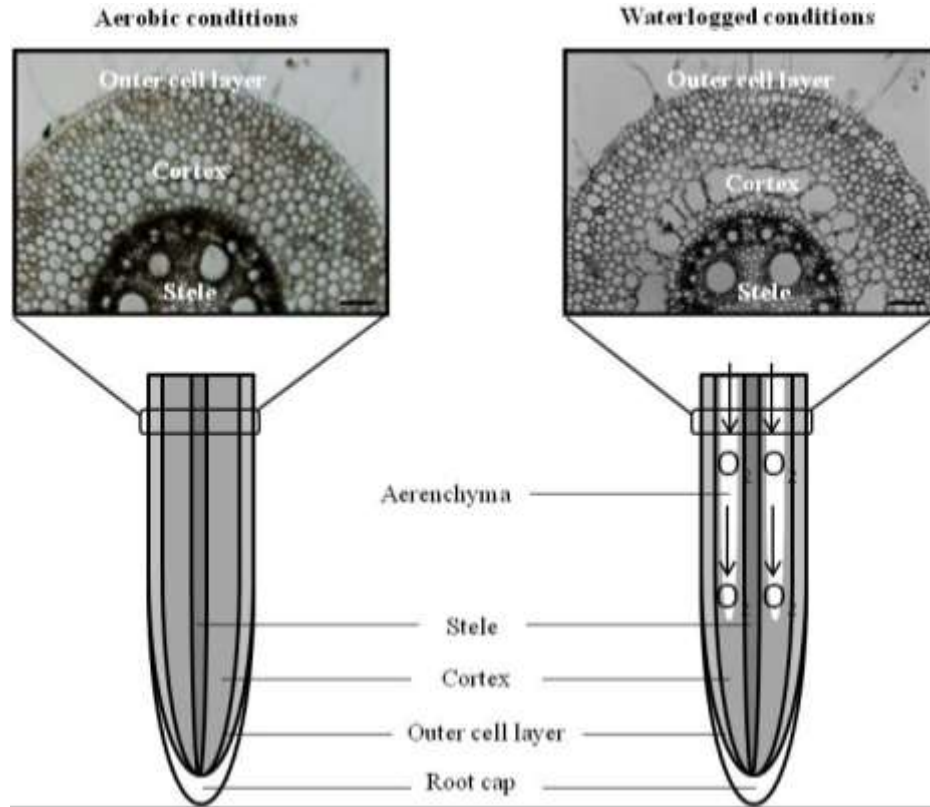
**Key words:** Aerenchyma, maize root cortex, waterlogging, ethylene, programmed cell death.

## INTRODUCTION

Respiration is one of the plant physiological processes, which is defined as the exchange of gases between air and cells within tissues. Oxygen and carbon dioxide are considered as the most important gases for respiration which are diffused in an opposite direction into and out the plant. Under natural conditions, oxygen and carbon dioxide are transported into or out of the plant's root via soil pores filled with air. Respiration is very sensitive to waterlogged conditions because the excess of water reduces the exchange of gases between the plant and the atmosphere. When the soil pores are filled with air, oxygen can easily diffuse into the plant's root but this diffusion is decreased when the soil pores are filled with

water. Indeed, the diffusion of gases through air is  $10^4$  fold faster than in water (Colmer and Voesenek, 2009). Additionally to the scarcity of oxygen in submerged conditions, the available oxygen will be also consumed by the microorganisms' resident in the soil. As a result, oxygen level decreases in underwater tissues. However, the carbon dioxide level increases due to the microbial and root respirations (Colmer and Voesenek, 2009). This deficiency of oxygen alters nutrient and water uptake in water-stressed plants causing the diminution of the total root volume (Bailey-Serres and Voesenek, 2008). Most of the higher plants are very sensitive to waterlogged conditions and their growth and yields can be negatively

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**Figure 1.** *Zea mays* roots grown under two different conditions; aerobic and waterlogged conditions. The cross-sections show the inducible lysigenous aerenchyma in maize roots under waterlogged conditions. Scale bar: 0.1 mm (Rajhi et al., 2011).

impacted. Plants cannot survive for long periods under these conditions because oxygen quantity is depleted in flooded soil within 48 h (Purvis and Williamson 1972; Fausey and McDonald, 1985). Plant tolerance to low oxygen availability differs between the species. In fact, only few plants can grow in waterlogged soils, such as rice which is known to be highly tolerant to flooding (Mustroph and Albercht, 2003). To escape the oxygen shortage problem, several transformations at the anatomical, morphological and metabolic levels take place in immersed tissues. The formation of aerenchyma is considered as the main important adaptation to the waterlogged conditions. It can be formed in shoots and roots of a large number of plant species such as maize (He et al., 1996a). Many reviews are emphasized on the morphological, anatomical, pharmacological and molecular studies of aerenchyma formation. In this review, the recent discoveries related to aerenchyma formation in maize roots under waterlogged stress is summarized.

## TYPES OF AERENCHYMA

Aerenchyma is a tissue composing of longitudinal gas

spaces separated by the strands of living cells, found in the root cortex of waterlogged plants as shown in Figure 1. Aerenchyma is formed by one of the two well described mechanisms: schizogeny or lysigeny. Schizogenous aerenchyma is formed by creating gas spaces between cells as a result of highly-regulated cell separation and differential cell expansion, without the death of the cells (Laan et al., 1989). Lysigenous aerenchyma is formed by creating gas spaces as a result of programmed cell death (PCD) in the cortical region of the root (Gunawardena et al., 2001a). Lysigenous aerenchyma is observed in many crops such as flooding tolerant rice, moderately tolerant wheat and intolerant maize (Mustroph and Albercht, 2003). Aerenchyma can be formed by both mechanisms in some species such as *Sagittaria lancifolia* (Schussler and Longstreth, 1996). In maize roots, lysigenous aerenchyma is formed by the death of cells in the mid cortex in a zone behind the apical root. Lysegineous aerenchyma is developed by the digestion of the longitudinal and radial cells separated by live cells attaching the stele and epidermis (Gunawardena et al., 2001a). The walls and the contents of the digested cells completely disappeared (Gunawardena et al., 2001a; Drew et al., 1980). Aerenchyma can be induced in response to stress or constitutively formed. Lysigenous

aerenchyma in many of the wetland plants is developed constitutively in their roots under normal growth conditions such as rice and *Juncus effusus* and its formation is intensified when the soil is saturated with water. In the dryland (non-wetland) plants, such as maize, lysigenous aerenchyma is induced by waterlogging conditions (Drew et al., 1979), mechanical impedance, hypoxia (He et al., 1996b), drought (Zhu et al., 2010) and by nutrient deficiency (He et al., 1992; Vassilis et al., 2012; Postma and Lynch, 2011).

## INDUCTION OF AERENCHYMA BY EXTERNAL STIMULI

### Induction by waterlogging

Gas spaces aerenchyma in the root cortex is formed in response to hypoxia. One of the important functions of aerenchyma is to enhance oxygen transport, where the shortage of oxygen may prevent submerged root respiration (Drew et al., 1979).

### Induction by drought

In order to diminish root metabolic rate to provide greater plant growth and water acquisition, maize roots develop aerenchyma in response to drought stress. Under drought conditions, the biomass production and yield of maize genotypes, which develop more aerenchyma, had respectively five and eight times greater than genotypes which develop less aerenchyma (Zhu et al., 2010).

### Induction by nutrient deficiencies

Maize roots develop aerenchyma when the soil suffers from the deficiency of the nitrate, phosphate or sulphate (Konings and Verschuren 2003; Bouranis et al., 2003; Vassilis et al., 2012). The mechanisms involved in the formation of aerenchyma under these conditions are still unclear. Under nutrient deprivation (nitrate, phosphate and sulphate), signs of PCD were observed at 1 cm behind the root tip of six-day old maize seedlings. The PCD caused by the nutrient deficiency is called nPCD (Vassilis et al., 2012). It has been demonstrated that ROS production may also contribute to aerenchyma formation due to nutrient deficiency (Bouranis et al., 2003). Vassilis et al. (2012) demonstrated that ROS and calcium are involved in the initiation of PCD. In addition, the objective of the development of aerenchyma in response to phosphorous deprivation is the reduction of the respiration and phosphorous content in the root tissue (Postma and Lynch, 2011). Deficiencies of nitrate, phosphate or sulphate increase the sensitivity of cortical cells to ethylene which promotes more cells lysis (Drew

et al., 1979; Bouranis et al., 2003). Ethylene can be considered as a general stress hormone arbitrated responses to hypoxia, drought and a number of nutrient deficiencies and it plays a crucial role in aerenchyma formation in maize roots under different stress conditions (He et al., 1992; Schachtman and Goodger, 2008; Borch et al., 1999; Brown et al., 2003; Postma and Lynch, 2010).

## AERENCHYMA FUNCTION

When plants cannot receive sufficient oxygen quantity for respiration, they develop aerenchyma in their roots. Aerenchyma is very important for the survival of the plants under waterlogged conditions. It minimizes the consumption of total oxygen per unit surface of the root by the formation of air cavities in place of living cells. It is the principal oxygen reservoir and the best ventilation system in the immersed tissue. Aerenchyma allows the passage of gases in and out of tissues in plant roots, petioles and stems. Oxygen is provided to the roots by aerenchyma, while other gases (carbon dioxide, ethylene and methane) are transported from the soil and the root to the shoot and the atmosphere (Armstrong, 1979). The oxygen can be transported by simple diffusion or a consequence of pressure flow. It can be offered from photosynthesis or from the atmosphere. Aerenchyma plays an important role in protecting the root tip from the harmful effect of anoxic soils (such as phytotoxins and organic compounds,  $\text{Fe}^{2+}$  and  $\text{Mn}^{2+}$ ) by increasing the oxygen concentration of the rhizosphere (Mergemann and Sauter, 2000).

## MECHANISMS OF AERENCHYMA FORMATION

### Implication of ethylene in aerenchyma formation

Ethylene, the plant gaseous hormone, which is a simple hydrocarbon that can diffuse into and out of plant tissues from both endogenous and exogenous sources, plays a central role in hypoxic stress signaling (Watkins, 2006). Ethylene is produced from methionine that is first converted to S-adenosylmethionine (AdoMet) by S-adenosylmethionine synthase. AdoMet is then converted to 1-aminocyclopropane-1-carboxylate (ACC) by ACC synthase (ACS). ACC oxidase (ACO) generates ethylene by oxidizing ACC in a reaction that also produces carbon dioxide ( $\text{CO}_2$ ) and hydrogen cyanide (HCN). The plant hormone ethylene is implicated in regulating cell death processes (Jackson et al., 1985). Actually, many of the adaptive growth responses take place in response to ethylene which is cumulated in underwater tissues (Drew et al., 1981). This accumulation is due to the reduced diffusion from the plant to the surrounding water and the induction of the biosynthesis of this hormone by stress

conditions (Drew et al., 1981). This hormone plays a central role in aerenchyma formation in maize roots. Under hypoxic conditions, maize roots act in response by inducing the expression of the ethylene biosynthetic machinery leading to the increase in ethylene production (Geisler-Lee et al., 2010). The process of PCD, which appears to occur in the roots of maize during the formation of lysigenous aerenchyma, appears to be regulated by ethylene (Rajhi et al., 2011; Yamauchi et al., 2011; Takahashi et al., 2015). The hypoxic treatment increased ethylene production in maize roots several fold within 3 h when compared with aerobic conditions. A 5.8 fold was observed at six hours after treatment and the maximum of ethylene evolution (10 fold) was detected at 12 h after the treatment (Geisler-Lee et al., 2010). Treatment of maize roots with inhibitors of ethylene action (e.g., silver ions) or ethylene biosynthesis [e.g., aminoethoxyvinylglycine (AVG), aminoxyacetic acid (AOA) and cobalt chloride] effectively blocks aerenchyma formation under hypoxic conditions (Drew et al., 1981; Konings, 1982). The use of 1-methylcyclopropene (1-MCP), an inhibitor of ethylene perception, totally blocks aerenchyma formation in maize roots under hypoxic conditions (Rajhi et al., 2011). Moreover, aerenchyma can be induced by treatment with ethylene even under aerobic conditions (Jackson et al., 1985; Takahashi et al., 2015). In wheat, Lysigenous aerenchyma formation was induced by ACC treatment (Yamauchi et al., 2014). These observations indicate that ethylene works as a trigger for inducible lysigenous aerenchyma in maize roots.

## Aerenchyma formation via cell death

### Cell death signs

The first signs of cell death during aerenchyma formation were detected in maize cells. The nuclear DNA fragmentation is considered to be a distinctive characteristic of animal cells apoptosis. Analyze of *in situ* terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL) applied to maize roots treated by ethylene or hypoxia suggested internucleosomal cleavage of DNA (Gunawardena et al., 2001a). Cytoplasmic changes including plasma membrane invagination and the formation of vesicles was detected before chromatin condensation (Gunawardena et al., 2001a), which is considered to be the first event in animal apoptosis. The major distinctive event in animal cells apoptosis is the formation of apoptotic bodies. This event was also confirmed by Gunawardena et al. in (2001a). In fact, a cellular condensation, condensation of chromatin and the presence of intact organelles surrounded by membrane similar to apoptotic bodies were detected in maize roots treated by ethylene or hypoxia. Aerenchyma formation in maize roots induced by ethylene or hypoxia shows characteristics in part, similar to both apoptosis

and cytoplasmic cell death in animal cells. Aerenchyma formation in maize roots appears to be a form of programmed cell death (Campbell and Drew, 1983).

### Cell wall degradation

In the final stage of the formation of the lysigenous aerenchyma, the cell wall is enzymatically degraded. In the beginning, the localizations of esterified pectin and de-esterified pectin in cell wall of the maize cortex tissue are modified during cell death, then the cell wall is degraded by the combined actions of pectolytic, xylanolytic and cellulolytic enzymes (Gunawardena et al., 2001a). Indeed, the activities which are involved in loosening or degrading the cell wall including cellulase, xylanase and pectinase, are increased in maize roots under waterlogged conditions. Several genes related to cell wall loosening and degradation such genes encoding xyloglucan endotransglucosylase, polygalacturonase and cellulase were up-regulated specifically in the cortical root cells under waterlogged conditions and their expression is repressed by the treatment of 1-MCP, an inhibitor of ethylene perception, under the same conditions (Rajhi et al., 2011). Both, the treatment of maize roots under hypoxic conditions or with a high ethylene concentrations, can induce remarkable augmentation in cellulase activity within 3 days (He et al., 1994) as well as increases in pectinase and xylanase activities (Bragina et al., 2003). Besides, it has been established that a xyloglucan endotransglycolase homolog in maize, which is induced by both ethylene and flooding, is related to aerenchyma formation (Sachs et al., 1996). In fact, treatment with an ethylene biosynthesis inhibitor, aminoxyacetic acid (AOA), under flooded conditions avoided the spread of aerenchyma in maize roots and totally restrained the accumulation of XET mRNA.

### Involvement of reactive oxygen species in aerenchyma formation

Recently many reports show that reactive oxygen species (ROS) play an important role in plant cell death, defense and growth (Steffens and Sauter, 2009; Yoshioka et al., 2009; Steffens and Sauter 2011). ROS include molecules (hydrogen peroxide and ozone), ions (hypochlorites) and radicals (hydroxyl and superoxide). The major source of ROS in plants is the NADPH oxidase (NOX) that catalyze conversion of dioxygen ( $O_2$ ) to the superoxide radical ( $O_2^-$ ) which ultimately leads to the production of hydrogen peroxide ( $H_2O_2$ ). In plants, the NOX homologs have been named respiratory burst oxidase homologs (Rboh) and they are also involved in ROS production in response to pathogens, plant defense, development, hormone biosynthesis and cellular signal transduction (Sagi and Fluhr, 2001; Torres et al., 2002; Foreman et al., 2003). It has been demonstrated that the hydrogen peroxide operates as a signal for ethylene-induced epidermal cell

death (Steffens and Sauter, 2009, 2005). Additionally, the hydrogen peroxide stimulates aerenchyma in the rice stem (Steffens and Sauter, 2010). In rice, the NADPH oxidase controls the  $H_2O_2$  which regulates epidermal cell death before the emergence of adventitious roots (Steffens and Sauter, 2009, 2005). The inhibitor of NADPH activity diphenylene iodonium (DPI) decreased the rates of cell death in rice epidermal cells (Steffens and Sauter, 2009). In fact, the use of an ethylene-releasing compound, ethephon, enhances ROS generation and increases aerenchyma in rice. Under carbon starvation, the DPI inhibited the  $O_2^-$  production from NADPH oxidase (Rboh) and cell death in carrot (*Daucus carota*) cells (Chae and Lee, 2001). In wheat, the treatment of the roots with ACC and DPI partly suppressed the aerenchyma ACC-induced responses (Yamauchi et al., 2014). In addition, the pre-treatment of wheat seedlings with ACC increased the expression of three genes encoding respiratory burst oxidase homologue. Among three *TaRboh* genes the expression level of the *TaRboh* gene was highest in the first seminal roots immediately after pre-treatment with ACC. However, *TaRboh* expression was highest at 72 h after initiation of growth under stagnant conditions (Yamauchi et al., 2014). In maize seedlings, treatment with 1-MCP repressed the expression of Rboh gene in the root cortical cells under waterlogged conditions. The expression of Rboh gene under hypoxic conditions is highest in cortical cells than in stellar or outer layer cells. Treatment of maize seedlings with DPI under waterlogged conditions reduced aerenchyma formation. This indicates that Rboh in maize is involved in aerenchyma formation specifically in the cortex of the root (Rajhi et al., 2011).

On the other hand, the expression of the gene encoding metallothionein, which works as a ROS scavenger (Wong et al., 2004), was repressed under waterlogged conditions in the cortex of maize roots and that the repression seemed to be ethylene dependent. The cortical cell-specific down-regulation of the maize metallothionein gene may contribute to higher accumulation of the RBOH-produced  $H_2O_2$ , which induces cell death in the cortical cells for lysigenous aerenchyma formation (Rajhi et al., 2011). Interestingly, the rice Metallothionein2b (MT2b) gene is down-regulated in response to ethylene and  $H_2O_2$  in epidermal cells, thereby amplifying the accumulation of  $H_2O_2$  produced by NADPH oxidase, to induce cell death (Steffens and Sauter, 2009, 2010). These observations raise the possibility that, waterlogging-induced up-regulation of Rboh is involved in  $H_2O_2$  production and the  $H_2O_2$  induces aerenchyma cell death in root cortical cells, which is controlled by ethylene.

### Implication of calcium signaling

Calcium-dependent signaling pathways are involved in the process of lysigenous aerenchyma formation in maize

roots (He et al., 1996b). Many studies have suggested that the cytosolic calcium ion ( $Ca^{2+}$ ) functions as a second messenger for signaling pathways in response to oxygen deprivation (Subbaiah et al., 1994; Tsuji et al., 2000; Baxter-Burrell et al., 2002).  $Ca^{2+}$  signaling may also be involved in aerenchyma formation in maize roots (He et al., 1996b). Several genes implicated in calcium signaling, whose expressions were significantly induced specifically in the maize root cortex under the waterlogged conditions were identified such as genes encoding EF hand family protein (Calceineurin B like), calmodulin like protein, C2 domain containing protein and EH domain containing protein. The calcium-dependent protein kinase activates Rboh by phosphorylation of its N-terminal region (Kobayashi et al., 2007). It has been reported that an interaction between the  $Ca^{2+}$  signaling and the RBOH-mediated  $H_2O_2$  production might be important for the programmed cell death in the root cortical cells (Rajhi et al., 2011).

### CONCLUSION

In conclusion, this study aimed to (i) clarify the meaning of aerenchyma formation and its role under different stresses especially under waterlogging conditions and (ii) try to understand the mechanism of formation of air spaces in the root cortical cells.

### CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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