



Regulation of root traits for internal aeration and tolerance to soil waterlogging-flooding stress

Yamauchi, Takaki; Colmer, Timothy D.; Pedersen, Ole; Nakazono, Mikio

Published in:
Plant Physiology

DOI:
[10.1104/pp.17.01157](https://doi.org/10.1104/pp.17.01157)

Publication date:
2018

Document version
Publisher's PDF, also known as Version of record

Citation for published version (APA):
Yamauchi, T., Colmer, T. D., Pedersen, O., & Nakazono, M. (2018). Regulation of root traits for internal aeration and tolerance to soil waterlogging-flooding stress. *Plant Physiology*, 176(2), 1118-1130.
<https://doi.org/10.1104/pp.17.01157>

Regulation of Root Traits for Internal Aeration and Tolerance to Soil Waterlogging-Flooding Stress¹[OPEN]

Takaki Yamauchi,^{a,2} Timothy D. Colmer,^b Ole Pedersen,^{b,c} and Mikio Nakazono^{a,b,3}

^aGraduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya 464-8601, Japan

^bUWA School of Agriculture and Environment, Faculty of Science, The University of Western Australia, Crawley, Western Australia 6009, Australia

^cFreshwater Biological Laboratory, Department of Biology, University of Copenhagen, 2100 Copenhagen, Denmark

ORCID IDs: 0000-0002-6772-6506 (T.Y.); 0000-0002-3383-9596 (T.D.C.); 0000-0002-0827-946X (O.P.); 0000-0001-7119-2052 (M.N.).

Root acquisition of water and nutrients is essential for plant growth and crop productivity (Lynch, 2015). An improved understanding of root system development and functioning, to identify root traits contributing to crop yields in various scenarios, is a research frontier that might enable a second Green Revolution needed to sustain world food security (Lynch, 2007). Roots are challenged by various abiotic and biotic constraints in soils, with water status of too little or too much being a major factor resulting in plant stress. Changing rainfall patterns have resulted in increased flood events in many regions, so that the development of flood-tolerant crops is a priority (Bailey-Serres et al., 2012). Water-saturated soils (i.e. waterlogged soils) are often anoxic, so that roots of poorly adapted species suffer oxygen deficiency that reduces respiration and results in a severe energy crisis, whereas well-adapted wetland species can thrive (Bailey-Serres and Voesenek, 2008). The detrimental impact on upland crops of soil waterlogging can be substantial (e.g. wheat [*Triticum aestivum*]; Setter and Waters, 2003; Herzog et al., 2016).

Root morphological and anatomical traits determine root growth and functioning in anoxic waterlogged soils. An increased number of newly emerged adventitious roots can compensate, at least partially, for the growth inhibition or even death of distal portions of roots present when waterlogging occurs (Jackson and Drew, 1984). Many plant species produce adventitious roots (Visser and Voesenek, 2004), with some emerging into the soil, others along the soil surface, and during deeper floods some even grow into the water column;

the latter are referred to as aquatic adventitious roots. The formation of aerenchyma (Fig. 1), the induction of a barrier to restrict radial oxygen loss (ROL) to the soil (Fig. 1), and the rate of respiratory consumption along roots together largely determine the internal oxygen supply along roots and, thus, the energy status and growth in anoxic waterlogged soils (Armstrong, 1979).

Aerenchyma provides an internal path for low-resistance gas-phase diffusion of oxygen into and along roots (Fig. 1A). Root growth into anoxic waterlogged soils depends upon the distance to which adequate oxygen can reach to sustain the respiratory demands of the root apex as it grows farther away from the shoot base and the source of the oxygen

ADVANCES

- Signaling during root aerenchyma formation in rice involves respiratory burst oxidase homolog-mediated reactive oxygen species production, activated by Ca²⁺-dependent protein kinases, as elucidated by laser microdissection of specific root tissues and transcript analyses.
- Quantitative trait loci mapping of maize x *Zea nicaraguensis* progenies revealed loci associated with aerenchyma formation and tight radial O₂ loss (ROL) barrier induction in roots, as well as adventitious rooting.
- Root ROL barrier induction is associated with increased expression of suberin-related genes in rice, and root barrier induction is responsive to phytotoxins (products of anaerobic microorganism metabolism) in waterlogged soils.
- Aquatic adventitious roots contribute to tolerance of long-term partial shoot submergence of some wetland plants, and these roots can be capable of photosynthesis.

¹ This work was partly supported by the Japan Society for the Promotion of Science (KAKENHI grant 15H04434) to M.N. and by a UWA Research Collaboration Award to T.D.C., O.P., and M.N.

² Current address: Graduate School of Agricultural and Life Sciences, University of Tokyo, Bunkyo, Tokyo 113-8657, Japan.

³ Address correspondence to nakazono@agr.nagoya-u.ac.jp. T.Y., T.D.C., O.P., and M.N. contributed equally to the writing of the article.

[OPEN] Articles can be viewed without a subscription.

www.plantphysiol.org/cgi/doi/10.1104/pp.17.01157

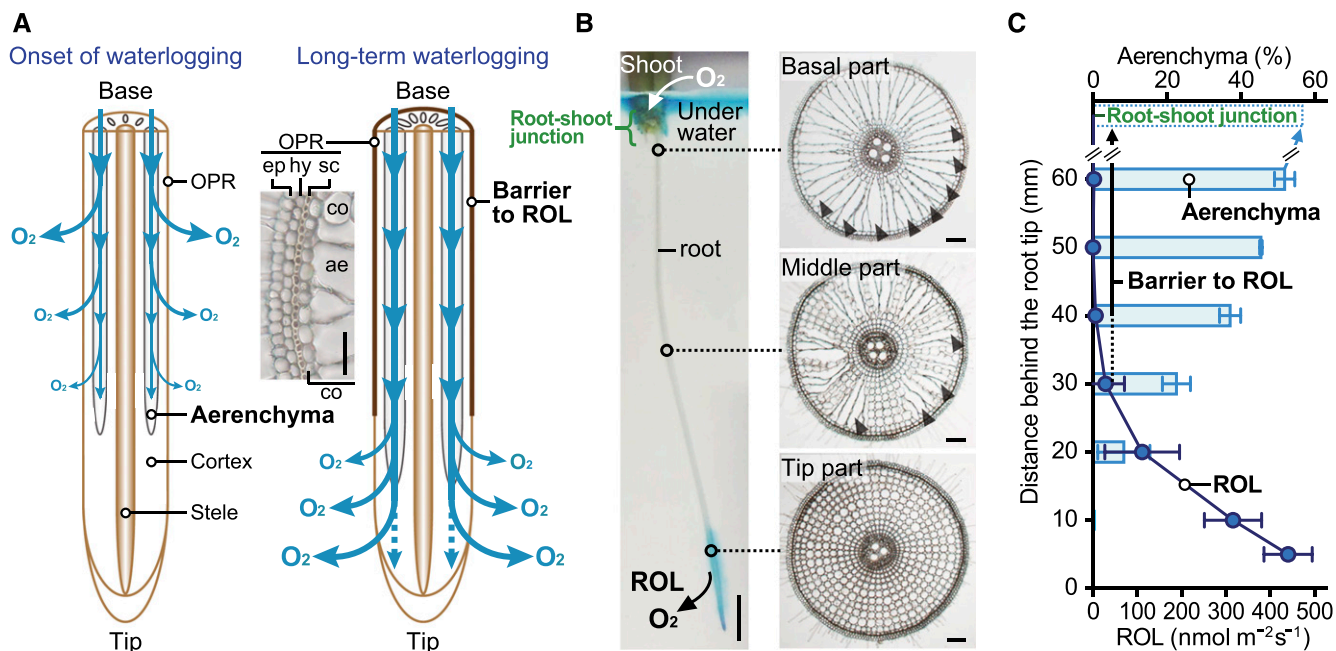


Figure 1. Internal oxygen transport along roots via aerenchyma and enhancement of oxygen diffusion to the root tip by a barrier to ROL in the basal root zones. A, Scheme of aerenchyma and spatial patterns of ROL along roots of rice. Rice roots constitutively form some aerenchyma in well-drained soil but not a tight ROL barrier, so that ROL from basal zones is substantial. During waterlogging, roots develop more aerenchyma and a tight barrier to ROL is induced. OPR, outer part of the root; ep, epidermis; hy, hypodermis/exodermis; sc, sclerenchyma; co, cortex; ae, aerenchyma. Bar = 100 μm . B, ROL from rice adventitious roots was visualized by staining with Methylene Blue in an oxygen-free medium. Blue color indicates oxygen, which diffused outward from the root surface. Cross sections are from just behind the tip (apex), middle, and basal parts of adventitious roots of rice grown under stagnant conditions (oxygen-free rooting medium, shoot in air). Lysigenous aerenchyma is indicated by arrowheads. Bar in the left image (Methylene Blue staining) = 10 mm; bars in the right images (root cross sections) = 100 μm . C, Percentage of aerenchyma of root cross-sectional area and profile of ROL along adventitious roots under stagnant conditions. Nine-day-old aerobically grown rice seedlings were further grown under stagnant conditions for 14 d, and 110- to 130-mm-long adventitious roots, which had emerged under stagnant conditions, were subjected to the experiments. Values are means \pm SD ($n = 3$). Aerenchyma started to form between 10 and 20 mm behind the tip, and the amount of aerenchyma increased toward the root base. Tissue porosity resulting from cubic cell packing would facilitate oxygen diffusion in the tip region. By contrast, the ROL was greatest near the root tip and least in the basal zones due to the induction of a tight barrier to ROL in the outer part of the root; the ROL barrier starts to form at 20 to 30 mm behind the root tip. These two traits facilitate the internal oxygen diffusion from shoots to root tips of wetland plants (e.g. rice) in waterlogged soil. Photographs and unpublished data are from T. Yamauchi and M. Nakazono.

(Armstrong, 1979). Thus, roots of greater gas-filled volume (i.e. greater porosity) can achieve longer lengths in waterlogged soil than roots of low porosity (Armstrong, 1979; Justin and Armstrong, 1987). Importantly, oxygen within the root aerenchyma not only diffuses in the longitudinal direction to the root tip; some of the oxygen is consumed by the root cells and some is also lost radially (i.e. ROL) to the rhizosphere (Fig. 1A). Many wetland plants, including rice (*Oryza sativa*), develop a barrier to ROL in the basal zone of roots, so that ROL is restricted and oxygen diffusion is promoted along the aerenchyma toward the root tip (Fig. 1A; Armstrong, 1979; Colmer, 2003b).

This Update focuses on the root morphological and anatomical traits determining oxygen supply to the distal tips of roots in waterlogged soils and the regulation of these traits during acclimation to

waterlogging-flooding stress. Metabolic acclimations to hypoxia (low oxygen) and anoxia (zero oxygen) also are important for plants during waterlogging-flooding stress, and these are considered elsewhere (Gibbs and Greenway, 2003; Greenway and Gibbs, 2003; Bailey-Serres and Voeselek, 2008; Voeselek and Bailey-Serres, 2015). Recent findings on aerenchyma formation, root ROL barrier induction, and the functioning of aquatic adventitious roots are summarized to highlight advances in knowledge of the signaling pathways underpinning root plasticity to acclimate to waterlogging-flooding events. The use of laser microdissection to enable transcript profiling of specific tissues has delivered insights into ethylene and reactive oxygen species (ROS) signaling pathways for aerenchyma formation in the root cortex and into suberin biosynthesis for ROL barrier formation in the root hypodermis/exodermis.

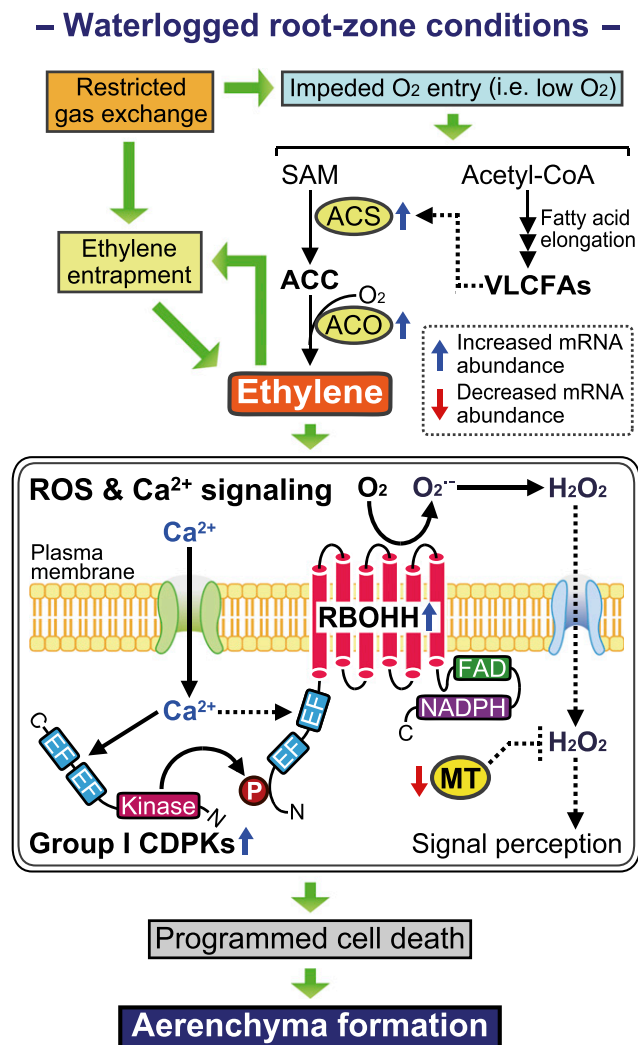


Figure 2. Model of the signaling processes for inducible aerenchyma formation in rice roots, based mainly on recent findings by Yamauchi et al. (2015, 2017a, 2017b). Under waterlogged (or stagnant) conditions, the entry (diffusion) of oxygen from the atmosphere into soil is impeded by restricted gas exchange, which results in low oxygen levels, whereas ethylene levels in roots are elevated due to the restricted gas exchange. Transcript abundances of the genes encoding the ethylene biosynthetic enzymes 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS) and ACC oxidase (ACO) are increased in hypoxic tissues, and the resulting increase in enzyme levels would enhance ethylene biosynthesis in roots, provided that some oxygen is available to ACO. Ethylene entrapment due to the restricted gas exchange further enhances ethylene accumulation in roots. In addition, the biosynthesis of very-long-chain fatty acids (VLCFAs) is enhanced under stagnant conditions, and VLCFAs are suggested to have a role in promoting *ACS1* expression in rice roots by an as yet unknown mechanism. In root cortical cells, ethylene enhances the expression of a gene encoding the respiratory burst oxidase homolog (RBOH) isoform H (RBOHH), which converts oxygen to the superoxide anion radical ($O_2^{\bullet-}$) in the apoplast. Cytosolic Ca^{2+} influx from the apoplast is stimulated in hypoxic roots. RBOHH is phosphorylated and activated by group I Ca^{2+} -dependent protein kinases (CDPKs) such as CDPK5 and CDPK13. The apoplastic $O_2^{\bullet-}$ production mediated by RBOH (e.g. RBOHH) results in an increase in hydrogen peroxide (H_2O_2) levels in the apoplast and/or cytosol. In addition, reduction of the expression of genes encoding type

Moreover, quantitative trait locus (QTL) analysis using mapping populations of barley (*Hordeum vulgare*) and cucumber (*Cucumis sativus*), and most notably produced from wide hybridization of a wetland wild relative *Zea nicaraguensis* with the upland crop maize (*Zea mays ssp. mays*), have yielded genetic information on important traits of aerenchyma formation, ROL barrier formation, and adventitious rooting.

AERENCHYMA FORMATION

Aerenchyma Enables the Movement of Oxygen from Shoots into Roots in Waterlogged Soil

Aerenchyma forms in primary tissues (primary aerenchyma) and in secondary tissues (secondary aerenchyma; Yamauchi et al., 2013; Takahashi et al., 2014). In general, primary aerenchyma is further classified into lysigenous aerenchyma and schizogenous aerenchyma (Justin and Armstrong, 1987). Lysigenous aerenchyma is formed by the death and lysis of cortical cells in roots (Fig. 1B) or parenchyma cells in shoots, whereas schizogenous aerenchyma in roots is formed by the separation of adjacent files of cells through differential division and/or expansion of cortical cells (Justin and Armstrong, 1987; Seago et al., 2005; Takahashi et al., 2014). Lysigenous aerenchyma formation has been studied at the molecular level, whereas schizogenous aerenchyma formation has not, so we focus in this Update on processes resulting in lysigenous aerenchyma, which is the type in roots of rice and other cereals.

Lysigenous aerenchyma forms in roots of a broad range of species, including many agriculturally important plants such as cereals. In rice, lysigenous aerenchyma forms not only in roots but also in the leaf lamina midrib, sheath, and stem internodes (Colmer and Pedersen, 2008). In roots of some wetland plants, such as rice, aerenchyma is constitutively formed under well-drained soil conditions, and its formation is further induced upon soil waterlogging (Fig. 1A). The former and latter are designated as constitutive and inducible aerenchyma formation, respectively (Colmer and Voesenek, 2009). Roots of nonwetland plants, such as maize and wheat, form inducible aerenchyma under waterlogged soil conditions but do not generally form constitutive aerenchyma under well-drained soil conditions (Colmer and Voesenek, 2009). Constitutive aerenchyma formation contributes to waterlogging tolerance, as evident for *Z. nicaraguensis* accessions with a higher degree of constitutive aerenchyma formation (Mano and Omori, 2013b). Interestingly, the death of root cortical cells (sometimes called root cortical senescence) is enhanced in maize and barley under drought or

1 metallothionein (MT1), which acts as a ROS scavenger, may contribute to promote ROS accumulation in the root cortical cells. Together, the increased amounts of apoplastic and/or intracellular ROS ($O_2^{\bullet-}$ and/or H_2O_2) induce PCD and subsequent lysigenous aerenchyma formation in the root cortex.

when nutrient deficient (e.g. nitrogen or phosphorus deficiencies; Lynch, 2015; Schneider et al., 2017b). The death of root cortical cells reduces the respiratory costs and nutrient requirements of the root (per unit of length), thereby releasing resources to sustain some root growth under drought or nutrient-deficient conditions (Lynch, 2015; Schneider et al., 2017a, 2017b).

The formation of aerenchyma enhances the oxygen status of roots, but there is a diffusion gradient along the length of the root so that oxygen declines with distance from the root-shoot junction (Fig. 1A; Armstrong, 1979). In addition to this longitudinal oxygen gradient along the root axis, microelectrode radial profiling of oxygen also demonstrated marked differences in oxygen concentrations between adjacent tissues (Armstrong et al., 1994; Gibbs et al., 1998). In general, radial oxygen profiles indicate epidermal/hypodermal uptake of oxygen from the cortex (or from the external medium when oxygen is present there), with elevated oxygen levels in the porous cortex (when supplied via aerenchyma) and steeply declining oxygen in the dense stelar tissues, which also can have a higher respiratory demand. Severe hypoxia, or even anoxia, occurred in the stelar tissues of roots in a hypoxic medium (Gibbs et al., 1998) and also in roots reliant on the internal movement of oxygen via aerenchyma when the partial pressure of oxygen of the shoots was reduced to 10 kPa (Armstrong et al., 1994). Stellar anoxia can adversely impact ion transport (e.g. in maize [Gibbs et al., 1998] and barley [Kotula et al., 2015]). The implications of these findings are that a narrow stele diameter can be of adaptive significance for waterlogging tolerance as roots with a shorter diffusion path length into the stele, and greater cortex (oxygen source)-to-stele (oxygen sink) tissue ratio, would avoid anoxia until oxygen availability declined to lower levels than for roots with a wider stele (Armstrong and Beckett, 1987).

The Process of Ethylene-Dependent Lysigenous Aerenchyma Formation and Its Adaptive Significance

Inducible aerenchyma formation involves programmed cell death (PCD), which is triggered by ethylene (Drew et al., 1979, 2000). This, in turn, raised the question of how the gaseous phytohormone ethylene can initiate the PCD of specific cell types (i.e. parenchyma cells in shoots or cortical cells in roots) and, indeed, only some of these cells in a coordinated fashion. Recent physiological and molecular studies address this question.

Under waterlogged conditions, ethylene accumulates in roots due to impeded gas movement to the rhizosphere and the enhancement of ethylene biosynthesis in the hypoxic roots (Fig. 2; Voesenek and Sasidharan, 2013; Sasidharan and Voesenek, 2015). Ethylene is produced from *S*-adenosyl-Met in two steps by the successive actions of ACS and ACO (Fig. 2). The step involving ACO requires oxygen for the conversion of ACC to ethylene. The K_m of ACO for oxygen is 0.5 to

0.6 kPa, measured in vitro for extracts from sunflower (*Helianthus annuus*) seedling roots (Finlayson and Reid, 1994). One highly relevant in vivo example of the response to oxygen of ethylene production is for adventitious roots of maize, in which it was almost completely halted in anoxia (nutrient solution flushed with N₂), whereas ethylene production was stimulated at 1 kPa oxygen to be above that in controls with 21 kPa oxygen (Jackson et al., 1985).

Roots of nonwetland (i.e. upland) plants hardly form aerenchyma under aerobic conditions (if water and nutrients are adequate; Lynch, 2015). The onset of soil waterlogging, therefore, may result in tissue anoxia and damage the tips and other distal parts of these roots of relatively low gas-filled porosity before aerenchyma can form (Yamauchi et al., 2014). By contrast, aerenchyma is constitutively formed in roots of many wetland plants (Armstrong, 1979; Colmer and Pedersen, 2008; Colmer and Voesenek, 2009); thus, some oxygen is present farther along roots after the onset of waterlogging so that respiration can continue, which would aid survival. Constitutive aerenchyma formation might not only be beneficial for tissue survival following the onset of waterlogging while aerenchyma forms, but the internal aeration capacity also would provide oxygen required for ethylene production (see preceding paragraph) needed to trigger the subsequent ethylene-dependent aerenchyma formation. However, a greater amount of aerenchyma also would facilitate some venting of this ethylene from the roots (Visser and Pierik, 2007); such venting would only impact aerenchyma formation if ethylene then remained below the threshold concentration required, but this seems unlikely. Exogenously supplied ACC increased ethylene production and aerenchyma formation in rice roots under aerobic conditions (Yamauchi et al., 2015, 2016), demonstrating that a low level of ACC production is likely rate limiting for ethylene biosynthesis in these aerobic roots. On the other hand, the levels of ethylene production in roots of rice and maize reflected the levels of ACO expression; a higher ACO expression level in rice roots than in maize roots under aerated conditions was associated with a more pronounced increase of ethylene production in rice roots than in maize roots immediately after the onset of low-oxygen conditions (Yamauchi et al., 2016).

In rice roots, the expression levels of ACS1 and ACO5 are most strongly increased under stagnant conditions (Yamauchi et al., 2015). Stagnant conditions, here and when used elsewhere in this Update, refers to deoxygenated nutrient solution containing 0.1% (w/v) agar, a treatment that mimics the changes in gas composition (low oxygen and elevated ethylene and CO₂) typically found in waterlogged soils (Wiengweera et al., 1997). Recent studies of a rice mutant have shown a link between VLCFA (20 or more carbons) metabolism and ethylene biosynthesis. The rice *reduced culm number1* (*rcn1*) mutant has a single nucleotide substitution in the gene encoding an ATP-binding cassette (ABC) transporter subfamily G, RCN1/OsABCG5. Although the

substrates of RCN1/OsABCG5 have not been determined, *rcn1* accumulates fewer of the major suberin monomers originating from VLCFAs (Shiono et al., 2014a). The induction of *ACS1* expression is almost completely suppressed in *rcn1* roots, in which saturated VLCFAs of 24, 26, and 28 carbons (i.e. C24, C26, and C28) were significantly lower than in the wild-type roots (Yamauchi et al., 2015). Exogenously supplied VLCFA (C26) increases *ACS1* expression and induces aerenchyma formation in *rcn1* roots. Furthermore, short-term treatment (24–48 h) of wild-type rice seedlings with the fatty acid elongase (i.e. β -ketoacyl-CoA synthase) inhibitor cafenstrole significantly reduced *ACS1* expression, ethylene accumulation, and aerenchyma formation in adventitious roots under stagnant conditions (Yamauchi et al., 2015). These results indicate that VLCFAs are directly or indirectly involved in stimulating ethylene biosynthesis and, thus, contribute to inducible aerenchyma formation in rice roots when in stagnant conditions (Fig. 2).

Unlike the regulation of inducible aerenchyma formation in roots (discussed above), understanding is much less for the regulation of constitutive aerenchyma formation under aerobic conditions. Yukiyooshi and Karahara (2014) reported that the ethylene perception inhibitor 1-methylcyclopropene (1-MCP) partially suppresses constitutive aerenchyma formation in rice roots grown on an agar slab gel. This finding suggests that ethylene is involved not only in inducible aerenchyma formation but also in constitutive aerenchyma formation. This evidence was confirmed by Yamauchi et al. (2016), who found that 1-MCP also partially suppressed aerenchyma formation in rice roots in aerated nutrient solution. The formation of some aerenchyma even when roots were treated with 1-MCP (Yukiyooshi and Karahara, 2014; Yamauchi et al., 2016) could indicate that constitutive aerenchyma formation in rice roots is regulated by an ethylene-independent pathway, as well as the ethylene-dependent pathway, if the 1-MCP treatment blocked all perception of ethylene.

ROS and Ca²⁺ Signaling during Aerenchyma Formation: Regulators of PCD of Specific Root Cortical Cells

The PCD during lysigenous aerenchyma formation in roots (Rajhi et al., 2011; Yamauchi et al., 2014) and shoots (Parlanti et al., 2011; Steffens et al., 2011) involves ROS signaling. RBOH, a plant homolog of mammalian NADPH oxidase, converts oxygen to O₂^{•-} (Fig. 2). Expression analysis using laser-microdissected tissues of rice roots revealed that a gene encoding one RBOH isoform (RBOHH) is induced most strongly in cortical cells under stagnant conditions (Yamauchi et al., 2017b). Knockout of the *RBOHH* gene in rice, which was generated by CRISPR/Cas9, reduces both ROS accumulation and aerenchyma formation (Yamauchi et al., 2017b). In addition to ROS signaling, Ca²⁺-dependent signaling also is involved in PCD for aerenchyma

formation (He et al., 1996; Drew et al., 2000). In rice roots, genes encoding two isoforms of Ca²⁺-dependent protein kinases (CDPK5 and CDPK13) are strongly expressed in cortical cells of roots under stagnant conditions (Yamauchi et al., 2017b). Interestingly, CDPK5 or CDPK13 induces RBOHH-mediated ROS production when *CDPK5* or *CDPK13* is coexpressed with *RBOHH* in *Nicotiana benthamiana* leaves (Yamauchi et al., 2017b). This suggests that ROS production in rice roots is regulated through Ca²⁺-dependent activation of RBOHH (Fig. 2). The ethylene perception inhibitor 1-MCP suppresses the expression of *RBOHH* (but not the expression of *CDPK5* or *CDPK13*) and reduces inducible aerenchyma formation under stagnant conditions (Yamauchi et al., 2017b), suggesting that up-regulation of *RBOHH* expression by ethylene is required for the PCD during inducible aerenchyma formation in rice roots. However, it remains to be determined whether ethylene directly regulates the increase in cytosolic Ca²⁺ (e.g. by its influx from apoplast) under low-oxygen conditions. In maize roots, expression of the *ZmRBOHH* gene, the closest homolog of *OsRBOHH*, is induced during aerenchyma formation under waterlogged conditions, and its expression is suppressed by 1-MCP treatment (Rajhi et al., 2011). Taken together, and although only a couple of species have been studied in-depth, we expect that the control of lysigenous aerenchyma formation through an ethylene, Ca²⁺, and ROS signaling cascade initiating selective PCD of cortical cells is likely a common feature in roots of plants in the family Poaceae (Gramineae).

RBOHH transcript abundance increases in roots of rice and maize under stagnant and waterlogged conditions, not only in the cortical cells but also in other root tissues, although their transcript levels are highest in cortical cells (Rajhi et al., 2011; Yamauchi et al., 2017b). The ROS signaling, therefore, involves components in addition to RBOHH, with MT, a Cys-rich small protein that acts as a ROS scavenger, being a key factor (Fig. 2). In maize roots, the expression level of a gene encoding *MT1* decreases in the cortical cells, but it is maintained in the stelar cells and in the outer cell layers of the roots under waterlogged conditions (Rajhi et al., 2011; Yamauchi et al., 2011). Similarly, *MT1* genes in rice roots are strongly expressed in the outer cell layers of the roots (Yamauchi et al., 2017a), where the expression of *RBOHH* and *CDPKs* also is detected under stagnant conditions (Yamauchi et al., 2017b). These findings suggest that differences in expression levels of the *MT1* genes determine the fate of cells for PCD in roots during aerenchyma formation; when *MT1* protein levels are low, then PCD proceeds. Similarly, in the parenchyma cells of rice internodes, H₂O₂-mediated aerenchyma formation is accelerated by the knock-down of *MT2b* (Steffens et al., 2011). Both *MT1* and *MT2* proteins conserve two Cys-rich domains, which are responsible for the metal-binding and ROS-scavenging activities (Hassinen et al., 2011). In adventitious roots of rice, the expression levels of some *MT1* genes are higher than those of *MT2* genes (Yamauchi et al., 2017a). By

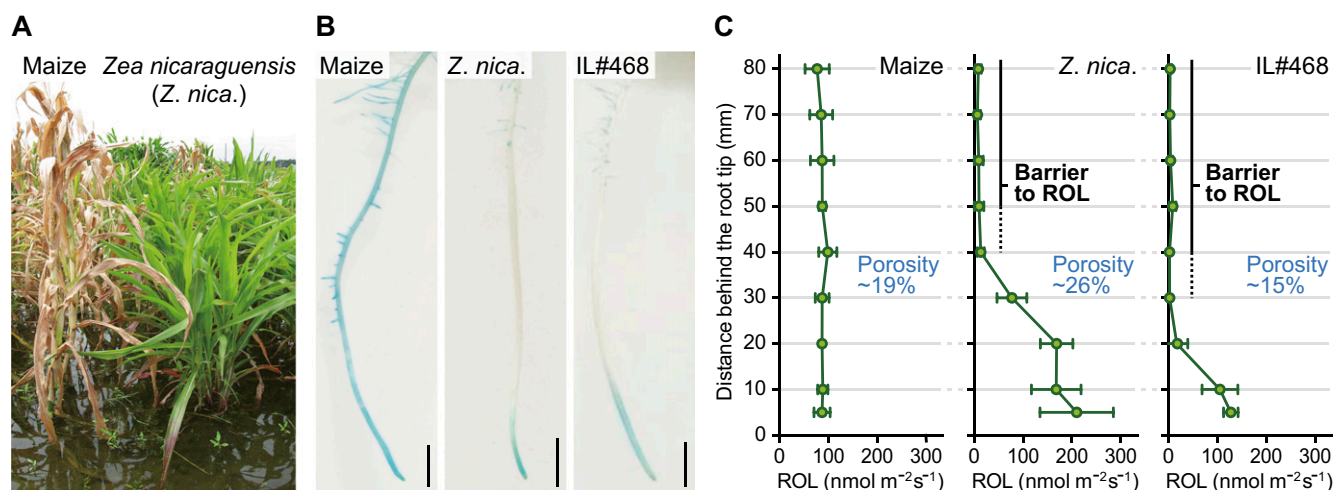


Figure 3. Waterlogging tolerance of *Z. nicaraguensis* and ROL barrier formation in roots as well as in a chromosome segment introgression line in maize. A, Waterlogging tolerance in the field is higher in *Z. nicaraguensis* than in maize (photograph courtesy of Drs. K. Kawaguchi, A. Oyanagi, F. Abe, and Y. Mano). B and C, Patterns of ROL along roots of maize, *Z. nicaraguensis*, and the chromosome segment introgression line #468 (IL#468), which possesses the locus for ROL barrier formation of *Z. nicaraguensis* in the genetic background of maize, under stagnant conditions (O₂-free rooting medium, shoot in air). B, ROL from adventitious roots visualized by Methylene Blue staining in an oxygen-free medium. Blue color indicates oxygen that has diffused outward from roots. Bars = 10 mm. C, Profiles of ROL, measured using a root-sleeving oxygen electrode, along intact adventitious roots in an oxygen-free medium with the shoot in air. Values are means \pm sd ($n = 4$). The ROL was low from the basal zones of roots of *Z. nicaraguensis* and IL#468 due to the induction of a tight ROL barrier, which starts to form at 20 to 40 mm behind the tips. Note that the distances of the apical regions where ROL occurs from roots of *Z. nicaraguensis* and IL#468 are not completely matched between the Methylene Blue staining (B) and the measurements using root-sleeving oxygen electrodes (C), as this can differ for different roots/batches of plants, but in all cases the basal regions had low ROL. Maize, by contrast, does not form a tight ROL barrier in roots. The higher root porosity of *Z. nicaraguensis* than of IL#468 is expected to contribute to the higher ROL levels in the root tips of *Z. nicaraguensis*. Photographs in B and data in C are reproduced from Watanabe et al. (2017) with permission from John Wiley & Sons.

contrast to roots, in the stem of rice, *MT2* genes are expressed higher than *MT1* genes (Zhou et al., 2006). Taken together, *MT1* proteins may play a major role in regulating ROS levels in roots, whereas in stems (internodes), it appears that the *MT2* proteins play the major role. Functional analysis of *MT1* is required to understand the cell type-specific control of ROS accumulation and ROS-induced cortical cell death during aerenchyma formation in roots of the Poaceae.

Genetic Basis of the Trait for Root Aerenchyma Formation

QTL analysis of constitutive aerenchyma formation under nonwaterlogged (i.e. drained) conditions using a BC₂F₁ or BC₄F₁ mapping population produced by crossing maize and *Z. nicaraguensis*, a wild relative that shows higher tolerance than maize to soil waterlogging (Fig. 3A), revealed QTLs for constitutive aerenchyma formation located in four chromosome regions: two on chromosome 1 and one each on chromosomes 5 and 8 (Mano and Omori, 2008, 2009; Mano et al., 2016). In barley, a major QTL for inducible aerenchyma formation in roots that explained 44% of the phenotypic variance is located on chromosome 4H, identified for a doubled haploid population produced from Yerong (waterlogging-tolerant) and Franklin (waterlogging-sensitive) varieties (Zhang et al., 2016).

QTL analysis of inducible aerenchyma formation in roots also has been conducted using a doubled haploid population from barley (variety Franklin) and *Hordeum spontaneum* (accession TAM407227; Zhang et al., 2017c). Interestingly, a major QTL for inducible aerenchyma formation in roots was identified from the Franklin/TAM407227 population, which also mapped on chromosome 4H and at the same, or nearest, position to the QTL for this trait in the Yerong/Franklin population (Zhang et al., 2016, 2017c). Moreover, the QTL for inducible aerenchyma formation on chromosome 4H mapped to the same area as a major QTL for waterlogging tolerance identified from the Franklin/TAM407227 population (Zhang et al., 2017c). Further studies are needed to identify the candidate genes underlying these QTLs for root aerenchyma formation and to study the molecular pathways related to the actions of these genes.

ROOT ROL BARRIER FORMATION

Root Barriers Can Restrict ROL from Basal Zones When in Waterlogged Soils

Roots of many wetland plants possess a barrier to restrict ROL from basal zones, which, in turn, enhances the longitudinal diffusion of oxygen along the aerenchyma toward the root tip (Fig. 1; Armstrong, 1979;

Colmer, 2003b). High resistance to radial oxygen diffusion may result from suberin in hypodermal/exodermal cell walls (e.g. in several wetland species; Armstrong et al., 2000; Soukup et al., 2007; Garthwaite et al., 2008; Kotula et al., 2009a). Respiratory activity in the outer cell layers also consumes oxygen (Armstrong, 1979; Armstrong et al., 2000; Garthwaite et al., 2008). The ROL barrier was demonstrated in roots of *Phragmites australis* by microelectrode radial oxygen profiling, which showed a marked drop in oxygen across the hypodermis/exodermis in the basal portions (i.e. 100 mm behind the root tip) of the roots, demonstrating the strong impedance to radial diffusion, which, together with oxygen consumption in the outer cell layers, resulted in almost zero ROL despite the relatively high oxygen concentration within the aerenchyma and the steep gradient to the anoxic external medium (Armstrong et al., 2000). The ROL barrier, in addition to restricting oxygen loss, also could reduce the entry of soil phytotoxins (e.g. Fe^{2+}) into roots in waterlogged soils (Armstrong, 1979; Armstrong and Armstrong, 2005). Importantly, the greater diffusive resistance across the ROL barrier cells appears not to impede the active uptake of nutrients (e.g. NO_3^- ; Rubinigg et al., 2002) and might have relatively small consequence for water mass flow (Garthwaite et al., 2006; Kotula et al., 2009b); however, data on these aspects are few, and roots with strongly induced tight ROL barriers should be evaluated for nutrient uptake capacity and for radial hydraulic conductivity.

The first quantitative spatial patterns of ROL from roots of rice, which demonstrated a barrier to ROL, were published almost 50 years ago (Armstrong, 1971). The root ROL barrier was shown to occur in several wetland species, and the significance for root aeration was modeled (Armstrong, 1979). The discovery that the rice root ROL barrier is responsive to growth conditions, being absent or only weak in aerated conditions but with a tight ROL barrier formed in roots when in stagnant or waterlogged conditions (Colmer et al., 1998; Colmer, 2003a), sparked renewed interest in this root trait. That is, the radial permeability of the outer part of the root is responsive to environmental conditions in rice and also in roots of some other wetland species, whereas a root ROL barrier forms constitutively in yet other wetland species (McDonald et al., 2002; Colmer, 2003b).

The signaling pathway for root ROL barrier induction remains to be discovered. Ethylene sensing triggers the formation of inducible aerenchyma (Fig. 2 and previous section), but ethylene does not induce the root ROL barrier in rice (Colmer et al., 2006). Growth under stagnant conditions induces ROL barrier development (Colmer, 2003a), but low oxygen alone imposed using N_2 -flushed nutrient solution is not enough to signal ROL barrier development, nor was the combination of low oxygen and elevated ethylene (Colmer et al., 2006). CO_2 also can increase to high levels in waterlogged soils (Greenway et al., 2006), but high (10 kPa) CO_2 did not induce barrier formation in rice (Colmer et al., 2006).

Barrier induction is weak in shorter roots but strong in longer roots, indicating that root tissue age/development stage influences the ROL barrier (Colmer et al., 2006; Shiono et al., 2011). Barrier induction commences within the first day upon exposure of a previously aerated root system to stagnant conditions (Shiono et al., 2011). Hypodermal/exodermal cell walls develop electron-dense materials during the first stages of ROL barrier formation (Shiono et al., 2011). A transcriptome analysis using laser-microdissected tissues of the outer cell layers of rice roots revealed that many genes involved in suberin biosynthesis (but not lignin biosynthesis) were strongly up-regulated during ROL barrier formation in rice (Shiono et al., 2014b). Moreover, malic acid and VLCFAs accumulate in the rice roots forming a ROL barrier, suggesting that malic acid is used for the biosynthesis of fatty acids, which, in turn, provide substrates for suberin biosynthesis (Kulichikhin et al., 2014). Interestingly, various compounds in waterlogged soils resulting from the metabolism of anaerobic microorganisms can induce a ROL barrier in roots of wetland species; these compounds include sulfide (Armstrong and Armstrong, 2005), Fe^{2+} (Mongon et al., 2014), and organic acids (Armstrong and Armstrong, 2001; Kotula et al., 2014). Further research is needed to elucidate the signaling events and biochemical regulation of root ROL barrier formation.

Genetic Basis of the Trait for Root ROL Barrier Formation

An inducible root ROL barrier forms in some waterlogging-tolerant wild relatives of upland crops, such as *Hordeum marinum* (McDonald et al., 2001; Garthwaite et al., 2003; Kotula et al., 2017) and *Z. nicaraguensis* (Abiko et al., 2012; Watanabe et al., 2017). The ability to hybridize these waterlogging-tolerant species with their crop relatives presents an opportunity for crop improvement and for studies of the genetic regulation of traits contributing to waterlogging tolerance. One example of an attempt to transfer an inducible ROL barrier to roots of wheat is the wide hybridization of *H. marinum* with wheat to produce amphiploids and disomic chromosome addition lines (Malik et al., 2011; Konnerup et al., 2017). However, although a root ROL barrier was partially expressed in some amphiploids, six of a possible seven disomic chromosome addition lines from such an amphiploid did not display the root ROL barrier trait (Malik et al., 2011; Konnerup et al., 2017). A more promising example is *Z. nicaraguensis* chromosome segment introgression lines in maize (Mano and Omori, 2013a). Several traits related to waterlogging tolerance have been assessed in these lines (Mano and Omori, 2013a); our focus here is on the inducible tight ROL barrier formation in roots of *Z. nicaraguensis*, a trait lacking in maize (Fig. 3, B and C; Abiko et al., 2012). The recent discovery that a chromosome segment introgression line expresses tight ROL barrier formation in adventitious

roots (Fig. 3, B and C; Watanabe et al., 2017) is an exciting development toward a better understanding of this root trait and for the capacity to incorporate it into maize via breeding. The segment was from the short arm of chromosome 3 and was ~22.6 Mb, so fine-mapping work is needed to develop molecular markers for use in marker-assisted breeding of this root trait, for which phenotyping is somewhat laborious, as well as to help in the eventual identification of the candidate gene(s). The use of mutant lines and transgenic plants to test specific candidate genes, with phenotyping using the oxygen indicator dye Methylene Blue followed by quantitative measurements using root-sleeving oxygen electrodes (as described by Watanabe et al. [2017]; Fig. 3, B and C), should enable the continuation of the recent gains in knowledge of the root ROL barrier trait.

ADVENTITIOUS ROOT FORMATION

Formation of Adventitious Roots as a Response to Soil Waterlogging and Deeper Floods

In waterlogged soils, the existing root system can suffer from a lack of oxygen, and the resulting low ATP production as oxidative phosphorylation ceases, unless aerenchyma is present (Bailey-Serres and Voesenek, 2010). New adventitious roots, containing aerenchyma, are formed from the stem in order to restore root functioning (i.e. water and nutrient uptake as well as anchorage; Visser and Voesenek, 2004; Sauter, 2013; Steffens and Rasmussen, 2016). When standing water occurs during deeper floods, adventitious roots also can emerge from the submerged portion of the stem.

The accumulation of auxin and ethylene in stem tissues can trigger the signaling cascade for adventitious root outgrowth in many dicots (Visser and Voesenek, 2004; Vidoz et al., 2010) and in monocots (e.g. rice; Steffens et al., 2006). Deepwater rice has been used as a model to unravel the signaling that results in the growth of adventitious roots from primordia that are constitutively formed at each node. The essential role of ethylene in adventitious root outgrowth from nodes of deepwater rice stems and a mechanical signal from the growing root tip to the overlying epidermis, in combination, result in the death of the epidermal cells overlying the growing root tip (Steffens et al., 2012). Moreover, the generation of ROS is involved in the signaling of epidermal cell death (Steffens et al., 2012). Steffens and Rasmussen (2016) recently presented a conceptual model on signaling and the formation of adventitious roots in response to flooding stress.

Genetic Basis of the Trait for Flooding-Induced Adventitious Root Formation

Adventitious root formation is controlled by complex genetic programs at each step, such as the initiation of root primordia, root emergence, and subsequent growth. Genetic factors controlling adventitious root

formation have been identified in Arabidopsis (*Arabidopsis thaliana*) and rice, and the regulatory networks have been reviewed elsewhere (Bellini et al., 2014).

Many species respond to soil flooding by the outgrowth of an increased number of adventitious roots. QTLs for flooding-induced adventitious root formation have been identified in maize (inbred Na4; Mano et al., 2005b), *Zea mays* ssp. *huehuetenangensis* (Mano et al., 2005a), and *Z. nicaraguensis* (Mano et al., 2009). These studies used, respectively, F₂ mapping populations produced from maize inbred lines B64 and Na4, from maize inbred line B64 and *Z. mays* ssp. *huehuetenangensis*, or a BC₃F₁ mapping population produced from maize inbred line Mi29 and *Z. nicaraguensis*. QTLs for adventitious root formation induced by soil flooding (i.e. the shallow water layer above a waterlogged soil) were located on chromosomes 3, 7, and 8 (Mano et al., 2005b), on chromosomes 4 and 8 (Mano et al., 2005a), and on chromosomes 3, 7, and 8 (Mano et al., 2009), respectively. A comparison of QTL positions between different mapping populations indicated that some QTL positions overlapped (Mano et al., 2009).

Using mapping populations produced from the cucumber waterlogging-tolerant line Zaoer-N (with greater flooding-induced adventitious root formation) and the waterlogging-sensitive line Pepino (with fewer flooding-induced adventitious roots), QTL analysis was carried out to map the chromosomal region(s) controlling adventitious root formation during soil waterlogging (Xu et al., 2017). The QTLs controlling increased adventitious root formation under waterlogged soil conditions were located on chromosomes 3, 5, and 6. RNA sequencing analysis revealed that 15 genes within the region of the major-effect QTL located on chromosome 6 (ARN6.1) were differentially expressed in response to soil waterlogging (Xu et al., 2017). Functional analyses of the differentially expressed genes will be a promising approach for the identification of gene(s) influencing adventitious root formation for the ARN6.1 locus.

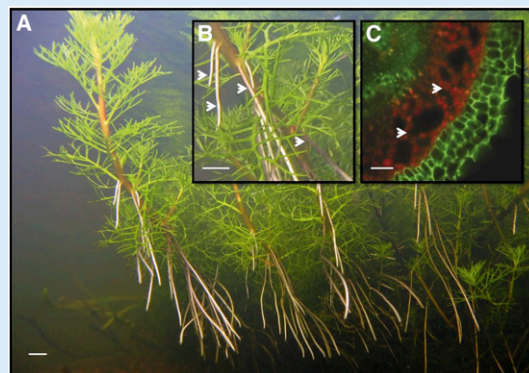
The development of maize, or cucumber, near-isogenic lines that possess these QTLs associated with adventitious roots would be a promising approach to identify the candidate genes regulating the outgrowth of these roots and to further evaluate the beneficial effect of flooding-induced adventitious root formation for waterlogging tolerance of these two species.

Aquatic Adventitious Roots

Amphibious plants often respond to floods resulting in partial shoot submergence by producing large numbers of adventitious roots also from the higher nodes along the stems in addition to the basal nodes (previous section); a well-known example is deepwater rice (Kende et al., 1998). These aquatic adventitious roots extend into the water column and take up nutrients dissolved in the floodwater (Cumbus and Robinson, 1977; Khan et al., 1982). Partially submerged

BOX 1. Photosynthetic Aquatic Adventitious**Roots**

In addition to nutrient and O₂ uptake from the floodwater, aquatic adventitious roots may also produce O₂ in underwater photosynthesis. Chloroplasts develop in adventitious roots of several species, but few studies have quantified the underwater photosynthesis by these roots. As examples, *Tecticornia pergranulata* (Rich et al., 2008) and *Meionectes brownii* (Rich et al., 2011) form photosynthetic aquatic adventitious roots. In the case of *T. pergranulata*, the photosynthetic rate (surface area basis) of these roots was 2-fold higher than that of the submerged succulent shoots, whereas net underwater photosynthesis on area basis of roots of *M. brownii* was typically less than 10% of that of the aquatic leaves. The aquatic adventitious roots of *M. brownii*, however, did not contribute to the O₂ status in the main stem regardless of whether these roots were photosynthetic or not (Rich et al., 2011). Underwater photosynthesis has not been assessed for other species forming aquatic adventitious roots; thus, the contribution of such roots to plant carbohydrate and/or O₂ status during submergence stress cannot yet be generalized.



BOX 1 Figure. Photographs: (A) *Meionectes brownii* possessing numerous aquatic adventitious roots when growing in a flooded habitat. (B) The roots are initially white (left arrows) when first produced but many roots subsequently turn pink (middle arrow) or greenish-grey (right arrow) and become photosynthetic (see text in BOX 1). (C) Some aquatic roots contain numerous chloroplasts which are primarily located in the cortex and are visible as red autofluorescent organelles (arrows) under UV light; example shown is a root of *Tecticornia pergranulata*. Photographs in A and B are by Ole Pedersen and, in C, are reproduced from Rich et al. (2008) with permission from John Wiley & Sons. Scale bars are 10 mm (A and B) or 50 μ m (C).

Solanum dulcamara showed a strong positive correlation between the uptake of phosphate from the floodwater and the total mass of aquatic adventitious roots (Zhang et al., 2017a). Moreover, partially submerged plants that were allowed to form plenty of aquatic adventitious roots showed higher transpiration and more growth than plants in which the formation of aquatic adventitious roots was experimentally stunted (Zhang et al., 2017a). The advantages summarized above only appeared during prolonged partial submergence (3 and 4 weeks). The vigorous growth of aquatic adventitious roots in *S. dulcamara* ceased under complete submergence and could not be fully restored by increasing floodwater oxygen (Zhang et al., 2017b). Plant carbohydrate status also influenced the growth of aquatic adventitious roots, but poor carbohydrate status prior to submergence could be partly alleviated via high light during submergence (Zhang et al., 2017b), presumably leading to higher underwater net photosynthesis (Colmer et al., 2011) and, thus, de novo carbohydrate production. Thus, the formation of aquatic adventitious roots depends on environmental factors such as floodwater depth (i.e. whether part of the stem is submerged or not), oxygen concentration, levels of light, and likely also dissolved CO₂ concentrations (the latter two influence

carbohydrate status) during floods resulting in plant submergence.

Aquatic adventitious roots also can enhance the internal aeration of the main stem of some submerged plants. Completely submerged *Alternanthera philoxeroides* with aquatic adventitious roots maintained stem tissue oxygen in darkness at 3.4 kPa, as opposed to only 1 kPa in stems where roots had been pruned (Ayi et al., 2016). The roots are possibly more gas permeable than the stem tissues and represent a large surface area for gases to equilibrate between the plant body and the floodwater, and since the adventitious roots are well connected with the stem via aerenchyma, oxygen that enters from the floodwater can diffuse into the stem (Ayi et al., 2016). Interestingly, aquatic adventitious roots also may produce oxygen in underwater photosynthesis (Rich et al., 2008, 2011; see BOX on photosynthetic aquatic adventitious roots).

Aquatic adventitious roots can experience dramatic fluctuations in tissue oxygen status during a diurnal cycle. In a field situation, aquatic adventitious roots of *Tecticornia pergranulata* cycled between periods of several hours of anoxia in the cortex during the night followed by steep increases in tissue oxygen upon sunrise (up to 15 kPa at 3 h after sunrise) as oxygen was produced by the completely submerged shoot (Pedersen

et al., 2006). The fluctuations in aquatic root oxygen status experienced by *Meionectes brownii* were even more dramatic: in the cortex reaching up to 43 kPa in the early afternoon, which then declined during the night to as low as 3 kPa just prior to sunrise (Rich et al., 2013). Similarly, for submerged plants with adventitious roots completely buried in anoxic soil, the tissues experience large diurnal fluctuations in oxygen status, as shown for submerged paddy rice, with long periods of anoxia during the night and steep increases in root oxygen (up to 14 kPa at midday) during daylight (Winkel et al., 2013), or for the aquatic plant *Lobelia dortmanna*, where root oxygen cycled between 8 kPa (late night) and 21 kPa (late afternoon; Sand-Jensen et al., 2005). The influence on root functioning of these large daily changes in oxygen status can be profound (e.g. root extension occurred only during the light period, when oxygen was available, and ceased during the night, when ethanol production commenced for submerged rice; Waters et al., 1989) and requires further study.

PERSPECTIVES ON FUTURE RESEARCH

This Update has focused on root aeration traits of aerenchyma, ROL barrier induction, and adventitious roots; here, we consider future research on these traits (for other traits, see Gibbs and Greenway, 2003; Greenway and Gibbs, 2003; Bailey-Serres and Voese-nek, 2008; Colmer and Voese-nek, 2009; Shabala et al., 2014).

Studies on aquatic adventitious roots are few, and research is needed on nutrient and water uptake from the surrounding water and on the roles of these roots in hormone production and carbohydrate and oxygen production/uptake/loss to fully understand the eco-physiological significance of these roots for plants with partially or fully submerged shoots. Moreover, the influence on aquatic root functioning of marked daily cycles in oxygen status (see preceding section) and of radial gradients in oxygen across the roots, which can result in an anoxic or severely hypoxic stele (Armstrong and Beckett, 1987), requires further elucidation.

Recent physiological and molecular biological studies have revealed important aspects of the signaling pathways involved in the formation of root aerenchyma, induction of a tight ROL barrier, and outgrowth of adventitious roots. This Update has focused on progress in understanding the signaling and genetic regulation of these root traits, but the need for additional physiological research on the responses of roots of various species to soil phytotoxins and root functioning in nutrient and water uptake from waterlogged soils also is emphasized (Shabala et al., 2014). The influence of a tight ROL barrier, as well as root morphology (including the role of lateral roots), on the acquisition of nutrients and water during soil waterlogging and upon subsequent drainage (including the recovery growth of roots) requires further study. In relation to signaling during root acclimation to

OUTSTANDING QUESTIONS

- Only some root cortical cells undergo programmed cell death during aerenchyma formation. What signaling mechanism determines the different fates of cortical cells during aerenchyma formation in roots of the Poaceae (Gramineae)?
- Constitutive aerenchyma formation in rice roots under aerobic conditions is regulated through ethylene-dependent and ethylene-independent pathways. Which signals control the ethylene-independent pathway?
- What is the signaling pathway for the inducible tight barrier to ROL in response to soil waterlogging in roots of rice and other wetland plants?
- Which gene(s) within the quantitative trait loci regions identified for aerenchyma formation, tight ROL barrier induction, and adventitious rooting confers these root traits?
- How widely occurring are photosynthetic aquatic adventitious roots and what are the contributions of these roots to shoot water, nutrient, and hormone status during flooding stress?

waterlogging, significant progress has been made in demonstrating the relationship between Ca^{2+} and ROS signaling for inducible aerenchyma formation and its regulation at the molecular level (Yamauchi et al., 2017b). However, key regulators (e.g. transcription factors) controlling these root traits remain to be identified. For this purpose, transcriptome analyses combined with laser microdissection were carried out, and many regulatory genes associated with lysigenous aerenchyma formation in maize roots (Rajhi et al., 2011; Takahashi et al., 2015) and tight ROL barrier formation in rice roots (Shiono et al., 2014b) were identified. Functional analyses of the identified regulatory genes will add significantly to our understanding of the molecular mechanisms controlling the formation of lysigenous aerenchyma and also of the tight ROL barrier.

Further analyses of QTLs for root aeration traits are another powerful tool to identify the key regulator genes. Identification of the responsible genes for these QTLs will make great progress in our understanding of the molecular mechanisms that control the root aeration traits under soil waterlogging. Currently, Mano et al. (2016) have been narrowing down the QTL regions in *Z. nicaraguensis* chromosomes by fine-mapping and developing near-isogenic lines of maize (inbred line Mi29), each possessing smaller chromosome segment(s) with one or more QTLs for these waterlogging-tolerance-related root traits, and furthermore have

been producing maize lines pyramiding these QTLs. The QTL pyramiding lines in maize possessing *Z. nicaraguensis* traits for constitutive aerenchyma, the inducible tight ROL barrier, and adventitious root formation will be expected to improve the waterlogging tolerance of maize. To this end, field testing will be needed to quantify the breeding value of these root traits in various waterlogging-flooding regimes and soil types for the above maize lines as well as for breeding programs and genetic studies of waterlogging tolerance in other crops.

ACKNOWLEDGMENTS

We thank Kentaro Kawaguchi, Atsushi Oyanagi, Fumitaka Abe, Yoshiro Mano, and Kohtaro Watanabe for providing some images used in Figure 3. We also thank Yoshiro Mano and Fumie Omori for discussion and critical reading of the article.

Received August 16, 2017; accepted November 3, 2017; published November 8, 2017.

LITERATURE CITED

- Abiko T, Kotula L, Shiono K, Malik AI, Colmer TD, Nakazono M (2012) Enhanced formation of aerenchyma and induction of a barrier to radial oxygen loss in adventitious roots of *Zea nicaraguensis* contribute to its waterlogging tolerance as compared with maize (*Zea mays* ssp. *mays*). *Plant Cell Environ* **35**: 1618–1630
- Armstrong J, Armstrong W (2001) Rice and *Phragmites*: effects of organic acids on growth, root permeability, and radial oxygen loss to the rhizosphere. *Am J Bot* **88**: 1359–1370
- Armstrong J, Armstrong W (2005) Rice: sulfide-induced barriers to root radial oxygen loss, Fe²⁺ and water uptake, and lateral root emergence. *Ann Bot* **96**: 625–638
- Armstrong W (1971) Radial oxygen losses from intact rice roots as affected by distance from the apex, respiration and waterlogging. *Physiol Plant* **25**: 192–197
- Armstrong W (1979) Aeration in higher plants. *Adv Bot Res* **7**: 225–332
- Armstrong W, Beckett PM (1987) Internal aeration and the development of stelar anoxia in submerged roots: a multishelled mathematical model combining axial diffusion of oxygen in the cortex with radial losses to the stele, the wall layers and the rhizosphere. *New Phytol* **105**: 221–245
- Armstrong W, Cousins D, Armstrong J, Turner DW, Beckett PM (2000) Oxygen distribution in wetland plant roots and permeability barriers to gas-exchange with the rhizosphere: a microelectrode and modelling study with *Phragmites australis*. *Ann Bot* **86**: 687–703
- Armstrong W, Strange ME, Cringle S, Beckett PM (1994) Microelectrode and modelling study of oxygen distribution in roots. *Ann Bot* **74**: 287–299
- Ayi Q, Zeng B, Liu J, Li S, van Bodegom PM, Cornelissen JHC (2016) Oxygen absorption by adventitious roots promotes the survival of completely submerged terrestrial plants. *Ann Bot* **118**: 675–683
- Bailey-Serres J, Lee SC, Brinton E (2012) Waterproofing crops: effective flooding survival strategies. *Plant Physiol* **160**: 1698–1709
- Bailey-Serres J, Voisenek LACJ (2008) Flooding stress: acclimations and genetic diversity. *Annu Rev Plant Biol* **59**: 313–339
- Bailey-Serres J, Voisenek LACJ (2010) Life in the balance: a signaling network controlling survival of flooding. *Curr Opin Plant Biol* **13**: 489–494
- Bellini C, Pacurar DI, Perrone I (2014) Adventitious roots and lateral roots: similarities and differences. *Annu Rev Plant Biol* **65**: 639–666
- Colmer TD (2003a) Aerenchyma and an inducible barrier to radial oxygen loss facilitate root aeration in upland, paddy and deep-water rice (*Oryza sativa* L.). *Ann Bot* **91**: 301–309
- Colmer TD (2003b) Long-distance transport of gases in plants: a perspective on internal aeration and radial oxygen loss from roots. *Plant Cell Environ* **26**: 17–36
- Colmer TD, Cox MCH, Voisenek LACJ (2006) Root aeration in rice (*Oryza sativa*): evaluation of oxygen, carbon dioxide, and ethylene as possible regulators of root acclimatizations. *New Phytol* **170**: 767–777
- Colmer TD, Gibberd MR, Wiengweera A, Tinh TK (1998) The barrier to radial oxygen loss from roots of rice (*Oryza sativa* L.) is induced by growth in stagnant solution. *J Exp Bot* **49**: 1431–1436
- Colmer TD, Pedersen O (2008) Oxygen dynamics in submerged rice (*Oryza sativa*). *New Phytol* **178**: 326–334
- Colmer TD, Voisenek LACJ (2009) Flooding tolerance: suites of plant traits in variable environments. *Funct Plant Biol* **36**: 665–681
- Colmer TD, Winkel A, Pedersen O (2011) A perspective on underwater photosynthesis in submerged terrestrial wetland plants. *AoB Plants* **2011**: plr030
- Cumbus IP, Robinson LW (1977) The function of root systems in mineral nutrition of watercress (*Rorippa nasturtium-aquaticum* (L.) Hayek). *Plant Soil* **47**: 395–406
- Drew MC, He CJ, Morgan PW (2000) Programmed cell death and aerenchyma formation in roots. *Trends Plant Sci* **5**: 123–127
- Drew MC, Jackson MB, Giffard S (1979) Ethylene-promoted adventitious rooting and development of cortical air spaces (aerenchyma) in roots may be adaptive responses to flooding in *Zea mays* L. *Planta* **147**: 83–88
- Finlayson SA, Reid DM (1994) Influence of CO₂ on ACC oxidase activity from roots of sunflower (*Helianthus annuus*) seedlings. *Phytochemistry* **35**: 847–851
- Garthwaite AJ, Armstrong W, Colmer TD (2008) Assessment of O₂ diffusivity across the barrier to radial O₂ loss in adventitious roots of *Hordeum marinum*. *New Phytol* **179**: 405–416
- Garthwaite AJ, Steudle E, Colmer TD (2006) Water uptake by roots of *Hordeum marinum*: formation of a barrier to radial O₂ loss does not affect root hydraulic conductivity. *J Exp Bot* **57**: 655–664
- Garthwaite AJ, von Bothmer R, Colmer TD (2003) Diversity in root aeration traits associated with waterlogging tolerance in the genus *Hordeum*. *Funct Plant Biol* **30**: 875–889
- Gibbs J, Greenway H (2003) Mechanisms of anoxia tolerance in plants. I. Growth, survival and anaerobic catabolism. *Funct Plant Biol* **30**: 1–47
- Gibbs J, Turner DW, Armstrong W, Darwent MJ, Greenway H (1998) Response to oxygen deficiency in primary maize roots. I. Development of oxygen deficiency in the stele reduces radial solute transport to the xylem. *Aust J Plant Physiol* **25**: 745–758
- Greenway H, Armstrong W, Colmer TD (2006) Conditions leading to high CO₂ (>5 kPa) in waterlogged-flooded soils and possible effects on root growth and metabolism. *Ann Bot* **98**: 9–32
- Greenway H, Gibbs J (2003) Mechanisms of anoxia tolerance in plants. II. Energy requirements for maintenance and energy distribution to essential processes. *Funct Plant Biol* **30**: 999–1036
- Hassinen VH, Tervahauta AI, Schat H, Kärenlampi SO (2011) Plant metallothioneins: metal chelators with ROS scavenging activity? *Plant Biol (Stuttg)* **13**: 225–232
- He CJ, Morgan PW, Drew MC (1996) Transduction of an ethylene signal is required for cell death and lysis in the root cortex of maize during aerenchyma formation induced by hypoxia. *Plant Physiol* **112**: 463–472
- Herzog M, Striker GG, Colmer TD, Pedersen O (2016) Mechanisms of waterlogging tolerance in wheat: a review of root and shoot physiology. *Plant Cell Environ* **39**: 1068–1086
- Jackson MB, Drew MC (1984) Effects of flooding on growth and metabolism of herbaceous plants. In TT Koslowski, ed, *Flooding and Plant Growth*. Academic Press, Orlando, FL, pp 47–128
- Jackson MB, Fenning TM, Drew MC, Saker LR (1985) Stimulation of ethylene production and gas-space (aerenchyma) formation in adventitious roots of *Zea mays* L. by small partial pressures of oxygen. *Planta* **165**: 486–492
- Justin SHFW, Armstrong W (1987) The anatomical characteristics of roots and plant response to soil flooding. *New Phytol* **106**: 465–495
- Kende H, van der Knaap E, Cho HT (1998) Deepwater rice: a model plant to study stem elongation. *Plant Physiol* **118**: 1105–1110
- Khan MR, Ventura W, Vergara BS (1982) Uptake through aquatic roots and distribution of ¹⁵N-tagged ammonium in deepwater rice. In Proceedings of the 1981 International Deepwater Rice Workshop. International Rice Research Institute, Los Banos, Philippines, pp 321–326
- Konnerup D, Malik AI, Islam AKMR, Colmer TD (2017) Evaluation of root porosity and radial oxygen loss of disomic addition lines of *Hordeum marinum* in wheat. *Funct Plant Biol* **44**: 400–409
- Kotula L, Clode PL, Striker GG, Pedersen O, Läuchli A, Shabala S, Colmer TD (2015) Oxygen deficiency and salinity affect cell-specific ion concentrations in adventitious roots of barley (*Hordeum vulgare*). *New Phytol* **208**: 1114–1125

- Kotula L, Colmer TD, Nakazono M** (2014) Effects of organic acids on the formation of the barrier to radial oxygen loss in roots of *Hordeum marinum*. *Funct Plant Biol* **41**: 187–202
- Kotula L, Ranathunge K, Schreiber L, Steudle E** (2009a) Functional and chemical comparison of apoplastic barriers to radial oxygen loss in roots of rice (*Oryza sativa* L.) grown in aerated or deoxygenated solution. *J Exp Bot* **60**: 2155–2167
- Kotula L, Ranathunge K, Steudle E** (2009b) Apoplastic barriers effectively block oxygen permeability across outer cell layers of rice roots under deoxygenated conditions: roles of apoplastic pores and of respiration. *New Phytol* **184**: 909–917
- Kotula L, Schreiber L, Colmer TD, Nakazono M** (2017) Anatomical and biochemical characterisation of a barrier to radial O₂ loss in adventitious roots of two contrasting *Hordeum marinum* accessions. *Funct Plant Biol* **44**: 845–857
- Kulichikhin K, Yamauchi T, Watanabe K, Nakazono M** (2014) Biochemical and molecular characterization of rice (*Oryza sativa* L.) roots forming a barrier to radial oxygen loss. *Plant Cell Environ* **37**: 2406–2420
- Lynch JP** (2007) Roots of the second Green Revolution. *Aust J Bot* **55**: 493–512
- Lynch JP** (2015) Root phenes that reduce the metabolic costs of soil exploration: opportunities for 21st century agriculture. *Plant Cell Environ* **38**: 1775–1784
- Malik AI, Islam AKMR, Colmer TD** (2011) Transfer of the barrier to radial oxygen loss in roots of *Hordeum marinum* to wheat (*Triticum aestivum*): evaluation of four *H. marinum*-wheat amphiploids. *New Phytol* **190**: 499–508
- Mano Y, Muraki M, Fujimori M, Takamizo T, Kindiger B** (2005a) Identification of QTL controlling adventitious root formation during flooding conditions in teosinte (*Zea mays* ssp. *huhuetenangensis*) seedlings. *Euphytica* **142**: 33–42
- Mano Y, Omori F** (2008) Verification of QTL controlling root aerenchyma formation in a maize × teosinte "*Zea nicaraguensis*" advanced backcross population. *Breed Sci* **58**: 217–223
- Mano Y, Omori F** (2009) High-density linkage map around the root aerenchyma locus *Qaer1.06* in the backcross populations of maize Mi29 × teosinte "*Zea nicaraguensis*." *Breed Sci* **59**: 427–433
- Mano Y, Omori F** (2013a) Flooding tolerance in interspecific introgression lines containing chromosome segments from teosinte (*Zea nicaraguensis*) in maize (*Zea mays* subsp. *mays*). *Ann Bot* **112**: 1125–1139
- Mano Y, Omori F** (2013b) Relationship between constitutive root aerenchyma formation and flooding tolerance in *Zea nicaraguensis*. *Plant Soil* **370**: 447–460
- Mano Y, Omori F, Loaisiga CH, Bird RMck** (2009) QTL mapping of above-ground adventitious roots during flooding in maize × teosinte "*Zea nicaraguensis*" backcross population. *Plant Root* **3**: 3–9
- Mano Y, Omori F, Muraki M, Takamizo T** (2005b) QTL mapping of adventitious root formation under flooding conditions in tropical maize (*Zea mays* L.) seedlings. *Breed Sci* **55**: 343–347
- Mano Y, Omori F, Tamaki H, Mitsuhashi S, Takahashi W** (2016) DNA marker-assisted selection approach for developing flooding-tolerant maize. *Jpn Agric Res Q* **50**: 175–182
- McDonald MP, Galwey NW, Colmer TD** (2001) Waterlogging tolerance in the tribe Triticeae: the adventitious roots of *Critesion marinum* have a relatively high porosity and a barrier to radial oxygen loss. *Plant Cell Environ* **24**: 585–596
- McDonald MP, Galwey NW, Colmer TD** (2002) Similarity and diversity in adventitious root anatomy as related to root aeration among a range of wetland and dryland grass species. *Plant Cell Environ* **25**: 441–451
- Mongon J, Konnerup D, Colmer TD, Rerkasem B** (2014) Responses of rice to Fe²⁺ in aerated and stagnant conditions: growth, root porosity and radial oxygen loss barrier. *Funct Plant Biol* **41**: 922–929
- Parlanti S, Kudahettige NP, Lombardi L, Mensuali-Sodi A, Alpi A, Perata P, Pucciariello C** (2011) Distinct mechanisms for aerenchyma formation in leaf sheaths of rice genotypes displaying a quiescence or escape strategy for flooding tolerance. *Ann Bot* **107**: 1335–1343
- Pedersen O, Vos H, Colmer TD** (2006) Oxygen dynamics during submergence in the halophytic stem succulent *Halosarcia pergranulata*. *Plant Cell Environ* **29**: 1388–1399
- Rajhi I, Yamauchi T, Takahashi H, Nishiuchi S, Shiono K, Watanabe R, Mliki A, Nagamura Y, Tsutsumi N, Nishizawa NK, et al** (2011) Identification of genes expressed in maize root cortical cells during lysigenous aerenchyma formation using laser microdissection and microarray analyses. *New Phytol* **190**: 351–368
- Rich SM, Ludwig M, Colmer TD** (2008) Photosynthesis in aquatic adventitious roots of the halophytic stem-succulent *Tecticornia pergranulata* (formerly *Halosarcia pergranulata*). *Plant Cell Environ* **31**: 1007–1016
- Rich SM, Ludwig M, Pedersen O, Colmer TD** (2011) Aquatic adventitious roots of the wetland plant *Meionectes brownii* can photosynthesize: implications for root function during flooding. *New Phytol* **190**: 311–319
- Rich SM, Pedersen O, Ludwig M, Colmer TD** (2013) Shoot atmospheric contact is of little importance to aeration of deeper portions of the wetland plant *Meionectes brownii*; submerged organs mainly acquire O₂ from the water column or produce it endogenously in underwater photosynthesis. *Plant Cell Environ* **36**: 213–223
- Rubinigg M, Stulen I, Elzenga JTM, Colmer TD** (2002) Spatial patterns of radial oxygen loss and nitrate net flux along adventitious roots of rice raised in aerated or stagnant solution. *Funct Plant Biol* **29**: 1475–1481
- Sand-Jensen K, Pedersen O, Binzer T, Borum J** (2005) Contrasting oxygen dynamics in the freshwater isoetid *Lobelia dortmanna* and the marine seagrass *Zostera marina*. *Ann Bot* **96**: 613–623
- Sasidharan R, Voeselek LACJ** (2015) Ethylene-mediated acclimations to flooding stress. *Plant Physiol* **169**: 3–12
- Sauter M** (2013) Root responses to flooding. *Curr Opin Plant Biol* **16**: 282–286
- Schneider HM, Postma JA, Wojciechowski T, Kuppe C, Lynch JP** (2017a) Root cortical senescence improves growth under suboptimal availability of N, P, and K. *Plant Physiol* **174**: 2333–2347
- Schneider HM, Wojciechowski T, Postma JA, Brown KM, Lücke A, Zeisler V, Schreiber L, Lynch JP** (2017b) Root cortical senescence decreases root respiration, nutrient content and radial water and nutrient transport in barley. *Plant Cell Environ* **40**: 1392–1408
- Seago JL Jr, Marsh LC, Stevens KJ, Soukup A, Votrubová O, Enstone DE** (2005) A re-examination of the root cortex in wetland flowering plants with respect to aerenchyma. *Ann Bot* **96**: 565–579
- Setter TL, Waters I** (2003) Review of prospects for germplasm improvement for waterlogging tolerance in wheat, barley and oats. *Plant Soil* **253**: 1–34
- Shabala S, Shabala L, Barcelo J, Poschenrieder C** (2014) Membrane transporters mediating root signalling and adaptive responses to oxygen deprivation and soil flooding. *Plant Cell Environ* **37**: 2216–2233
- Shiono K, Ando M, Nishiuchi S, Takahashi H, Watanabe K, Nakamura M, Matsuo Y, Yasuno N, Yamanouchi U, Fujimoto M, et al** (2014a) RCN1/OsABCG5, an ATP-binding cassette (ABC) transporter, is required for hypodermal suberization of roots in rice (*Oryza sativa*). *Plant J* **80**: 40–51
- Shiono K, Ogawa S, Yamazaki S, Isoda H, Fujimura T, Nakazono M, Colmer TD** (2011) Contrasting dynamics of radial O₂-loss barrier induction and aerenchyma formation in rice roots of two lengths. *Ann Bot* **107**: 89–99
- Shiono K, Yamauchi T, Yamazaki S, Mohanty B, Malik AI, Nagamura Y, Nishizawa NK, Tsutsumi N, Colmer TD, Nakazono M** (2014b) Microarray analysis of laser-microdissected tissues indicates the biosynthesis of suberin in the outer part of roots during formation of a barrier to radial oxygen loss in rice (*Oryza sativa*). *J Exp Bot* **65**: 4795–4806
- Soukup A, Armstrong W, Schreiber L, Franke R, Votrubová O** (2007) Apoplastic barriers to radial oxygen loss and solute penetration: a chemical and functional comparison of the exodermis of two wetland species, *Phragmites australis* and *Glyceria maxima*. *New Phytol* **173**: 264–278
- Steffens B, Geske T, Sauter M** (2011) Aerenchyma formation in the rice stem and its promotion by H₂O₂. *New Phytol* **190**: 369–378
- Steffens B, Kovalev A, Gorb SN, Sauter M** (2012) Emerging roots alter epidermal cell fate through mechanical and reactive oxygen species signaling. *Plant Cell* **24**: 3296–3306
- Steffens B, Rasmussen A** (2016) The physiology of adventitious roots. *Plant Physiol* **170**: 603–617
- Steffens B, Wang J, Sauter M** (2006) Interactions between ethylene, gibberellin and abscisic acid regulate emergence and growth rate of adventitious roots in deepwater rice. *Planta* **223**: 604–612
- Takahashi H, Yamauchi T, Colmer TD, Nakazono M** (2014) Aerenchyma formation in plants. *Plant Cell Monogr* **21**: 247–265
- Takahashi H, Yamauchi T, Rajhi I, Nishizawa NK, Nakazono M** (2015) Transcript profiles in cortical cells of maize primary root during ethylene-induced lysigenous aerenchyma formation under aerobic conditions. *Ann Bot* **115**: 879–894

- Vidocz ML, Loreti E, Mensuali A, Alpi A, Perata P (2010) Hormonal interplay during adventitious root formation in flooded tomato plants. *Plant J* **63**: 551–562
- Visser EJW, Pierik R (2007) Inhibition of root elongation by ethylene in wetland and non-wetland plant species and the impact of longitudinal ventilation. *Plant Cell Environ* **30**: 31–38
- Visser EJW, Voeselek LACJ (2004) Acclimation to soil flooding—sensing and signal-transduction. *Plant Soil* **254**: 197–214
- Voeselek LACJ, Bailey-Serres J (2015) Flood adaptive traits and processes: an overview. *New Phytol* **206**: 57–73
- Voeselek LACJ, Sasidharan R (2013) Ethylene—and oxygen signalling—drive plant survival during flooding. *Plant Biol (Stuttg)* **15**: 426–435
- Watanabe K, Takahashi H, Sato S, Nishiuchi S, Omori F, Malik AI, Colmer TD, Mano Y, Nakazono M (2017) A major locus involved in the formation of the radial oxygen loss barrier in adventitious roots of teosinte *Zea nicaraguensis* is located on the short-arm of chromosome 3. *Plant Cell Environ* **40**: 304–316
- Waters I, Armstrong W, Thomson CJ, Setter TL, Adkins S, Gibbs J, Greenway H (1989) Diurnal changes in radial oxygen loss and ethanol metabolism in roots of submerged and non-submerged rice seedlings. *New Phytol* **113**: 439–451
- Wiengweera A, Greenway H, Thomson CJ (1997) The use of agar nutrient solution to simulate lack of convection in waterlogged soils. *Ann Bot* **80**: 115–123
- Winkel A, Colmer TD, Ismail AM, Pedersen O (2013) Internal aeration of paddy field rice (*Oryza sativa*) during complete submergence—importance of light and floodwater O₂. *New Phytol* **197**: 1193–1203
- Xu X, Ji J, Xu Q, Qi X, Chen X (2017) Inheritance and quantitative trait loci mapping of adventitious root numbers in cucumber seedlings under waterlogging conditions. *Mol Genet Genomics* **292**: 353–364
- Yamauchi T, Fukazawa A, Nakazono M (2017a) *METALLOTHIONEIN* genes encoding ROS scavenging enzymes are down-regulated in the root cortex during inducible aerenchyma formation in rice. *Plant Signal Behav* **12**: e1388976
- Yamauchi T, Rajhi I, Nakazono M (2011) Lysigenous aerenchyma formation in maize root is confined to cortical cells by regulation of genes related to generation and scavenging of reactive oxygen species. *Plant Signal Behav* **6**: 759–761
- Yamauchi T, Shimamura S, Nakazono M, Mochizuki T (2013) Aerenchyma formation in crop species: a review. *Field Crops Res* **152**: 8–16
- Yamauchi T, Shiono K, Nagano M, Fukazawa A, Ando M, Takamura I, Mori H, Nishizawa NK, Kawai-Yamada M, Tsutsumi N, et al (2015) Ethylene biosynthesis is promoted by very-long-chain fatty acids during lysigenous aerenchyma formation in rice roots. *Plant Physiol* **169**: 180–193
- Yamauchi T, Tanaka A, Mori H, Takamura I, Kato K, Nakazono M (2016) Ethylene-dependent aerenchyma formation in adventitious roots is regulated differently in rice and maize. *Plant Cell Environ* **39**: 2145–2157
- Yamauchi T, Watanabe K, Fukazawa A, Mori H, Abe F, Kawaguchi K, Oyanagi A, Nakazono M (2014) Ethylene and reactive oxygen species are involved in root aerenchyma formation and adaptation of wheat seedlings to oxygen-deficient conditions. *J Exp Bot* **65**: 261–273
- Yamauchi T, Yoshioka M, Fukazawa A, Mori H, Nishizawa NK, Tsutsumi N, Yoshioka H, Nakazono M (2017b) An NADPH oxidase RBOH functions in rice roots during lysigenous aerenchyma formation under oxygen-deficient conditions. *Plant Cell* **29**: 775–790
- Yukiyoshi K, Karahara I (2014) Role of ethylene signalling in the formation of constitutive aerenchyma in primary roots of rice. *AoB Plants* **6**: plu043
- Zhang Q, Huber H, Beljaars SJM, Birnbaum D, de Best S, de Kroon H, Visser EJW (2017a) Benefits of flooding-induced aquatic adventitious roots depend on the duration of submergence: linking plant performance to root functioning. *Ann Bot* **120**: 171–180
- Zhang Q, Huber H, Boerakker JWT, Bosch D, de Kroon H, Visser EJW (2017b) Environmental factors constraining adventitious root formation during flooding of *Solanum dulcamara*. *Funct Plant Biol* **44**: 858–866
- Zhang X, Fan Y, Shabala S, Koutoulis A, Shabala L, Johnson P, Hu H, Zhou M (2017c) A new major-effect QTL for waterlogging tolerance in wild barley (*H. spontaneum*). *Theor Appl Genet* **130**: 1559–1568
- Zhang X, Zhou G, Shabala S, Koutoulis A, Shabala L, Johnson P, Li C, Zhou M (2016) Identification of aerenchyma formation-related QTL in barley that can be effective in breeding for waterlogging tolerance. *Theor Appl Genet* **129**: 1167–1177
- Zhou G, Xu Y, Li J, Yang L, Liu JY (2006) Molecular analyses of the metallothionein gene family in rice (*Oryza sativa* L.). *J Biochem Mol Biol* **39**: 595–606