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Regulation of root adaptive anatomical and morphological traits during low soil oxygen

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Summary
Flooding causes oxygen deprivation in soils. Plants adapt to low soil oxygen availability by changes in root morphology, anatomy, and architecture to maintain root system functioning. Essential traits include aerenchyma formation, a barrier to radial oxygen loss, and outgrowth of adventitious roots into the soil or the floodwater. We highlight recent findings of mechanisms of constitutive aerenchyma formation and of changes in root architecture. Moreover, we use modelling of internal aeration to demonstrate the beneficial effect of increasing cortex-to-stele ratio on sustaining root growth in waterlogged soils. We know the genes for some of the beneficial traits, and the next step is to manipulate these genes in breeding in order to enhance the flood tolerance of our crops.

I. Introduction
Many regions of the world are experiencing floods at greater frequencies, so crops are needed that are more tolerant of soil waterlogging (Pedersen et al., 2017). Waterlogged soils are typically devoid of oxygen ($O_2$), yet roots require $O_2$ for respiration. A lack of energy results in growth impairment, reduced nutrient ion uptake, inability for cell maintenance, cell damage, and even death. These adverse effects on roots impact on shoot growth and crop yield (e.g. wheat ($Triticum aestivum$); Herzog et al., 2016).

Root morphological and anatomical traits contribute to plant adaptation to waterlogged soils. Acclimation to low $O_2$ status and the associated energy crisis have been reviewed elsewhere (Bailey-Serres & Voesenek, 2008; Schmidt et al., 2018; Armstrong et al., 2019). Outgrowth of adventitious roots, into the soil, along the surface or into the floodwater, is a common feature of tolerant plants. Aerenchyma, developed constitutively and/or induced...
upon soil waterlogging, enhances the internal supply of O₂ along roots. Features such as a greater cortex-to-stele ratio (CSR) and a smaller surface area to volume (SA : V) also promote the diffusion of O₂ along roots. In addition, a barrier within the outer cell layers to prevent radial O₂ loss (ROL) from the cortex to the rhizosphere further enhances O₂ movement to the growing apex of roots in waterlogged soils.

This Tansley insight highlights recent findings on the key root system architecture and root anatomical traits (see Box 1), including work on the development of constitutive aerenchyma and to unravel the signalling pathways involved in acclimation to soil waterlogging. Moreover, we demonstrate the beneficial effects of the various traits to root growth in waterlogged soil by presentation of outputs from a mathematical model of internal aeration.

II. Role and molecular mechanism of constitutive aerenchyma formation

Formation of gas-filled aerenchyma in the root cortex is the mechanism by which internal O₂ supply can increase because O₂ diffuses substantially faster in gas phase than in the liquid phase. In the family Poaceae, aerenchyma is formed by the autolysis of root cortical cells (lysigenous aerenchyma; see Yamauchi et al., 2018). In some wetland plants, such as rice (Oryza sativa), lysigenous aerenchyma is formed constitutively (i.e. even in drained soil), and its formation is further induced by soil waterlogging. Thus, constitutive and inducible aerenchyma formation can be distinguished (Colmer & Voesenek, 2009). Several other plants, in addition to rice, have constitutively formed aerenchyma; Zea nicaraguensis, a wetland relative of maize (Zea mays subsp. mays), is an interesting example. When the roots of Z. nicaraguensis are first exposed to soil waterlogging, O₂ can immediately move internally to the root tips (Gong et al., 2019). Moreover, constitutively formed aerenchyma accelerates inducible aerenchyma formation (Gong et al., 2019). Inducible aerenchyma formation is stimulated by ethylene, and the production of ethylene requires O₂ (see Yamauchi et al., 2018); roots with constitutive aerenchyma experience hypoxia, but avoid anoxia, and ethylene then triggers inducible aerenchyma formation. The beneficial effect of pre-existing aerenchyma was also demonstrated by artificially inducing aerenchyma in wheat roots under aerated conditions by application of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) prior to imposing stagnant, deoxygenated conditions; aerenchyma in the ACC pretreated roots promoted internal O₂ movement to the root tips (Yamauchi et al., 2014). Therefore, constitutive aerenchyma formation in roots is of adaptive significance for plants faced with transient soil waterlogging. In addition to aerenchyma formation, the ACC pretreatment to wheat roots increased expression of genes for ethanol fermentation (Yamauchi et al., 2014); ethylene ‘priming’ of tissues can enhance acclimation to subsequent anoxia (Hartman et al., 2019).

The pathway controlling inducible aerenchyma formation in roots is ethylene dependent and is also regulated by reactive oxygen species (ROS; Yamauchi et al., 2017, 2018). In wheat roots, nitric oxide levels increase under hypoxia, and this appears to stimulate ethylene production, ROS generation, and cortical cell death for inducible aerenchyma formation (Wany et al., 2017). In rice, constitutive aerenchyma formation in roots is regulated by both ethylene-dependent and ethylene-independent pathways (Yamauchi et al., 2018). Until recently, we had poor knowledge of how constitutive aerenchyma development is regulated. Yamauchi et al. (2019b) found that constitutive aerenchyma formation in rice roots is regulated by auxin signalling. The auxin signalling is mediated by auxin/indole-3-acetic acid protein (AUX/IAA; e.g. IAA13) and auxin response factor (ARF; e.g. ARF19). Auxin signalling mediated by these proteins stimulates the expression of a transcription factor called lateral organ boundaries domain (LBD)-containing protein 1–8 (LBD1–8), which regulates the initiation of both constitutive aerenchyma formation and lateral root development in rice roots (Fig. 1a). It is likely that optimal levels of auxin differ for triggering constitutive aerenchyma formation in the cortical cells and lateral root development in the pericycle cells (Fig. 1a; Yamauchi et al., 2019b). Further studies are needed to understand how constitutive aerenchyma formation and lateral root development are differently regulated by LBD1–8 in the cortical cells and pericycle cells, respectively. Moreover, it would be of interest to determine whether aerenchyma-related auxin signalling is ethylene independent.

III. A large cortex-to-stele ratio enables deeper root penetration in anoxic soils

Aerenchyma formation and a barrier to ROL are traits determining the maximum root length in waterlogged soils (Armstrong, 1979; Yamauchi et al., 2018). In addition, the CSR (area based) can also increase in roots as a response to waterlogging (Yamauchi et al., 2019a). A greater cross-sectional area of the porous cortex, compared with dense tissues of the stele, promotes internal O₂ diffusion and root extension into anoxic soils (Sundgren et al., 2018). We have quantified the CSR trait using a modified version of the root aeration model by Armstrong (1979) in order to estimate maximum root length (Supporting Information Methods S1). Maximum root length is determined by the diffusion path length, which is in turn influenced by the overall root gas-filled porosity as well as by root O₂ consumption rate, both of which change with the proportions of the metabolically active stelar tissues and less active cortex tissue. Our results demonstrate the beneficial effect of increasing CSR on the maximum length achieved by waterlogged roots, both for roots without a barrier to ROL (Fig. 2b) or with a barrier to ROL (Fig. 2c). Our modelling indicates that species with an inherently small CSR (e.g. maize and wheat) may benefit substantially from only small increases in the CSR, whereas rice already has a large CSR with already near-maximal benefits on maximum root length (Fig. 2b,c). Plasticity to increase CSR is thus a trait contributing to waterlogging tolerance in some species; and from the limited data available, such plasticity appears present in at least some dryland crops, as well as large CSR being a key feature of rice (Fig. 2a; Yamauchi et al., 2019a).
Box 1 Root traits involved in plant adaptation to flooding.

Soil waterlogging and partial shoot submergence affect the root system. Roots need oxygen (O2) for respiration, which provides the energy (ATP) required for cell maintenance, growth, and to transport nutrient ions. When the soil becomes waterlogged, or if deeper floods also partially submerge the shoots, roots need to continue functioning so as to sustain the shoots with nutrients and water. The existing aerobically grown roots need to survive and acclimate if possible, whereas new adventitious roots better adapted to low soil O2 can also emerge from stem nodes. Some roots can also emerge into the water above the soil. Signalling processes trigger acclimations (see following lists and Figs 1, 2). Acclimations that increase internal O2 movement enhance root growth into anoxic waterlogged soils.

**Aerobically grown roots that then experience waterlogging**
- If O2/energy deficient, anaerobic metabolism is initiated to produce some ATP to enable short-term survival.
- Constitutive aerenchyma enables immediate internal movement of O2 and continued functioning.
- Inducible aerenchyma formation further enhances internal O2 movement.
- Formation of secondary aerenchyma (e.g. aerenchymatous phellem, a spongy tissue filled with gas spaces) in roots, root nodules, stem, and hypocotyl of some plants (e.g. legumes) enhances gas exchange between belowground tissues and atmosphere.
- Induction of a radial O2 loss (ROL) barrier facilitates longitudinal O2 diffusion, and may also impede phytotoxin entry.
- Changes in root growth direction may facilitate acquisition of O2, which may be present in the shallow soil layers.

**Newly developed/emerged adventitious roots**
- Changes in root architecture lead, first, to fewer and shorter laterals, thus reducing the demand for O2 from the main axis, and, second, to wide growth angles, resulting in superficial roots near the soil surface where some O2 may be present.
- Aerenchyma formation enhances supply of O2 to the root tips.
- ROL barrier formation further enhances O2 diffusion to root tips.
- A large cortex-to-stele ratio in adventitious roots enables greater internal O2 movement.
- Thick roots are less prone to ROL than thin roots are, as a proportion of internal O2 available.
- Adventitious roots develop aboveground and access O2 in the floodwater.

*Box Fig. 1* Rice (upland, lowland, or deepwater): When soil is waterlogged, the existing, aerobically grown roots acclimate to remain viable by enhancing O2 supply through formation of aerenchyma and a barrier to ROL. Moreover, development and emergence of thicker adventitious roots with a large cortex-to-stele ratio, larger aerenchyma, and ROL barrier are enhanced. When deeper floods partially submerge the shoot, internodes of deepwater rice elongate and adventitious roots emerge at the nodes into the floodwater (‘aquatic roots’). Arabidopsis: Roots grow sideways upon hypoxia, which may avoid deeper anoxic soil layers. Formation and growth of lateral roots is inhibited during hypoxia.
IV. Thick roots are less prone to radial oxygen loss than thin roots

The SA : V of roots is a trait that responds to soil waterlogging. Thin roots have a high SA : V, implying that O₂ within aerenchyma of thin roots is more prone to radial diffusion into anoxic soils, at least for roots lacking a barrier to ROL. Rice, maize, and wheat all respond to waterlogging by forming new adventitious roots with a lower SA : V, owing to these new roots being of greater diameter than the thinner existing roots. We have performed a quantitative analysis based on whole root cross-sectional areas in photographs presented by Yamauchi et al. (2019a). In the case of rice, total root cross-sectional area is approximately two-fold higher for roots developed under stagnant, deoxygenated conditions than for the thinner roots in aerated nutrient solution (Yamauchi et al., 2019a), causing a reduction in SA : V of 24%; consequently, this would reduce ROL by 24% in the absence of a barrier to ROL (if ROL per unit root surface area were equivalent in the two cases). Plasticity in the root SA : V trait is greatest for maize (35% reduction in stagnant, deoxygenated solution compared with aerated nutrient solution) and smaller for wheat (16% reduction). However, since neither maize (Abiko et al., 2012) nor wheat (Malik et al., 2001) can form a barrier to ROL, any reduction in SA : V would be of importance for maintaining internal, longitudinal, O₂ diffusion along roots towards the tips when in anoxic waterlogged soil.

V. Environmental triggering of root radial oxygen loss barrier formation

ROL reduces the distance that O₂ reaches via diffusion along the aerenchyma, which in turn restricts root growth in waterlogged soils. The roots of many wetland species, however, form a barrier near the root exterior that substantially reduces ROL from the basal zones (Armstrong, 1979; Colmer, 2003). Suberin deposited in the walls of hypodermis/exodermis cells likely contributes to the ROL barrier (Armstrong et al., 2000; Soukup et al., 2007; Garthwaite et al., 2008; Kotula et al., 2009). Interestingly, an ROL barrier is formed constitutively in roots of some species, whereas it is induced by soil waterlogging in others; this second category includes rice (Colmer, 2003). Although ethylene triggers aerenchyma formation in roots of rice, it does not signal root ROL barrier formation (Colmer et al., 2006). Some compounds produced by anaerobic bacteria in waterlogged soils, such as low molecular weight carboxylic acids, sulphides, or reduced iron (‘soil phytotoxins’), can trigger the formation of a root ROL barrier (Armstrong & Armstrong, 1999; Kotula et al., 2014; Mongon et al., 2014). Studies have typically used high concentrations of phytotoxins (though still environmentally relevant), with root growth adversely affected. A recent study of rice demonstrated triggering of root ROL barrier formation by sub-toxic concentrations of carboxylic acids (Colmer et al., 2019). Concentrations of <0.05 mM of four organic acids triggered an ROL barrier in roots of rice, and there was no adverse effect on root tissue respiration (Colmer et al., 2019).

The role of suberin in the root ROL barrier is supported by observations of upregulation of suberin biosynthesis-related genes in whole root segments (Colmer et al., 2019), as well as in tissues sampled from the outer cell layers of roots of rice (Shiono et al., 2014). However, suberin lamellae also occur in roots of species that do not form a barrier to ROL (Abiko et al., 2012; Watanabe et al., 2017); the O₂ permeability of these suberin lamellae may be determined by the suberin monomer composition and/or the structure (Watanabe et al., 2017). In summary, the root ROL barrier enhances the role of aerenchyma to promote longitudinal diffusion of O₂ within roots towards the tip, and thus root growth in anoxic waterlogged soils (compare the modelled lengths of roots in Fig. 2b,c).

VI. Changes in root architecture in response to flooding

Root system architecture is determined by the formation, elongation, and the growth angle of roots. Recent studies revealed that all three parameters are changed under flooding conditions, resulting in an overall altered root architecture. The formation of adventitious roots and the ability to form aerenchyma are major quantitative traits of flooding tolerance (Mustroph, 2018), and many flooding-adapted plants form dormant adventitious root primordia that emerge upon flooding. This process is regulated via ethylene functioning as a ‘flooding signal’ and results in development of porous roots that functionally support or replace the original root system (Dawood et al., 2016). In maize, adventitious root formation and survival of long-term waterlogging were promoted by ZmEREB180 (Yu et al., 2019), a member of group VII ethylene response factors (ERFVIIIs) that act as key transcriptional regulators of low-O₂ responses in plants. Interestingly, ZmEREB180 also reduced oxidative damage and promoted expression of metabolic hypoxia genes during submergence, indicating that developmental and metabolic adaptations to flooding are coordinately controlled. Adventitious roots improve nutrient uptake and plant fitness, particularly during long-term flooding (Zhang et al., 2017). In Arabidopsis, ERFVIIIs in hypoxic lateral root primordia prevent transcription of auxin-controlled developmental genes, thereby suppressing lateral root development (Shukla et al., 2019). The growth of adventitious roots and repression of lateral root development under low-O₂ conditions change the root system architecture with a shift from belowground to stem-borne, sometimes floating, roots.

VII. Avoidance responses of roots

Flooding is a compound stress involving low light, slow gas diffusion with reduced O₂ availability, and accumulation of ethylene followed by re-oxygenation during de-submergence. Root systems respond in several ways to improve O₂ supply to the growing root tip. Adaptations of aquatic adventitious roots are aimed at avoiding soil anoxia and low redox potential E₄₋. The avoidance of such a hostile environment leads to growth toward the soil surface rather than downward into soil. An
example of avoidance responses is the reversible adjustment of the root growth angle in rice, where adventitious roots emerging from the stem nodes grow upwards in the dark and downwards

in the light, mediated by both red and blue light receptors (Lin & Sauter, 2018; Fig. 1b). This growth behaviour suggests that darkness signals soil areas with low O₂ tension that must be
avoided, whereas light indicates proximity to the air that will rapidly desiccate the roots during partial submergence and must therefore also be avoided; both behaviours result in roots growing close to the soil surface. Another example of an avoidance response was described for Arabidopsis roots (Eysholdt-Derzso & Sauter, 2017). When exposed to hypoxia, the primary root growth angle changed to direct root elongation to the side, thereby also avoiding deeper, and hence more hypoxic, soil layers. The angle of root bending is antagonistically determined by hypoxia and by hypoxia-induced ERFVIIs, particularly RAP2.12. Hypoxia reduces abundance of the polar auxin transporter PIN2 that drives basipetal auxin transport, which leads to auxin accumulation in the root tip, whereas ERFVIIs reduce auxin activity, indicating that the avoidance of hypoxia is driven by controlled polar auxin transport. Similarly, the growth angle of adventitious roots in rice appears to be determined by an auxin gradient across the root tip (Lin & Sauter, 2019; Fig. 1b), suggesting that auxin is a key hormone for the adjustment of the root structure to hypoxic conditions.

VIII. Conclusion

Flooding results in an overall altered root system that ranges from changes in root system architecture, with more roots growing in the upper soil layer where some O₂ may be present, to new roots growing in the floodwater (‘aquatic roots’). Root structure is also altered, including thicker roots (lower SA : V), aerenchyma (lower resistance to internal O₂ diffusion), an ROL barrier (greater retention of O₂), and a higher CSR (proportionally less tissue of high respiratory demand and of low gas-filled porosity). These responses and traits all enhance O₂ supply to root tissues and some reduce O₂ loss to the soil. Recent research has revealed important roles of auxin in flooding-adaptive responses of roots in addition to the roles of ethylene. Auxin signalling contributes to constitutive aerenchyma formation and controls lateral root growth during hypoxia, whereas polar auxin transport via PIN efflux carriers determines root growth direction in flooding-associated hypoxia and changes in light conditions in floodwaters. We know the genes for some but not all of these traits, and the next step is to manipulate
these genes in breeding in order to enhance the flood tolerance of our crops.

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Author contributions
OP, MS, TDC and MN planned and designed this review. OP, MS, TDC and MN wrote the manuscript.

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**Supporting Information**

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

**Methods S1** Modelling of maximum length of adventitious roots.

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See also the Editorial on this article by Sasidharan et al., 229: 5–7.