Invited Review

Climate-smart crops: key root anatomical traits that confer flooding tolerance

Takaki Yamauchi^{*1,2,5)}, Koji Noshita^{1,3,4)} and Nobuhiro Tsutsumi²⁾

¹⁾ Japan Science and Technology Agency, PRESTO, Kawaguchi, Saitama 332-0012, Japan

²⁾ Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo, Tokyo 113-8657, Japan

³⁾ Department of Biology, Kyushu University, Fukuoka, Fukuoka 819-0395, Japan

⁴⁾ Plant Frontier Research Center, Kyushu University, Fukuoka, Fukuoka 819-0395, Japan

⁵⁾ Present address: Bioscience and Biotechnology Center, Nagoya University, Furo-cho, Chikusa, Nagoya, Aichi 464-8601, Japan

Plants require water, but a deficit or excess of water can negatively impact their growth and functioning. Soil flooding, in which root-zone is filled with excess water, restricts oxygen diffusion into the soil. Global climate change is increasing the risk of crop yield loss caused by flooding, and the development of flooding tolerant crops is urgently needed. Root anatomical traits are essential for plants to adapt to drought and flooding, as they determine the balance between the rates of water and oxygen transport. The stele contains xylem and the cortex contains aerenchyma (gas spaces), which respectively contribute to water uptake from the soil and oxygen supply to the roots; this implies that there is a trade-off between the ratio of cortex and stele sizes with respect to adaptation to drought or flooding. In this review, we analyze recent advances in the understanding of root anatomical traits that confer drought and/or flooding tolerance to plants and illustrate the trade-off between cortex and stele sizes. Moreover, we introduce the progress that has been made in modelling and fully automated analyses of root anatomical traits and discuss how key root anatomical traits can be used to improve crop tolerance to soil flooding.

Key Words: aerenchyma to cortex ratio (ACR), cortex to stele ratio (CSR), drought, modelling, root anatomical phenotyping, water and oxygen transport, xylem vessels.

Introduction

Root anatomical traits are essential for plants to survive in nature because some of them determine the balance between the rates of water and oxygen transport. Roots mainly consist of three concentric cell layers, i.e. epidermis, cortex, and stele (Coudert *et al.* 2010, Dolan *et al.* 1993). More specifically cortex includes endodermis and hypodermis and/or exodermis, and in some cases the sclerenchyma (**Fig. 1**) (Lux *et al.* 2004, Taleisnik *et al.* 1999). The stele contains xylem vessels and phloem tissue which contribute to water and nutrients transport (Petricka *et al.* 2012). The main root of *Arabidopsis* (*Arabidopsis thaliana*) has a single cortex cell layer (when the endodermis is not taken into account) produced by a single asymmetric cell division of cortex/endodermis initial cells (Dolan and Okada 1999, Petricka *et al.* 2012). On the other hand, the sequential cell divisions of cortex/endodermis initial cells generate several cortex cell layers in rice (*Oryza sativa*) (**Fig. 1**) (Kamiya *et al.* 2003). Similar to rice, the roots

of most plant species, e.g. sorghum (*Sorghum bicolor*) (**Fig. 1**), have more than two cortex cell layers (Justin and Armstrong 1987).

Plants require water for growth and functioning, even though the majority of the water is used for transpiration (Yoo *et al.* 2009). Plant productivity is contributed by photosynthetic carbon assimilation (Lawson and Blatt 2014, Tanaka *et al.* 2019), and thus roots must efficiently take up water from the soil to support photosynthesis for effective plant growth (Leaky *et al.* 2019). In general, plants with large aboveground parts develop thick roots with large xylem vessels (Wahl and Ryser 2000), possibly to maintain the high root hydraulic conductivity required to efficiently move water from the soil to photosynthetic tissues. Indeed, the diameters of roots and xylem vessels are gradually enlarged with increasing shoot dry weight (Yu *et al.* 2015). Root anatomical traits, however, are not exclusively determined by the water requirements for plant growth, as soil water availability by plants is often restricted by environmental factors, such as drought, which is a deficit of water required by plants in the soil (Lloyd-Hughes 2014).

Soil flooding, which is defined as the root-zone being filled with (excess) water (Sasidharan *et al.* 2017), severely restricts oxygen diffusion from the air into the soil (Colmer and Flowers 2008). As the slow diffusion rate

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*Corresponding author (e-mail: atkyama@agr.nagoya-u.ac.jp)

Fig. 1. Root tissue structures in upland versus wetland species. Root cross-sections at 10 mm from the shoot-root junctions of sorghum (inbred line BTx623, left) and rice (cv. Nipponbare, right) grown under aerated nutrient solutions. Bars = 100 μ m. Dotted lines show that upland and wetland species have a larger proportion of stele and cortex, respectively. Wetland species generally have clear sclerenchyma cell layer(s) with a thick cell wall, even though sclerenchyma is also defined in roots of some upland species, including sorghum (asterisk; Galamay *et al.* 1991). In a strict sense, the endodermis, sclerenchyma, and hypodermis/exodermis are included in parts of cortex (Lux *et al.* 2004). ep; epidermis, hy; hypodermis, co; cortex, en; endodermis, st; stele, xy; xylem, ae; aerenchyma, ex; exodermis, sc; sclerenchyma.

in flooded soils results in an inadequate supply of oxygen into submerged tissues, root cells are often damaged by severe hypoxia or anoxia (Drew 1997). Unlike the blood circulatory system in animals, plants have no active oxygen dispersal mechanisms, and thus oxygen is predominately moved throughout plants by diffusion (Armstrong and Armstrong 2014). Lysigenous aerenchyma is a large internal gas space created by the death and lysis of the parenchyma (cortical) cells (Fig. 1) (Jackson and Armstrong 1999). In rice, lysigenous aerenchyma in roots, leaf midrib, sheath, and stem internodes compose an internal path for gas diffusion in gas phase speeding up longitudinal and radial diffusion of oxygen 10,000-fold (Colmer and Pedersen 2008). The families of Poaceae and Fabaceae (both include agronomically important crops) form lysigenous aerenchyma in the roots (Justin and Armstrong 1987), but the levels and/or timing of its formation differ among the species (Colmer and Voisenek 2009, Yamauchi *et al.* 2018).

Aerenchyma formation begins at the apical part of the roots and gradually extends towards the basal part of the roots, forming a longitudinal path for gas diffusion in flooded soil (Fig. 2) (Armstrong 1979, Colmer 2003b). The roots of some wetland species, such as rice, forms a barrier to radial oxygen loss (ROL) in the outer part of the roots to restrict oxygen diffusion from the root surfaces to rhizosphere, and thus oxygen movement through the aerenchyma is further promoted toward the root tips (Fig. 2) (Colmer 2003a, Ejiri *et al.* 2020, Kotula *et al.* 2009, Kulichikhin *et al.* 2014, Shiono *et al.* 2014). Recent findings have demonstrated that aerenchyma and ROL bar-

Fig. 2. Longitudinal path for water and oxygen movement along the roots of upland versus wetland species under soil flooding. Functions of xylem vessels, aerenchyma and a barrier to radial oxygen loss (ROL) barrier in the roots of upland and wetland species. Aerenchyma formation begins at the apical part of the roots and gradually expands towards the basal parts of the roots. Some wetland species, e.g. rice, form ROL barrier in the outer part of the roots to restrict oxygen leakage from the root surfaces into the rhizosphere. Soil water is transported to unsubmerged aerial tissues through xylem vessels, whereas oxygen in the air is transported to submerged roots and rhizosphere through aerenchyma. The dashed lines indicate oxygen diffusion through intercellular spaces in the cortex. Root cross-sections at 10 mm from the shoot-root junctions of sorghum (inbred line BTx623, left) and rice (cv. Nipponbare, right) grown under oxygen-deficient nutrient solutions. Bars = 100 μ m.

rier enhance oxygen diffusion not only in the main roots but also in lateral roots (Pedersen *et al.* 2021b). Together, wetland species can more efficiently transport oxygen into the root tips than upland species under soil flooding due to the formation of aerenchyma (Fig. 2).

Global climate change will increase the risk of flooding during crop production (Bailey-Serres *et al.* 2012, Voisenek and Bailey-Serres 2015). Thus, it is urgent to develop effective approaches for the improvement of crop tolerance to flooding. To do this, it is extremely important to understand how plants adapt to flooding. On the other hand, we should not overlook the trade-offs among the adaptive traits, as flooding is not a constant condition, but saturates drained soil suddenly (Bailey-Serres *et al.* 2012). In this review, we provide an overview of root anatomical traits that help plants adapt to soil flooding. Root anatomical traits associated with drought avoidance are also discussed to illustrate the trade-off between the ratio of cortex and stele sizes, which is a key trait to adapt to drought or flooding. We focus on the root anatomical traits in rice, wheat (*Triticum aestivum*), and maize (*Zea mays* ssp. *mays*), and briefly introduce a modelling approach that is useful for evaluating longitudinal patterns of root anatomical traits. We also summarize the recent advances in root phenotyping techniques and introduce our progress

on the development of a fully automated image analysis for root anatomical traits in sorghum. Taken together, we propose using the key root anatomical traits to improve crop tolerance to soil flooding, beyond the limitations stemming from the adaptive trade-off.

Contribution of root tissue size to the adaptation to drought and flooding

Root thickness has been considered to be one of the essential traits for the tolerance to both drought and flooding (Colmer 2003b, Price *et al.* 2002). The deep and thick root systems that allow for the uptake of water from deep soils are important for the drought avoidance in rice (Price *et al.* 2002). In most cases, thicker roots with larger stele and xylem areas are associated with deep rooting phenotypes, as xylem vessels in the stele are required for water uptake to increase root hydraulic conductivity (Price *et al.* 2002, Uga *et al.* 2008). Recent findings in maize lines show that root thickness itself is not related to rooting depth (Vanhees *et al.* 2020). Similarly, thicker roots are believed to enhance longitudinal oxygen diffusion by reducing the resistance to gas-phase diffusion (Colmer 2003b). Indeed, wetland species (e.g. some *Carex* species) generally have thicker and more porous roots than species found in non-flooded soils (Visser *et al.* 2000a, 2000b). Recently, Pedersen *et al.* (2021a) suggest that thick roots having smaller surface area to volume ratio (SA:V) is less prone to lose oxygen from the root surface (i.e. ROL) than thin roots. While this concept can explain why having thicker root diameters are more adaptive to soil flooding, SA:V might be affected by the proportion of cortex volume, given that the porous cortex is the main path for oxygen movement (Colmer 2003b). Similarly, smaller SA:V (thicker root diameters) may be less prone to lose water from the root surface, and thus it may also contribute to drought adaptation.

Several lines of evidence suggest that root tissue size is associated with tolerance to drought and flooding, even though the proportion of root tissue size is also considered in some cases (Burton *et al.* 2013, Colmer 2003b, Price *et al.* 2002). **Fig. 3A–3C** shows the cross-sections at most basal parts of adventitious roots of upland wheat and maize, and wetland rice grown under aerated solution and nitrogen-flashed stagnant deoxygenated solution, which mimic the aerobic drained soils and anoxic flooded soils, respectively (Wiengweera *et al.* 1997). If root tissue size is itself essential for flooding tolerance, then rice roots must have largest cortex and aerenchyma areas. However, results show that rice actually has much smaller cortex, stele, and aerenchyma areas than maize, which has largest shoot length and dry weight (Yamauchi *et al.* 2019b), except for the area of aerenchyma under aerated conditions (**Fig. 3D–3F**). This observation suggests that root tissue size is biased by the implication of plant size or biomass, and that the proportion of root tissue size is also needed to be considered.

Adaptive significance of aerenchyma formation

Aerenchyma formation is essential for tolerance to drought/water stress (Lynch 2018, 2019). Recombinant inbred lines of maize with a higher proportion of the cortex occupied by aerenchyma have more shoot biomass and greater yields than those with a lower proportion of aerenchyma under water stress (Zhu *et al.* 2010). The high-aerenchyma lines have less root respiration and deeper rooting systems compared with low-aerenchyma lines (Zhu *et al.* 2010), suggesting that aerenchyma formation is beneficial to maize by reducing the metabolic cost of soil water exploration under drought conditions (Lynch 2018, 2019). The deep rooting phenotype was demonstrated to be an important factor for drought avoidance in rice (Uga *et al.* 2013). Given that aerenchyma formation reduces the metabolic cost of root elongation, substantial aerenchyma formation in rice roots might also contribute to drought tolerance by supporting the deep rooting systems. In addition to the reduction in respiration, converting living cortical cells to gas spaces may allow the plant to reinvest its nutrients into the growing root tips (Lynch 2018).

Under soil flooding, aerenchyma formation and ROL barrier formation (reviewed in this issue by Ejiri *et al.* 2021) largely contributing to the internal oxygen supply along the roots (**Fig. 2**) (Colmer 2003b). Moreover, aerenchyma reduces the respiratory costs of roots under flooding (Armstrong 1979, Colmer 2003b). Aerenchyma constitutively forms in the roots of wetland species under aerobic (drained soil) conditions, whereas its formation in the roots of both wetland and upland species are induced in response to the oxygen-deficiency under flooded conditions (**Fig. 2**) (Colmer and Voisenek 2009). Indeed, substantial aerenchyma was detected in the roots of rice, but not wheat or maize under aerated conditions (**Fig. 3F**) (Yamauchi *et al.* 2019b). Constitutive aerenchyma formation is considered one of the key root traits for flooding tolerance, as oxygen diffuses into the roots through aerenchyma as soon as soil flooding begins (Colmer and Voisenek 2009). Some barley (*Hordeum vulgare*) cultivars form aerenchyma (root cortical senescence) under aerobic conditions to reduce the metabolic costs of root growth rather than to enhance oxygen diffusion (Schneider *et al.* 2017a, 2017b). Aerenchyma detected in wheat roots under aerated conditions might be related to the root cortical senescence (**Fig. 3A, 3F**). The typical values for the percentages of gas-filled spaces in adventitious roots of upland and wetland grass species are well described in terms of root porosity (% gas volume/root volume), which results from the combination of aerenchyma with intercellular gas spaces; 1–8% and 8–45% for upland and wetland species under aerobic conditions, and 3–27% and 15–55% for upland and wetland species under oxygen deficient conditions (Colmer 2003b, Herzog *et al.* 2016).

The adaptive significance of constitutive aerenchyma

Fig. 3. The anatomical traits of adventitious roots of wheat, maize, and rice. Cross-sections at 10 mm from the shoot-root junctions of adventitious roots of wheat (A), maize (B), and rice (C) under aerated or stagnant conditions. Nine-day-old aerobically-grown seedlings of wheat (cv. Bobwhite), maize (inbred line B73), and rice (cv. Nipponbare) were further grown under aerated or stagnant conditions for 7 days (Yamauchi *et al.* 2019b). Cortex cell layers and lysigenous aerenchyma are indicated by magenta dashed lines and blue arrowheads, respectively. Bars = 100 μ m. Areas of cortex (D), stele (E), aerenchyma (F), and the ratio of cortex to stele (G), aerenchyma to cortex (H), and aerenchyma to whole root (I) of wheat, maize, and rice roots. Adventitious (Adv.) root lengths (J) and numbers (K) of wheat, maize, and rice were reconstructed by Yamauchi *et al.* (2019b). Boxplots show the median (horizontal lines), 25th to 75th percentiles (edges of the boxes), minimum to maximum (edges of the whiskers), and mean values (dots in the boxes) [D–I (n = 9) and J, K (n = 15)]. Significant differences between aerated and stagnant conditions at $P < 0.01$ (two-sample *t* test) are denoted by *. Different lowercase letters denote significant differences among different species ($P < 0.05$, one-way ANOVA and then Tukey's test for multiple comparisons). Red and blue letters indicate the multiple comparisons for the data in aerated and stagnant conditions, respectively.

formation has been demonstrated using accessions of a wild relative of maize, *Zea nicaraguensis*, adapted to the frequently flooded northwest coastal plain of Nicaragua (Iltis and Benz 2000, Mano and Omori 2007). These accessions have a high potential to form constitutive aerenchyma in roots, and this is indeed associated with their higher tolerance to soil flooding (Mano and Omori 2013). Moreover, introgression of four quantitative trait loci (QTL) for constitutive aerenchyma formation from *Z. nicaraguensis* confers tolerance to the onset of oxygen deficiency in the maize inbred line Mi29 (Gong *et al.* 2019). The recent advances in QTL analyses on constitutive aerenchyma formation is reviewed in this issue by Mano and Nakazono (2021). As the regulatory mechanisms of constitutive aerenchyma and inducible aerenchyma in rice roots are at least partly different (Yamauchi *et al.* 2017, 2019a, 2020a),

the genetic loci that determine the two types of aerenchyma formation might be independent.

Currently, the contribution of lysigenous aerenchyma formation to the tolerance of soil flooding in legumes (Fabaceae) is poorly understood (Striker and Colmer 2017). On the other hand, secondary aerenchyma (phellem), which is a white and spongy tissue formed in the stem, hypocotyl and roots (Striker *et al.* 2015, Takahashi *et al.* 2014, 2018, Yamauchi *et al.* 2013), enhances oxygen diffusion from aerial tissues to the submerged tissues of soybean (*Glycine max*) and forage legume *Melilotus siculus* (Shimamura *et al.* 2003, Verboven *et al.* 2012). Although lysigenous aerenchyma formation in the roots of recombinant inbred lines of *Lotus japonicus* is correlated with some root traits, e.g. root length or root dry mass, it is not correlated with the shoot dry mass under soil flooding (Striker *et al.* 2014).

This observation suggests that lysigenous aerenchyma formation in the roots of *L. japonicus* might be responsible for the reduction in the metabolic cost of enhancing root growth. Further studies are required to understand the adaptive significance of lysigenous aerenchyma formation in legumes.

Trade-off between cortex and stele: benefits in adaptation to soil flooding

The size and/or proportion of xylem vessels in the stele and aerenchyma in the cortex is essential for tolerance to drought and flooding, respectively (Colmer 2003b, Uga *et al.* 2008, Yamauchi *et al.* 2018), and this suggests that there is an adaptive trade-off between cortex and stele sizes, as an increase in the proportion of stele simultaneously implies a decrease in the proportion of cortex and vice versa. In other words, cortex to stele ratio (CSR) is associated with how well plants adapt to drought and flooding; low and high CSR should be related to the adaptation to drought and flooding, respectively. Recently, we demonstrated that the roots of wetland rice have much higher CSR than do those of upland wheat and maize (Fig. 3G) (Yamauchi *et al.* 2019b). Moreover, CSR in the roots of all three plant species increases in response to oxygen deficiency under stagnant conditions (Yamauchi *et al.* 2019b).

The adaptive significance of higher CSR to soil flooding could consist of two main aspects, that is, to provide more space for cortical cell death (i.e. aerenchyma formation) and to avoid the occurrence of anoxia in stelar cells. As shown in Fig. 3H, the aerenchyma to cortex ratio (ACR) in rice roots is 1.8- and 1.4-fold higher than that in wheat and maize roots, respectively, whereas the aerenchyma to whole root ratio (AWR) in rice roots was 2.2- and 1.6-fold higher, respectively, under stagnant conditions (Fig. 3I). As CSR is 3.4- and 4.0-fold higher in rice roots than wheat and maize roots, respectively (Fig. 3G), a high proportion of cortex (high CSR) in rice roots enhances the proportion of aerenchyma in the roots (high AWR) (Yamauchi *et al.* 2019b). Intercellular spaces in the cortex also enhance oxygen diffusion (Fig. 2) (Armstrong 1971, Yamauchi *et al.* 2019b), and thus a high CSR with large gas spaces in the roots must be essential for adaptation to soil flooding. Indeed, thicker rice roots with higher CSR and larger cortex area can transport more oxygen into the root tips than thinner rice roots (Yamauchi *et al.* 2019b). Interestingly, modelling analyses demonstrated that increasing CSR has beneficial effects on the predicted maximum root lengths during soil flooding, both for the roots with and without the formation of ROL barriers (Pedersen *et al.* 2021a). In line with this hypothesis, rice has longer adventitious roots than do wheat or maize under stagnant conditions (Fig. 3J).

Another line of evidence demonstrates that plants with smaller steles (i.e. higher CSR) better adapt to soil flooding (Armstrong *et al.* 2019, Colmer and Greenway 2011). Measurements of radial oxygen profiles in maize roots revealed

high oxygen levels in the porous cortex and much lower levels in the dense stelar tissues (Armstrong *et al.* 1994, 2000, Gibbs *et al.* 1998). Indeed, the oxygen available to stelar cells is insufficient to support aerobic respiration under flooding (Colmer and Greenway 2011). Oxygen deficiency in the stelar cells of maize and barley restricts the loading of potassium and chloride ions into xylems (maize; Gibbs *et al.* 1998, barley; Kotula *et al.* 2015), and thus a smaller stele size associated with larger porous-cortex size is advantageous for plant adaptation to flooding as it reduces the risk of ‘anoxic core’ development in the stele (Armstrong *et al.* 2019). Roots of the wetland species generally have a smaller proportion of steles compared to those of upland species (McDonald *et al.* 2002), further supporting this idea.

Trade-off between cortex and stele: disadvantages in adaptation to soil flooding?

While aerenchyma formation helps plants adapt to both drought and flooding, there remains a question whether cortical cell death leads to a decrease in radial transport of water and nutrients. Interestingly, the surviving strands in the cortex of aerenchymatous roots (Fig. 2) provide an apoplastic pathway for potassium ions from the root surface to the xylem vessels of maize roots (Drew and Saker 1986, Jackson and Armstrong 1999). Moreover, aerenchyma does not form in the apical parts of the roots (Fig. 2), where water and nutrients are efficiently taken up (Geldner 2013, Yamauchi *et al.* 2019a). The radial hydraulic conductivity and phosphate and nitrate transports are reduced by the formation of aerenchyma in the excised barley roots (Schneider *et al.* 2017b), but a simulation study revealed that this reduction had a small effect on the nutrient uptake under suboptimal availability of nitrogen, phosphorus, and potassium (Schneider *et al.* 2017a). Together, aerenchyma formation itself cannot be disadvantageous for plant growth under any environmental conditions.

A study of several grass species revealed that plant heights are positively correlated with the stele and xylem areas (Wahl and Ryser 2000), indicating that smaller water and nutrient transport capacities caused by the smaller xylem areas are disadvantageous for plant growth. As shown in Fig. 3E, the stele area in rice roots is much smaller than those in wheat and maize roots, suggesting that there is an adaptive trade-off between the efficient oxygen transport achieved by high CSR associated with a large cortex and aerenchyma, and inefficient water and nutrients uptake through small stele and xylem vessels. On the other hand, it may be that large xylems are not required for better growth under flooding where soil pores are filled with enough water, given that large xylems are required to acquire deep soil water (Price *et al.* 2002, Uga *et al.* 2008). However, this hypothesis does not fully explain why paddy rice can grow vigorously under flooding. One way to overcome this situation is to increase the number of

roots, which can compensate for the less effective water and nutrients uptake. Indeed, rice has 3.9- and 3.2-fold more adventitious roots than do wheat and maize, respectively, under stagnant conditions (Fig. 3K) (Yamauchi *et al.* 2019b). Interestingly, the patterns of differences in CSR and adventitious root numbers among the three species were similar (Fig. 3G, 3K). The wetland species *Rumex palustris* has more adventitious roots than the non-wetland species *Rumex thyrsoiflorus* under stagnant conditions (Visser *et al.* 1995, 1996). These evidences suggest that a large number of roots in flooding-tolerant genotypes compensate for the less efficient water and nutrients uptake due to a high CSR.

Consideration of longitudinal patterns of root anatomical traits: a modelling approach

Root cells are generated from the stem cells at the root apex, and thus rates of cell proliferation and subsequent cell elongation determine the rates of root elongation into the soil (Motte *et al.* 2019). Although the distance from the root tips roughly reflects the time at which the cells emerged, an age-dependent evaluation is required for deeper understanding of root developmental processes. Aerenchyma gradually grows towards the basal part of the roots (Fig. 2) (Armstrong 1979, Colmer 2003b), suggesting that its formation at the same distances from the root tips correspond to different time points after root cell emergence among the roots with different elongation rates (Evans 2003).

We recently demonstrated that the proportion of aerenchyma formation in rice roots fits well to the Gompertz growth model (Yamauchi *et al.* 2020b), a sigmoid model commonly used to interpret population mortality or growth (Gompertz 1825, Tjørve and Tjørve 2017). By using the regression models, we converted the patterns of aerenchyma formation at the distances from the root tips to those of aerenchyma formation at times after the root cell emergences (Yamauchi *et al.* 2020b). The results show that the patterns of aerenchyma formation in the wild-type rice and rice mutant, in which constitutive aerenchyma formation is reduced and the elongation rate is enhanced by defects in the auxin signaling, differ between the distance-dependent and time (age)-dependent evaluations; the time (age)-dependent model reveals that aerenchyma formation in roots of the auxin-signaling mutant is reduced due to the delayed initiation of its formation rather than suppression of the formation throughout the roots (Yamauchi *et al.* 2020b).

As shown in Fig. 4A, aerenchyma formation (indicated by AWR, i.e. aerenchyma to whole root ratio) in rice roots both under aerated and stagnant conditions fit well to the Gompertz model. Accounting for the elongation rates (approximately 0.6 mm h⁻¹ for both under aerated and stagnant conditions), the calculated distances at each time point were used as the explanatory variables (*x* values) in the regression models, and then time-dependent

Fig. 4. Conversion of distant-dependent aerenchyma formation to time (age)-dependent aerenchyma formation in rice roots. Nine-day-old aerobically-grown rice seedlings were further grown under aerated or stagnant conditions for 7 days (Yamauchi *et al.* 2019b). (A) Aerenchyma to whole root ratio (AWR) at each position of the adventitious roots. Gompertz model was fitted using all AWR values as the response variables, and distances from the root tips as the explanatory variables. SSgompertz function in the R stats package (version 3.5.2) was used for the fitting. Each dot indicates the AWR value, and the dots with gray borders indicate the mean values ($n = 9$). Significant differences between aerated and stagnant conditions at $P < 0.01$ (two-sample *t* test) are denoted by *. (B) Age-dependent aerenchyma formation. The AWRs were simulated using equations of the Gompertz models shown in (A) using the distances from the root tips as the explanatory variables, which were calculated by three different elongation rates (0.3, 0.6, or 0.9 mm h⁻¹).

patterns of AWR were predicted (Fig. 4B). The patterns of time-dependent AWR simulated by using two other postulated elongation rates (0.3 or 0.9 mm h⁻¹) revealed that the patterns of time (age)-dependent aerenchyma formation are largely affected by elongation rates (Fig. 4B). These results indicate that the age-dependent aerenchyma formation (and also other root anatomical traits) may not be the same among different genotypes with different root elongation rates, even though the patterns of distance-dependent aerenchyma formation are exactly the same. This kind of modeling approaches will be useful for accurately comparing the longitudinal patterns of root anatomical traits

among different genotypes (e.g. mutant lines or crop cultivars).

Advances in the phenotyping of root anatomical traits

Roots are hidden in the soil matrix, making it challenging to assess their growth patterns. Recent advances in root phenotyping technologies have allowed us to accelerate the understanding of adaptive root traits to improve crop tolerance to abiotic stresses (reviewed in this issue by Takahashi and Pradal 2021). Progress in X-ray computed tomography and magnetic resonance imaging enables to determine three-dimensional patterns of root systems in the soil in a nondestructive way (Atkinson *et al.* 2019, Teramoto *et al.* 2020). However, phenotyping root anatomy remains a challenge in these systems (Atkinson *et al.* 2019). On the other hand, extracting regional data from root cross-sectional images is very time consuming. In most cases, root researchers use image processing softwares, e.g. ImageJ or Photoshop, for quantification by manually assigning root tissues, but in some cases, the root cross-sectional images are adjusted to black and white by thresholding, and the numbers of pixels is quantified (Maricle and Lee 2002). Even with image processing softwares, the non-automated analyses are not suitable for processing a large number of images, e.g. for QTL analysis or genome-wide association studies (GWAS) of root anatomical traits.

Burton *et al.* (2012) established the software *RootScan* which enables semi-automated image analysis of the anatomical features of root cross-sections. *RootScan* can propose candidate regions of root tissues, e.g. stele, cortex, xylem, and aerenchyma, based on an image processing pipeline, and then those regions can be manually validated with the graphical user interface (GUI). *RootScan* is useful for high-throughput analyses, and it has been applied in GWAS on root anatomical traits in maize and barley (Oyiga *et al.* 2020, Saengwilai *et al.* 2016). Several similar tools with *RootScan*, such as PHIV-RootCell (Lartaud *et al.* 2015) and RootAnalyzer (Chopin *et al.* 2015), are proposed to analyze root anatomical properties. Nevertheless, a fully automated analysis of root cross-sectional images may help for application of root anatomical traits for future breeding of abiotic stress tolerance.

Recent advances in deep neural network models enable us to perform these fully automated image analyses of root anatomical properties. Object detection methods, such as Faster-RCNN (Ren *et al.* 2017) and YOLO v4 (<https://github.com/AlexeyAB/darknet>), can be used to determine the numbers, approximate sizes and shapes of cells, lacunae, etc. as bounding boxes that fence the region of interests (ROIs) in root cross-sectional images. Indeed, Wang *et al.* (2020) demonstrated that the numbers and diameters of stele and xylems can be quantified from root cross-sectional images of rice using Faster-RCNN trained on relatively small datasets (fewer than 400 images). On the

Fig. 5. Qualitative results of the segmentation of root anatomical structures. The original images (left) were obtained from the root cross-sections, which were prepared from the adventitious roots of hydroponically-grown sorghum seedlings. The ground truth mask images (center) were prepared by manual labeling. The results of the prediction (right) show that the U-Net model successfully captured region of the epidermis, cortex, stele, aerenchyma, and xylem. Each row corresponds to a root cross-section specimen.

other hand, U-Net (Ronneberger *et al.* 2015) and DeepLabv3+ (Chen *et al.* 2018) are widely used semantic segmentation models that classify each image at the pixel level to some categories can be applied to quantify areas and outline shapes. However, it is difficult to distinguish among instances of the same class that adjoined each other, as semantic segmentation models focus on pixel classification. Although larger datasets with detailed annotation are required, instance segmentation methods, such as Mask R-CNN (He *et al.* 2020), enable pixel-wise annotations of adjoining instances, making it possible to perform single-cell scale quantifications of anatomical properties.

As shown in Fig. 5, fully automated image analysis using deep learning techniques will realize segmentation of the root tissues without any user feedbacks. Using U-Net trained on 25 cross-sectional images of sorghum roots, the epidermis, cortex, stele, and xylems were successfully segmented (mean intersection-over-union; mIoU = 0.8879). To improve the segmentation quality of aerenchyma and also the other four tissues, more trained data with annotations for the tissues on the root cross-sectional images are needed.

Conclusion and future perspective

This review summarizes progress in understanding of the root anatomical traits that are benefits and/or disadvantages in plants to adapt to soil flooding. Based on the evidence available, we propose that the key root anatomical traits, i.e. CSR and ACR, are good indicators for evaluating the capacity of adaptation to drought and flooding (Fig. 6).

Fig. 6. Root anatomical structures associated with plant adaptation to drought and flooding. Roots with low CSR associated with large stele (xylem) would efficiently acquire water in the dry soil. Roots with high cortex to stele ratio (CSR) associated with a large cortex (aerenchyma) can efficiently supply oxygen to submerged root tips in the flooded (wet) soil. A high aerenchyma to cortex ratio (ACR) can reduce the metabolic cost of roots in both the dry and wet soils. Moreover, a high ACR enhances oxygen diffusion into the root tips in the wet soil. Asterisk indicates that aerenchyma formation could be advantageous for some upland crops to grow under nutrient-deficient conditions, even in the drained soil. ae; aerenchyma, xy; xylem, co; cortex, st; stele.

Recently, this idea has been supported by the finding that CSR and ACR are correlated with adaptation of wild Poaceae species to the gradients of soil water (Yamauchi *et al.* 2021). High ACR is required to reduce metabolic costs and to enhance oxygen diffusion within plants during flooding (Colmer 2003b). High CSR is important for efficiently transporting oxygen into the root tips through aerenchyma (Colmer 2003b), and preventing ‘anoxic core’ development, which makes water and nutrient loading into xylems less efficient (Armstrong *et al.* 2019). Given that the roots of paddy rice have a much higher CSR and ACR than do those of upland crops, sorghum, wheat, and maize (Figs. 1–3), genetic improvements in CSR and ACR in upland crops could be an attractive approach for flooding tolerance breeding.

Climate change is increasing the risk of crop yield loss by flooding events, so that it is urgent to improve crop tolerance to flooding and make crop production more sustainable (Bailey-Serres *et al.* 2012, Voesenek and Bailey-Serres 2015). Flooding is not constant; it suddenly saturates the drained soils (Bailey-Serres *et al.* 2012), and thus the plasticity of root anatomical traits might be essential for plants to adapt to climate changes. Interestingly, the CSR and ACR in the roots of both upland and wetland species increase under stagnant deoxygenated conditions (Fig. 3G, 3H), suggesting that the roots of most plant species can alter their CSR and ACR in response to flooding, even though the response levels may be different among genotypes. Given that having a high CSR with small xylem vessels is disadvantageous for plant growth under the drained or dry soil, CSR with high plasticity could be extremely

important for a plant’s response to intermittent flooding caused by climate change.

Recent advances in physiological and molecular biological studies have revealed the signaling pathways involved in the root aerenchyma formation (Pedersen *et al.* 2021a, Yamauchi *et al.* 2018), whereas those involved in regulating the balance between cortex and stele areas remain unclear. Thus, it is necessary to identify the genetic loci that control CSR, which might be associated with the size and/or number of cortical cells and/or stelar cells. Moreover, these loci may also be applied in the future to improve crop tolerance to soil flooding. For these purposes, genetic analyses, e.g. QTL analysis and GWAS, on CSR by using plant species with large genetic diversities are needed. GWAS combined with high-throughput image analyses of root cross-sections was used in maize and barley (Oyiga *et al.* 2020, Saengwilai *et al.* 2016). On the other hand, sorghum may be also useful for the studies on root anatomical traits because the mapping populations and germplasm collections with wide genetic diversity have already undergone QTL mapping and GWAS, respectively (Kajiya-Kanegae *et al.* 2020, Sakamoto *et al.* 2019).

Age-dependent evaluation is required for conducting detailed comparisons of root anatomical traits among the different genotypes with different root elongation rates. Distance-to-time conversion using Gompertz growth model enabled us to compare a variety of root elongation rates and confirm the age-dependent aerenchyma formation in rice roots (Fig. 4) (Yamauchi *et al.* 2020b). This kind of modelling approaches will be useful for accurately evaluating the longitudinal patterns of root anatomical traits in diverse genotypes. On the other hand, extracting areas, lengths or numbers of cells/tissues from the root cross-sectional images is time-consuming and laborious. While semi-automated root cross-sectional image analyses have accelerated research on root anatomical traits (Burton *et al.* 2012, Chopin *et al.* 2015, Lartaud *et al.* 2015), fully automated root cross-sectional image analyses (Fig. 5) will also help with future efforts to improve abiotic stress tolerance in crops. Genetic analyses of root anatomical traits, such as CSR and ACR, combined with modelling and/or image analyses will be a powerful tool for identifying the key genes/loci for breeding of stress-tolerant crops.

Author Contribution Statement

T.Y. and K.N. wrote the first draft of the manuscript. T.Y. performed the data and modelling analyses. K.N. performed the automated image analysis. N.T. supervised the research, read the manuscript, and provided comments for improvement of the manuscript.

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Literature Cited

- 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., M.E. Strange, S. Cringle and P.M. Beckett (1994) Microelectrode and modelling study of oxygen distribution in roots. *Ann. Bot.* 74: 287–299.
- Armstrong, W., D. Cousins, J. Armstrong, D.W. Turner and P.M. Beckett (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. and J. Armstrong (2014) Plant internal oxygen transport (diffusion and convection) and measuring and modelling oxygen gradients. *Plant Cell Monogr.* 21: 267–297.
- Armstrong, W., P.M. Beckett, T.D. Colmer, T.L. Setter and H. Greenway (2019) Tolerance of roots to low oxygen: ‘Anoxic’ cores, the phytoglobin-nitric oxide cycle, and energy or oxygen sensing. *J. Plant Physiol.* 239: 92–108.
- Atkinson, J.A., M.P. Pound, M.J. Bennett and D.M. Wells (2019) Uncovering the hidden half of plants using new advances in root phenotyping. *Curr. Opin. Biotechnol.* 55: 1–8.
- Bailey-Serres, J., S.C. Lee and E. Brinton (2012) Waterproofing crops: effective flooding survival strategies. *Plant Physiol.* 160: 1698–1709.
- Burton, A.L., M. Williams, J.P. Lynch and K.M. Brown (2012) *RootScan*: software for high-throughput analysis of root anatomical traits. *Plant Soil* 357: 189–203.
- Burton, A.L., K.M. Brown and J.P. Lynch (2013) Phenotypic diversity of root anatomical and architectural traits in *Zea* species. *Crop Sci.* 53: 1042–1055.
- Chen, L.C., Y. Zhu, G. Papandreou, F. Schroff and H. Adam (2018) Encoder-decoder with atrous separable convolution for semantic image segmentation. *Lect. Notes Comput. Sci. (including Subser. Lect. Notes Artif. Intell. Lect. Notes Bioinformatics)* 11211: 833–851.
- Chopin, J., H. Laga, C.Y. Huang, S. Heuer and S.J. Miklavcic (2015) RootAnalyzer: a cross-section image analysis tool for automated characterization of root cells and tissues. *PLoS ONE* 10: e0137655.
- Colmer, T.D. (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, T.D. (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, T.D. and O. Pedersen (2008) Oxygen dynamics in submerged rice (*Oryza sativa*). *New Phytol.* 178: 326–334.
- Colmer, T.D. and T.J. Flowers (2008) Flooding tolerance in halophytes. *New Phytol.* 179: 964–974.
- Colmer, T.D. and L.A.C.J. Voesenek (2009) Flooding tolerance: suites of plant traits in variable environments. *Funct. Plant Biol.* 36: 665–681.
- Colmer, T.D. and H. Greenway (2011) Ion transport in seminal and adventitious roots of cereals during O₂ deficiency. *J. Exp. Bot.* 62: 39–57.
- Coudert, Y., C. Périn, B. Courtois, N.G. Khong and P. Gantet (2010) Genetic control of root development in rice, the model cereal. *Trends Plant Sci.* 15: 219–226.
- Dolan, L., K. Janmaat, V. Willemsen, P. Linstead, S. Poethig, K. Roberts and B. Scheres (1993) Cellular organization of the *Arabidopsis thaliana* root. *Development* 119: 71–84.
- Dolan, L. and K. Okada (1999) Signalling in cell type specification. *Semin. Cell Dev. Biol.* 10: 149–156.
- Drew, M.C. (1997) Oxygen deficiency and root metabolism: injury and acclimation under hypoxia and anoxia. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48: 223–250.
- Drew, M.C. and L.R. Saker (1986) Ion transport to the xylem in aerenchymatous roots of *Zea mays* L. *J. Exp. Bot.* 37: 22–33.
- Ejiri, M., Y. Sawazaki and K. Shiono (2020) Some accessions of amazonian wild rice (*Oryza glumaepatula*) constitutively form a barrier to radial oxygen loss along adventitious roots under aerated conditions. *Plants (Basel)* 9: 880.
- Ejiri, M., T. Fukao, T. Miyashita and K. Shiono (2021) A barrier to radial oxygen loss helps the root system cope with waterlogging-induced hypoxia. *Breed. Sci.* 71: 40–50.
- Evans, D.E. (2003) Aerenchyma formation. *New Phytol.* 161: 35–49.
- Galamay, T.O., Y. Kono, A. Yamauchi and M. Shimizu (1991) Comparative root anatomy of seminal and nodal root axes of summer cereals with special reference to the development of hypodermis and cortical sclerenchyma. *Jpn. J. Crop Sci.* 60: 184–190.
- Geldner, N. (2013) The endodermis. *Annu. Rev. Plant Biol.* 64: 531–558.
- Gibbs, J., D.W. Turner, W. Armstrong, M.J. Darwent and H. Greenway (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.
- Gompertz, B. (1825) On the nature of the function expressive of the law of human mortality, and on a new mode of determining the value of life contingencies. *Philos. Trans. R. Soc. Lond., B, Biol. Sci.* 182: 513–583.
- Gong, F., H. Takahashi, F. Omori, W. Wang, Y. Mano and M. Nakazono (2019) QTLs for constitutive aerenchyma from *Zea nicaraguensis* improve tolerance of maize to root-zone oxygen deficiency. *J. Exp. Bot.* 70: 6475–6487.
- He, K., G. Gkioxari, P. Dollár and R. Girshick (2020) Mask R-CNN. *IEEE Trans. Pattern Anal. Mach. Intell.* 42: 386–397.
- Herzog, M., G.G. Striker, T.D. Colmer and O. Pedersen (2016) Mechanisms of waterlogging tolerance in wheat—a review of root and shoot physiology. *Plant Cell Environ.* 39: 1068–1086.
- Iltis, H.H. and B.F. Benz (2000) *Zea nicaraguensis* (Poaceae), a new teosinte from Pacific coastal Nicaragua. *Novon* 10: 382–390.
- Jackson, M.B. and W. Armstrong (1999) Formation of aerenchyma and the processes of plant ventilation in relation to soil flooding and submergence. *Plant Biol.* 1: 274–287.
- Justin, S.H.F.W. and W. Armstrong (1987) The anatomical characteristics of roots and plant response to soil flooding. *New Phytol.* 106: 465–495.
- Kajiya-Kanegae, H., H. Takanashi, M. Fujimoto, M. Ishimori, N. Ohnishi, F.W. Wacera, E.A. Omollo, M. Kobayashi, K. Yano, M. Nakano *et al.* (2020) RAD-seq-based high-density linkage map construction and QTL mapping of biomass-related traits in sorghum using the Japanese landrace Takakibi NOG. *Plant Cell*

- Physiol. 61: 1262–1272.
- Kamiya, N., J.I. Itoh, A. Morikami, Y. Nagato and M. Matsuoka (2003) The *SCARECROW* gene's role in asymmetric cell divisions in rice plants. *Plant J.* 36: 45–54.
- Kotula, L., K. Ranathunge, L. Schreiber and E. Steudle (2009) 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., P.L. Clode, G.G. Striker, O. Pedersen, A. Läuchli, S. Shabala and T.D. Colmer (2015) Oxygen deficiency and salinity affect cell-specific ion concentrations in adventitious roots of barley (*Hordeum vulgare*). *New Phytol.* 208: 1114–1125.
- Kulichikhin, K., T. Yamauchi, K. Watanabe and M. Nakazono (2014) Biochemical and molecular characterization of rice (*Oryza sativa* L.) roots forming a barrier to radial oxygen loss. *Plant Cell Environ.* 37: 2406–2420.
- Lartaud, M., C. Perin, B. Courtois, E. Thomas, S. Henry, M. Bettembourg, F. Divol, N. Lanaud, F. Artus, C. Bureau *et al.* (2015) Phiv-Rootcell: a supervised image analysis tool for rice root anatomical parameter quantification. *Front. Plant Sci.* 5: 790.
- Lawson, T. and M.R. Blatt (2014) Stomatal size, speed, and responsiveness impact on photosynthesis and water use efficiency. *Plant Physiol.* 164: 1556–1570.
- Leaky, A.D.B., J.N. Ferguson, C.P. Pignon, A. Wu, Z. Jin, G.L. Hammer and D.B. Lobell (2019) Water use efficiency as a constraint and target for improving the resilience and productivity of C₃ and C₄ crops. *Annu. Rev. Plant Biol.* 70: 781–808.
- Lloyd-Hughes, B. (2014) The impracticality of a universal drought definition. *Theor. Appl. Climatol.* 117: 607–611.
- Lux, A., L. Miroslava, J. Abe and S. Morita (2004) Root cortex: structural and functional variability and responses to environmental stress. *Root Res.* 13: 117–131.
- Lynch, J.P. (2018) Rightsizing root phenotypes for drought resistance. *J. Exp. Bot.* 69: 3279–3292.
- Lynch, J.P. (2019) Root phenotypes for improved nutrient capture: an underexploited opportunity for global agriculture. *New Phytol.* 223: 548–564.
- Mano, Y. and F. Omori (2007) Breeding for flooding tolerant maize using “teosinte” as a germplasm resource. *Plant Root* 1: 17–21.
- Mano, Y. and F. Omori (2013) Relationship between constitutive root aerenchyma formation and flooding tolerance in *Zea nicaraguensis*. *Plant Soil* 370: 447–460.
- Mano, Y. and M. Nakazono (2021) Genetic regulation of root traits for soil flooding tolerance in genus *Zea*. *Breed. Sci.* 71: 30–39.
- Maricle, B.R. and R.W. Lee (2002) Aerenchyma development and oxygen transport in the estuarine cordgrasses *Spartina alterniflora* and *S. anglica*. *Aquat. Bot.* 74: 109–120.
- McDonald, M.P., N.W. Galwey and T.D. Colmer (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.
- Motte, H., S. Vanneste and T. Beeckman (2019) Molecular and environmental regulation of root development. *Annu. Rev. Plant Biol.* 70: 465–488.
- Oyiga, B.C., J. Palczak, T. Wojciechowski, J.P. Lynch, A.A. Naz, J. Léon and A. Ballvora (2020) Genetic components of root architecture and anatomy adjustments to water-deficit stress in spring barley. *Plant Cell Environ.* 43: 692–711.
- Pedersen, O., M. Sauter, T.D. Colmer and M. Nakazono (2021a) Regulation of root adaptive anatomical and morphological traits during low soil oxygen. *New Phytol.* 229: 42–49.
- Pedersen, O., Y. Nakayama, H. Yasue, Y. Kurokawa, H. Takahashi, A.H. Floytrup, F. Omori, Y. Mano, T.D. Colmer and M. Nakazono (2021b) Lateral roots, in addition to adventitious roots, form a barrier to radial oxygen loss in *Zea nicaraguensis* and a chromosome segment introgression line in maize. *New Phytol.* 229: 94–105.
- Petricka, J.J., C.M. Winter and P.N. Benfey (2012) Control of *Arabidopsis* root development. *Annu. Rev. Plant Biol.* 63: 563–590.
- Price, A.H., J.E. Cairns, P. Horton, H.G. Jones and H. Griffiths (2002) Linking drought-resistance mechanisms to drought avoidance in upland rice using a QTL approach: progress and new opportunities to integrate stomatal and mesophyll responses. *J. Exp. Bot.* 53: 989–1004.
- Ren, S., K. He, R. Girshick and J. Sun (2017) Faster R-CNN: towards real-time object detection with region proposal networks. *IEEE Trans. Pattern Anal. Mach. Intell.* 39: 1137–1149.
- Ronneberger, O., P. Fischer and T. Brox (2015) U-net: Convolutional networks for biomedical image segmentation. *Lect. Notes Comput. Sci. (including Subser. Lect. Notes Artif. Intell. Lect. Notes Bioinformatics)* 9351: 234–241.
- Saengwilai, P., H. Schneider, E.A. Nord, J.M. Johnson, J. Foerster, S.M. Kaeppler, K.M. Brown and J.P. Lynch (2016) Phenotypic variation and genome-wide association study of root anatomical traits in maize (*Zea mays* L.). *ProQuest Number*: 10005940: 116.
- Sakamoto, L., H. Kajiya-Kanegae, K. Noshita, H. Takanashi, M. Kobayashi, T. Kudo, K. Yano, T. Tokunaga, N. Tsutsumi and H. Iwata (2019) Comparison of shape quantification methods for genomic prediction, and genome-wide association study of sorghum seed morphology. *PLoS ONE* 14: e0224695.
- Sasidharan, R., J. Bailey-Serres, M. Ashikari, B.J. Atwell, T.D. Colmer, K. Fagerstedt, T. Fukao, P. Geigenberger, K.H. Hebelstrup, R.D. Hill *et al.* (2017) Community recommendations on terminology and procedures used in flooding and low oxygen stress research. *New Phytol.* 214: 1403–1407.
- Schneider, H.M., J.A. Postma, T. Wojciechowski, C. Kuppe and J.P. Lynch (2017a) Root cortical senescence improves growth under suboptimal availability of N, P, and K. *Plant Physiol.* 174: 2333–2347.
- Schneider, H.M., T. Wojciechowski, J.A. Postma, K.M. Brown, A. Lücke, V. Zeisler, L. Schreiber and J.P. Lynch (2017b) Root cortical senescence decreases root respiration, nutrient content and radial water and nutrient transport in barley. *Plant Cell Environ.* 40: 1392–1408.
- Shimamura, S., T. Mochizuki, Y. Nada and M. Fukuyama (2003) Formation and function of secondary aerenchyma in hypocotyl, roots and nodules of soybean (*Glycine max*) under flooded conditions. *Plant Soil* 251: 351–359.
- Shiono, K., T. Yamauchi, S. Yamazaki, B. Mohanty, A.I. Malik, Y. Nagamura, N.K. Nishizawa, N. Tsutsumi, T.D. Colmer and M. Nakazono (2014) 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.
- Striker, G.G., C. Casas, M.E. Manzur, R.A. Ploschuk and J.J. Casal (2014) Phenomic networks reveal largely independent root and shoot adjustment in waterlogged plants of *Lotus japonicus*. *Plant Cell Environ.* 37: 2278–2293.
- Striker, G.G., N.L. Teakle, T.D. Colmer and E.G. Barrett-Lennard (2015) Growth responses of *Melilotus siculus* accessions to combined salinity and root-zone hypoxia are correlated with differences in tissue ion concentrations and not differences in root

- aeration. *Environ. Exp. Bot.* 109: 89–98.
- Striker, G.G. and T.D. Colmer (2017) Flooding tolerance of forage legumes. *J. Exp. Bot.* 68: 1851–1872.
- Takahashi, H., T. Yamauchi, T.D. Colmer and M. Nakazono (2014) Aerenchyma formation in plants. *Plant Cell Monogr.* 21: 247–265.
- Takahashi, H., Q. Xiaohua, S. Shimamura, A. Yanagawa, S. Hiraga and M. Nakazono (2018) Sucrose supply from leaves is required for aerenchymatous phellem formation in hypocotyl of soybean under waterlogged conditions. *Ann. Bot.* 121: 723–732.
- Takahashi, H. and C. Pradal (2021) Root phenotyping: important and minimum information required for root modeling in crop plants. *Breed. Sci.* 71: 109–116.
- Taleisnik, E., G. Peyrano, A. Córdoba and C. Arias (1999) Water retention capacity in root segments differing in the degree of exodermis development. *Ann. Bot.* 83: 19–27.
- Tanaka, Y., S. Adachi and W. Yamori (2019) Natural genetic variation of the photosynthetic induction response to fluctuating light environment. *Curr. Opin. Plant Biol.* 49: 52–59.
- Teramoto, S., S. Takayasu, Y. Kitomi, Y. Arai-Sanoh, T. Tanabata and Y. Uga (2020) High-throughput three-dimensional visualization of root system architecture of rice using X-ray computed tomography. *Plant Methods* 16: 66.
- Tjørve, K.M.C. and E. Tjørve (2017) The use of Gompertz models in growth analyses, and new Gompertz-model approach: An addition to the Unified-Richards family. *PLoS ONE* 12: e0178691.
- Uga, Y., K. Okuno and M. Yano (2008) QTLs underlying natural variation in stele and xylem structures of rice root. *Breed. Sci.* 58: 7–14.
- Uga, Y., K. Sugimoto, S. Ogawa, J. Rane, M. Ishitani, N. Hara, Y. Kitomi, Y. Inukai, K. Ono, N. Kanno *et al.* (2013) Control of root system architecture by *DEEPER ROOTING 1* increases rice yield under drought conditions. *Nat. Genet.* 45: 1097–1102.
- Vanhees, D.J., K.W. Loades, A.G. Bengough, S.J. Mooney and J.P. Lynch (2020) Root anatomical traits contribute to deeper rooting of maize under compacted field conditions. *J. Exp. Bot.* 71: 4243–4257.
- Verboven, P., O. Pedersen, E. Herremans, Q.T. Ho, B.M. Nicolai, T.D. Colmer and N. Teakle (2012) Root aeration via aerenchymatous phellem: three-dimensional micro-imaging and radial O₂ profiles in *Melilotus siculus*. *New Phytol.* 193: 420–431.
- Visser, E.J.W., C.J. Heijink, K.J.G.M. van Hout and L.A.C.J. Voesenek (1995) Regulatory role of auxin in adventitious root formation in two species of *Rumex*, differing in their sensitivity to waterlogging. *Physiol. Plant.* 93: 116–122.
- Visser, E.J.W., G.M. Bögemann, C.W.P.M. Blom and L.A.C.J. Voesenek (1996) Ethylene accumulation in waterlogged *Rumex* plants promotes formation of adventitious roots. *J. Exp. Bot.* 47: 403–410.
- Visser, E.J.W., G.M. Bögemann, H.M. Van de Steeg, R. Pierik and C.W.P.M. Blom (2000a) Flooding tolerance of *Carex* species in relation to field distribution and aerenchyma formation. *New Phytol.* 148: 93–103.
- Visser, E.J.W., T.D. Colmer, C.W.P.M. Blom and L.A.C.J. Voesenek (2000b) Changes in growth, porosity, and radial oxygen loss from adventitious roots of selected mono- and dicotyledonous wetland species with contrasting types of aerenchyma. *Plant Cell Environ.* 23: 1237–1245.
- Voesenek, L.A.C.J. and J. Bailey-Serres (2015) Flood adaptive traits and processes: an overview. *New Phytol.* 206: 57–73.
- Wahl, S. and P. Ryser (2000) Root tissue structure is linked to ecological strategies of grasses. *New Phytol.* 148: 459–471.
- Wang, C., X. Li, D. Caragea, R. Bheemanahallia and S.V.K. Jagadish (2020) Root anatomy based on root cross-section image analysis with deep learning. *Comput. Electron. Agric.* 175: 105549.
- Wiengweera, A., H. Greenway and C.J. Thomson (1997) The use of agar nutrient solution to simulate lack of convection in waterlogged soils. *Ann. Bot.* 80: 115–123.
- Yamauchi, T., S. Shimamura, M. Nakazono and T. Mochizuki (2013) Aerenchyma formation in crop species: a review. *Field Crops Res.* 152: 8–16.
- Yamauchi, T., M. Yoshioka, A. Fukazawa, H. Mori, N.K. Nishizawa, N. Tsutsumi, H. Yoshioka and M. Nakazono (2017) An NADPH oxidase RBOH functions in rice roots during lysigenous aerenchyma formation under oxygen-deficient conditions. *Plant Cell* 29: 775–790.
- Yamauchi, T., T.D. Colmer, O. Pedersen and M. Nakazono (2018) Regulation of root traits for internal aeration and tolerance to soil waterlogging-flooding stress. *Plant Physiol.* 176: 1118–1130.
- Yamauchi, T., A. Tanaka, H. Inahashi, N.K. Nishizawa, N. Tsutsumi, Y. Inukai and M. Nakazono (2019a) Fine control of aerenchyma and lateral root development through AUX/IAA- and ARF-dependent auxin signaling. *Proc. Natl. Acad. Sci. USA* 116: 20770–20775.
- Yamauchi, T., F. Abe, N. Tsutsumi and M. Nakazono (2019b) Root cortex provides a venue for gas-space formation and is essential for plant adaptation to waterlogging. *Front. Plant Sci.* 10: 259.
- Yamauchi, T., A. Tanaka, N. Tsutsumi, Y. Inukai and M. Nakazono (2020a) A role for auxin in ethylene-dependent inducible aerenchyma formation in rice roots. *Plants (Basel)* 9: 610.
- Yamauchi, T., M. Nakazono, Y. Inukai and N. Tsutsumi (2020b) Distance-to-time conversion using Gompertz model reveals age-dependent aerenchyma formation in rice roots. *Plant Physiol.* 183: 1424–1427.
- Yamauchi, T., O. Pedersen, M. Nakazono and N. Tsutsumi (2021) Key root traits of Poaceae for adaptation to soil water gradients. *New Phytol.* (in press.)
- Yoo, C.Y., H.E. Pence, P.M. Hasegawa and M.V. Mickelbart (2009) Regulation of transpiration to improve crop water use. *Crit. Rev. Plant Sci.* 28: 410–431.
- Yu, P., F. Hochholdinger and C. Li (2015) Root-type-specific plasticity in response to localized high nitrate supply in maize (*Zea mays*). *Ann. Bot.* 116: 751–762.
- Zhu, J., K.M. Brown and J.P. Lynch (2010) Root cortical aerenchyma improves the drought tolerance of maize (*Zea mays* L.). *Plant Cell Environ.* 33: 740–749.