

# Flooding-adaptive root and shoot traits in rice

Chen Lin<sup>A,B,\*</sup> , Zhao Zhang<sup>A</sup>, Xuwen Shen<sup>A</sup>, Dan Liu<sup>B,C</sup>  and Ole Pedersen<sup>C,D,\*</sup> 

For full list of author affiliations and declarations see end of paper

**\*Correspondence to:**

Chen Lin

Key Laboratory of Plant Functional Genomics of the Ministry of Education, Yangzhou University, Yangzhou 225009, China  
Email: [clin@yzu.edu.cn](mailto:clin@yzu.edu.cn)

Ole Pedersen

Freshwater Biological Laboratory, Department of Biology, University of Copenhagen, Universitetsparken 4, Copenhagen 2100, Denmark  
Email: [opedersen@bio.ku.dk](mailto:opedersen@bio.ku.dk)

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## ABSTRACT

Wetland plants, including rice (*Oryza* spp.), have developed multiple functional adaptive traits to survive soil flooding, partial submergence or even complete submergence. In waterlogged soils and under water, diffusion of O<sub>2</sub> and CO<sub>2</sub> is extremely slow with severe impacts on photosynthesis and respiration. As a response to shallow floods or rising floodwater, several rice varieties, including deepwater rice, elongate their stems to keep their leaves above the water surface so that photosynthesis can occur unhindered during partial submergence. In stark contrast, some other varieties hardly elongate even if they become completely submerged. Instead, their metabolism is reduced to an absolute minimum so that carbohydrates are conserved enabling fast regrowth once the floodwater recedes. This review focuses on the fascinating functional adaptive traits conferring tolerance to soil flooding, partial or complete submergence. We provide a general analysis of these traits focusing on molecular, anatomical and morphological, physiological and ecological levels. Some of these key traits have already been introgressed into modern high-yielding genotypes improving flood tolerance of several cultivars used by millions of farmers in Asia. However, with the ongoing changes in climate, we propose that even more emphasis should be placed on improving flood tolerance of rice by breeding for rice that can tolerate longer periods of complete submergence or stagnant flooding. Such tolerance could be achieved via additional tissues; i.e. aquatic adventitious roots relevant during partial submergence, and leaves with higher underwater photosynthesis caused by a longer gas film retention time.

**Keywords:** aerenchyma formation, flooding tolerance, gas film, *Oryza sativa*, ROL barrier, root traits, stem elongation, submergence.

## Introduction

Flooding is one of the most severe natural disasters threatening the food supply worldwide, and both frequency and severity have increased in the past decades (Pedersen *et al.* 2017). Flooding can cause severe damage to both the root and shoot systems of plants, and the soil root system is particularly prone to flooding stress due to the reduced gas diffusion rate in waterlogged soils (Armstrong 1979). Waterlogged soils prevent plants from obtaining sufficient O<sub>2</sub> for root respiration from the rhizosphere since the O<sub>2</sub> is consumed by microorganisms, and slow diffusion restricts the replenishment of O<sub>2</sub> from the atmosphere above (Ponnamperuma 1972). Consequently, root tissues become severely hypoxic, or even anoxic, with consequences for water and nutrient uptake (Pedersen *et al.* 2021; Tong *et al.* 2023). Deeper floods inundating the shoot result in additional stress caused by the slow gas exchange under water (CO<sub>2</sub> for photosynthesis and O<sub>2</sub> for respiration (Colmer and Pedersen 2008)) as well as the reduction in light (resulting from reflection and absorption (Kirk 1994)). The resulting decline in carbohydrate production further accelerates tissue death (Voesenek and Bailey-Serres 2009; Kurokawa *et al.* 2018).

Wetland plants, including rice (*Oryza* spp.), have developed multiple functional adaptive traits to survive soil flooding, partial submergence, or even complete submergence. In shallow floods, several rice varieties elongate their stems to keep their leaves above the water surface (known as snorkelling) so that photosynthesis can occur unhindered

(Hattori *et al.* 2009). Aerenchyma is another important trait present in the roots, stems and leaves (Yamauchi *et al.* 2016), where it serves as a low-resistance pathway for O<sub>2</sub> diffusion to the submerged parts of the plant. However, if O<sub>2</sub> becomes insufficient to fuel aerobic respiration, rice can switch to anaerobic respiration allowing the plant to produce energy in the absence of molecular O<sub>2</sub> (Lee *et al.* 2014). During partial submergence, a brand new root system develops in some rice types, and these roots emerge from the stem in the water and are referred to as aquatic adventitious roots (Lorbiecke and Sauter 1999; Lin *et al.* 2021). In addition to nutrient and water uptake, aquatic adventitious roots also help anchoring the plant as the original soil roots are perishing due to lack of O<sub>2</sub>. Therefore, rice is used as a model plant to study the molecular mechanisms of plant tolerance to flooding since responses to flooding vary among the different rice varieties.

This review focuses on the fascinating functional adaptive traits conferring tolerance to soil flooding as well as partial or complete submergence. We provide a general analysis of these traits at the molecular, anatomical and morphological, physiological and ecological levels. Finally, we identify a number of traits to focus on in the quest of breeding for more flood-resilient rice cultivars in order to tackle the ongoing climate changes.

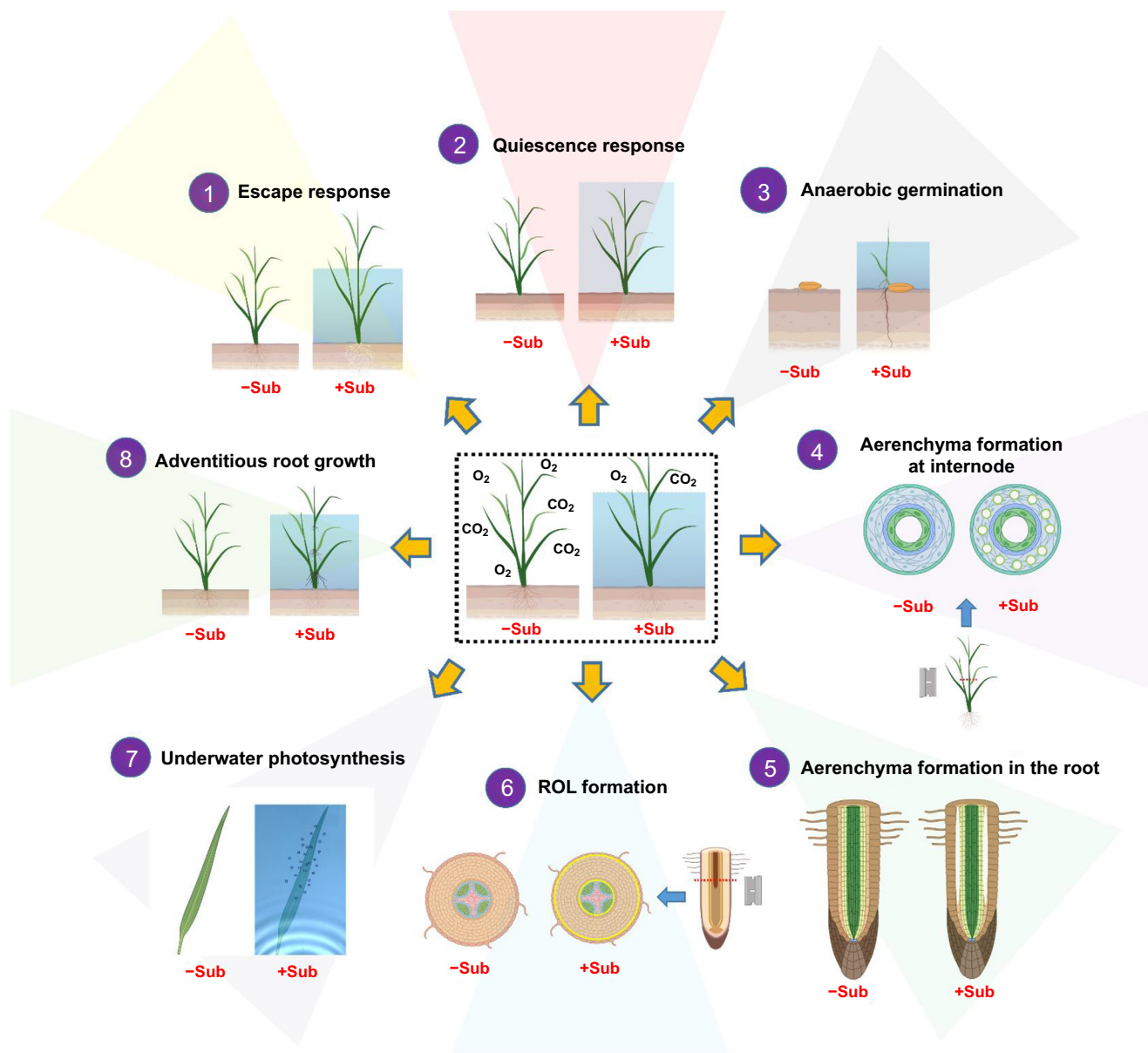
## Escape response to flooding

Growing along river or lake banks with fluctuating water levels, deepwater rice has a striking capacity for stem and leaf elongation to keep track with rising floodwaters. This capacity allows deepwater rice to remain in contact with atmospheric O<sub>2</sub> and CO<sub>2</sub> so that respiration and photosynthesis are maintained even during severe floods (Fig. 1) (Raskin and Kende 1984a; Bleecker *et al.* 1986; Catling 1992; Lorbiecke and Sauter 1999; Nagai *et al.* 2020; Lin *et al.* 2023). This kind of ‘escape response’ to rising floodwater is classified as low-O<sub>2</sub> escape syndrome (LOES) (Voeselek and Bailey-Serres 2015). Stem elongation mainly occurs at the intercalary meristem of the second and third internode (Kende *et al.* 1998; Nagai and Ashikari 2023), and the involvement of gibberellin acids (GA) in regulating stem elongation has been extensively studied (Raskin and Kende 1984b; van der Knaap *et al.* 2000; Hattori *et al.* 2009; Nagai *et al.* 2020). Compared to paddy rice (cv. T65), deepwater rice (cv. C9285) accumulated much more GA<sub>1</sub> and GA<sub>4</sub> after being exposed to partial submergence (Hattori *et al.* 2009; Nagai *et al.* 2020). Three major quantitative trait loci (QTLs) are involved in stem elongation with QTL12 having the largest effect (Hattori *et al.* 2009). Two ERFVII transcription factors, i.e. SNORKEL1 and SNORKEL2, play a significant role in controlling stem elongation, but ethylene rather than GA induces the expression of SNORKEL1 and SNORKEL2, revealing a function of SNORKEL1 and SNORKEL2 in the interplay between ethylene and GA signalling (Hattori *et al.* 2009).

GAs activate the intercalary meristem of the rice stem and regulate the stem cell elongation during submergence. The GA biosynthesis gene *SEMIDWARF1* (*SD1*) controls the stem elongation in deepwater rice (Kuroha *et al.* 2018), and it has been shown that the domestication of paddy rice resulted in the loss of function of this allele (Sasaki *et al.* 2002). The ethylene-responsive transcription factor OsEIL1a activates the *SD1* haploid gene in deepwater rice cv. C9285 upon submergence, and *SD1* further stimulates the biosynthesis of GA<sub>4</sub>, enabling the internode elongation (Kuroha *et al.* 2018). Evolutionary analysis revealed that the characteristic *SD1* haploid gene of deepwater rice was originally evolved from *Oryza rufipogon* (Kuroha *et al.* 2018). Excitingly, two genes controlling internode elongation in response to GA acting in an antagonistic manner have recently been discovered (Nagai *et al.* 2020). In the presence of GA, the expression of *ACCELERATOR OF INTERNODE ELONGATION 1* (*ACE1*) gene was highly induced, and cell division in the region of intercalary meristem was further activated, eventually leading to internode elongation. By contrast, *DECELERATOR OF INTERNODE ELONGATION 1* (*DEC1*), encoding a zinc-finger transcription factor, suppressed internode elongation. Based on the analysis of genetic diversity, *ACE1* and *DEC1* were both frequently engaged in domesticating shorter plants to improve lodging resistance in rice populations as well as taller rice plants that were suitable to grow in deepwater habitats (Nagai *et al.* 2020). In addition, QTLs governing internode elongation during the early vegetative stage with relative minor effect were discovered on chromosomes 2 and 4 in deepwater rice, despite that the associated genes have not been cloned within the QTLs (Nagai *et al.* 2012).

## Quiescence response to flooding

Quiescence enables plants to survive flooding via carbohydrate conservation. In other words, in deep but short floods, it might be more fruitful to wait out the submergence event rather than investing carbohydrates in elongation (see above) and then run out of reserves before surfacing. FR13A is a flood-tolerant Indian landrace of rice (Xu *et al.* 2006; Bailey-Serres *et al.* 2010), and via map-based positional cloning, *SUBMERGENCE1A* (*SUB1A*) was identified to play a major role in its flood tolerance via repression of shoot elongation (Xu *et al.* 2006). This strategy is referred to as low-O<sub>2</sub> quiescence syndrome (LOQS), and it reduces metabolism and preserves energy reserves during submergence (Fig. 1) (Voeselek and Bailey-Serres 2009, 2015). It has also been shown that the action of *SUB1A* reduces the rate of starch hydrolysis and accumulation of ethanol, lactate, and alanine as well as metabolic by-products of amino acids (Fukao *et al.* 2006; Barding *et al.* 2012). FR13A and the related nearly isogenic lines are all able to survive complete submergence for more than 2 weeks (Das *et al.* 2005; Fukao *et al.* 2006). Once the floodwater subsides, plants recover to grow due to



**Fig. 1.** Adaptive traits conferring flood tolerance in rice. (1) Escape response: stem elongation occurs when the intercalary meristem is activated and cell division takes place in response to ethylene and GA during submergence. (2) Quiescence response: *SUB1A* boosts GA signalling repressors Slender Rice-I (SLR1) and SLR1 Like-I (SLRL1), and reduces the expression of the GA-induced genes. In addition, the growth rate and metabolism are reduced. (3) Anaerobic germination: anaerobic germination helps rice to germinate under water and promotes elongation of the coleoptile. (4) Aerenchyma formation at the internode and (5) in the roots facilitates rapid O<sub>2</sub> diffusion to submerged tissues. (6) The root barrier to radial O<sub>2</sub> loss: formation of a root barrier to radial O<sub>2</sub> loss prevents O<sub>2</sub> loss to the rhizosphere and enhances the O<sub>2</sub> status in roots. (7) Underwater photosynthesis: superhydrophobic leaf cuticles retain a gas film enabling gas exchange with the floodwater, which enhances underwater photosynthesis and respiration. (8) Aquatic adventitious root growth: aquatic adventitious roots emerge and grow in the water in response to partial submergence and take over the function of the soil root system. Several components are created with BioRender.com. ROL, radial O<sub>2</sub> loss.

the better carbohydrate status, and since they have only elongated little during the submergence event, they are also much less prone to lodging (Ismail *et al.* 2013).

The ability of paddy rice to tolerate submergence is consequently greatly enhanced by the *SUB1A* gene. *SUB1A*,

a member of the ERFVII transcription factor family, has been extensively studied to understand the physiological and molecular mechanisms of submergence tolerance in rice, and it has been found that *SUB1A* restricts ethylene biosynthesis, and reduces metabolism and energy consumption early in the

submergence event (Tamang and Fukao 2015; Fukao *et al.* 2019). Furthermore, *SUB1A* suppresses shoot elongation and prevents excessive energy consumption by reducing the response to GA and by activating brassinosteroid biosynthesis (Fukao and Bailey-Serres 2008; Schmitz *et al.* 2013). Moreover, *SUB1A* stimulates the onset of mitosis and increases the expression of *phosphorylated protein kinase 3* (MPK3), and it has therefore been proposed that *SUB1A* also promotes flooding tolerance by controlling MPK3 activity in rice (Singh and Sinha 2016). During flooding, *SUB1A* also enhances leaf gas film thickness and retention time (see section on underwater photosynthesis) (Chakraborty *et al.* 2021).

Following de-submergence, rice also suffers from oxidative stress followed by rapid increase in cell dehydration (Fukao and Xiong 2013). Interestingly, *SUB1A* increases plant survival through detoxification of reactive O<sub>2</sub> species (ROS) during the recovery phase after a submergence event. However, the decrease in ABA caused by submergence is not due to *SUB1A* (Fukao *et al.* 2011). *SUB1A* likewise promotes photosynthesis and metabolic recovery from flooding damage during de-submergence (Alpuerto *et al.* 2016). It has been shown that *SUB1A* contributes to the rapid restoration of PSII performance, such as Fv/Fm,  $\Phi$ PSII and metabolism of protein and amino acids including arginine, phenylalanine, proline, threonine and valine. The ERFVII family proteins often serve as substrates of the N-degron pathway in an O<sub>2</sub>-dependent manner (Bailey-Serres *et al.* 2012), but the *SUB1A* protein is not a substrate for the N-degron pathway (Gibbs *et al.* 2011). Recent research has demonstrated that the direct interaction of N-terminus and C-terminus of *SUB1A* helps stabilising the protein and prevents degradation via the N-degron pathway (Lin *et al.* 2019). Transcriptomic analysis also supports the role of *SUB1A* in rapid recovery during submergence and de-submergence (Jung *et al.* 2010; Locke *et al.* 2018).

Ectopic expression of *SUB1A* results in GA-insensitive phenotype plants. With submergence, *SUB1A* boosted the abundance of the GA signalling repressors Slender Rice-1 (SLR1) and SLR1 Like-1 (SLRL1), and remarkably reduced the expression of the GA-induced genes (Fukao and Bailey-Serres 2008). Ethylene, which promotes the expression of *SUB1A*, further stimulates the increased abundance of GA signalling inhibitory factors and decreases GA responsiveness (Fukao and Bailey-Serres 2008). By contrast, GA responsiveness and stem elongation was enhanced by ethylene in waterlogging-intolerant rice varieties (Fukao and Bailey-Serres 2008). The above shows that *SUB1A* enhances the abundance of the GA signal repressors SLR1 and SLRL1, which in turn decreases GA responsiveness by ethylene during flooding (Fukao and Bailey-Serres 2008).

In conclusion, the quiescence response coded by *SUB1* has been found to be effective at all growth stages from early seedling stage to about a week before flowering, and therefore new *Sub1* varieties have been spreading fast and are now grown by millions of farmers in Asia (Ismail *et al.* 2013).

## Anaerobic germination and anaerobic seedling development

Anaerobic germination and anaerobic seedling development are extremely important for wetland plants, given that these traits enable seedlings to grow rapidly above the water and take in O<sub>2</sub> from the atmosphere (Fig. 1). Anaerobic germination and anaerobic seedling development are both linked with rapid seed germination and coleoptile elongation, with the activities of alcoholic fermentation enzymes taking over energy production from aerobic respiration (Miro and Ismail 2013; Miro *et al.* 2017; Yu *et al.* 2021). The majority of crops are unable to germinate underwater due to the limited ATP produced by glycolysis and ethanolic fermentation (Yu *et al.* 2021), and it is particularly the root development and growth, which are hampered by low O<sub>2</sub> availability (Sauter 2013; Pedersen *et al.* 2021). Therefore, the ability of rice to germinate underwater is unique, and the majority of rice varieties can germinate under severe hypoxia, or even anoxia, and they also extend their coleoptile to a certain degree, but they fail to produce proper leaves and roots (Alpi and Beevers 1983; Miro and Ismail 2013; Yu *et al.* 2021). However, the coleoptile of some wild rice relatives elongates prior to the development of a root system when it sprouts in wet soils (Fig. 1), allowing the coleoptile, (which is of high tissue porosity; see section on aerenchyma below) to reach the surface of the water where it can obtain O<sub>2</sub> from the air; the O<sub>2</sub> subsequently diffuses downwards to support root development (Lee and Lin 1995; Ella and Setter 1999; Magneschi *et al.* 2009). Anaerobic germination and anaerobic seedling development are highly beneficial to agricultural production, since direct-seeded rice reduces labour costs and water requirements for rice transplanting (Ismail *et al.* 2012; Tuong *et al.* 2015).

*Anaerobic Germination 1* (AG1) derived from a Japanese landrace Khao Hlan On (KHO) is so far the most important QTL related with tolerance to anaerobic germination and anaerobic seedling development (Angaji *et al.* 2010; Kretzschmar *et al.* 2015). Several other QTLs with major and minor impacts on anaerobic germination have been identified through a variety of genetic linkage mapping analyses (Ling *et al.* 2004; Angaji *et al.* 2010; Baltazar *et al.* 2019). *Trehalose-6-phosphate phosphatase 7* (TPP7), which was found on chromosome 9 in KHO, has ultimately been identified as *Anaerobic Germination 1* (AG1) (Kretzschmar *et al.* 2015). Trehalose-6-phosphate (T6P) helps monitoring the amount of sucrose, the coordination of carbon catabolism and the transfer of carbon from source to sink tissues (Yadav *et al.* 2014; Figueroa and Lunn 2016). Interestingly, the energy sensor sucrose non-fermenting 1-related protein kinase 1 (SnRK1A) is inhibited by increased T6P levels (Zhang *et al.* 2009). Under water, the activation of the  $\alpha$ -Amylase activities in the early coleoptile improves the trehalose content in the seed coleoptile and it also enhances

anaerobic germination and elongation (Kretzschmar *et al.* 2015). Consequently, chromosomal deletion, which include the loss of the *TPP7* gene, results in an impaired anaerobic germination tolerance of the high-yield cv. IR64 (Kretzschmar *et al.* 2015).

Anaerobic germination involves multiple processes, and rapid starch breakdown is the most important among them to maintain the metabolism of carbohydrates and supply energy for germination (Ella and Setter 1999; Ismail *et al.* 2009; Magneschi *et al.* 2009). It has been shown that enzyme activity of  $\alpha$ -Amylase was increased to promote starch breakdown (Bailey-Serres and Chang 2005; Ismail *et al.* 2012). Moreover, alcohol dehydrogenase (ADH), a key enzyme in the anaerobic pathway of carbohydrate metabolism, was highly activated during anaerobic germination (Waters *et al.* 1991; Gibbs *et al.* 2000; Ismail *et al.* 2012). In addition, the enzyme activity for cellular expansion and cell wall loosening was enhanced (Choi *et al.* 2003; Ismail *et al.* 2009), and the level of GA was increased (Gu *et al.* 2010).

At the molecular level, the calcineurin b-like interacting protein kinase 15 (CIPK15) is seen as a central hub in response to low-O<sub>2</sub> conditions and sugar starvation. The expression of *CIPK15* is highly induced in embryonic tissues of the rice seeds (Lee *et al.* 2009; Yim *et al.* 2012). CIPK15 further promotes the activation of the energy sensor SnRK1A, which amplifies transcription of specific amylase genes in the starchy endosperm enabling the nutrient transport from the endosperm to the embryo (Lee *et al.* 2009; Yu *et al.* 2015, 2021). Both hypoxia and sucrose starvation promote the expression of *SnRK1A* (Ramon *et al.* 2019). SnRK1A phosphorylates the transcription factor MYB SUCROSE 1 (MYBS1) and activated MYBS1 interacts with the TA box at the promoter region of  *$\alpha$ -Amylase* ( *$\alpha$ -Amy*) genes. MYBS2 competes with MYBS1 for the promoter binding of  *$\alpha$ -Amy* gene, and SnRK1A-INTERACTING NEGATIVE REGULATORS (SKIN1/2) negatively regulate the expression of MYBS1 and  *$\alpha$ -Amy3* (Zhang *et al.* 2009; Chen *et al.* 2019).  $\alpha$ -Amylase hydrolyses seed starch to provide sugars serving as substrate for fermentation-mediated energy production to support anaerobic germination and anaerobic seedling development underwater. CIPK15 also contributes to the upregulated expression of *ADH* genes (Lee *et al.* 2009; Miro and Ismail 2013), which is required to maintain the regeneration of NAD<sup>+</sup> in anaerobic respiration. The coupling of SnRK1A-dependent starch catabolism and anaerobic metabolism supplies energy for the elongation of underwater coleoptiles (Lee *et al.* 2014).

The regulation of anaerobic germination and anaerobic seedling development is a quantitative trait and 11 significant marker-associated sites have been discovered through a genome-wide associated study of 273 Japonica rice varieties (Nghie *et al.* 2019). Based on RNA-Seq analysis, a total number of 26 genes associated with the establishment of cell membrane and cell wall during embryonic sheath elongation have been identified (Hsu and Tung 2017).

However, further investigation is still required to pinpoint the regulatory mechanisms governing this feature particularly to flooding-tolerant genotypes. In molecular breeding, the discovery of novel QTLs for anaerobic tolerance during germination aids in the understanding of the genetic and molecular bases of anaerobic tolerance and then these potent QTLs can be used for molecular marker-assisted selection in agriculture.

## Aerenchyma formation and O<sub>2</sub> diffusion

Aerenchyma forms a low-resistance pathway for gas diffusion between roots and shoot with O<sub>2</sub> diffusion downwards and CO<sub>2</sub> and CH<sub>4</sub> diffusing in the opposite direction. Aerenchyma formation in rice is a result of programmed cell death in roots, stem and leaf tissues, and programmed cell death has been studied in great detail (see Nishiuchi *et al.* 2012 for a thorough review on the topic). When soil flooding kicks in, O<sub>2</sub> initially present in the soil pores is soon consumed by plant roots and soil microbes rendering the soil anoxic after a few days (Ponnamperuma 1972). Therefore, the only source of O<sub>2</sub> to the roots is diffusion of molecular O<sub>2</sub> from shoot to root via internal gas spaces (Drew *et al.* 2000; Colmer 2003a; Yamauchi *et al.* 2018). The development of aerenchyma in rice tissues greatly reduces the resistance to long-distance O<sub>2</sub> diffusion and in combination with the barrier to radial O<sub>2</sub> loss (see next section) oxygenation of root tissues is sustained under soil flooding (Fig. 1). Rice forms two types of aerenchyma; i.e. constitutive aerenchyma that is also formed in drained soils, and inducible aerenchyma forming as a response to soil flooding (Yukiyoshi and Karahara 2014; Yamauchi *et al.* 2016).

Aerenchyma is constitutively formed in rice under aerobic, drained conditions. The molecular mechanism behind constitutive aerenchyma formation has long been a puzzle, and only recently it was discovered that formation of constitutive aerenchyma is mediated by auxin (Yamauchi *et al.* 2019). Auxin/indole-3-acetic acid protein (AUX/IAA; IAA) and auxin response factor (ARF)-mediated signalling are essential both for aerenchyma and lateral root formation. ARF-dependent transcriptional regulation is repressed by IAA. IAA13 belongs to the IAA family, and lack of IAA13 (*iaa13* mutant) results in a phenotype of reduced aerenchyma and lateral root formation (Yamauchi *et al.* 2019). ARF19 belongs to the ARF family, and it was further identified as the target of IAA13, and LBD1-8 (a protein containing a lateral organ boundary domain, LBD) was subsequently identified as the target of ARF19 (Yamauchi *et al.* 2019). IAA13, ARF19 and LBD1-8 are all highly expressed in the root cortex, indicating that they are involved in co-regulating the formation of constitutive aerenchyma. Importantly, overexpression of LBD1-8 in the *iaa13* background fully restored the formation of constitutive aerenchyma, and auxin

transport inhibitors prevented the formation of aerenchyma, while the application of natural auxin reversed the inhibitory effect (Yamauchi et al. 2019). The current evidence indicates that constitutive aerenchyma formation is under the control of auxin signalling via AUX/IAA and ARF module (Yamauchi et al. 2019). Interestingly, recent research implies that there is a direct link between constitutive aerenchyma and plant tolerance to waterlogging. Despite sharing the same potential to form inducible aerenchyma, maize (*Zea mays*) and wheat (*Triticum aestivum*) both have relatively little waterlogging tolerance probably due to the absence of constitutive aerenchyma, which is thought to facilitate oxygenation of root tissues at the onset of soil flooding and prior to the formation of inducible aerenchyma (Colmer and Voesenek 2009; Yamauchi et al. 2018).

Formation of inducible aerenchyma under waterlogged conditions is mainly controlled by the ethylene and ROS signalling pathways (Yamauchi et al. 2017). There is always a low, constitutive production of ethylene in rice roots, but in a drained soil ethylene is lost to the rhizosphere via radial diffusion. However during waterlogging, outward diffusion is impeded and ethylene accumulates in the tissue where it triggers programmed cell death as well as an increased ethylene production (Yamauchi et al. 2018). In contrast, ROS are mainly produced by NADPH oxidase and respiratory burst oxidase homologues (RBOH), triggering signal transduction in different processes in plants (Mhamdi and Van Breusegem 2018; Mittler et al. 2022). It has recently been shown that the RBOH subtype (RBOHH) plays an important role in ethylene-induced root aerenchyma formation (Yamauchi et al. 2017). In cortex cells of rice roots, hypoxia induces the expression of the *CDPK5* and *CDPK13*, which encode calcium-dependent protein kinases, and the co-expression of *RBOHH* with *CDPK5* or *CDPK13* results in ROS accumulation in infiltrated tobacco (*Nicotiana tabacum*) leaves (Yamauchi et al. 2017, 2018). However, inhibition of RBOH activity or cytosolic calcium influx abolishes the aerenchyma formation induced by ethylene, and knocking out *RBOHH* reduces the accumulation of ROS and induces the formation of aerenchyma in rice roots (Yamauchi et al. 2017). These results indicate that under hypoxic conditions, *RBOHH*-mediated ROS production is crucial for ethylene-induced root aerenchyma formation through *CDPK5* and *CDPK13* in rice (Yamauchi et al. 2017).

In addition to the root, rice plants also form aerenchyma in the internode during partial or complete submergence. Aerenchyma formation in the internode also facilitates  $O_2$  diffusion from shoot to root and thereby enhances flood tolerance. The aerenchyma is ubiquitously present in each internode of the deepwater rice cv. Pin Gaew 56 (PG56) (Steffens et al. 2011) with pronounced developmental gradients of inducible aerenchyma formation from old to younger internodes; the nodes do not form aerenchyma (Steffens et al. 2011). Two lowland rice types also exhibit

aerenchyma development at the internode, albeit to a lesser degree (Steffens et al. 2011). Treatment with the ethylene-releasing compound ethephon or submergence both promoted aerenchyma formation in different rice genotypes (Steffens et al. 2011), and anatomical analysis indicated that pre-aerenchymal cells contain less starch, no chloroplasts and have thinner cell walls (Steffens et al. 2011). In addition, high levels of singlet  $O_2$  and  $H_2O_2$  were observed in these pre-aerenchymal cells as compared with other parenchymal cells. Interestingly, the formation of singlet  $O_2$  and  $H_2O_2$  promoted by ethephon is essential for the formation of aerenchyma. This is further supported by the enhanced aerenchyma formation in the rice mutant of the  $H_2O_2$  scavenger -MT2b (Steffens et al. 2011).

### Formation of a root barrier to radial $O_2$ loss

Constitutive as well as inducible aerenchyma are key traits for root growth in flooded soils, but without the inducible barrier to radial  $O_2$  loss, maximum root length is significantly restricted. Even with aerenchyma constituting 50% of the root cross-sectional area, the root of rice would not grow much longer than 150 mm (see modelling of maximum root length in Pedersen et al. 2021). In order to grow longer roots,  $O_2$  must be retained inside the root tissue in order to diffuse all the way to the root tip, since cell divisions and continuous root extension cannot take place in the absence of molecular  $O_2$  (Armstrong and Webb 1985). Fortunately, rice roots can form a barrier to radial  $O_2$  loss in the proximal parts of the mature adventitious roots to prevent  $O_2$  loss to the anoxic rhizosphere (Fig. 1) (Colmer 2003b).

In rice, the root barrier to radial  $O_2$  loss is inducible. Paddy rice growing in non-flooded soils do not form a barrier to radial  $O_2$  loss, but the formation is triggered as soon as the soil becomes waterlogged (Colmer et al. 1998; Colmer 2003b; Ejiri et al. 2021). The functional properties; i.e. high resistance to  $O_2$  diffusion in the outer part of the root can be observed already after 6 h of exposure to anoxia and after 24 h, the barrier is complete (Shiono et al. 2011). The environmental signalling for barrier formation is surprisingly not related to low  $O_2$ , high  $CO_2$  or high ethylene (Colmer et al. 2006), which would otherwise characterise an anoxic soil. Instead, it has been shown that  $H_2S$  (Peralta Ogorek et al. 2023a),  $Fe^{2+}$  (Mongon et al. 2014) and low-molecular-weight carboxylic acids (Colmer et al. 2019) can all act as environmental signals for barrier formation. These chemical compounds are all produced by anaerobic bacteria in anoxic soils, indicating that the barrier formation relies on functional anoxia of the microbial community in the rhizosphere.

Although the induction of the barrier to radial  $O_2$  loss is swift and functionally in place within 24 h of exposure to soil flooding, cell wall modifications of the outer part of the root are not yet detectable with histochemical staining. Cell

wall depositions of suberin and/or lignin in the outer part of the root are thought to form the functional biochemical components of the barrier (Schreiber 1999; Kotula *et al.* 2009; Watanabe *et al.* 2017). Suberin is mainly deposited in the exodermal cell walls (Kotula *et al.* 2009; Nishiuchi *et al.* 2021), whereas lignin is mainly found in the sclerenchyma (Armstrong 2000; Soukup *et al.* 2007). The molecular mechanisms controlling barrier formation have not yet been fully elucidated. However, laser microdissection enabled isolation of the outer part of the root and subsequent tissue-specific transcriptome analysis showed that flooding resulted in upregulation of two genes involved in the suberin biosynthesis (cytochrome P450 (OsCYP86B3) and ABC transporter (OsABCG5)) suggesting that these molecular regulators are essential for the development of the barrier (Shiono *et al.* 2014). This study also showed that the expression of different transcription factors, including WRKY, NAC and MYB, increased under conditions of soil flooding (Shiono *et al.* 2014), and these transcription factors might directly or indirectly contribute to the development of radial O<sub>2</sub> loss barriers. Given the lack of relevant rice mutants, functional studies of these genes associated with barrier development still need investigation. There are also studies indicating that lignin plays an important role in the formation of a barrier to radial O<sub>2</sub> loss (Kotula *et al.* 2009; Shiono *et al.* 2011; Abiko *et al.* 2012). A very recent study showed that lignin-related genes were upregulated during barrier formation as a response to external H<sub>2</sub>S application with 11 genes involved in the phenylpropanoid and/or lignin metabolic processes (Peralta Ogorek *et al.* 2023a). Moreover, genes involved in the biosynthesis of lignin were also upregulated as a response to low molecular carboxylic acids and the subsequent formation of a barrier to radial O<sub>2</sub> loss (Colmer *et al.* 2019). Nevertheless, the roles of suberin and lignin in barrier formation have not yet been fully elucidated.

It has very recently been shown that the formation of the barrier to radial O<sub>2</sub> loss relies on ABA signal transduction. The application of the ABA biosynthesis inhibitor FLU prevented the formation of the barrier, as well as in the rice mutant, *osaba1* (Shiono *et al.* 2022). An impaired ROL barrier was observed in the rice mutant with a defective ABA biosynthesis gene (*osaba1*), and the barrier was made fully functional by adding exogenous ABA to the root medium. These findings suggest that ABA is an inducer of suberin lamellae formation as visualised by more pronounced suberin depositions in the exodermis (Shiono *et al.* 2022). In order to better understand how the barrier is regulated at the molecular level, it thus seems appropriate to look into these ABA-related genes.

In addition to the primary role of the barrier to radial O<sub>2</sub> loss; i.e. maintaining high O<sub>2</sub> status of the root tissues, the barrier also has additional functions in roots of rice. Gas diffusion is generally restricted by the barrier as shown for H<sub>2</sub> and water vapour (Peralta Ogorek *et al.* 2021) and also H<sub>2</sub>S (Peralta Ogorek *et al.* 2023a). Moreover, apoplastic

movements of H<sub>2</sub>O (Song *et al.* 2022), Na<sup>+</sup> (Krishnamurthy *et al.* 2011) and Fe<sup>2+</sup> (Jimenez *et al.* 2021) are also greatly restricted by the barrier and therefore, this trait can be considered a jack of all trades with its multiple functional roles (Peralta Ogorek *et al.* 2023b).

An often-overlooked trait also restricting radial O<sub>2</sub> loss from roots under soil flooding is a low surface area to volume ratio. The thicker the root, the smaller the relative surface from which O<sub>2</sub> can be lost to the anoxic rhizosphere, and therefore a thick root will lose less O<sub>2</sub> compared with a thin root if the concentration gradient from tissue to environment is the same (Pedersen *et al.* 2021). Paddy rice forms thick adventitious roots as a response to soil flooding (Lorbiecke and Sauter 1999), and thick roots in combination with a tight barrier to radial O<sub>2</sub> loss is thus an excellent combination of root traits serving to conserve O<sub>2</sub> within the cortical tissues.

### Aquatic adventitious root growth as response to partial submergence

Adventitious root development is a key trait conferring flood tolerance since these roots replace the impaired soil root system during submergence. Even if the existing soil root system has high amounts of cortical aerenchyma, molecular diffusion of O<sub>2</sub> can be too slow to satisfy tissue demand when the distance is long as would be the case if a large portion of the shoot is submerged into water. However, the primordia of adventitious root growth are constitutively formed at the node as part of the normal developmental process (Lorbiecke and Sauter 1999; Steffens and Rasmussen 2016). During flooding, entrapped ethylene in the stem tissues promotes the formation of adventitious roots at the nodes (Fig. 1). In rice, the epidermal cell death above the root primordia is coordinated with adventitious root growth (Steffens and Sauter 2005), both of which are mediated by ethylene, ROS and mechanical signalling (Steffens and Sauter 2009; Steffens *et al.* 2012). During flooding, the expression of the ethylene biosynthesis genes, *ACO1* and *EOL1*, are highly induced in the epidermal cells above the adventitious root tips, and ethylene accumulation stimulates NADPH oxidase activity and elevates biosynthesis of H<sub>2</sub>O<sub>2</sub> (Steffens and Sauter 2009; Steffens *et al.* 2012). Meanwhile, the expression of the H<sub>2</sub>O<sub>2</sub> scavenger gene, *MT2b*, is inhibited, further promoting ethylene and H<sub>2</sub>O<sub>2</sub> signalling transduction (Steffens and Sauter 2009). Ethylene also promotes the production of ROS in the adventitious root primordia, and mechanical signals and ROS are both required for the emergence of adventitious roots as mechanical signals locally provide spatial information triggering programmed cell death of epidermal cells (Steffens *et al.* 2012). While GA alone is not functional, it acts synergistically with ethylene to induce the formation of adventitious roots (Steffens *et al.* 2006), but

adventitious root growth induced by ethylene is suppressed by exogenous application of ABA (Steffens *et al.* 2006).

The lack of O<sub>2</sub> in flooded soils damages the soil roots and eventually they become dysfunctional and perish. However, the new adventitious roots developed by deepwater rice as responses to partial submergence are primarily formed in the floodwater and are therefore referred to as aquatic adventitious roots. These roots are capable of obtaining O<sub>2</sub> from the floodwater since they do not form a barrier to radial O<sub>2</sub> loss (the barrier is very weak) rendering these roots permeable to O<sub>2</sub> (Lin *et al.* 2021). Deepwater rice (Lin *et al.* 2023) and paddy rice (Inouye and Mochizuki 1980) form two types of adventitious roots differing in morphology and timing of emergence. First, relatively thin roots emerge as fast as 3 days after exposure to partial submergence, and these continue to grow for about 2 weeks, reaching a maximum length of 200 mm (Lin *et al.* 2023). Second, another thicker type then forms some days later, reaching 350 mm and continues to grow for 6–8 weeks. This second type can continue to form from new or existing primordia following periods of recurrent flooding, whereas the first thinner type only grows during the first flood event and dies if they become de-submerged (Lin *et al.* 2023). The upregulation of key genes between thin and thick adventitious roots indicates a common contribution in activating meristems in aquatic adventitious roots, enhancing their response to flooding. Morphological and anatomical analyses indicate that thick adventitious are more suitable for long-term floods than the thinner, but the exact importance for water and nutrient uptake of these two types of roots awaits further investigation (Lin *et al.* 2023).

## Underwater photosynthesis during submergence

The terrestrial leaves of rice can photosynthesise under water although at a much lower rate than in air. The lower photosynthetic rate is due to the extremely slow gas diffusion in water resulting in CO<sub>2</sub> limitation of photosynthesis and possibly also photorespiration caused by internal build-up of photosynthetically produced O<sub>2</sub> (Colmer *et al.* 2011). Both processes restrict underwater photosynthesis in rice to maximum rates of about 25% of that attained in air, but the achieved photosynthetic rates during partial or complete submergence are normally below 10% of those in air (Winkel *et al.* 2016), and consequently, the production of organic carbon is limited. However, the molecular O<sub>2</sub> produced in underwater photosynthesis can diffuse over long distances internally via the well-developed aerenchyma providing a low-resistance pathway (Pedersen *et al.* 2021). Therefore, molecular O<sub>2</sub> can sustain aerobic processes in tissues as far away as in the root tips (Yamauchi *et al.* 2018).

A crucial leaf trait enabling underwater photosynthesis is the superhydrophobic leaf cuticle that retains a thin gas film during partial or complete submergence (Fig. 1). Leaf

gas films are visible as a silvery sheen from the submerged parts of the leaves (Raskin and Kende 1983), and they prevent flooding of the stomata, which are positioned in the grooves both on the ad- and abaxial side of the plicate leaves (Lauridsen *et al.* 2014). Hydrophobicity of rice leaves is the result of three leaf traits: (1) at the macro level by the plicate leaf structure, where the deep grooves prevent water from wetting the bottom of the grooves; (2) at the micro level where the leaf papillae add extra 3D structure to the leaf surface; and (3) at the nano level caused by the wax platelets forming the cuticle surface (Bhushan *et al.* 2009). In addition to preventing flooding of the stomata and the sub-stomatal cavities, the leaf gas films present a large surface area for gas exchange with the surrounding floodwater (Verboven *et al.* 2014). Once CO<sub>2</sub> is trapped inside the gas film, it can quickly diffuse to the nearest stomata and enter the leaf; the diffusion inside the gas film is 10 000-fold faster than in the floodwater. Similarly, O<sub>2</sub> produced in photosynthesis can swiftly diffuse into the gas film and further dissolve in the floodwater via the vast surface area presented by the gas film, and both processes contribute to establishing photosynthetic rates, which are about 5-fold higher in the presence of leaf gas films than without at environmentally relevant CO<sub>2</sub> concentrations in the floodwater (Pedersen *et al.* 2009).

Regrettably, the superhydrophobic features of the leaf cuticle are gradually lost over a submergence event and therefore the leaf gas films also disappear. Four genotypes of completely submerged rice all lost their leaf gas films within 4–7 days of submergence under field conditions (Winkel *et al.* 2014). Following loss of gas films, underwater net photosynthesis also declined significantly, and chlorophyll senescence accelerated. In this study, gas film retention time was not related to the *SUB1* locus, which was represented in two of the four tested genotypes (FR13A in which the *SUB1* locus occurs naturally and Swarna-SUB1 where *SUB1* had been introgressed) (Winkel *et al.* 2014). In the interim, a later study found that leaf gas film thickness was significantly higher in *SUB1* genotypes than genotypes not containing the *SUB1* locus, and it seemed related to lower expression of *LGF1* gene (Kurokawa *et al.* 2018) in the latter (Chakraborty *et al.* 2021). For rice submerged in a field situation, the benefits of superhydrophobic leaves and the associated leaf gas films as related to underwater photosynthesis and respiration are restricted to about 1 week of complete submergence, whereas under controlled laboratory conditions with submergence into clean artificial floodwater, the gas films can persist longer (Pedersen *et al.* 2009).

## Perspectives

Since cultivated rice was domesticated from wild ancestors that were true wetland plants, *Oryza sativa* (Asian rice) and *Oryza glaberrima* (African rice) both possess root traits

enabling growth in waterlogged soils. However, higher aerenchyma formation (constitutive as well as inducible), a large cortex to stele ratio, and an even tighter barrier to radial O<sub>2</sub> loss could all improve growth in flooded paddy soils and even holds the potential to reduce greenhouse gas emissions (Jimenez and Pedersen 2023). Importantly, it was recently shown that some of these root traits (e.g. the barrier to radial O<sub>2</sub> loss and thick adventitious roots) formed during soil flooding also prime the root system of rice for a subsequent drought (Song *et al.* 2023; Peralta Ogorek *et al.* 2023c) a situation that often occurs in the farmer's field. Multiple genes or QTLs underlying the control of these key root traits can be introgressed into modern high-yielding cultivars via pyramiding producing novel cultivars that can withstand multiple abiotic stresses, but this approach requires that the genes or QTLs are known (Lin *et al.* 2022), so much more research focusing on genetic regulation of quantitative root traits are needed.

In deeper floods resulting in partial or complete submergence, focus needs to be more on shoot traits enabling growth and survival during deep flood events. We therefore suggest further investigating the genes in the two QTLs regulating adventitious root growth in deepwater rice. The functions of both types of aquatic adventitious roots with respect to water and nutrient uptake during partial submergence have to be further studied to justify introducing aquatic adventitious roots as a flood tolerance trait in rice cultivars for areas experiencing shallow flooding causing partial submergence of paddy rice for months (Kuanar *et al.* 2017). Similarly, much more focus is needed on the superhydrophobic properties of the rice cuticle and why hydrophobicity, and therefore also the beneficial leaf gas films, are lost after only a few days of submergence (Winkel *et al.* 2014). It is interesting that initial leaf gas film thickness is linked to SUB1 (Chakraborty *et al.* 2021), but the mechanism remains unknown. We propose to investigate the role of leaf morphology since research has shown that the vast majority of the gas volume is retained within the grooves of the plicate leaves (Lauridsen *et al.* 2014), and therefore leaves with deeper grooves and steeper angles within each groove may retain gas films longer than leaves with a more flattened surfaces.

In this review, we have summarised the many root and shoot traits in rice conferring tolerance to waterlogging or submergence. However, unlike rice, the majority of the world's crop plants are very susceptible to different types of flooding, and therefore more research and integration are needed to apply this knowledge from rice to improve flood tolerance in other crops such as maize, wheat, barley (*Hordeum vulgare*) and soybean (*Glycine max*).

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**Data availability.** Data sets supporting the analyses of this review are made available in the original papers.

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**Author affiliations**

<sup>A</sup>Key Laboratory of Plant Functional Genomics of the Ministry of Education, Yangzhou University, Yangzhou 225009, China.

<sup>B</sup>Plant Developmental Biology and Plant Physiology, University of Kiel, Am Botanischen Garten 5, Kiel 24118, Germany.

<sup>C</sup>Freshwater Biological Laboratory, Department of Biology, University of Copenhagen, Universitetsparken 4, Copenhagen 2100, Denmark.

<sup>D</sup>School of Biological Sciences, The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia.