Ethylene-Mediated Acclimations to Flooding Stress[1]

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Flooding is detrimental for plants, primarily because of restricted gas exchange underwater, which leads to an energy and carbohydrate deficit. Impeded gas exchange also causes rapid accumulation of the volatile ethylene in all flooded plant cells. Although several internal changes in the plant can signal the flooded status, it is the pervasive and rapid accumulation of ethylene that makes it an early and reliable flooding signal. Not surprisingly, it is a major regulator of several flood-adaptive plant traits. Here, we discuss these major ethylene-mediated traits, their functional relevance, and the recent progress in identifying the molecular and signaling events underlying these traits downstream of ethylene. We also speculate on the role of ethylene in postsubmergence recovery and identify several questions for future investigations.

Come gather round people wherever you roam
And admit that the waters around you have grown
And accept it that soon you'll be drenched to the bone
If your time to you is worth saving
And you'd better start swimming or you'll sink like a stone
For the times they are a-changing…

THE TIMES THEY ARE A-CHANGIN'
Words and Music by Bob Dylan
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Fifty years later, these prophetic words from Dylan's legendary song strangely ring true. We live in an increasingly wetter world. Flooding events have become more frequent, severe, and unpredictable, a trend that is linked to climate change (Arnell and Liu, 2001; Hirabayashi et al., 2013). Not only do they destroy human lives, but also, they affect plants, on which we depend so much. Flooding negatively affects plant biodiversity, natural species distribution, and global food production because of crop losses (Silvertown et al., 1999; Normile, 2008), because most terrestrial plants, including major crops, are extremely sensitive to wet conditions. It might seem counterintuitive that a molecule so biologically benign and indispensable for plant growth and function is harmful when present in excess. However, this is attributed to the fact that water is an extremely poor medium for gas diffusion.

Hampered gas exchange with flooded organs leads to restriction of two vital plant processes: photosynthesis and respiration. The problem is compounded by stagnant and/or turbid floodwaters, because this further restricts the availability of light and oxygen. An energy crisis quickly results owing to an imbalance between energy production and consumption, ultimately causing plant mortality. Flooding survival tactics in the plant kingdom vary widely and include several morphological, anatomical, physiological, and molecular changes that can prolong survival and even facilitate permanent habitation in wet environments (Voesenek and Bailey-Serres, 2015). Initiation of these changes requires accurate and timely perception of water inundation to initiate adaptive responses. The term flooding encompasses both waterlogging and submergence. Waterlogging implies soil flooding, where only roots are exposed to wet conditions. Submergence also immerses the shoot (partially or wholly). Unless otherwise specified, this nomenclature will be used throughout this article.

ETHYLENE: AN EARLY AND RELIABLE SIGNAL

Other than O_2 and CO_2, restricted gas diffusion in flooded plant organs also affects the dynamics of another volatile: ethylene. Internal changes in oxygen and ethylene are considered primary signals triggering plant-adaptive responses to flooding. However, the temporal and spatial dynamics of these two gases during the course of a flooding event can be very distinct (Voesenek and Sasidharan, 2013). Even in nonflooded plants, steep oxygen gradients occur in organs, such as seeds and fruits, because of their density and high metabolic demand (Van Dongen and Licausi, 2015). Flooding causes a decrease in oxygen availability to all plant cells. However, this drop in oxygen levels is not uniform and can vary, especially between the shoot and root (Fig. 1). Different oxygen
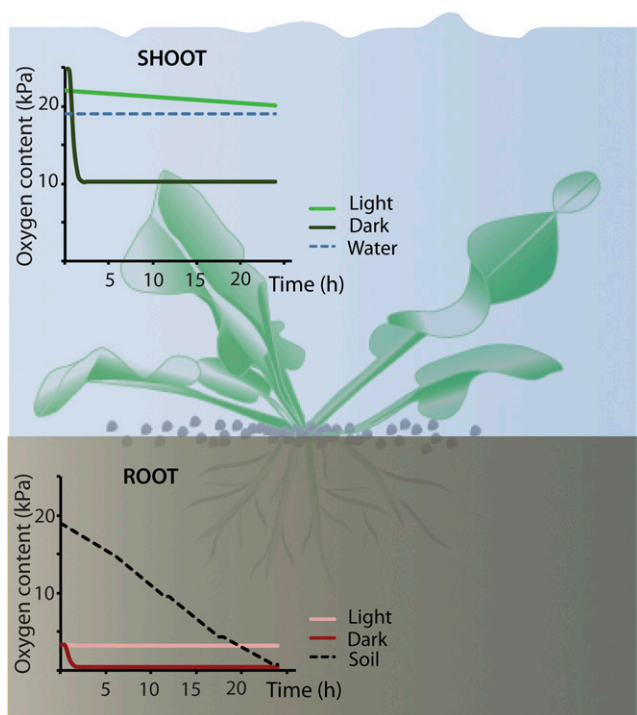
Figure 1. Oxygen dynamics during flooding. The root and shoot of a flooded plant have very different oxygen dynamics. Depicted are the general trends in endogenous oxygen levels in the shoot and root of plants when submerged in the light or in darkness. Also shown are typical oxygen concentrations in the surrounding floodwater and soil. Generalized trend lines shown are based on measurements on submerged Arabidopsis plants over a 24-h period (Vashisht et al., 2011).

dynamics between these two organs are caused by their direct environments. Flooded roots in waterlogged soils are rapidly depleted of oxygen because of microbial and root respiration (Vashisht et al., 2011). Root oxygen content is consequently strongly dependent on photosynthetically derived oxygen from the shoot when the plant is completely submerged or oxygen that diffuses into an emerged shoot. The flow of oxygen from the shoot to the root is, in turn, influenced by source to sink strength, tissue porosity, and root respiratory demand. Submerged shoots are surrounded by water that is relatively more oxygen replete (Vashisht et al., 2011), especially upper layers that are in contact with the atmosphere (Setter et al., 1987). Internal oxygen levels in the shoot are determined by light availability, presence of leaf gas films, and leaf traits that facilitate underwater photosynthesis and inward diffusion of oxygen, even under restricted conditions of the aquatic environment (Mommer et al., 2004; Pedersen et al., 2009). In fact, in planta oxygen measurements show that, in the presence of sufficient illumination, internal oxygen content can stay at normoxic values in submerged shoots (Vashisht et al., 2011; van Veen et al., 2013).

All cells of a plant are capable of synthesizing ethylene, and endogenous levels are largely determined by biosynthesis rates and amounts lost by diffusion to

the external environment. Measurements of endogenous ethylene concentrations in flooded plant organs all consistently report rapid (within 1 h) elevation of ethylene to physiologically saturating (1 μL L^{-1}) concentrations after the onset of flooding (Voesenek and Sasidharan, 2013). Flooding-induced increases in the expression and activity of the ethylene biosynthetic enzymes 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase and ACC synthase have been reported in several species (Van Der Straeten et al., 2001; Lee et al., 2011; van Veen et al., 2013). However, it is ethylene's sluggish outward diffusion in water that causes its fast physical entrapment and accumulation to saturating levels in flooded tissues.

This rapid buildup upon flooding, independent of most environmental conditions, makes ethylene a reliable and timely signal conveying flooding stress ahead of the onset of hypoxic and anoxic conditions. It is, therefore, not surprising that ethylene is a key regulator of several flood-adaptive traits.

FLOODING

Waterlogging

Although soil flooding directly exposes only plant roots to the stress, whole-plant functioning is affected in the absence of timely stress perception and initiation of appropriate adaptive responses (Sauter, 2013). As soil microbes and roots rapidly consume the remaining oxygen in the waterlogged soil, roots switch to inefficient anaerobic fermentation to generate ATP needed for proper functioning. Ultimately, available carbohydrate reserves are used, and as anoxia sets in, starvation, impaired membrane integrity, and entry of phytotoxic compounds from the waterlogged soil can all combine to severely compromise root growth and function. The resulting inability to transport water and nutrients also affects shoot function, resulting in symptoms such as wilting, senescence, and death. Adaptive traits that improve aeration, thereby preventing root anoxia, are, therefore, critical to maintain root function and whole-plant survival of waterlogging. These traits include the formation of a suberin/lignin barrier in the root that prevents radial loss of oxygen to enhance its delivery to the root tip (Shiono et al., 2011), increased formation of air spaces (aerenchyma) that increases organ porosity and root aeration (Takahashi et al., 2014), and formation of aerenchyma-rich adventitious roots (ARs; Sauter, 2013).

Submergence

Complete submergence is even more detrimental, completely cutting off plant access to the aerial environment and seriously compromising photosynthesis (Voesenek and Bailey-Serres, 2015). As with waterlogging, strategies to cope with submergence are directed toward improving aeration. An escape strategy involving directed shoot growth out of floodwaters restores atmospheric contact (Hattori et al., 2011;

Sasidharan et al., 2013; van Veen et al., 2013). After oxygen entry into the shoot, oxygenation of the rest of the plant is facilitated by aerenchymatous tissue (Pierik et al., 2009). Some species improve underwater photosynthesis rates aided by specialized leaf traits and gas films (Mommer and Visser, 2005). If the floods are deep, the energy-depleting escape strategy is not beneficial, because plant growth will not result in emergence. In this case, restricted growth is an alternative strategy that economizes on reserves for postsubmergence growth reestablishment (Fukao et al., 2006; Sasidharan et al., 2013; van Veen et al., 2013).

Ethylene is an important regulator of several of the aforementioned traits, including adaptive systemic responses of the shoot upon waterlogging. During waterlogging, the ethylene precursor ACC produced in the flooded root is transported through the xylem stream to the shoot (Jackson, 2002). Here, oxygen-mediated conversion of ACC to ethylene triggers the adaptive changes typically observed in shoots of waterlogged plants, such as shoot aerenchyma formation and leaf nastic movements (Jackson, 2002).

Ethylene-Mediated Flooding-Adaptive Traits

Aerenchyma: Airing the Plant

Aerenchymatous tissue can develop in both roots and shoots depending on the species and environmental conditions (Colmer and Pedersen, 2008; Parlanti et al., 2011; Steffens et al., 2011). These air-filled breaches extending throughout the inner plant connect flooded parts with those still in aerial contact and greatly improve internal aeration. It also reduces the number of oxygen-consuming cells, which is obviously advantageous in low-oxygen environments. Even during complete submergence, aerenchymatous tissue could facilitate access to available oxygen sources, such as the relatively oxygen-rich floodwater, gas films on submerged leaves, and oxygen produced by underwater photosynthesis. Two main types of aerenchymatous tissue are lysigenous (formed by regulated cortical cell death) and schizogenous (involving cell separation during tissue development; Takahashi et al., 2014). Both types can be either constitutive or inducible upon flooding. Constitutive aerenchyma is found in both wetland and nonwetland plants and can be further enhanced by flooding (Drew et al., 2000; Yamauchi et al., 2014). In other species, waterlogging induces aerenchyma formation (Rajhi et al., 2011; Yamauchi et al., 2014). Most research on aerenchyma formation has focused on lysigenous aerenchyma, which will be discussed here. Ethylene is an important hormonal signal triggering flooding-induced lysigenous aerenchyma formation in several species studied, including rice (Oryza sativa; Fukao and Bailey-Serres, 2008a; Sauter, 2013), maize (Zea mays; Rajhi et al., 2011), and wheat (Triticum aestivum; Yamauchi et al., 2014).

Aerenchyma formation can be induced in maize roots by ethylene application, and conversely, inhibitors of ethylene biosynthesis or perception can block the process in flooded or hypoxic roots (Rajhi et al., 2011). In rice, constitutive aerenchyma forms during normal development but gets further augmented in response to flooding and hypoxia in a process that is ethylene dependent (Takahashi et al., 2014; Yukiyoshi and Karahara, 2014). Ethylene was so far not considered relevant to the formation of constitutive aerenchyma, but a recent report suggests otherwise. In a so-called sandwich method, Japonica rice caryopses were germinated between two agar slabs. This allowed application of different chemicals to different sides of the same emergent root. ACC treatment only on one side resulted in a higher percentage of aerenchyma in the ACC-exposed developing root, whereas 1-methyl-cyclopropane (an ethylene perception inhibitor) elicited a reverse trend (Yukiyoshi and Karahara, 2014). The ethylene dependency and inducibility of aerenchyma formation can strongly vary between genotypes (Parlanti et al., 2011; Yin et al., 2013). For example, the rice varieties FR13A and Arborio Precoce both show constitutive aerenchyma in leaf sheaths that is further enhanced by submergence. However, ethylene signaling was implicated in aerenchyma formation only in the Arborio Precoce variety (Parlanti et al., 2011).

Downstream of the ethylene-signaling module, several signaling components have been identified using pharmacological approaches in maize roots (Fig. 2). In these experiments, manipulation of Ca^{2+} levels and use of chemicals interfering with phosphorylation and phosphoinositide signaling helped identify a signaling pathway leading from ethylene and involving heterotrimeric G proteins, protein phosphorylation, and Ca^{2+} as essential signaling components (Drew et al., 2000). Reactive oxygen species (ROS) are another important component in the ethylene-mediated signaling network. In rice internodes, ethylene-induced formation of stem aerenchyma involved increased superoxide radicals and hydrogen peroxide in preaerenchyma cells. Genetic manipulation and exogenous application of hydrogen peroxide showed that ROS could induce aerenchyma in a dose-dependent manner (Steffens et al., 2011). In wheat roots as well, ethylene-mediated aerenchyma formation was dependent on controlled ROS production by NADPH oxidases (Yamauchi et al., 2014). ROS are important components in cell death signaling, and their regulated generation might be important in triggering physiological cell death to form aerenchyma. The demise of specific cells in the root cortex by genetically programmed cell death is the terminal step in aerenchyma formation. In maize cells, the process has been followed in detail using light and electron microscopy and revealed the distinct stages of controlled cell death during aerenchyma formation (Gunawardena et al., 2001). These start with plasma membrane invagination and vesicle formation followed by nuclear events, such as
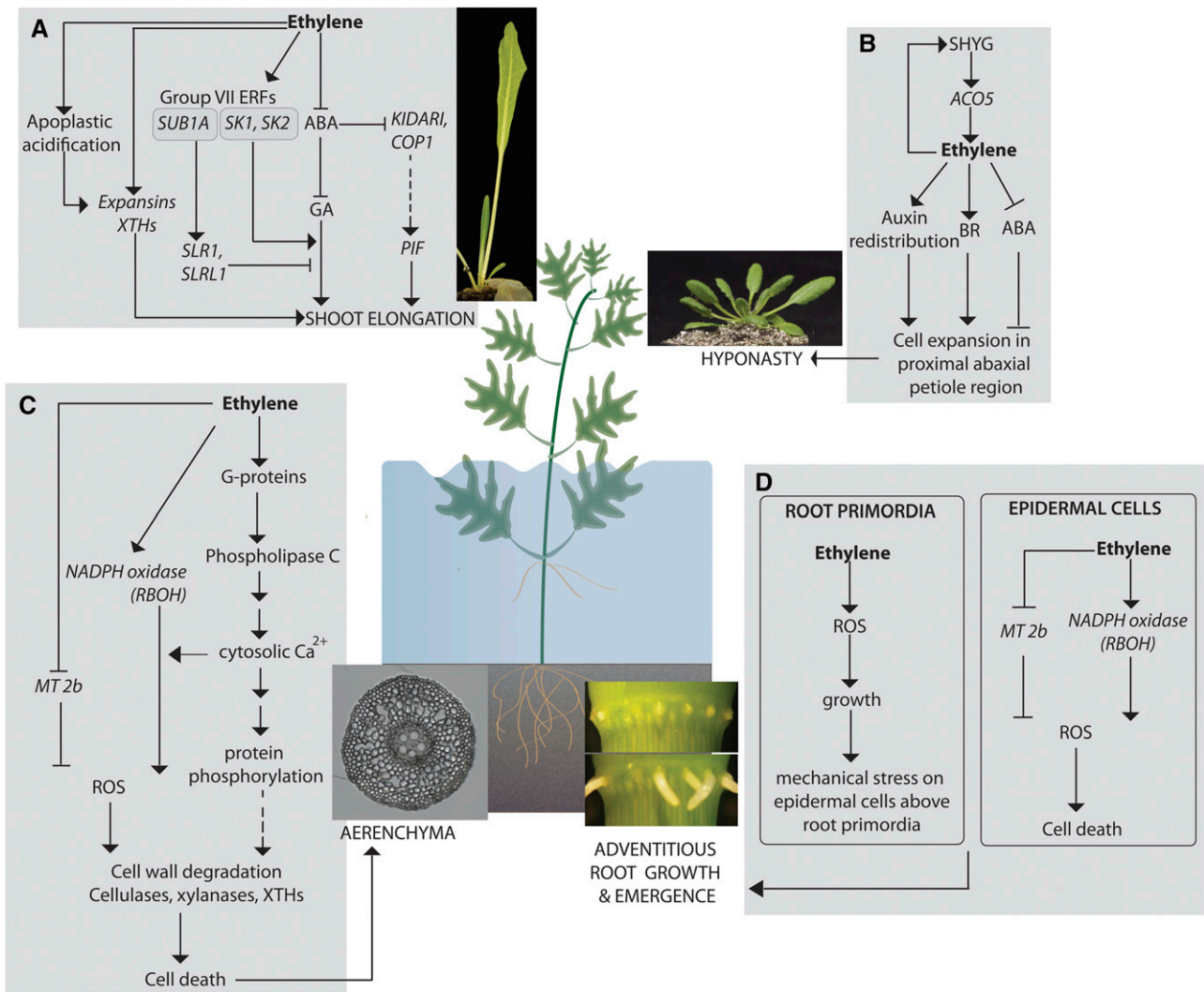
Figure 2. Ethylene-mediated flood-adaptive traits. An overview of the ethylene signaling networks regulating flooding-induced shoot elongation (A), hyponasty (B), aerenchyma (C), and AR growth (D). Depicted are generalized schemes based on studies in one or more species. Interactions and hierarchy of signaling components can vary depending on species. Images shown are *R. palustris* (A), Arabidopsis (B), barley (*Hordeum vulgare*) root cross sections (C), and rice stem nodes (D). Photographs courtesy of Shiono Katsuhiro (B) and Bianka Steffens (D). *COP1, CONSTITUTIVE PHOTOMORPHOGENIC1*; *MT2B, METALLOTHIONEIN 2B*; *PIF, PHYTOCHROME INTERACTING FACTOR*; *RBOH, RESPIRATORY BURST OXIDASE HOMOLOG*; *SLR1, SLENDER RICE1*; *SLRL1, SLENDER RICE-LIKE1*; *XTH, XYLOGLUCAN ENDOTRANSGLUCOSYLASE/HYDROLASE*.

chromatin condensation and DNA fragmentation, and ultimately, cell wall breakdown and collapse of the entire cell, leaving behind empty air spaces. Cell wall degradation involves ethylene-mediated increases in activities of enzymes, such as cellulases, pectinases, and xylanases (Bragina et al., 2003; Xu et al., 2013).

Recent microarray studies using microdissected cortical cells from maize root tips and 1-methylcyclopropane have identified ethylene-mediated transcriptomic changes occurring in preaerenchymatous cortical cells (Rajhi et al., 2011). Major changes were observed in functional gene categories associated with ethylene signaling, ROS metabolism, cell wall degradation, and calcium signaling, providing support to the signaling network constructed from the more physiological studies mentioned above.

What determines the lysis of only specific cortical cells is still unclear. Preaerenchymatous cells in rice have distinct characteristics, including low starch, thinner cell walls, less chlorophyll, and higher amounts of ROS (Steffens et al., 2011). However, details of how and when this cellular identity is established and what marks these cells for their final fate need to be determined. One possibility is the differential ethylene sensitivity of preaerenchyma cells, but this remains to be verified.

Advantageous: Adventitious Roots

These postembryonic roots originating from shoots and the upper parts of the original roots are observed in several species, including rice, *Solanum dulcamara*, *Rumex* spp., and tomato (*Solanum lycopersicum*) upon flooding (Visser et al., 1996; Steffens et al., 2006; Vidoz et al., 2010; Dawood et al., 2014). Aerenchyma-rich ARs improve shoot-root gas diffusion and can completely replace flood-damaged soil-borne roots. Ethylene is important for AR formation, although its role can differ depending on the species (McDonald and Visser, 2003; Steffens et al., 2006; Vidoz et al., 2010). For example, in waterlogged *Rumex palustris*, AR formation is mediated by ethylene-induced increase in the auxin sensitivity of root-forming tissue (Visser et al., 1996). In tomato, waterlogging-induced AR formation requires ethylene perception by the Never Ripe receptor. Elevated ethylene levels stimulate auxin transport to the shoot, where an induction of *ACC synthase* genes results in increased stem ethylene. This then directs auxin flow toward the submerged stem to initiate AR growth. Accordingly, an inhibition of auxin transport hampers normal adventitious rooting (Vidoz et al., 2010).

Flooding-induced AR growth from preexisting root primordia, such as in rice, requires penetration of overlying cell layers. Studies in deep-water rice internodes have unraveled how AR growth from root primordia is coordinated with the death of overlying epidermal cells to facilitate AR emergence. Physiological experiments have convincingly shown the primary role of ethylene in triggering AR growth and epidermal cell death, and both processes are synergistically enhanced by GA and inhibited by abscisic acid (ABA; Steffens and Sauter, 2005; Steffens et al., 2006). Although microarray studies revealed that the epidermal cells overlaying root primordia have a very distinct cellular identity (Steffens and Sauter, 2009), the precise signal that caused only these specific cells to die remained unknown. It is now clear that this trigger is the mechanical stimulus provided by the underlying root primordia when they start growing (Steffens et al., 2012). The growing ARs exert a mechanical force on the epidermal cells overlying them in a process that also requires ethylene-mediated ROS formation (Fig. 2). Interestingly, ROS or ethylene alone could only induce ectopic cell death when a dummy force was also present. These studies provide a clear example of how a mechanical force can provide the spatial signal required to localize ethylene action to a targeted cluster of cells during flooding when ethylene accumulates equally in all flooded cells.

Rising to the Occasion: Shoot Hyponasty

Upward leaf movement or hyponasty is considered an important acclimation to flooding and results from an unequal growth rate of the cells on the abaxial and adaxial sides of the affected organ (Cox et al., 2004; Polko et al., 2012). Flooding-induced shoot hyponasty has been observed in species, such as *Rumex* spp. and Arabidopsis (*Arabidopsis thaliana*), in response to flooding (Cox et al., 2003; Lee et al., 2011; Rauf et al., 2013). During the course of a flooding event, as floodwaters rise, hyponastic growth, especially in such rosette species, would elevate the leaves above the water. When submerged, the almost vertical reorientation of *Rumex* spp. leaves also directs subsequent shoot elongation on the shortest path out of the water.

In *R. palustris*, early hyponastic growth upon submergence is a prerequisite for subsequent shoot elongation to outgrow floodwaters (Cox et al., 2003). In Arabidopsis, such flooding-induced petiole elongation is absent, but hyponasty is observed upon both waterlogging and complete submergence (Lee et al., 2011; Rauf et al., 2013). In both *R. palustris* and Arabidopsis, hyponastic growth is driven by ethylene, and exogenous application of ethylene mimics this trait, even in the absence of flooding (Millenaar et al., 2005; Heydarian et al., 2010). Increased ethylene biosynthesis in waterlogged Arabidopsis is linked to transcript accumulation of *1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID OXIDASE5* (*ACO5*). *ACO5* is a direct target of the NAC (for no apical meristem [NAM], Arabidopsis transcription activation factor [ATAF], and cup-shaped cotyledon [CUC2]) transcription factor SPEEDY HYPONASTIC GROWTH (SHYG) that is induced in shoots upon waterlogging. *SHYG* itself is ethylene inducible, although it is unclear what causes its early induction in the shoots upon root flooding. Accordingly, waterlogging fails to induce a wild-type hyponastic response in *shyg* and *aco5* mutants (Rauf et al., 2013). In *R. palustris*, ethylene regulates rapid hyponastic growth by interaction with auxin and ABA (Cox et al., 2004). Within the first 1 h of submergence, a dramatic ethylene-mediated depletion of ABA is required to prevent the inhibitory effect of this hormone on the initiation, speed, and maintenance of hyponastic growth. Ethylene also promotes a lateral redistribution of auxin to the outer cell layers in the petiole (Cox et al., 2004), likely causing differential growth because of expansion of specific cells. Indeed, in Arabidopsis petioles, ethylene causes expansion of cells in a proximal 3- to 4-mm zone in the abaxial epidermal cell layer coinciding with the selective expression of cell wall-modifying expansins and a transverse growth-promoting orientation of cortical microtubules (Polko et al., 2012; Rauf et al., 2013). In *R. palustris*, a third hormone, GA, positively regulates the speed of hyponasty. However, this is attributed to GA already present in the petioles, because submergence-induced GA levels increase only after the onset of hyponasty (Benschop et al., 2006). Studies in Arabidopsis have also added brassinosteroids (BRs) downstream of ethylene. In the *ROTUNDIFOLIA3* mutant, affected in a cytochrome P450 involved in BR biosynthesis, ethylene was unable to induce differential cell expansion and therefore, leaf hyponasty. Similar observations were made upon chemical perturbation of BR biosynthesis, supporting the involvement of BR action in ethylene-mediated hyponasty (Polko et al., 2013; Fig. 2).

The Right Time to Grow: Ethylene-Regulated Shoot Elongation

Depending upon the flooding regime, plants in such hydrological niches show distinct growth responses classified into two antithetical strategies: escape and quiescence (Voesenek and Bailey-Serres, 2015). Entrapped ethylene is the primary regulator for both of these strategies, invoking species-dependent stimulated or restricted shoot growth.

Escape

A robust escape response is observed in many species inhabiting niches with prolonged and shallow flooding. This brisk growth of the youngest leaves keeps them ahead of the rising floodwaters and in atmospheric contact. In the well-studied models of rice and *Rumex* spp., the hormonal trinity of ethylene, GA, and ABA forms the conserved regulatory core of this response (Fig. 2).

In *R. palustris*, the escape response can be replicated by ethylene application and conversely, strongly dampened by inhibition of ethylene perception (Cox et al., 2004; Heydarian et al., 2010). Detailed growth kinetics, physiological analyses, and endogenous hormone measurements coupled with genome-wide transcriptome profiling have facilitated a detailed reconstruction of the timeline of molecular events underlying this impressive growth response (van Veen et al., 2013; Voesenek and Bailey-Serres, 2015). Ethylene rapidly accumulates to saturating levels (>1 μL L^{-1}) in submerged *R. palustris*. Although measurements indicate that this takes up to 1 h, ethylene-mediated effects are detected much earlier. Ethylene-induced petiole cell wall acidification occurs within 20 min after submergence (Vreeburg et al., 2005). This sets the optimal milieu for the activities of cell wall-modifying proteins, like expansins and xyloglucan endotransglucosylase/hydrolases, that mediate cellular expansion (Sasidharan et al., 2011). Within the first 1 h, ethylene also causes a massive (up to 80%) depletion of endogenous ABA, which is critical to evince shoot elongation. This is mediated by a down-regulation of the anabolic enzyme *9-cis-epoxycarotenoid dioxygenase* and an up-regulation of the catabolic enzyme *ABA-8-hydroxylase* (Benschop et al., 2005; van Veen et al., 2013). ABA does not curb early ethylene-induced apoplastic acidification or the up-regulation of specific expansin genes (Vreeburg et al., 2005). However, the maintained block on ABA by ethylene is essential to permit GA-mediated growth stimulation, which occurs after 4 to 5 h of submergence (Benschop et al., 2006; van Veen et al., 2013). In this later phase, flooded petioles also show increased expression of genes associated with shade avoidance and photomorphogenesis. These genes include orthologs of the Arabidopsis E3-ubiquitin ligase CONSTITUTIVE PHOTOMORPHOGENIC1, the basic helix-loop-helix protein KIDARI, and PHYTOCHROME INTERACTING FACTORS, which have established roles in promoting growth-related events linked to light signaling. However, in submerged *R. palustris*, the accumulation of these transcripts is not associated with changes in the light environment but instead, requires ethylene-mediated ABA reduction (van Veen et al., 2013). Interestingly, ABA depletion mediated by ethylene was found to be key factor regulating natural variation in flooding-induced shoot elongation in natural accessions of *R. palustris* (Chen et al., 2010).

Deep-water rice escapes from submergence with a spectacular growth response (20–25 cm d^{-1}), which allows the hollow rice stem to stay above water and aerate the rest of the plant (Hattori et al., 2009). Flooding-induced intralacunar accumulation of ethylene is the primary regulator of this internodal elongation (Hattori et al., 2009). As in *R. palustris*, this positive effect of ethylene is the result of modulation of the contrasting effects of ABA and GA on shoot elongation (Fukao and Bailey-Serres, 2008a). Ethylene's primary effects in this respect are 2-fold. First, it causes depletion of ABA by regulation of ABA metabolism; second, it promotes GA-mediated internodal elongation by increasing tissue sensitivity to and biosynthesis of GA (Hoffmann-Benning and Kende, 1992; Saika et al., 2007). GA is essential for internodal elongation, and flooded rice has increased endogenous levels of bioactive GA$_1$ (Hoffmann-Benning and Kende, 1992). Exogenous ABA application restricts elongation because of reduced tissue responsiveness to GA. Farther downstream, induction of the expression and activity of expansins facilitates cellular expansion and growth (Choi et al., 2003). Shoot elongation is a quantitative trait attributed primarily to the *SNORKEL* (*SK*) locus on chromosome 12, which together with two other loci on chromosomes 1 and 3, can account for the full escape response (Hattori et al., 2008, 2009, 2011). The *SK* locus encodes two ethylene-inducible group VII ETHYLENE RESPONSE FACTOR (ERF) transcription factors: SK1 and SK2. Expression of these genes in nondeep-water rice triggers internodal elongation, even in nonflooded plants (Hattori et al., 2009). How signaling from the *SK*s leads to downstream events culminating in shoot elongation is unclear. Quantitative trait loci analyses on GA-controlled responses of deep-water rice internodes indicate that signaling downstream of the *SK*s and the other uncharacterized quantitative trait loci regions coordinately affects GA biosynthesis (Ayano et al., 2014; Nagai et al., 2014).

Quiescence

The escape strategy is only beneficial if leaves emerge, thereby restoring gas exchange with the atmosphere. Additionally, this response must bring the leaves above the water before depletion of existing carbohydrate reserves, and the shoot must be porous enough to act as a snorkel for the rest of the submerged

plant (Pierik et al., 2009; Akman et al., 2012). When floods are transient or too deep to outgrow, the quiescence strategy is favored (Fukao et al., 2006; Akman et al., 2012; van Veen et al., 2013). The *Rumex* sp. *Rumex acetosa* and lowland rice are well-studied examples of quiescent behavior, where restriction of growth and other energetically expensive processes allow conservation of resources until the floodwaters recede. In *R. acetosa*, submergence causes an active restriction of petiole growth (van Veen et al., 2013). Although ethylene accumulates to saturating levels, there is no ABA down-regulation or GA increase (Benschop et al., 2005). Instead, *R. acetosa* displays metabolic adjustments, consistent with energy conservation (van Veen et al., 2013).

The quiescent characteristics of lowland rice are attributed to the *SUBMERGENCE1* (*SUB1*) locus originally identified in the submergence tolerant FR13A landrace. This locus encodes two to three (*SUB1A*, *SUB1B*, or *SUB1C*) transcription factors also belonging to the group VII ERF family. Rice varieties possessing the *SUB1A* gene are tolerant to complete submergence and survive such conditions for up to 2 weeks (Fukao et al., 2006; Xu et al., 2006; Perata and Voesenek, 2007). Comparison of near-isogenic lines differing only in the possession of the SUB1 locus revealed the mechanism by which *SUB1A* confers submergence tolerance (Fukao et al., 2006, 2011). Submergence causes massive accumulation of *SUB1A* transcripts, much higher than can be replicated with ethylene application alone. It is presumed that *SUB1A* is also positively regulated by other flooding-associated signals, such as low oxygen or starvation. Interestingly, *SUB1A* induction feeds back on ethylene biosynthesis, and it dampens both ethylene production and responsiveness (Fukao et al., 2006), thereby curbing ethylene-mediated shoot elongation in *SUB1A*-containing lines. *SUB1A* enhances transcript abundance of Slender Rice1 and Slender Rice-Like1, which negatively regulate GA responses (Fukao and Bailey-Serres, 2008b). The presence of *SUB1A*, therefore, restricts growth by blocking GA-mediated activation of growth-promoting genes. Submergence-induced or ectopic expression of *SUB1A* has revealed that it is responsible for the lower expression of genes associated with cell elongation, starch metabolism, and induction of fermentation genes (Fukao et al., 2006). *SUB1A*-mediated tolerance, therefore, stems from curbing carbohydrate use and preventing an energy crisis during submergence.

ETHYLENE AND PLANT OXYGEN SENSING

Arabidopsis group VII ERFs have also been intensively studied in the context of their regulatory role in acclimative responses to flooding and hypoxic stress (Sasidharan and Mustroph, 2011; Bailey-Serres et al., 2012; Van Dongen and Licausi, 2015). Arabidopsis has five group VII ERFs: Related to APETALA2 12 (RAP2.12), RAP2.2, RAP2.3, Hypoxia responsive1 (HRE1), and HRE2

(Bailey-Serres et al., 2012). At least four (except RAP2.3) members have been shown to be redundantly involved in the regulation of hypoxia-responsive gene expression and survival (Papdi et al., 2008; Hinz et al., 2010; Licausi et al., 2010; Hess et al., 2011). All five Arabidopsis group VII ERFs possess a signature N-terminal motif that makes them susceptible to oxygen-dependent degradation through the N-end rule pathway (NERP) of targeted proteolysis (Gibbs et al., 2011; Licausi et al. 2011; Bailey-Serres et al., 2012). This conserved protein degradation pathway links the fate of a protein to its N-end terminus. Recent studies in Arabidopsis have shown that group VII ERF abundance and consequently, hypoxia responses are regulated by the oxygen-dependent degradation of group VII ERFs through the NERP (Gibbs et al., 2011; Licausi et al., 2011; Sasidharan and Mustroph, 2011). In these proteins possessing the characteristic N-terminal sequence starting with Met-Cys, the cleavage of the terminal Met exposes the Cys residue. During normoxic conditions, the oxidation of this Cys commits the protein to degradation through the NERP. A drop in oxygen levels limits the degradation of these proteins, allowing them to move to the nucleus and switch on target gene expression, including anaerobic metabolism and other survival-related genes (Gibbs et al., 2011; Licausi et al., 2011). Experiments have also established that, during normoxia, RAP2.12 can escape degradation because of its association with plasma membrane proteins Acyl CoA Binding Protein1 (ACBP1) and ACBP2. Hypoxia triggers RAP2.12 dissociation and translocation to the nucleus to initiate target gene expression (Licausi et al., 2011).

Interestingly, the N termini of the rice SKs deviate from the conserved N-terminal degrons associated with the NERP, and experiments have established that SUB1A is not an N-end rule substrate (Gibbs et al., 2011). It is speculated that this N-end rule independence of SUB1A coupled with its ethylene inducibility would initiate quiescence-related energy management before the onset of hypoxia and result in higher submergence tolerance. A detailed update on the understanding of group VII ERFs and the N-end rule-mediated mechanism of oxygen sensing can be found elsewhere (Gibbs et al., 2015).

AFTER THE FLOODS: ETHYLENE AND POSTSUBMERGENCE ACCLIMATION

When floodwaters subside, energy-depleted plant tissues acclimated to low-oxygen and low-light conditions are abruptly reexposed to the terrestrial environment. Reaeration is typically associated with an increased formation of reactive ROS molecules and harmful metabolites. Normally, ROS production occurs as part of normal cellular metabolism and is kept in check with an active scavenging system (Blokhina and Fagerstedt, 2010). However, flooding stress disrupts this carefully maintained homeostasis. This is evidenced by the high levels of ROS-related lipid peroxidation and cellular damage observed during

reoxygenation (Fukao et al., 2011). Root cell membrane damage resulting from this lipid peroxidation is likely the cause of another frequently observed symptom of reaeration: dehydration stress. Despite excessive water in the soil, some plants display symptoms of water deficit after reoxygenation, such as wilted leaves, and up-regulate dehydration-responsive genes (Setter et al., 2010; Fukao et al., 2011; Tamang et al., 2014; Tsai et al., 2014). These symptoms could be caused by reduced hydraulic conductance of flooded roots (Rodríguez-Gamir et al., 2011). Flooding tolerance, therefore, involves surviving not just submergence but also, thereafter, limiting dehydration and oxidative stress and recovering growth and photosynthesis. The molecular responses and signaling events occurring in plants postflooding have received scant attention, and also, the possible role of ethylene after desubmergence has received little attention. Although ethylene trapped by floodwaters would escape from submerged plant organs upon desubmergence, studies report that ethylene biosynthesis increases during reoxygenation (Voesenek et al., 2003; Tsai et al., 2014). An assessment of ethylene production after desubmergence in several species revealed that the strongest response was in flooding-escape species, assigning it a functional significance (Voesenek et al., 2003). This ethylene production would allow shoot elongation to continue, even after the leaf tips have reemerged. However, postsubmergence ethylene signaling might also be of relevance in nonescape species, albeit for different functional reasons, such as improving postsubmergence recovery. In Arabidopsis, reoxygenation is associated with increased expression of ethylene biosynthetic enzymes (Tsai et al., 2014). Furthermore, the ethylene-insensitive (ein) mutants *ein2-5* and *ein3eil1* showed increased sensitivity to postanoxic stress. This could be linked to the impaired regulation of many functional gene clusters associated with ABA biosynthesis, dehydration, and heat shock proteins (Tsai et al., 2014). The submergence tolerance of SUB1 rice also extends to better postsubmergence recovery. The ethylene-inducible *SUB1A* gene mediates improved tolerance to dehydration and oxidative stress by inducing genes associated with ROS amelioration and acclimation to dehydration (Fukao et al., 2011). Although it is apparent that ethylene modulates plant responses postsubmergence, there is plenty to be investigated. The role of ethylene in regulating postsubmergence-mediated drought responses, the interaction with ROS, and ethylene's possibly distinct roles in postsubmergence recovery of quiescent/escape species are some interesting aspects for future research.

FUTURE OUTLOOK

Studies so far have linked ethylene primarily to the regulation of morphological and anatomical traits that improve aeration in a flooded plant. These traits are triggered early upon flooding before the onset of severe oxygen deprivation. Because of the oxygen dependency of ethylene biosynthesis, ethylene is not considered an important regulator of anoxia tolerance. However, recent studies showing improved anoxia survival in ethylene-pretreated plants (van Veen et al., 2013) challenge this perception. Indeed, ethylene signaling may be of little relevance during anoxia, but its presence in the early stages of flooding could already prime for forthcoming oxygen deprivation. Ethylene, therefore, seems to mediate plant responses to all stages of a flooding event. The challenge of future studies will be to further unravel the distinct molecular events occurring in each of these flooding-related phases. The molecular basis of ethylene priming, the downstream events that ethylene mediates, especially through various ERFs, its interaction with other flooding-associated signals, such as ROS, sugars, and nitric oxide, and its regulation of postsubmergence recovery are just some of the pertinent research areas pending additional investigation.

LITERATURE CITED

Akman M, Bhikharie AV, McLean EH, Boonman A, Visser EJW, Schranz ME, van Tienderen PH (2012) Wait or escape? Contrasting submergence tolerance strategies of *Rorippa amphibia*, *Rorippa sylvestris* and their hybrid. Ann Bot (Lond) **109:** 1263–1276

Arnell N, Liu C (2001) Hydrology and water resources. *In* JJ McCarthy, OF Canziani, NA Leary, DJ Dokken, KS White, eds, Climate Change 2001: Impacts, Adaptation and Vulnerability. Cambridge University Press, Cambridge, UK, pp 191–234

Ayano M, Kani T, Kojima M, Sakakibara H, Kitaoka T, Kuroha T, Angeles-Shim RB, Kitano H, Nagai K, Ashikari M (2014) Gibberellin biosynthesis and signal transduction is essential for internode elongation in deepwater rice. Plant Cell Environ **37:** 2313–2324

Bailey-Serres J, Fukao T, Gibbs DJ, Holdsworth MJ, Lee SC, Licausi F, Perata P, Voesenek LACJ, van Dongen JT (2012) Making sense of low oxygen sensing. Trends Plant Sci **17:** 129–138

Benschop JJ, Bou J, Peeters AJ, Wagemaker N, Gühl K, Ward D, Hedden P, Moritz T, Voesenek LA (2006) Long-term submergence-induced elongation in *Rumex palustris* requires abscisic acid-dependent biosynthesis of Gibberellin1. Plant Physiol **141:** 1644–1652

Benschop JJ, Jackson MB, Gühl K, Vreeburg RAM, Croker SJ, Peeters AJM, Voesenek LACJ (2005) Contrasting interactions between ethylene and abscisic acid in *Rumex* species differing in submergence tolerance. Plant J **44:** 756–768

Blokhina O, Fagerstedt KV (2010) Oxidative metabolism, ROS and NO under oxygen deprivation. Plant Physiol Biochem **48:** 359–373

Bragina TV, Rodionova NA, Grinieva GM (2003) Ethylene production and activation of hydrolytic enzymes during acclimation of maize seedlings to partial flooding. Russ J Plant Physiol **50:** 794–798

Chen X, Pierik R, Peeters AJM, Poorter H, Visser EJW, Huber H, de Kroon H, Voesenek LACJ (2010) Endogenous abscisic acid as a key switch for natural variation in flooding-induced shoot elongation. Plant Physiol **154:** 969–977

Choi D, Lee Y, Cho HT, Kende H (2003) Regulation of expansin gene expression affects growth and development in transgenic rice plants. Plant Cell **15:** 1386–1398

Colmer TD, Pedersen O (2008) Oxygen dynamics in submerged rice (*Oryza sativa*). New Phytol **178:** 326–334

Cox MCH, Benschop JJ, Vreeburg RAM, Wagemaker CA, Moritz T, Peeters AJM, Voesenek LACJ (2004) The roles of ethylene, auxin, abscisic acid, and gibberellin in the hyponastic growth of submerged *Rumex palustris* petioles. Plant Physiol **136**: 2948–2960

Cox MCH, Millenaar FF, Van Berkel YE, Peeters AJ, Voesenek LA (2003) Plant movement. Submergence-induced petiole elongation in *Rumex palustris* depends on hyponastic growth. Plant Physiol **132**: 282–291

Dawood T, Rieu I, Wolters-Arts M, Derksen EB, Mariani C, Visser EJW (2014) Rapid flooding-induced adventitious root development from preformed primordia in *Solanum dulcamara*. AoB Plants **6**: plt058

Drew MC, He CJ, Morgan PW (2000) Programmed cell death and aerenchyma formation in roots. Trends Plant Sci **5**: 123–127

Fukao T, Bailey-Serres J (2008a) Ethylene - A key regulator of submergence responses in rice. Plant Sci **175**: 43–51

Fukao T, Bailey-Serres J (2008b) Submergence tolerance conferred by Sub1A is mediated by SLR1 and SLRL1 restriction of gibberellin responses in rice. Proc Natl Acad Sci USA **105**: 16814–16819

Fukao T, Xu K, Ronald PC, Bailey-Serres J (2006) A variable cluster of ethylene response factor-like genes regulates metabolic and developmental acclimation responses to submergence in rice. Plant Cell **18**: 2021–2034

Fukao T, Yeung E, Bailey-Serres J (2011) The submergence tolerance regulator SUB1A mediates crosstalk between submergence and drought tolerance in rice. Plant Cell **23**: 412–427

Gibbs DJ, Conde JV, Berckhan S, Prasad G, Mendiondo GM, Holdsworth MJ (2015) Group VII ethylene response factors coordinate oxygen and nitric oxide signal transduction and stress responses in plants. Plant Physiol **169**: 23–31

Gibbs DJ, Lee SC, Isa NM, Gramuglia S, Fukao T, Bassel GW, Correia CS, Corbineau F, Theodoulou FL, Bailey-Serres J, et al (2011) Homeostatic response to hypoxia is regulated by the N-end rule pathway in plants. Nature **479**: 415–418

Gunawardena AHLAN, Pearce DM, Jackson MB, Hawes CR, Evans DE (2001) Characterisation of programmed cell death during aerenchyma formation induced by ethylene or hypoxia in roots of maize (*Zea mays* L.). Planta **212**: 205–214

Hattori Y, Nagai K, Ashikari M (2011) Rice growth adapting to deepwater. Curr Opin Plant Biol **14**: 100–105

Hattori Y, Nagai K, Furukawa S, Song XJ, Kawano R, Sakakibara H, Wu J, Matsumoto T, Yoshimura A, Kitano H, et al (2009) The ethylene response factors SNORKEL1 and SNORKEL2 allow rice to adapt to deep water. Nature **460**: 1026–1030

Hattori Y, Nagai K, Mori H, Kitano H, Matsuoka M, Ashikari M (2008) Mapping of three QTLs that regulate internode elongation in deepwater rice. Breed Sci **58**: 39–46

Hess N, Klode M, Anders M, Sauter M (2011) The hypoxia responsive transcription factor genes ERF71/HRE2 and ERF73/HRE1 of Arabidopsis are differentially regulated by ethylene. Physiol Plant **143**: 41–49

Heydarian Z, Sasidharan R, Cox MCH, Pierik R, Voesenek LACJ, Peeters AJM (2010) A kinetic analysis of hyponastic growth and petiole elongation upon ethylene exposure in *Rumex palustris*. Ann Bot (Lond) **106**: 429–435

Hinz M, Wilson IW, Yang J, Buerstenbinder K, Llewellyn D, Dennis ES, Sauter M, Dolferus R (2010) Arabidopsis RAP2.2: an ethylene response transcription factor that is important for hypoxia survival. Plant Physiol **153**: 757–772

Hirabayashi Y, Mahendran R, Koirala S, Konoshima L, Yamazaki D, Watanabe S, Kim H, Kanae S (2013) Global flood risk under climate change. Nat Clim Chang **3**: 816–821

Hoffmann-Benning S, Kende H (1992) On the role of abscisic Acid and gibberellin in the regulation of growth in rice. Plant Physiol **99**: 1156–1161

Jackson MB (2002) Long-distance signalling from roots to shoots assessed: the flooding story. J Exp Bot **53**: 175–181

Lee SC, Mustroph A, Sasidharan R, Vashisht D, Pedersen O, Oosumi T, Voesenek LACJ, Bailey-Serres J (2011) Molecular characterization of the submergence response of the *Arabidopsis thaliana* ecotype Columbia. New Phytol **190**: 457–471

Licausi F, Kosmacz M, Weits DA, Giuntoli B, Giorgi FM, Voesenek LACJ, Perata P, van Dongen JT (2011) Oxygen sensing in plants is mediated by an N-end rule pathway for protein destabilization. Nature **479**: 419–422

Licausi F, van Dongen JT, Giuntoli B, Novi G, Santaniello A, Geigenberger P, Perata P (2010) HRE1 and HRE2, two hypoxia-inducible ethylene response factors, affect anaerobic responses in Arabidopsis thaliana. Plant J **62**: 302–315

McDonald MP, Visser EJW (2003) A study of the interaction between auxin and ethylene in wild type and transgenic ethylene-insensitive tobacco during adventitious root formation induced by stagnant root zone conditions. Plant Biol **5**: 550–556

Millenaar FF, Cox MCH, van Berkel YE, Welschen RAM, Pierik R, Voesenek LACJ, Peeters AJM (2005) Ethylene-induced differential growth of petioles in Arabidopsis. Analyzing natural variation, response kinetics, and regulation. Plant Physiol **137**: 998–1008

Mommer L, Pedersen O, Visser EJW (2004) Acclimation of a terrestrial plant to submergence facilitates gas exchange under water. Plant Cell Environ **27**: 1281–1287

Mommer L, Visser EJW (2005) Underwater photosynthesis in flooded terrestrial plants: a matter of leaf plasticity. Ann Bot (Lond) **96**: 581–589

Nagai K, Kondo Y, Kitaoka T, Noda T, Kuroha T, Angeles-Shim RB, Yasui H, Yoshimura A, Ashikari M (2014) QTL analysis of internode elongation in response to gibberellin in deepwater rice. AoB Plants **6**: plu028

Normile D (2008) Agricultural research. Reinventing rice to feed the world. Science **321**: 330–333

Papdi C, Abrahám E, Joseph MP, Popescu C, Koncz C, Szabados L (2008) Functional identification of Arabidopsis stress regulatory genes using the controlled cDNA overexpression system. Plant Physiol **147**: 528–542

Parlanti S, Kudahettige NP, Lombardi L, Mensuali-Sodi A, Alpi A, Perata P, Pucciariello C (2011) Distinct mechanisms for aerenchyma formation in leaf sheaths of rice genotypes displaying a quiescence or escape strategy for flooding tolerance. Ann Bot (Lond) **107**: 1335–1343

Pedersen O, Rich SM, Colmer TD (2009) Surviving floods: leaf gas films improve O₂ and CO₂ exchange, root aeration, and growth of completely submerged rice. Plant J **58**: 147–156

Perata P, Voesenek LACJ (2007) Submergence tolerance in rice requires Sub1A, an ethylene-response-factor-like gene. Trends Plant Sci **12**: 43–46

Pierik R, van Aken JM, Voesenek LACJ (2009) Is elongation-induced leaf emergence beneficial for submerged *Rumex* species? Ann Bot (Lond) **103**: 353–357

Polko JK, Pierik R, van Zanten M, Tarkowská D, Strnad M, Voesenek LACJ, Peeters AJM (2013) Ethylene promotes hyponastic growth through interaction with ROTUNDIFOLIA3/CYP90C1 in Arabidopsis. J Exp Bot **64**: 613–624

Polko JK, van Zanten M, van Rooij JA, Marée AFM, Voesenek LACJ, Peeters AJM, Pierik R (2012) Ethylene-induced differential petiole growth in *Arabidopsis thaliana* involves local microtubule reorientation and cell expansion. New Phytol **193**: 339–348

Rajhi I, Yamauchi T, Takahashi H, Nishiuchi S, Shiono K, Watanabe R, Mliki A, Nagamura Y, Tsutsumi N, Nishizawa NK, et al (2011) Identification of genes expressed in maize root cortical cells during lysigenous aerenchyma formation using laser microdissection and microarray analyses. New Phytol **190**: 351–368

Rauf M, Arif M, Fisahn J, Xue GP, Balazadeh S, Mueller-Roeber B (2013) NAC transcription factor speedy hyponastic growth regulates flooding-induced leaf movement in *Arabidopsis*. Plant Cell **25**: 4941–4955

Rodríguez-Gamir J, Ancillo G, González-Mas MC, Primo-Millo E, Iglesias DJ, Forner-Giner MA (2011) Root signalling and modulation of stomatal closure in flooded citrus seedlings. Plant Physiol Biochem **49**: 636–645

Saika H, Okamoto M, Miyoshi K, Kushiro T, Shinoda S, Jikumaru Y, Fujimoto M, Arikawa T, Takahashi H, Ando M, et al (2007) Ethylene promotes submergence-induced expression of OsABA8ox1, a gene that encodes ABA 8'-hydroxylase in rice. Plant Cell Physiol **48**: 287–298

Sasidharan R, Mustroph A (2011) Plant oxygen sensing is mediated by the N-end rule pathway: a milestone in plant anaerobiosis. Plant Cell **23**: 4173–4183

Sasidharan R, Mustroph A, Boonman A, Akman M, Ammerlaan AMH, Breit T, Schranz ME, Voesenek LACJ, van Tienderen PH (2013) Root transcript profiling of two *Rorippa* species reveals gene clusters associated with extreme submergence tolerance. Plant Physiol **163**: 1277–1292

Sasidharan R, Voesenek LACJ, Pierik R (2011) Cell wall modifying proteins mediate plant acclimatization to biotic and abiotic stresses. Crit Rev Plant Sci **30**: 548–562

Sauter M (2013) Root responses to flooding. Curr Opin Plant Biol **16**: 282–286

Setter TL, Bhekasut P, Greenway H (2010) Desiccation of leaves after de-submergence is one cause for intolerance to complete submergence of the rice cultivar IR 42. Funct Plant Biol **37**: 1096–1104

Setter TL, Kupkanchanakul T, Kupkanchanakul K, Bhekasut P, Wiengweera A, Greenway H (1987) Concentrations of CO_2 and O_2 in floodwater and in internodal lacunae of floating rice growing at 1-2 metre water depths. Plant Cell Environ **10**: 767–776

Shiono K, Ogawa S, Yamazaki S, Isoda H, Fujimura T, Nakazono M, Colmer TD (2011) Contrasting dynamics of radial O2-loss barrier induction and aerenchyma formation in rice roots of two lengths. Ann Bot (Lond) **107**: 89–99

Silvertown J, Dodd ME, Gowing DJG, Mountford JO (1999) Hydrologically defined niches reveal a basis for species richness in plant communities. Nature **400**: 61–63

Steffens B, Geske T, Sauter M (2011) Aerenchyma formation in the rice stem and its promotion by H_2O_2. New Phytol **190**: 369–378

Steffens B, Kovalev A, Gorb SN, Sauter M (2012) Emerging roots alter epidermal cell fate through mechanical and reactive oxygen species signaling. Plant Cell **24**: 3296–3306

Steffens B, Sauter M (2005) Epidermal cell death in rice is regulated by ethylene, gibberellin, and abscisic acid. Plant Physiol **139**: 713–721

Steffens B, Sauter M (2009) Epidermal cell death in rice is confined to cells with a distinct molecular identity and is mediated by ethylene and H_2O_2 through an autoamplified signal pathway. Plant Cell **21**: 184–196

Steffens B, Wang J, Sauter M (2006) Interactions between ethylene, gibberellin and abscisic acid regulate emergence and growth rate of adventitious roots in deepwater rice. Planta **223**: 604–612

Takahashi H, Yamauchi T, Colmer T, Nakazono M (2014) *In* JT van Dongen, F Licausi, eds, Aerenchyma Formation in Plants, Vol 21,. Springer, Vienna, pp 247–265

Tamang BG, Magliozzi JO, Maroof MAS, Fukao T (2014) Physiological and transcriptomic characterization of submergence and reoxygenation responses in soybean seedlings. Plant Cell Environ **37**: 2350–2365

Tsai KJ, Chou SJ, Shih MC (2014) Ethylene plays an essential role in the recovery of Arabidopsis during post-anaerobiosis reoxygenation. Plant Cell Environ **37**: 2391–2405

Van Der Straeten D, Zhou Z, Prinsen E, Van Onckelen HA, Van Montagu MC (2001) A comparative molecular-physiological study of submergence response in lowland and deepwater rice. Plant Physiol **125**: 955–968

Van Dongen JT, Licausi F (January 12, 2015) Oxygen sensing and signaling. Annu Rev Plant Biol 10.1146/annurev-arplant-043014-114813

van Veen H, Mustroph A, Barding GA, Vergeer-van Eijk M, Welschen-Evertman RAM, Pedersen O, Visser EJW, Larive CK, Pierik R, Bailey-Serres J, et al (2013) Two *Rumex* species from contrasting hydrological niches regulate flooding tolerance through distinct mechanisms. Plant Cell **25**: 4691–4707

Vashisht D, Hesselink A, Pierik R, Ammerlaan JMH, Bailey-Serres J, Visser EJW, Pedersen O, van Zanten M, Vreugdenhil D, Jamar DCL, et al (2011) Natural variation of submergence tolerance among *Arabidopsis thaliana* accessions. New Phytol **190**: 299–310

Vidoz ML, Loreti E, Mensuali A, Alpi A, Perata P (2010) Hormonal interplay during adventitious root formation in flooded tomato plants. Plant J **63**: 551–562

Visser E, Cohen JD, Barendse G, Blom C, Voesenek L (1996) An ethylene-mediated increase in sensitivity to auxin induces adventitious root formation in flooded *Rumex palustris Sm*. Plant Physiol **112**: 1687–1692

Voesenek LACJ, Bailey-Serres J (2015) Flood adaptive traits and processes: an overview. New Phytol **206**: 57–73

Voesenek LACJ, Jackson MB, Toebes AHW, Huibers W, Vriezen WH, Colmer TD (2003) De-submergence-induced ethylene production in *Rumex palustris*: regulation and ecophysiological significance. Plant J **33**: 341–352

Voesenek LACJ, Sasidharan R (2013) Ethylene—and oxygen signalling—drive plant survival during flooding. Plant Biol (Stuttg) **15**: 426–435

Vreeburg RAM, Benschop JJ, Peeters AJM, Colmer TD, Ammerlaan AH, Staal M, Elzenga TM, Staals RH, Darley CP, McQueen-Mason SJ, et al (2005) Ethylene regulates fast apoplastic acidification and expansin A transcription during submergence-induced petiole elongation in *Rumex palustris*. Plant J **43**: 597–610

Xu K, Xu X, Fukao T, Canlas P, Maghirang-Rodriguez R, Heuer S, Ismail AM, Bailey-Serres J, Ronald PC, Mackill DJ (2006) Sub1A is an ethylene-response-factor-like gene that confers submergence tolerance to rice. Nature **442**: 705–708

Xu QT, Fan HY, Jiang Z, Zhou ZQ, Yang L, Mei FZ, Qu LH (2013) Cell wall degradation and the dynamic changes of Ca2+ and related enzymes in the developing aerenchyma of wheat (Triticum aestivum L.) under waterlogging. Acta Biol Hung **64**: 328–340

Yamauchi T, Watanabe K, Fukazawa A, Mori H, Abe F, Kawaguchi K, Oyanagi A, Nakazono M (2014) Ethylene and reactive oxygen species are involved in root aerenchyma formation and adaptation of wheat seedlings to oxygen-deficient conditions. J Exp Bot **65**: 261–273

Yin D, Chen S, Chen F, Jiang J (2013) Ethylene promotes induction of aerenchyma formation and ethanolic fermentation in waterlogged roots of *Dendranthema spp*. Mol Biol Rep **40**: 4581–4590

Yukiyoshi K, Karahara I (2014) Role of ethylene signalling in the formation of constitutive aerenchyma in primary roots of rice. AoB Plants **6**: plu043