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Flood adaptive traits and processes: an overview

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Summary

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Unanticipated flooding challenges plant growth and fitness in natural and agricultural ecosystems. Here we describe mechanisms of developmental plasticity and metabolic modulation that underpin adaptive traits and acclimation responses to waterlogging of root systems and submergence of aerial tissues. This includes insights into processes that enhance ventilation of submerged organs. At the intersection between metabolism and growth, submergence survival strategies have evolved involving an ethylene-driven and gibberellin-enhanced module that regulates growth of submerged organs. Opposing regulation of this pathway is facilitated by a subgroup of ethylene-response transcription factors (ERFs), which include members that require low O₂ or low nitric oxide (NO) conditions for their stabilization. These transcription factors control genes encoding enzymes required for anaerobic metabolism as well as proteins that fine-tune their function in transcription and turnover. Other mechanisms that control metabolism and growth at seed, seedling and mature stages under flooding conditions are reviewed, as well as findings demonstrating that true endurance of submergence includes an ability to restore growth following the deluge. Finally, we highlight molecular insights obtained from natural variation of domesticated and wild species that occupy different hydrological niches, emphasizing the value of understanding natural flooding survival strategies in efforts to stabilize crop yields in flood-prone environments.

*These authors contributed equally to this work.

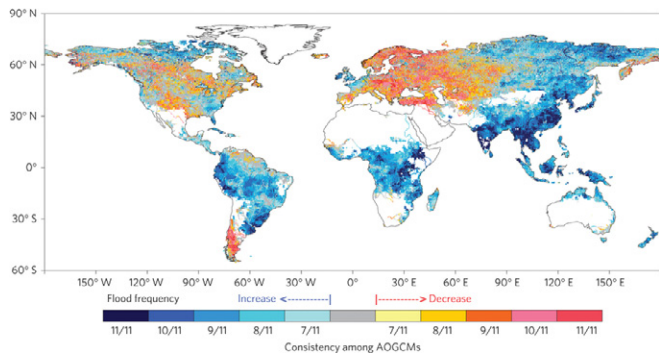


Fig. 1 Predicted change in flooding frequency in the 21st Century assuming increased mean temperature based on projected limited mitigation of CO₂ emissions in the representative concentration pathway (RCP) 8.5 scenario (reprinted with permission from Nature Climate Change (Hirabayashi *et al.*, 2013), copyright 2013). The color scale indicates the consistency of projections with the 11 atmosphere–ocean general circulation models (AOGCMs). For example, regions with greater frequency of floods predicted by all models are shown in dark blue and lower frequency of floods in dark red.

I. Introduction

Excess of water or floods can negatively impact agricultural yields by delaying planting, reducing vigor, altering development and increasing susceptibility to diseases. The financial impact of floods on agriculture is difficult to calculate, but insurance payouts to farmers in the USA for flooding damage averaged \$24 billion (\$US) yr⁻¹ between 2001 and 2011 (Bailey-Serres *et al.*, 2012a). Inundation events also effect the distribution and diversity of species in natural ecosystems (Silvertown *et al.*, 1999). It is expected that flooding frequency will rise in Southeast Asia, southern India, East Africa, Siberia and northern parts of South America this century (IPCC, 2012; Hirabayashi *et al.*, 2013; Fig. 1).

Plants successfully occupy habitats with a wide spectrum of flooding regimes (i.e. continual, seasonal, ephemeral, shallow, deep). The remarkable variation in flooding tolerance and adaptive traits is exemplified by the unequal distribution of species in flood-prone Rhine River ecosystems (Vervuren *et al.*, 2003; Van Eck *et al.*, 2004; Voeselek *et al.*, 2004; van Eck *et al.*, 2006) and species of major tropical river floodplains such as the Amazon basin (Parolin *et al.*, 2004; Herrera, 2013) (Fig. 2). Naturally evolved flooding survival strategies of wild *Oryza* species are displayed in some domesticated rice (*Oryza sativa*) cultivars, particularly those capable of underwater elongating in deepwater paddies or tolerant of short-term complete submergence.

During floods, plants endure environmental perturbations such as a restricted access to atmospheric O₂ and CO₂, hampered outward diffusion of plant evolved ethylene (C₂H₄) (Voeselek & Sasidharan, 2013), electrochemical soil changes resulting in higher concentrations of toxic elements including manganese (Mn²⁺), iron (Fe²⁺) and sulfide (H₂S, HS⁻, S²⁻) (Bailey-Serres & Voeselek, 2008; Lamers *et al.*, 2012; Zeng *et al.*, 2012) and reduction in available light (Vervuren *et al.*, 2003). As a consequence, cells and tissues are exposed to pronounced internal variations in O₂ and CO₂, and elevation in ethylene as well as reactive nitrogen and reactive oxygen species (ROS). ROS are produced at the onset of flooding-induced O₂ deprivation as a

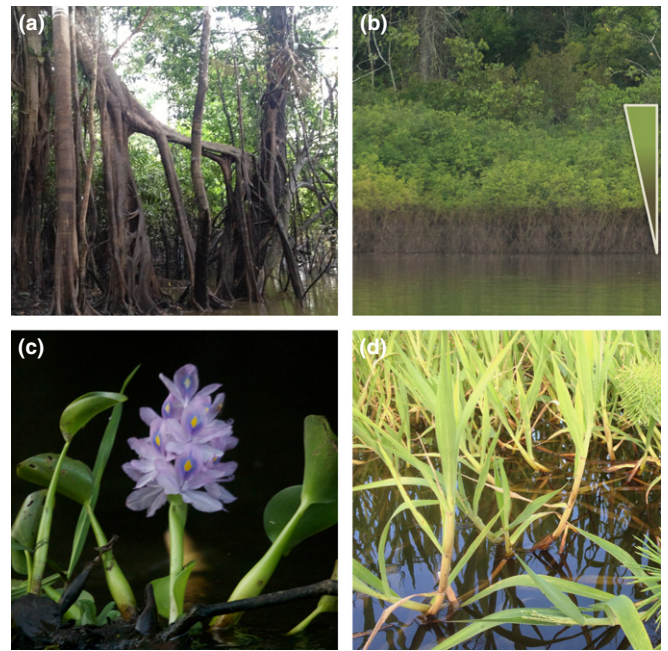


Fig. 2 Examples of plants or vegetation showing survival strategies upon flooding of the upper Amazon basin of Peru. (a) Aerial prop roots of a *Ficus* ssp. during the low water season (Marañón River tributary; photographer Julia Bailey-Serres (J.B-S.)). (b) Vigorous regrowth of dormant vegetation after desubmergence; an endurance strategy (Ucayali River Supay Caño; photographer J.B-S.). The triangle indicates the gradient of regrowth from first to last desubmerged branches. (c) Elongated petiole and floral stem of common water hyacinth (*Eichornia crassipes*); an escape strategy (Marañón River tributary; photographer Nicholas Serres). (d) Internode elongation of *Poaceae*; an escape strategy (Ucayali River; photographer J. B-S.).

consequence of the inhibition of mitochondrial electron transport and generation of superoxide that is converted to hydrogen peroxide by dismutation (Santosa *et al.*, 2007). Increases in superoxide and hydrogen peroxide are prevalent upon reoxygenation (Blokina & Fagerstedt, 2010; Steffens *et al.*, 2013; Fig. 3).

Although flooding is a compound stress, most research has focused on the induced energy and carbohydrate crisis caused by hampered oxidative phosphorylation and low rates of photosynthesis, respectively. Flood-tolerant plants are characterized by a continuum of survival strategies of which the low-O₂ escape syndrome (LOES) and low-O₂ quiescence syndrome (LOQS) are extremes (reviewed by Bailey-Serres & Voeselek, 2008, 2010; Voeselek & Bailey-Serres, 2013) (Fig. 3). During escape, various induced and/or constitutive traits interact in such a way that the rates of gas exchange between cells and the atmosphere above the water level increase. Escape phenotypes, not necessarily all present in one species, include upward bending of leaves (hyponasty), enhanced shoot elongation, formation of interconnected air-filled voids (aerenchyma), induction of barriers to radial O₂ loss (ROL) in roots, development of adventitious roots (ARs), formation of gas films on leaf surfaces, modifications of leaf anatomy and pressurized gas flow through porous tissues (Jackson & Armstrong, 1999; Colmer, 2003a; Mommer & Visser, 2005; Colmer & Pedersen, 2007; Polko *et al.*, 2011; Sauter, 2013). Of these traits, there is growing understanding of the developmental plasticity that drives aerenchyma and AR formation and elongation of aerial organs. All

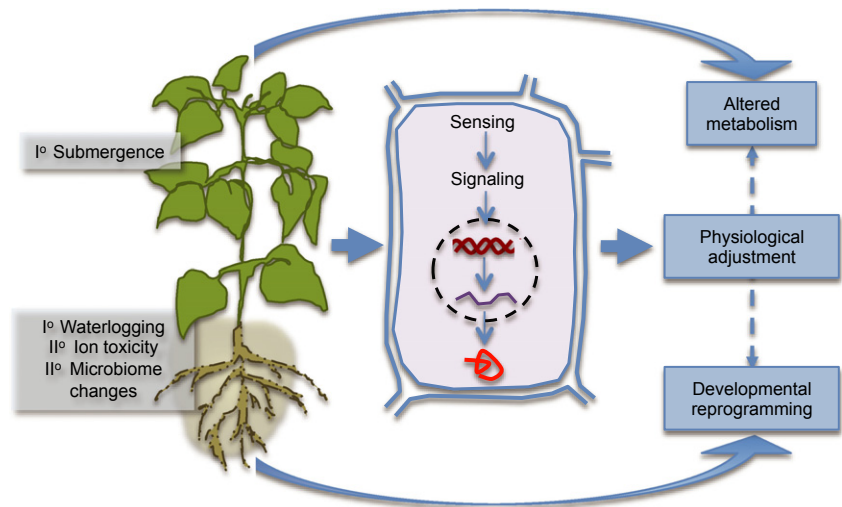


Fig. 3 Overview of flooding stimuli that trigger plasticity in plant physiology and development. The low-O₂ quiescence strategy (LOQS) is associated with plants that endure prolonged complete submergence. The low-O₂ escape strategy (LOES) is also triggered by submergence but is generally characterized by stimulated elongation growth of submerged organs, ultimately increasing gas exchange between aerial and submerged tissues. Additional traits are associated with LOES at the cellular level, including reduced leaf thickness and repositioning of chloroplasts to maximize light energy absorption and underwater photosynthesis. Plants with root systems that are water-saturated and often shared with O₂-consuming microbes can maintain gas exchange between roots and shoots if aerenchyma is constitutively formed or readily induced by waterlogging. C, carbon; N, nitrogen; NO, nitric oxide; ROS, reactive oxygen species; n/a, not applicable.

Primary Event	Secondary Effect	Signaling	Key Responses	Reaeration
Prolonged complete submergence	low CO ₂ in light low O ₂ in dark reduced C and N	ethylene reduced light low O ₂ NO ROS	Low-Oxygen Quiescence Strategy (LOQS) Quiescence of metabolism and growth; meristem or organ protection	ROS dehydration
Partial submergence	low CO ₂ in light low O ₂ in dark	ethylene reduced light	Low-Oxygen Escape Strategy (LOES) Escape by elongation of aerial organs	ROS
Root waterlogging	low O ₂ toxic ions reduced C and N	ethylene ROS low O ₂	Increased ventilation via aerenchyma or adventitious roots; Reduced O ₂ loss by barrier formation	ROS

involve ethylene, but the first two also involve ROS, whereas the latter is controlled by a hormonal network including abscisic acid (ABA) and gibberellin (GA).

In contrast to the energy-consuming LOES, LOQS manages metabolism and constrains growth. Generally, to maintain cellular homeostasis under low-O₂ stress, starch catabolism, glycolysis, ethanolic fermentation and a bifurcated tricarboxylic acid (TCA) cycle are used to fuel substrate-level ATP production and enable the synthesis of proteins involved in metabolite transport, ROS protection and chaperone activity (Bailey-Serres & Voesenek, 2008, 2010; Bailey-Serres *et al.*, 2012a). This is accompanied by reduction in bioenergy-consuming processes associated with cell division and growth, including DNA, protein, ribosome and cell wall synthesis. Genome-scale studies have demonstrated that increases in mRNAs associated with anaerobic metabolism, ROS protection and chaperone activity occur in plants with varying tolerance of prolonged submergence or O₂ deprivation (Christianson *et al.*, 2010; Mustroph *et al.*, 2010; Lee *et al.*, 2011; Nanjo *et al.*, 2011; Narsai & Whelan, 2013; Sasidharan *et al.*, 2013; van Veen *et al.*, 2013; Tamang *et al.*, 2014).

For both LOES and LOQS, controlled anaerobic metabolism is tantamount to survival of low-O₂ conditions. At the core of this mechanism is the evolutionarily conserved group VII ethylene-response factor (ERF-VII) transcription factors (TFs), which activate genes necessary for anaerobic metabolism in *Arabidopsis thaliana* (Gibbs *et al.*, 2011; Licausi *et al.*, 2011). The turnover of these proteins is dependent on specific features of their N-termini and a branch of the N-end rule pathway of targeted proteolysis. The similarity of ERF-VIIs and N-end rule pathway components in various species suggests that this mechanism is conserved across higher plants. A reason for this may be that hypoxic or even anoxic conditions occur in some tissues during the plant life cycle (i.e. seeds, fruits, tubers, meristems and anthers) (Borisjuk & Rolletschek, 2008; Licausi & Perata, 2009; Thiel *et al.*, 2011; Kelliher & Walbot, 2012, 2014). In fact, hypoxia is an important determinant of cell fate during anther development in maize (*Zea mays*; Kelliher & Walbot, 2012). As will be described, ERF-VII function is fine-tuned in *A. thaliana* and is a target of natural selection for successful LOES and LOQS in rice (Fukao *et al.*, 2006; Xu *et al.*, 2006; Hattori *et al.*, 2009).

Here we review advancements in the understanding of flooding survival strategies made over the past 10 yr. These involve knowledge gleaned at a variety of levels – morphological, physiological, cellular and molecular – through examination of wild, model and cultivated species. We highlight the importance of recovery from submergence and the emerging relationship of this capacity to survive subsequent periods of low water availability. Finally, we consider how genetic and phenotypic variation in flooding tolerance might be further exploited to improve resilience of crops to floods.

II. Root acclimations that promote root aeration

Plants benefit from O₂ diffusion from the shoot to the root to establish a higher O₂ concentration in the latter, even when petiole porosity is as low as *c.* 10%, as observed in *A. thaliana* (Vashisht *et al.*, 2010) [Correction added after online publication 7 January 2015: in the preceding sentence the word ‘root’ was changed to ‘petiole’ so the sentence now reads ‘...even when petiole porosity...’]. The source of this O₂ is either photosynthesizing submerged leaves or the atmosphere when parts of the shoot have emerged. The concentration of O₂ that can be maintained in the root tip meristem depends on three factors: internal O₂ diffusion (determined by the porosity of the tissue, the tortuosity of the longitudinally connected air channels, and the length of the diffusion path); loss of O₂ to the environment (i.e. radial oxygen loss (ROL)); and rate of respiration. Intercellular lacunae, defined as aerenchyma, increase tissue porosity and thereby contribute significantly to gas diffusion in and out of organs surrounded by water.

Aerenchyma is constitutively present in many wetland plants and inducible upon flooding stress in some wetland and many dryland species. It develops in existing root and shoot tissues, secondary tissues, or newly formed organs such as ARs. Formation is through programmed cell death (PCD) in the root cortex (lysigenous aerenchyma), separation of previously connected cells (schizogenous aerenchyma), or cell division and cell expansion without separation or lysis (expansigenous aerenchyma) (Colmer, 2003b; Evans, 2003; Seago *et al.*, 2005; Steffens *et al.*, 2011). These voids facilitate inward diffusion or pressurized flow-through (if a flow outlet is available) of O₂ in roots and outward diffusion of root-generated ethylene and soil-generated methane (Colmer, 2003a; Laanbroek, 2010). Effective aerenchyma enables roots to sustain oxidative phosphorylation and thus normal ATP concentrations (Drew *et al.*, 1985) and avoid growth limitation by elevated ethylene (Visser & Pierik, 2007). Despite the benefits it confers in flooded environments, aerenchyma is not constitutive in all plants. This reflects trade-offs between root porosity, mechanical strength, and radial nutrient transport (Striker *et al.*, 2007; Hu *et al.*, 2013).

There is a broad consensus that lysigenous aerenchyma formation in waterlogged roots is triggered by accumulation of ethylene (He *et al.*, 1996; Shiono *et al.*, 2007; Rajhi *et al.*, 2011; Yamauchi *et al.*, 2014). Interestingly, these longitudinally interconnected gas-filled pores also form as a result of mechanical impedance and deficiencies in nitrogen, phosphate or sulphate (He *et al.*, 1992; Bouranis *et al.*, 2003; Abiko & Obara, 2014). Flooding-induced formation of aerenchyma involves selective PCD, although certain

characteristics of apoptosis are lacking (Joshi & Kumar, 2012). Aerenchyma formation requires ethylene, Ca²⁺ and ROS signaling and ultimately cell wall degradation (Drew *et al.*, 2000; Evans, 2003; Fig. 4b).

Molecular studies of grasses have expanded our understanding of aerenchyma formation. A cell type-specific transcriptome analysis using waterlogged maize (*Zea mays*) roots demonstrated strong up-regulation of superoxide dismutase, cell wall-loosening enzymes, and several TFs in the aerenchyma-forming root cortex as compared with the stele. Significant down-regulation, predominantly in the cortex, was observed for a metallothionein gene encoding a ROS scavenging protein (Rajhi *et al.*, 2011). Aerenchyma formation in stems of deepwater rice (*Oryza sativa*) was also associated with an elevation in ROS and down-regulation of *METALLOTHIONEIN 2b* mRNA (Steffens *et al.*, 2011). A source of ROS in this process is probably the Ca²⁺-dependent plasma membrane-localized respiratory burst oxidase homologs (RBOHs), which promote apoplastic superoxide production to amplify ROS-mediated signaling. Wheat (*Triticum aestivum*) roots pretreated with the ethylene precursor 1-aminocyclopropanecarboxylic acid (ACC) induced high levels of *RBOH* transcripts and formed more aerenchyma upon waterlogging, but this was blocked by the NADPH oxidase inhibitor diphenyleneiodonium (DPI) (Yamauchi *et al.*, 2014). A study considering the development of aerenchyma within the leaf sheath of submerged rice also implicated ethylene and ROS in accessions that differ in elongation during submergence (Parlanti *et al.*, 2011). Both accessions displayed reduced aerenchyma formation when pretreated with DPI, again implicating NADPH oxidase-dependent ROS signaling. But inhibition of ethylene biosynthesis did not affect submergence-promoted aerenchyma formation in the nonelongating and submergence-tolerant landrace FR13A. This suggests that ROS is sufficient to trigger sheath aerenchyma development in FR13A.

Little is known of the process of schizogenous aerenchyma formation (Evans, 2003). In the liverwort *Marchantia polymorpha*, a T-DNA mutant (*nopperabo1 (nop1)*) was identified with impaired schizogenous intercellular space development. The causal protein, NOP1, localized near to the plasma membrane, is a plant U-box (PUB) E3 ubiquitin ligase, carrying tandem C-terminal ARMA-DILLO repeats (these are usually 40 amino acids long and proteins containing them have many tandemly repeated copies folded into a rigid protein domain). It was hypothesized that NOP1 regulates schizogenous formation of air chambers via a membrane-localized receptor-like kinase signaling pathway resulting in ubiquitylation and degradation of target proteins (Ishizaki *et al.*, 2013).

Aerenchyma facilitates relatively high O₂ concentrations in roots growing in anaerobic soils. However, because of the very steep radial O₂ diffusion gradient, this O₂ can be lost by outward diffusion into the soil (i.e. ROL). As the rate of this radial diffusion is inversely correlated to the resistance of the root to gas diffusion, high O₂ concentrations and thus longer root lengths can be maintained when ROL is prevented by a constitutive or inducible apoplastic barrier (Colmer, 2003a; De Simone *et al.*, 2003). The importance of a ROL barrier for waterlogging tolerance was demonstrated by the introduction of a barrier from a wild relative (*Hordeum marinum*) into wheat (*T. aestivum*) (Malik *et al.*, 2010).

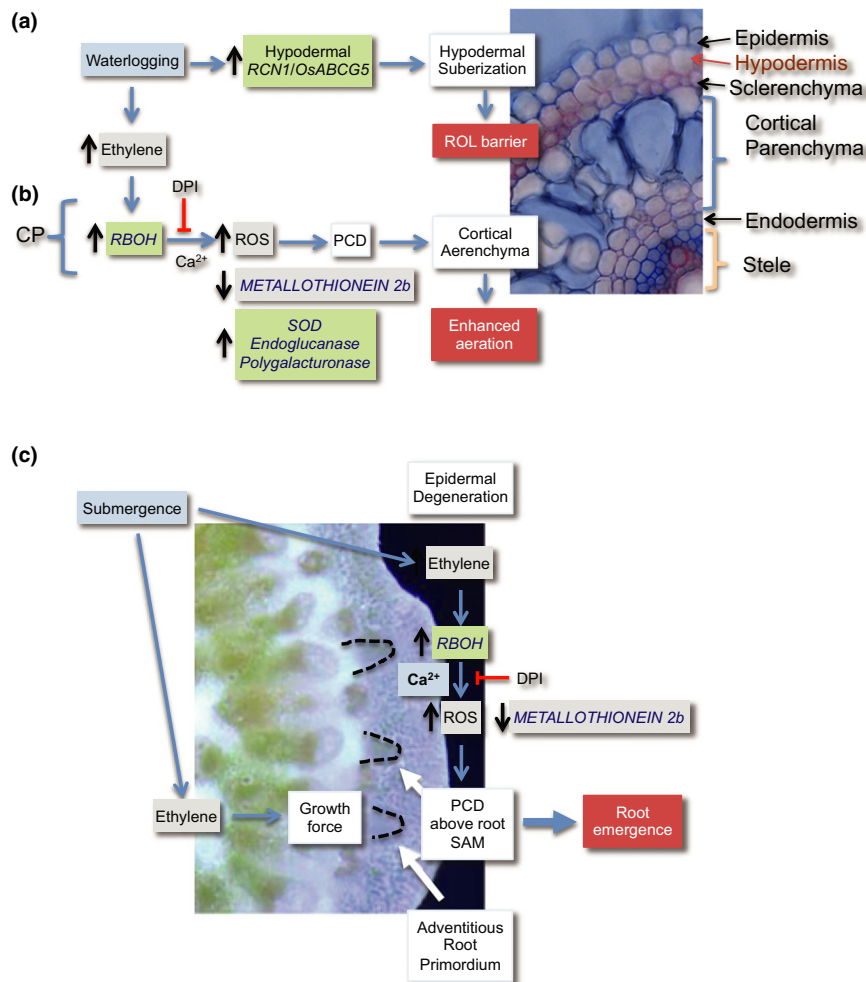


Fig. 4 Three examples of developmental plasticity associated with roots that are promoted when rice is flooded. (a) A radial O_2 loss (ROL) barrier and cortical aerenchyma form as a result of waterlogging to enhance the aeration of root meristems. The ROL is a consequence of deposition of lamellae of suberin between epidermal and hypodermal cells; it can also include lignification of the sclerenchyma/epidermal cells. Hypodermal cells that are suberized are referred to as the exodermis. ROL formation involves up-regulation of genes, including a hypodermal cell ATP-binding cassette (ABC) transporter (*REDUCED CULM NUMBER1 (RCN1)/OsABCG5*). (b) Aerenchyma forms as a consequence of programmed cell death (PCD) of cortical parenchyma (CP) cells. This ethylene-promoted process involves calcium (Ca^{2+}) flux, respiratory burst oxidases (RBOHs), and generation of reactive O_2 species (ROS). The process is inhibited by diphenylene iodonium (DPI), which inhibits RBOHs and other NADPH oxidases. The image was provided by Germain Pauluzzi. SOD, superoxide dismutase. (c) The emergence of preformed adventitious roots from submerged stem nodes is also mediated by ethylene and blocked by DPI. This developmental response involves localized epidermal cell degeneration that is driven by the localized force of the emerging root meristem. Processes in the nascent root and subtending epidermal cells are shown. SAM, shoot apical meristem. The micrograph is from Steffens *et al.* (2012) with permission (www.plantcell.org; © American Society of Plant Biologists).

The barrier is typically comprised of suberized lamellae that form in the exodermal/hypodermal space, particularly near to the root tip, and lignified sclerenchyma/epidermal cells. These constitute an efficient ROL barrier as well as an apoplastic blockade between living cells and the anaerobic and sometimes toxic soil environment (i.e. saline or highly reduced) (Armstrong *et al.*, 2000; Shiono *et al.*, 2011; Watanabe *et al.*, 2013). These modifications are also correlated with minimizing ROL in other waterlogging-tolerant plants such as wild *Zea nicaraguensis* (Abiko *et al.*, 2012).

Metabolite profiling of longitudinal sections of ARs of rice (*O. sativa*) growing under barrier-forming stagnant conditions revealed that malic acid and very long chain fatty acids accumulated. The concentrations increased from the root apex to the base, thus paralleling the development of the barrier (Kulichikhin *et al.*, 2014). Malic acid is a substrate for fatty acid biosynthesis and thus a

precursor for suberin formation. Molecular investigation of the short and shallow root phenotype of waterlogged *reduced culm number1 (rcn1)* mutants of rice led to the recognition of an ATP-binding cassette (ABC) transporter (*RCN1/OsABCG5*) proposed to export the very long chain fatty acids and/or their derivatives across the hypodermal plasma membrane into the apoplast where they serve as major components of suberin (Shiono *et al.*, 2014) (Fig. 4a). In comparison to the wild type, *rcn1-2* roots fail to develop effective suberized lamellae or a ROL barrier under deoxygenated conditions. Elevated ethylene, high CO_2 or low O_2 is not essential for barrier formation, but root exudates or cellular degradation products may be important (Colmer *et al.*, 2006; Garthwaite *et al.*, 2008).

In addition to increased porosity and a radial diffusion barrier, many species develop ARs from the hypocotyl or basal stem region

when waterlogged (Visser & Voeselek, 2005; Sauter, 2013). These can replace an existing and often deteriorating primary root system. Flood-induced ARs typically have higher porosities than the primary root system (Laan *et al.*, 1989) or ARs that form under well-aerated conditions (Visser *et al.*, 2000). ARs typically grow in the better aerated topsoil layers during waterlogging or float in flood waters (Dawood *et al.*, 2014). The capacity of some ARs to develop chloroplasts can provide an additional source of O₂ and carbohydrates (Rich *et al.*, 2008, 2012a).

At the cellular level, ethylene and auxin are often integral to flooding-induced AR formation (reviewed in Visser & Voeselek, 2005). However, in the case of flood-induced ARs from pre-existing primordia of rice stem nodes, it is ethylene and not auxin that signals activation of the cell cycle (Lorbiecke & Sauter, 1999), which is followed by formation of ROS as measured with electron paramagnetic resonance spectroscopy (Steffens *et al.*, 2013) (Fig. 4c). As observed for aerenchyma, this developmental process also involves the DPI-inhibited plasma membrane RBOHs (Steffens *et al.*, 2012). The emergence of the delicate AR primordia involves PCD of the overlying epidermal cells, which is mediated by ethylene-promoted ROS production. This PCD occurs in a remarkably spatially specific manner, its location being determined by the force exerted by the outgrowing meristems. The epidermal weakening can be elicited by application of ethylene along with the local force, indicating that a mechanical signal provides the necessary spatial resolution (Steffens *et al.*, 2012). The reductions in the *METALLOTHIONEIN 2b* mRNA also participate in nodal AR emergence, as a mutant of this ROS ameliorating protein showed enhanced force-induced epidermal PCD.

III. Regulating reaeration by active emergence in *Rumex palustris* and *Oryza sativa*

Internal aeration of roots via diffusion can take place in completely submerged plants with the O₂ arising from underwater photosynthesis in shoot mesophyll or AR cortical cells or the influx of O₂

from the water layer into the shoot. However, shoot to root aeration is far more efficient when shoots emerge above the floodwater (Rich *et al.*, 2012b; Herzog & Pedersen, 2014). For this reason, some plants from flood-prone environments have evolved the ability to elongate their porous shoots when underwater to facilitate a LOES. Because of the carbon costs involved, this trait is restricted to species or accessions/landraces from environments characterized by shallow, but relatively prolonged floods (Groeneveld & Voeselek, 2003; Voeselek *et al.*, 2004). In species examined to date, a hormonal hierarchy involving ethylene as a trigger, ABA as a repressor and GA/auxin as promoters is associated with modulation of underwater elongation growth (Figs 5, 6). Both in rice accessions from Asia and *Rumex* species from Rhine floodplains, differential regulation of this hierarchy is associated with a LOES and LOQS.

In the case of rice, accessions vary in the degree of underwater elongation of submerged stems and leaves. The LOES of 'deepwater' rice varieties is determined in large part by the *SNORKEL* (*SK*) locus, which encodes the two ERF-VII TFs *SK1* and *SK2* (Hattori *et al.*, 2009) (Fig. 6a). The ethylene-triggered induction of *SK1/2* during submergence promotes underwater growth of internodes, enabling plants to elongate underwater at a rate of 25 cm d⁻¹ and to heights of several meters. Two additional uncharacterized loci on chromosomes 1 and 3 are needed along with *SK1/2* for the full deepwater escape response. Recent comparison of near-isogenic lines (NILs) differing in the presence versus absence of the three deepwater quantitative trait loci (QTLs) (NIL1 + NIL3 + *SK1/SK2*) revealed that these loci control the up-regulation of mRNA encoding a rate-limiting GA20 oxidase (*GA20ox*), which correlates with increased concentrations of bioactive GA₁ and GA₄ in internode tissue, although the contributions of the individual loci remain unclear (Ayano *et al.*, 2014). Mutation of *GA3ox*, which acts after *GA20ox*, or disruption of genes required for GA responsiveness (i.e. the genes encoding the GA receptor Gibberellin-insensitive dwarf (*GID*) and proteins involved in its turnover) significantly limited underwater

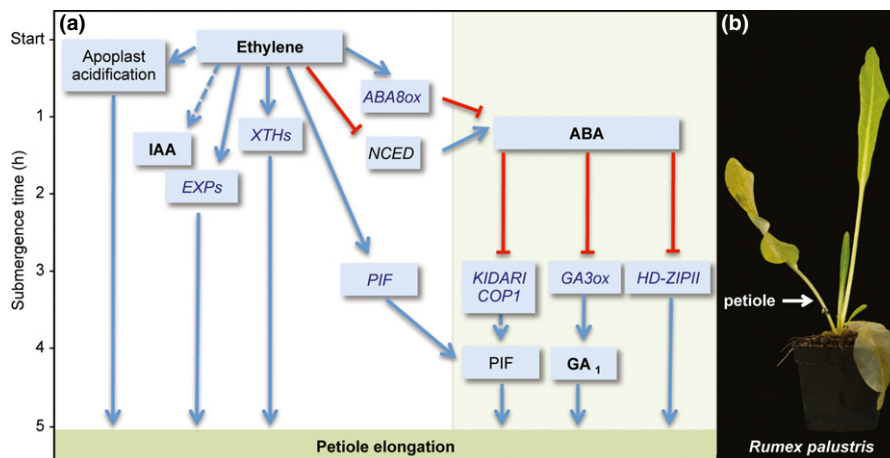


Fig. 5 Factors controlling underwater petiole elongation in *Rumex palustris*. (a) Time-scale of modulation of genes, hormones and cellular factors associated with promotion of petiole elongation in submerged plants. Pathways in the shaded region in green are abscisic acid (ABA) dependent. (b) Photograph following 7 d of submergence. Data are based on van Veen *et al.* (2013). *NCED*, 9-CIS-EPOXYCAROTENOID DIOXYGENASE; *ABA8ox*, ABA-8-HYDROXYLASE; *HD-ZIPII*, CLASS II HOMEODOMAIN-LEUCINE-ZIPPER; *COP1*, CONSTITUTIVE PHOTOMORPHOGENIC1; *EXPs*, EXPANSINs; *GA3ox*, GA3 OXIDASE; *GA₁*, gibberellin1; *IAA*, indole-3-acetic acid; *PIF*, PHYTOCHROME INTERACTING FACTORS; *XTHs*, XYLOGLUCANENDOTRANSGLUCOSYLASE-HYDROLASES.

apoplast is detected within 10 min (Vreeburg *et al.*, 2005). This reduction in apoplastic pH is optimal for the activity of cell wall-loosening proteins (Kende *et al.*, 2004). Elevated ethylene also stimulates, within 2 h of submergence, the abundance of mRNAs encoding cell wall-modifying proteins such as expansins (EXP) and xyloglucanotransglucosylase-hydrolases (XTH). This is concomitant with a reduction in mRNAs encoding 9-cis-epoxycarotenoid dioxygenase (NCED), which catalyzes the rate-limiting step in ABA biosynthesis (Benschop *et al.*, 2005; Vreeburg *et al.*, 2005; van Veen *et al.*, 2013). Analyses of petiole transcripts, using global mRNA-seq and targeted transcript analyses, further refined the understanding of this hierarchical response (van Veen *et al.*, 2013). mRNAs encoding ABA-8-hydroxylase, which converts ABA to inactive phaseic acid, are induced as early as 1 h after submergence (H. van Veen, pers. comm.). Finally, *PHYTOCHROME INTERACTING FACTORS* (*PIF*) mRNAs are up-regulated within 2–3 h.

Indications that auxin also plays a role, specifically in the early hours of submergence-induced petiole elongation, come from experiments on *R. palustris* in which the endogenous indole-3-acetic acid (IAA) concentration in petioles was lowered by leaf blade removal (Cox *et al.*, 2004). This treatment prolonged the lag-phase before petiole elongation from 2 to 8 h, an effect that could be rescued completely by addition of the IAA analog 1-naphthalene-acetic acid (NAA) (Cox *et al.*, 2006). Consistent with a role for auxin is the increase of the endogenous IAA concentration in both adaxial and abaxial epidermal petiole slices within 2 h of submergence (Cox *et al.*, 2004) and the up-regulation of several *AUX/IAA* genes in the petioles of *R. palustris* but not *R. acetosa* (van Veen *et al.*, 2013). Interestingly, auxin is necessary to regulate several *XTHs* in *A. thaliana* during shade-induced growth responses, a response that is phenotypically very similar to underwater petiole elongation (Pierik *et al.*, 2010; Keuskamp *et al.*, 2011).

The role of ABA in regulation of petiole growth is also evident from the observation that ABA concentrations declined by 80% within 1 h of submergence. This response seems to be a general reaction in *Rumex* as it was observed in all petioles, leaf blades and roots. The strong reduction of petiole elongation in submerged plants that received exogenous ABA demonstrates the importance of ABA-dependent regulation (Benschop *et al.*, 2005, 2006). After 3 h of submergence, the GA biosynthesis gene *GA3ox* and a set of genes known from photomorphogenic and shade avoidance responses (*KIDARI*, *CONSTITUTIVE PHOTOMORPHOGENESIS 1* (*COP1*) and *CLASS II HOMEODOMAIN-LEUCINE-ZIPPER* (*HD-ZIPII*)) were up-regulated (van Veen *et al.*, 2013). The first increase in bioactive GA₁ was observed after 4–5 h, too late to explain the initiation of underwater growth. This suggests that the initial elongation growth in *R. palustris* petioles operates independently of GA (Benschop *et al.*, 2006). The increase in the expression of typical shade avoidance-associated genes suggests a role for phytochrome-dependent signaling during underwater elongation. However, the decrease in the red : far red ratio (R : FR) from 2.2 to 1.6 that occurs during submergence was not sufficient to induce elongation and/or expression of photomorphogenesis genes under nonsubmerged conditions. Moreover, artificially filtering of FR wavelengths, resulting in higher R : FR ratios

underwater, did not reduce the elongation response. These data strongly suggest that the light signaling machinery is activated in submerged plants, independent of phytochrome signaling (van Veen *et al.*, 2013). Both *KIDARI* and *COP1* interact with *LONG HYPOCOTYL IN FAR-RED1* (*HFR1*). This basic helix-loop-helix TF forms heterodimers with *PIFs*, thus preventing their activation of target genes associated with enhanced shoot elongation (Galstyan *et al.*, 2011). *COP1* functions as an E3 ligase and targets, among other proteins, *HFR1* for degradation (Yang *et al.*, 2005). The heterodimerization of *KIDARI* with *HFR1* releases *PIFs* from inhibition (Hyun & Lee, 2006). We surmise that promotion of ethylene-regulated transcription of *PIFs* in combination with the increase of both *KIDARI* and *COP1* elevates concentrations of activated *PIFs*, leading to stimulation of growth-related processes (van Veen *et al.*, 2013).

Underwater shoot elongation has been studied in depth in two model plants: *R. palustris* and *O. sativa*. The conserved interaction between the hormones ethylene, ABA and GA perceives submergence and regulates the upsurge of cell expansion in both species. *ERF-VII* TFs regulate underwater growth in rice, whereas light-signaling genes regulate enhance shoot elongation in *R. palustris*. The latter observation demonstrates the downstream similarity of growth control during shade avoidance and underwater elongation.

IV. Limiting O₂ starvation with gas films and underwater photosynthesis

Submerged leaves develop diffusive boundary layers with a thickness very similar to that of leaves in air. However, the 10⁴-fold lower diffusion coefficient of gases in water causes a proportionally lower gas flux at a similar concentration gradient in the boundary layer of water-surrounded leaves. These boundary layers contribute a very large proportion of the resistance to CO₂ and O₂ exchange in submerged leaves, constraining photosynthesis and respiration (Pedersen *et al.*, 2013). Another limitation for underwater photosynthesis is the exponential decrease of light with depth (Colmer *et al.*, 2011). Dissolved organic matter and suspended particles further attenuate light penetration in floodwaters (Vervuren *et al.*, 2003). Consequently, net photosynthesis of submerged terrestrial leaves is often significantly reduced compared with aerial leaves. It is, however, also lower than the rates in leaves of aquatic plant species because of the general lack of beneficial leaf traits (Colmer *et al.*, 2011; Herrera, 2013). Nevertheless, in a range of terrestrial species submergence in the presence of light is beneficial, highlighting the importance of underwater photosynthesis for submergence survival (Vervuren *et al.*, 2003; Mommer *et al.*, 2006; Vashisht *et al.*, 2010; Lee *et al.*, 2011; Herrera, 2013).

In some submerged terrestrial plants, new leaves develop that are characterized by a higher specific leaf area, reoriented chloroplasts toward the epidermis of the leaf as well as thinner cuticles and cell walls (Mommer *et al.*, 2005b). Other relevant traits are the development of dissected leaves under water and the maintenance of gas films (Colmer *et al.*, 2011). All of these traits reduce diffusion resistance for CO₂ and thus increase the rates of underwater photosynthesis (Mommer *et al.*, 2005a). *Rumex palustris* develops

new leaves under water and acclimations in these leaves (Mommer *et al.*, 2005b) lead to a 38-fold decrease of the diffusion resistance to CO₂ under water (Mommer *et al.*, 2005a).

Many terrestrial plants have water-repellent or hydrophobic leaf surfaces that retain a thin layer of air (gas film) when submerged. This enlarges the gas–water interface and allows fast CO₂ diffusion within the air layer so that stomata can stay open. This was found to result in a 1.5–6-fold increase in the rate of underwater photosynthesis compared with leaves in which the gas film was removed, in laboratory and field studies (Colmer *et al.*, 2011; Winkel *et al.*, 2011, 2013, 2014; Pedersen *et al.*, 2013). There is evidence of variation in this trait in rice, with higher net underwater photosynthesis and longer leaf gas film retention in the submergence-tolerant landrace FR13A over a 12-d submergence period in plants grown in the field (Winkel *et al.*, 2014). Although gas film persistence was correlated with better maintenance of carbohydrates during submergence in FR13A, the duration of gas film retention was less in a Sub1 variety (Swarna-Sub1), indicating that genetic determinants other than the submergence-tolerant determinant *SUB1A* contribute to gas film formation or underwater photosynthesis.

Gas films and the above-mentioned morphological and anatomical leaf acclimations improve not only the inward diffusion of CO₂ in the light, but also the diffusion of O₂ from the water layer into the leaf in turbid waters or at night (Pedersen *et al.*, 2009; Verboven *et al.*, 2014). A novel function for leaf gas films was recently described for the annual legume *Melilotus siculus* submerged in saline waters. This species has gas films for the first 3 d of submergence on both leaf surfaces that not only improve gas exchange but also prevent salt intrusion (Teakle *et al.*, 2014).

V. Key metabolic acclimations to flooding and low-O₂ stress and their control

Dynamic changes in mRNAs and metabolites in response to submergence, waterlogging or hypoxia (typically <10% O₂ in surrounding air or aqueous media) have been evaluated in a wide range of species including *A. thaliana*, rice, other crops, deciduous trees and *Chlamydomonas* (reviewed by Sweetlove *et al.*, 2010; Grossman *et al.*, 2011; Narsai *et al.*, 2011; Bailey-Serres *et al.*, 2012b; Banti *et al.*, 2013; Kreuzwieser & Rennenberg, 2014; Mustroph *et al.*, 2014; Shingaki-Wells *et al.*, 2014). These surveys demonstrate both similarities and differences in metabolic acclimations. With near uniformity, environmental conditions with low O₂ elevate mRNAs encoding enzymes of an anaerobic metabolism module comprised of starch consumption (amylases), sucrose catabolism (sucrose synthase), glycolysis (phosphofructokinase), and pyruvate metabolism to ethanol (pyruvate decarboxylase (PDC)), alcohol dehydrogenase (ADH) or lactate (lactate dehydrogenase), as well as alanine (alanine aminotransferase), GABA (glutamate decarboxylase), succinate, and several glucogenic amino acids. The current hypothesis is that restriction of mitochondrial electron transport caused by limited O₂ availability is accompanied by a bifurcation of the TCA cycle that enhances production of ATP by the TCA enzyme succinyl-CoA ligase (Rocha *et al.*, 2010; Sweetlove *et al.*, 2010; Bailey-Serres *et al.*, 2012a). This scenario requires further confirmation by metabolic

flux analyses (Szecowka *et al.*, 2013), which might also consider how anaerobic metabolism is curtailed and its products consumed upon reoxygenation or desubmergence (Narsai *et al.*, 2009; Barding *et al.*, 2012, 2013; Mustroph *et al.*, 2014).

A fundamental advance was made with the recognition in *A. thaliana* that transcription of anaerobic metabolism genes is governed by O₂-regulated localization and turnover of the ERF-VIIs (Gibbs *et al.*, 2011; Licausi *et al.*, 2011). The underlying O₂ homeostasis sensing mechanism involves the arginine branch of the evolutionarily conserved N-end rule pathway of targeted proteolysis (Arg/N-end rule pathway), which has been summarized in several reviews (Bailey-Serres & Voeselek, 2010; Banti *et al.*, 2013; Licausi *et al.*, 2013; Gibbs *et al.*, 2014a). Briefly, there are five ERF-VIIs genes in *A. thaliana*. Three of these are constitutively expressed (*Related to AP2 12 (RAP2.12)*, *RAP2.2* and *RAP2.3*) and further up-regulated by darkness or ethylene, and the other two (*HYPOXIA RESPONSIVE ERF1/2*) are highly induced at transcriptional and translational levels by O₂ deprivation at multiple developmental stages. ERF-VII turnover by the Arg/N-end rule pathway occurs when both O₂ and nitric oxide (NO) are available, suggesting that ERF-VIIs participate in homeostatic O₂- and NO-sensing mechanisms (Gibbs *et al.*, 2011, 2014b; Licausi *et al.*, 2011). Reduction of O₂ by transfer of plants from normoxia to hypoxia or inhibition of NO accumulation by genetic or chemical means is sufficient to stabilize these proteins.

The core Arg/N-end pathway that regulates ERF-VII turnover is relatively well characterized (Fig. 7). The process requires a cysteine as the second residue of the protein and several enzymes: methionine aminopeptidase (MetAP), arginyl aminotransferase (ATE) and the N-recogin E3 ubiquitin-ligase (proteolysis 6 (PRT6)). MetAP-mediated removal of the initiator methionine yields an N-terminal Cys that is a substrate for oxidation. This may occur spontaneously, but is catalyzed by the O₂-dependent plant cysteine oxidase (PCO1/2), which oxidizes N-terminal, but not internal Cys residues (Weits *et al.*, 2014). Work in animals and now plants suggests that this modification probably requires both O₂ and NO (Gibbs *et al.*, 2014a). The presumed resultant NH₂-Cys-sulfinic or NH₂-Cys-sulfonic moiety structurally resembles an N-terminal aspartic acid that is recognized by ATE. This enzyme adds an N-terminal Arg from a tRNA^{ARG} to generate an NH₂-Arg-Cys^{ox} terminus. In these three steps, the tertiary destabilizing N-terminal Cys is converted to the secondary destabilizing N-terminal Cys^{ox} and finally to a primary destabilizing NH₂-Arg. The resultant N-degron is recognized by PRT6, a single-subunit Really Interesting New Gene (RING)-domain N-recogin coupled with an E2 ligase that adds ubiquitin to an internal lysine residue of the ERF-VII. This completes the marking of the protein for 26S proteasome-mediated destruction.

A challenging question is what cellular concentration of O₂ promotes stabilization and activation of these ERF-VIIs. Kosmacz *et al.* (2014) demonstrated that reduction of atmospheric O₂ below 10% increases the nuclear localization of RAP2.12. As O₂ concentrations decline, RAP2.12 is relocalized from the plasma membrane to the nucleus, concomitant with increased accumulation of hypoxia-responsive mRNAs. The proportion of cells with detectable nuclear localized RAP2.12-GFP increases significantly

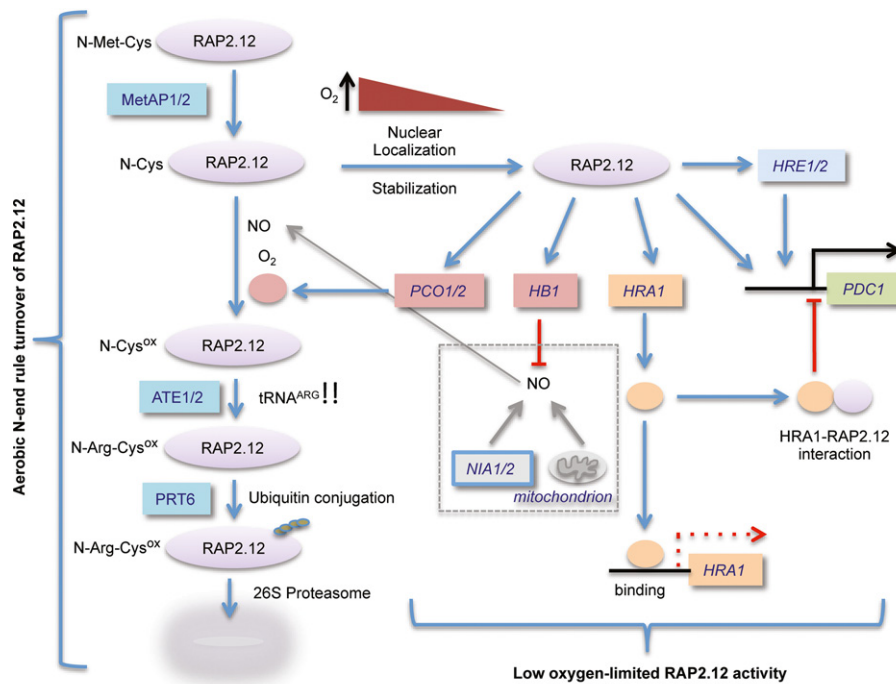


Fig. 7 Oxygen (O_2) sensing via the arginine branch of the N-end rule regulates positive and negative transcriptional regulation of core hypoxia response genes in *Arabidopsis thaliana*. The five group VII ethylene-responsive TFs (ERF-VIIs) of *A. thaliana*, which include Related to AP2 12 (RAP2.12), are turned over by the N-end rule pathway of targeted proteolysis (Gibbs *et al.*, 2011; Licausi *et al.*, 2011). This multistep pathway begins with initiator methionine removal by a Methionine aminopeptidase (MetAP1/2). The new amino-terminus, a cysteine residue, is a target for oxidation to Cys-sulfinic or Cys-sulfonic acid (Cys^{ox}), a reaction promoted by O_2 and nitric oxide (NO) generated from nitrate reductases (i.e. NIA1/2) (Gibbs *et al.*, 2014a,b) and possibly other sources. It is proposed that N-Cys^{ox}-RAP2.12 is sequentially modified by Arginyl aminotransferase1/2 (ATE1/2) and a ubiquitin E3 ligase (Proteolysis 6 (PRT6)), triggering degradation under aerobic conditions (Licausi *et al.*, 2011), whereas the oxidation of the N-Cys is limited when O_2 is limited. As O_2 concentrations in air decline below 10% or within 1 h of O_2 deprivation, RAP2.12 is stabilized as confirmed by accumulation of RAP2.12-GFP within nuclei (Kosmacz *et al.*, 2014). The red triangle is representative of the increase in stability as O_2 concentrations decline. Nuclear RAP2.12 is associated with increased core hypoxia-responsive gene transcript levels (Gibbs *et al.*, 2011; Licausi *et al.*, 2011). Directed studies recognize PYRUVATE DECARBOXYLASE1 (*PDC1*), HYPOXIA RESPONSIVE ERF1/2 (*HRE1/2*), trihelix TF HYPOXIA RESPONSE ATTENUATOR1 (*HRA1*), PLANT CYSTEINE OXIDASE1/2 (*PCO1/2*) and HEMOGLOBIN1 (*HB1*) as targets of RAP2.12 transcriptional activation (Giuntoli *et al.*, 2014; Klecker *et al.*, 2014; Weits *et al.*, 2014). HRE2 transcriptionally activates *PDC1* (Julia Bailey-Serres, unpublished). Blue arrows, transcriptional activation. Both HRA1 and PCO1/2 negatively regulate RAP2.12. HRA1 acts by direct interaction, limiting the transcriptional activation of *PDC1* (Giuntoli *et al.*, 2014). Dashed arrow, limited *HRA1* activation by RAP2.12 as a result of direct binding of HRA1 to its promoter. This relationship between HRA1 and RAP2.12 may enable a pulse of *PDC1* transcription. PCO1/2 inhibit RAP2.12 by catalyzing its N-Cys oxidation to initiate RAP2.12 turnover. The gray box highlights proposed regulation by NO, whereby *HB1* contributes to the fine-tuning of RAP2.12 accumulation by scavenging NO produced by NIA1/2 or mitochondria during O_2 deprivation. Arg, arginine.

within 3 h of exposure to 1% O_2 , with a significant decline in nuclear RAP2.12 with 3 h of reoxygenation (Kosmacz *et al.*, 2014).

The regulation of ERF-VII accumulation and activity is complex. In addition to the core Arg/N-end rule components and O_2 , there is involvement of PCO1/2 and NO. Disruption of *PCO1/2* limits the degradation of RAP2.12 and reduces submergence tolerance of rosette-stage plants (Weits *et al.*, 2014). Inhibition of NO accumulation by disruption of *NITRATE REDUCTASE1/2* (*NIR1/2*) or treatment with a chemical NO scavenger also stabilizes ERF-VIIs (Gibbs *et al.*, 2014b). Interestingly, PCO1/2, *NIR1/2* and *HEMOGLOBIN1* (*HB1*), the last encoding an NO scavenger, are all hypoxia-responsive genes (Mustroph *et al.*, 2009). Moreover, these genes are constitutively up-regulated in the *prt6-1* mutant, indicating that they are under Arg/N-end rule pathway control (Gibbs *et al.*, 2011). At least *PCO1/2* and *HB1* are under direct ERF-VII regulation (Klecker *et al.*, 2014; Weits *et al.*, 2014). As mitochondrial production of NO is elevated under hypoxia and regulated by HB1 (reviewed by

Bailey-Serres & Voeselek, 2008; Igamberdiev *et al.*, 2010; Hill, 2012), it can be proposed that HB1 contributes to regulation of ERF-VII accumulation by removal of NO during hypoxia (Fig. 7, dashed box). The role of HB1 and NO in the regulation of ERF-VII turnover as O_2 concentrations vary deserves further scrutiny.

There is cellular control of RAP2.12 by another RAP2.12-regulated and hypoxia-responsive gene. The trihelix TF hypoxia response attenuator 1 (*HRA1*) directly binds RAP2.12 and inhibits its transcriptional activation of *PDC1* and other hypoxia-responsive genes (Giuntoli *et al.*, 2014). This negative control occurs under low- O_2 conditions, but is restricted because *HRA1* binds its own promoter and thereby limits its activation by RAP2.12. It is envisioned that the coupling of RAP2.12 and *HRA1* function allows for pulsing of transcription of genes that promote anaerobic metabolism.

Other regulatory modules are relevant to low- O_2 survival in *A. thaliana* and possibly other plants. Another regulatory mode, limited to shoot tissue, involves the TF *PHOSPHATE*

STARVATION RESPONSE1 (*PHR1*) which is known to activate a phosphate starvation-response gene network (Klecker *et al.*, 2014). Under hypoxia, *PHR1* drives expression of galactolipid biosynthesis genes associated with modification of the composition of plastid membranes. It is hypothesized that disturbance of photosynthesis by hypoxia promotes chloroplast-to-nucleus signaling that triggers the *PHR1* network, thereby providing an adaptive benefit for low-O₂ or reoxygenation survival. A second module influenced by low O₂ is the low-energy signaling nexus that reprograms gene expression at transcriptional and translational levels, and impacts carbon and nitrogen metabolism, the cell cycle and development (Baena-Gonzalez *et al.*, 2007; Tomé *et al.*, 2014). Key components of the low-energy network include the conserved protein kinases sucrose nonfermenting-1-related protein kinase 1 (*SnRK1*) and target of rapamycin (*TOR*). The efficient anaerobic germination of rice seeds involves *SnRK1* and an upstream kinase (reviewed by Lee *et al.*, 2014). Future studies that take advantage of mutants, inducible RNAi lines and specific inhibitors of key components of these pathways will clarify their importance in flooding survival strategies.

VI. Managing quiescence in growth during submergence

A success story of modern agriculture is the introduction of the *SUB1* locus from the submergence-tolerant landrace FR13A of rice into popular high-yielding varieties and the rapid adoption of these 'Sub1' varieties in flood-prone regions in Asia (Bailey-Serres *et al.*, 2010; Ismail *et al.*, 2013; Singh *et al.*, 2013). The *SUB1* locus encodes two to three ERF-VII TFs including *SUB1A*, which regulates LOQS traits that prolong survival of short but deep submergence events (Fukao *et al.*, 2006; Xu *et al.*, 2006) as well as recovery and regrowth following desubmergence (Fukao *et al.*, 2011). The LOES provided by *SK1/SK2* is antithetical to the LOQS provided by the gene *SUB1A-1*.

The mechanisms of submergence tolerance determined by *SUB1A-1* have been intensively studied with a pair of NILs differing only in the region of chromosome 9 comprising the *SUB1* locus and transgenics that ectopically express *SUB1A-1*. These have revealed that *SUB1A* plays multiple roles during submergence and the desubmergence recovery period in shoot tissues. Its role during submergence centers on the same hormone triumvirate that regulates elongation growth in *R. palustris* and deepwater rice (Fig. 6).

As seen for *SK1/2*, submergence rapidly up-regulates *SUB1A-1* transcripts by over 200-fold (Fukao *et al.*, 2006). This is probably a response to the rapid entrapment of ethylene in submerged tissues. Factors such as low O₂ or low energy may also promote *SUB1A* transcript abundance during submergence, as the maximal induction by ethylene is far below that observed under submergence. As observed for deepwater rice, submergence promotes ethylene biosynthesis in lowland varieties, but this is significantly curbed in *SUB1A-1* genotypes (Fukao *et al.*, 2006). The increase in ethylene is associated with a decline in ABA content whether or not *SUB1A-1* is present (Fukao & Bailey-Serres, 2008). However, unlike deepwater rice which biosynthesizes GA in submerged tissue

(Hattori *et al.*, 2009; Ayano *et al.*, 2014), lowland rice shoots display little or no increase in GA₄ (Schmitz *et al.*, 2013). The limited GA biosynthesis in these lowland varieties during submergence may relate to the presence of the loss-of-function *sd-1* allele (*OsGA20ox2*). The deepwater elongating varieties C9285 and NIL1 + NIL3 + SK1/SK2, by contrast, possess a functional and submergence-induced *OsGAox2* allele that may be essential for their c. 8-fold increase in GA₁ and GA₄ (Ayano *et al.*, 2014).

In rice, GA₄ binds its receptor *GID* to promote its interaction with the DELLA domain-containing GRAS TF Slender rice1 (*SLR1*) which is bound to GA-responsive genes to limit their transcription. This stimulates ubiquitylation of *SLR1* by SCF^{SLY1/GID2}, a Skp1-Cullin-F box protein (SCF) E3 ligase that includes the F-box protein Gibberellin insensitive dwarf 2 (*GID2*) (Hauvermale *et al.*, 2012). Ubiquitylation of *SLR1* targets it for proteasome degradation, freeing *GID1* for reuse and enabling the transcriptional activation of the genes that had been *SLR1* bound. In this manner, GA₄ can promote the activation of genes associated with elongation growth. However, rice possesses a second GRAS domain protein, Slender rice1-like1 (*SLRL1*), which lacks a DELLA domain and is therefore not targeted for degradation in the same manner.

A role attributed to submergence-induced *SUB1A-1* is increased maintenance of *SLR1* and *SLRL1*. In lines possessing *SUB1A-1*, levels of *SLR1/SLRL1* mRNA and protein are enhanced by submergence or treatment with ACC (Fukao & Bailey-Serres, 2008). New data suggest that the up-regulation of *SLR1* protein and mRNA of the GA catabolic gene *GA2ox7* is mediated by enhanced brassinosteroid (BR) biosynthesis in *SUB1A-1* genotypes (Schmitz *et al.*, 2013). Submergence in the presence of the bioactive BR brassinolide inhibits elongation growth, with greater inhibition in *SUB1A-1* genotypes. This indicates that shoot growth regulation in submerged rice involves interplay between ethylene, BRs and GA.

Accompanying the ethylene-driven GA-promoted elongation of submerged shoots is the catabolism of starch and soluble sugars (Fukao *et al.*, 2006). This involves up-regulation of α -amylases in the leaf mesophyll which break down starch to sugars, for production of ATP by oxidative phosphorylation when O₂ is present or anaerobic metabolism if O₂ is limiting. Submerged rice leaves rapidly deplete the available starch with concomitant increases in amino acids, TCA intermediates and fermentation end-products (Barding *et al.*, 2012, 2013). Genotypes with submergence-inducible or constitutively expressed *SUB1A-1* induce lower levels of leaf α -*AMYLASE* transcripts (Fukao *et al.*, 2006; Fukao & Bailey-Serres, 2008). Consistent with the observations that the LOQS involves less mobilization of hydrolysable carbon, *SUB1A-1* lines restrict the rate and extent of starch hydrolysis and accumulate lower concentrations of ethanol, lactate and alanine as well as amino acid metabolic end-products (Fukao *et al.*, 2006; Barding *et al.*, 2012, 2013). This restriction on metabolism probably occurs at the level of mobilization of carbon reserves, as *SUB1A-1* genotypes accumulate higher levels of *PCD* and *ADH* transcripts and enzymes under submergence.

The LOQS of Sub1 rice extends beyond carbohydrate conservation. As noted in several studies, ethylene-driven declines in leaf

chlorophyll content occur more rapidly in genotypes lacking *SUB1A-1* (Ella *et al.*, 2003; Fukao *et al.*, 2006; Winkel *et al.*, 2014). Chlorophyll breakdown also occurs when rice plants are left in the dark for extended periods, a situation that can occur as a result of submergence in turbid waters. Remarkably, *SUB1A-1* transcript levels rose over 100-fold in leaves after 1 d of anticipated darkness (Fukao *et al.*, 2012), although levels of this transcript did not rise under a standard diurnal regime (Peña-Castro *et al.*, 2011). The dampening of dark-induced chlorophyll catabolism and a more rapid recovery of photosynthetic activity upon re-illumination were correlated with lower induction of transcripts associated with chlorophyll catabolism (Fukao *et al.*, 2012). Transcripts of *DELAY OF THE ONSET OF SENESCENCE (DOS)*, which encodes a zinc-finger TF that negatively regulates chlorophyll catabolism, were also significantly elevated in *SUB1A-1*, further indicating that *SUB1A* activity somehow dampens carbon and nitrogen mobilization under energy-limiting conditions (darkness and submergence).

Studies of flooding responses of *Rumex* and *Lotus* species indicate that quiescence and escape are not mutually exclusive. In *Lotus tenuis*, shoots elongate upon partial submergence but arrest growth when completely submerged, apparently switching from LOES to LOQS (Manzur *et al.*, 2009). This is associated with an elevated shoot porosity and limited consumption of soluble carbohydrates in the shoot/root crown. Lethal Time 50 (LT₅₀) analyses suggest that fully submerged *R. palustris* survives submergence in darkness just as well as its 'quiescent' relative *R. acetosa* (van Veen *et al.*, 2014). Interestingly, anoxia survival was improved when *R. palustris* plants were pretreated with ethylene, whereas that of *R. acetosa* was not (van Veen *et al.*, 2013). These observations indicate that the capacity for a LOES does not preclude the ability to invoke the LOQS. The petiole-specific activation of the signaling machinery previously associated with shade avoidance, independent of phytochromes, may underlie the regional-specific changes that manifest underwater elongation, reminiscent of the behavior of stem internode regions in deepwater rice. Contrasting adaptation strategies are also illustrated by tree species in the Amazonian floodplains, which can manage high metabolic levels that sustain growth during submergence (Herrera, 2013; Kreuzwieser & Rennenberg, 2014). In conclusion, although the LOES may result in a net consumption of carbohydrates in specific cells, it might be combined with LOQS determined by a threshold of O₂ or energy deficiency.

VII. After the deluge

Flooding events are frequently transient, as a result of agricultural management of floodwaters or natural drainage. Key to an effective waterlogging or submergence survival strategy is the ability to remain reproductively viable. The LOES enables flowering and fruiting to occur above the air–water interface. For plants with the LOQS, fitness requires the capacity to quickly resume growth, flower and set seeds after the inundation subsides. For either survival strategy, several factors are to be considered: control of ROS formed upon reoxygenation; return to homeostasis including recovery of photosynthetic activity and prevention of desiccation; and progression of development to reproduction.

Studies in an array of species and O₂ conditions have noted elevation of transcripts associated with response to oxidative stress, emphasizing their importance (Branco-Price *et al.*, 2008; Blokhina *et al.*, 2014). There are also many demonstrated instances of ROS production or damage in response to reoxygenation (reviewed by Fukao & Bailey-Serres, 2004; Bailey-Serres & Chang, 2005; Blokhina & Fagerstedt, 2010). It is hypothesized that, as cells transit from hypoxia to normoxia, there is a burst in superoxide production at complex III (cytochrome bc₁) of the mitochondrial electron transport chain, as a result of a lag in reactivation of complex IV (cytochrome *c* oxidase) (Santosa *et al.*, 2007). A deficiency in antioxidants or antioxidant enzymes upon reoxygenation would lead to damage of cellular membranes, which could impact cell integrity. Intriguingly, the burst in ROS emanating from mitochondria upon both O₂ deprivation and reoxygenation promotes the transient activation of MAP kinases that play key signaling roles in abiotic and biotic stress responses in *A. thaliana* (Chang *et al.*, 2012). Evidence of the importance of ROS management during reoxygenation comes from the finding that Sub1 rice minimizes ROS accumulation and leaf water loss during desubmergence recovery (Fukao *et al.*, 2011). Sub1 lines have higher levels of mRNAs associated with limitation of ROS accumulation before and during the recovery phase (Jung *et al.*, 2010; Mustroph *et al.*, 2010; Fukao *et al.*, 2011).

The transition from O₂-depleted to O₂-replete conditions will undoubtedly result in a rapid change in cellular redox status. Our understanding of low-O₂ sensing mediated by the Arg/N-end rule pathway indicates that ERF-VII N-terminal cysteine oxidation enhanced by PCO1/2 (Weits *et al.*, 2014) would result in rapid degradation of the TF, thereby switching off the synthesis of mRNAs encoding key enzymes of anaerobic metabolism. Reactive cysteines may be relevant to other rapid adjustments upon changes in O₂ availability and redox status upon reoxygenation. These could include regulation of other TFs, release of mRNAs that are sequestered from the translational apparatus and regulation of membrane transporters.

Desubmergence or reoxygenation of plants results in significant changes in gene transcript accumulation (e.g. studies by Branco-Price *et al.*, 2008; Tamang *et al.*, 2014; Tsai *et al.*, 2014). It is well established that ethylene evolution is enhanced in a species-specific way upon desubmergence (Voesenek *et al.*, 2003). In *A. thaliana* seedlings, reoxygenation promotes accumulation of transcripts associated with ethylene production (Branco-Price *et al.*, 2008); moreover, ethylene-insensitive mutants (*ein2-5* and *ein3 eil1*) are dysfunctional in up-regulation of many functional classes of gene during reoxygenation (Tsai *et al.*, 2014). This suggests that ethylene signaling plays a role during stress recovery, perhaps independent of the demise of ERF-VIIs.

Often submergence and waterlogging reduce maximum photosystem II (PSII) quantum efficiency (Kreuzwieser & Rennenberg, 2014), albeit the presence of leaf gas films on submerged leaves can limit this constraint (Winkel *et al.*, 2014). An underlying cause is the degradation of chlorophyll, which as mentioned is less pronounced during submergence or extended darkness in Sub1 rice (Fukao *et al.*, 2006, 2012). Other studies have recognized a correlation between submergence tolerance and the ability to

rapidly recover photosynthesis (Luo *et al.*, 2009, 2011), including the ability of some species to retain functional PSII complexes even after prolonged periods of submergence in darkness (i.e. certain species endemic to Amazonian river floodplains; reviewed by Parolin, 2008; Herrera, 2013). A better understanding of the mechanisms associated with chloroplast maintenance, modifications, or turnover in flooded tissues is needed.

VIII. Perspective

Examination of species endemic to environments that experience various flooding regimes as well as the semiaquatic crop rice has identified plastic developmental, physiological and molecular mechanisms that benefit survival. The combined recognition of a low-O₂ homeostasis mechanism that controls anaerobic metabolism in *A. thaliana* and key molecular and physiological processes from rice and wild species such as *R. palustris* provides knowledge that might be deployed to improve flooding survival of key crops. Here we propose two scenarios for 'waterproofing' plants.

The first scenario involves the manipulation of ERF-VII activity and turnover. The conservation of ERF-VIIs and Arg/N-end rule pathway (*MetAP*, *ATE1/2*, *PRT6* and *PCO1/2*), *HBI* and *HRA1* genes in diverse land plants indicates that the low-O₂ regulation of hypoxia-responsive genes is highly conserved. Moreover, the strong overlap in transcriptome responses to flooding/hypoxia across species and at multiple developmental stages (Mustroph *et al.*, 2010; Narsai *et al.*, 2011; van Veen *et al.*, 2013) suggests the manipulation of this core module is an avenue for improvement of flooding survival. The submergence resilience of *A. thaliana* genotypes with modified ERF-VII turnover, resulting from manipulation of the N-termini of the ERF-VIIs or the Arg/N-end rule pathway components (Gibbs *et al.*, 2011; Licausi *et al.*, 2011; Giuntoli *et al.*, 2014; Weits *et al.*, 2014), strongly indicates that this module can be used to enable LOQS. The results in *A. thaliana* are promising; approximately a doubling of LT₅₀ was observed in *prt6* mutants compared with wild type when submerged in darkness (R. Sasidharan, pers. comm.). Future translation of this knowledge to crops requires consideration of the native ERF-VIIs, the location and timing of their expression, proteins that control their activity, and direct gene targets. This need is exemplified by two recent findings: first, that HRA1 limits the activity of RAP2.12 (Giuntoli *et al.*, 2014) and, secondly, that the DELLAs Gibberellin insensitive (GAI) and Repressor of *ga1-3* (RGA) bind RAP2.3 to limit its binding to target gene sequences (Marín-de la Rosa *et al.*, 2014).

The second scenario involves the identification and transfer of survival solutions between cultivars and species. Within-species transfer is elegantly demonstrated in rice with the *SUB1A* and *SK1/2* genes. The submergence tolerance regulator *SUB1A-1* influences anaerobic metabolism but extends its impact to GA-mediated elongation growth. The remarkably antithetical regulation of GA biosynthesis and the apparent responsiveness associated with *SK1/SK2* of deepwater elongating rice emphasize that carbon-consumptive metabolism can be pushed to the other extreme to enable survival. Although the heterologous constitutive expression of *SUB1A-1* in *A. thaliana* did not yield submergence-tolerant plants,

the transgenics displayed increased GA-responsiveness and increased ABA responsiveness (Peña-Castro *et al.*, 2011), similar to *SUB1A-1* overexpression lines of rice (Fukao & Bailey-Serres, 2008). There are other demonstrations of the value of the between-species translation approach, as illustrated by waterlogging-tolerant *Hordeum marinum* (wild sea barley), a relative of wheat (*T. aestivum*). *Hordeum marinum* is characterized by higher root porosity and a stronger ROL barrier in the basal zones of ARs compared with wheat. *Hordeum marinum*-wheat amphiploids display higher root porosities and an effective ROL barrier, demonstrating the successful transfer of waterlogging tolerance traits to wheat (Malik *et al.*, 2010). Although details of the relevant molecular and developmental mechanisms remain uncharacterized, this example illustrates successful transfer of flood tolerance from wild relatives to crops cultivars. Continued identification of genetic diversity in flooding survival traits – acting at seed, seedling and reproductive stages and for a range of flooding regimes – is essential.

We advocate that a 'learn from nature' approach provides potential to identify and mobilize additional genes and pathways that provide effective survival strategies. These may be those lost during crop domestication or present only in species adapted to specific ecological niches. As already demonstrated, next-generation sequencing technologies coupled with physiological studies can be applied to wild species to glean insights into mechanisms not present in model species. The advancement of genomic methodologies and genome editing technologies provide exciting opportunities and the promise that insurance against unanticipated flooding can be effectively added to crops to improve yield stability.

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