Flooding Stress: Acclimations and Genetic Diversity

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Key Words

aerenchyma, anoxia, response strategy, hypoxia, reactive oxygen species, submergence

Abstract

Flooding is an environmental stress for many natural and man-made ecosystems worldwide. Genetic diversity in the plant response to flooding includes alterations in architecture, metabolism, and elongation growth associated with a low O_2 escape strategy and an antithetical quiescence scheme that allows endurance of prolonged submergence. Flooding is frequently accompanied with a reduction of cellular O_2 content that is particularly severe when photosynthesis is limited or absent. This necessitates the production of ATP and regeneration of NAD^+ through anaerobic respiration. The examination of gene regulation and function in model systems provides insight into low-O_2-sensing mechanisms and metabolic adjustments associated with controlled use of carbohydrate and ATP. At the developmental level, plants can escape the low-O_2 stress caused by flooding through multifaceted alterations in cellular and organ structure that promote access to and diffusion of O_2. These processes are driven by phytohormones, including ethylene, gibberellin, and abscisic acid. This exploration of natural variation in strategies that improve O_2 and carbohydrate status during flooding provides valuable resources for the improvement of crop endurance of an environmental adversity that is enhanced by global warming.

Contents

INTRODUCTION

Partial to complete flooding is detrimental for most terrestrial plants because it hampers growth and can result in premature death. Some plant species have a remarkable capacity to endure these conditions, and certain species can even grow vigorously in response to flooding. This interspecific variation has a strong impact on species abundance and distribution in flood-prone ecosystems worldwide (12, 31, 122, 138, 150, 151). Furthermore, flooding has a severe negative influence on the productivity of arable farmland because most crops are not selected to cope with flooding stress (121). The Intergovernmental Panel on Climate Change (IPCC) (**http://www.ipcc.ch**) reported that the anthropogenically induced change of world climate increases the frequency of heavy precipitation and tropical cyclone activity. This is likely to engender more frequent flooding events in river flood plains and arable farmland, particularly affecting the world's poorest farmers (1).

The observation that some plant species can cope with flooding stress and others cannot imposes the question of why a flooded environment is detrimental. The adversity is largely due to the dramatically reduced gas exchange between plants and their aerial environment during partial to complete submergence. Gases such as O_2, CO_2, and ethylene diffuse very slowly in water (46). Because of this tremendous barrier for gas diffusion, the cellular O_2 level can decline to concentrations that restrict aerobic respiration (39, 46). Depending on the tissue and light conditions, the cellular CO_2 level either increases in shoots in the dark and roots (47) or decreases in shoots in the light (83). The endogenous concentration of the gaseous plant hormone ethylene increases in tissues surrounded by water (59, 148). This accumulation activates adaptive signal transduction pathways, whereas similar concentrations hamper normal growth in many terrestrial plants (93). Furthermore, complete submergence decreases light intensity, dampening photosynthesis (141). A third major change in the flooded environment is the reduction of oxidized soil components to toxic concentrations (12). In summary, flooding is a compound stress in which the decline in molecular O_2 and thus the restriction of ATP synthesis and carbohydrate resources have major consequences for growth and survival. However, O_2 depletion is not the only active stress component, and often its impact is restricted to nonphotosynthesizing organs (84).

O_2 shortage (hypoxia/anoxia) is not restricted to flooding stress. It is a frequent metabolic status of cells during normal development, particularly in tissues with high cell density, a high O_2 demand, and/or restricted O_2 entry, such as meristems, seeds, fruits, and storage organs (43). Fundamental insight into the low O_2–sensing mechanism, downstream signal transduction, and metabolic alterations that promote survival is key to increased crop

Hypoxia (e.g., <20.9% and >0% O_2 at 20°C): characterized by increased anaerobic metabolism, increased ATP production via glycolysis owing to limited availability of O_2 for oxidative phosphorylation, and increased NAD^+ regeneration via lactate and ethanolic fermentation. Cellular ATP content is reduced

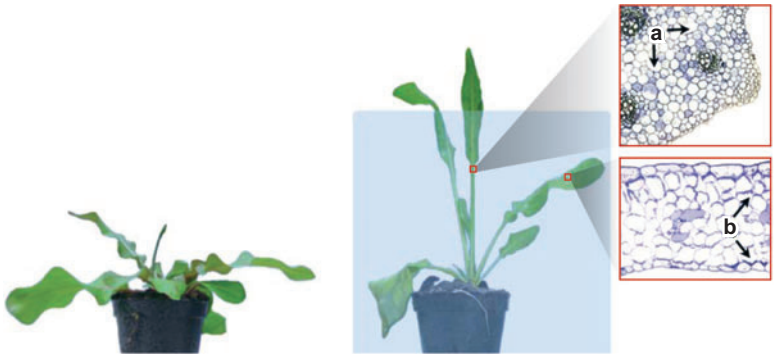
production in flood-prone environments and has wider implications for biologists (3, 43). Most studies on flooding stress have focused on relatively flood-tolerant species from genera such as *Oryza*, *Rumex*, and *Echinochloa*. Single species studies are valuable for an understanding of the regulation of various acclimations but less meaningful in an ecological perspective. Here genetic diversity in acclimations to flooding stress is discussed side by side with the molecular regulation of low-O_2 responses and flooding tolerance. Ultimately we aim to shed light on the genes, proteins, and processes controlling these phenotypes.

GENETIC DIVERSITY OF STRATEGIES TO SURVIVE FLOODING

Not all species in flood-prone environments are flood tolerant. Some species avoid flooding by completing their life cycle between two subsequent flood events, whereas flooding periods are survived by dormant life stages [e.g., *Chenopodium rubrum* thrives in frequently flooded environments by timing its growth between floods and producing seeds that survive flooding (134)]. Established plants also use avoidance strategies through the development of anatomical and morphological traits. This amelioration response, here called the low oxygen escape syndrome (LOES) (**Figure 1**), facilitates the survival of submerged organs. Upon complete submergence several species from flood-prone environments have the capacity to stimulate the elongation rate of petioles, stems, or leaves. This fast elongation can restore contact between leaves and air but can also result in plant death if energy reserves are depleted before emergence. Concomitant with high elongation rates, the leaves also develop a thinner overall morphology, develop thinner cell walls and cuticles, and reorient

Anoxia (e.g., 0% O_2 at 20°C): characterized by anaerobic metabolism, NAD^+ regeneration via lactate and ethanolic fermentation, and ATP production solely via glycolysis (2–4 mol ATP per mole hexose). Cellular ATP content is low, and ADP content is elevated

LOES: low-oxygen escape syndrome



Drained	Submerged	Trait
Low	High	Shoot elongation
Low	High	Aerenchyma
High	Low	Leaf thickness
Around intercellular spaces	Toward epidermis	Chloroplast position

Figure 1

Various species display the low-oxygen escape syndrome (LOES) when submerged. The syndrome includes enhanced elongation of internodes and petioles, the formation of aerenchyma in these organs (air spaces indicated by *arrows* labeled *a*), and increased gas exchange with the water layer through reduced leaf thickness and chloroplasts that lie directed toward the epidermis (indicated by *arrows* labeled *b*). Photographs are courtesy of Ronald Pierik, Liesje Mommer, Mieke Wolters-Arts, and Ankie Ammerlaan.

chloroplasts toward the leaf surface. These traits reduce the resistance for diffusion of CO_2 and O_2, facilitating inward diffusion and thereby improving underwater photosynthesis and aerobic metabolism (82, 83). Thus, the LOES improves the aeration of the plant, which is further enhanced by the relatively low resistance for internal gas diffusion owing to a system of interconnected gas conduits called aerenchyma, a property typical of many wetland plants (24, 33). These conduits are constitutive, induced in existing tissues (roots, petioles, stems) (33) or formed during the development of adventitious roots that arise from the root shoot junction or stem nodes (115, 142). In specialized cases the longitudinal diffusion of O_2 to the root apex is further enhanced by the development of a barrier to radial oxygen loss to minimize escape of O_2 to the surrounding environment (24, 25).

LOES is costly and will only be selected for in environments where the cost is outweighed by benefits such as improved O_2 and carbohydrate status, both contributing to a higher fitness (120). The flooding regime is an important determinant for selection in favor of or against LOES. A study on the distribution of species in the Rhine floodplains confirmed this hypothesis. Here LOES occurs predominantly in species from habitats characterized by prolonged, but relatively shallow, flooding events (150). However, the benefits of LOES do not outweigh the costs when the floods are too deep or ephemeral. These regimes favor a quiescence strategy characterized by limited underwater growth and conservation of energy and carbohydrates (39, 91). This strategy is a true tolerance mechanism, driven by adjustment of metabolism. With respect to low-O_2 stress, this includes the downregulation of respiration and limited stimulation of fermentation to create a positive energy budget when organ hypoxia starts (43, 148). The *SUB1A* gene of the polygenic rice (*Oryza sativa* L.) *Submergence1* (*Sub1*) locus was shown to confer submergence tolerance through a 'quiescence' strategy in which cell elongation and carbohydrate metabolism is repressed (41, 91, 159) (**Figure 2**). *SUB1A*, encodes an ethylene-responsive element (ERF) domain–containing transcription factor (41). The lack of *SUB1A-1* or the presence of a slightly modified allele is associated with reduced submergence tolerance and the induction of the LOES. This example demonstrates that environment-driven selection on a single locus can significantly alter survival strategy.

***Sub1*:** *Submergence1* polygenic locus of rice; determines submergence tolerance

Oxygen deficiency/ deprivation: the natural and experimental conditions in which cellular oxygen content is reduced but metabolic status is not determined

mtETC: mitochondrial electron transport chain

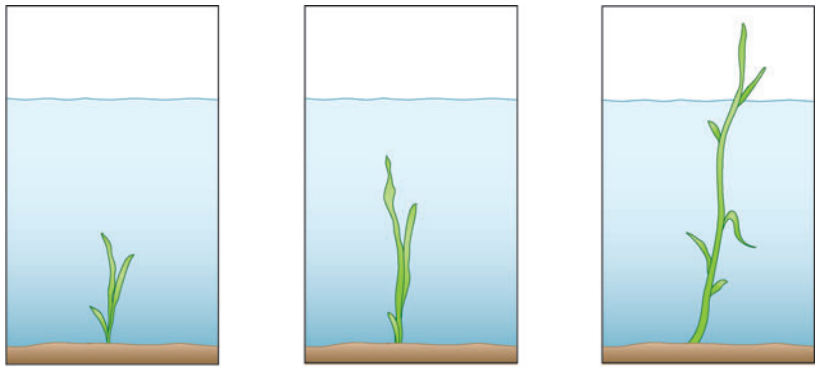
ROS: reactive oxygen species

ACCLIMATION TO FLOODING AT THE CELLULAR LEVEL

Overview of Cellular Adjustments to Oxygen Deprivation

During flooding, the onset of O_2 deprivation is rapid in the dark and in nonphotosynthetic cells. The reduced availability of O_2 as the final electron acceptor in the mitochondrial electron transport chain (mtETC) mediates a rapid reduction of the cellular ATP:ADP ratio and adenylate energy charge (AEC) ([ATP + 0.5 ADP]/[ATP+ADP+AMP]) (46). Cells cope with this energy crisis by relying primarily on glycolysis and fermentation to generate ATP and regenerate NAD^+, respectively. Whether a LOES or a quiescence response to flooding is activated, cellular acclimation to transient O_2 deprivation requires tight regulation of ATP production and consumption, limited acidification of the cytosol, and amelioration of reactive oxygen species (ROS) produced either as O_2 levels fall during flooding or upon reoxygenation after withdrawal of the flood water.

O_2 concentration is 20.95% at 20°C in air but ranges from 1 to 7% in the core of well-aerated roots, stems, tubers, and developing seeds (14, 44, 46, 107, 136, 137). Within a root, O_2 levels and consumption vary zonally; the highly metabolically active meristematic cells are in a continuous state of deficiency. Upon flooding, the ~10,000-fold-slower diffusion of O_2 in water rapidly limits its availability for mitochondrial respiration. This deprivation is progressively more severe as



	Lowland Rice		Deepwater Rice
	Tolerant	**Intolerant**	
Strategy	**Quiescence**	**LOES**	**LOES**
Sub1 haplotype	*SUB1A-1, SUB1B, SUB1C*	*SUB1B, SUB1C or SUB1A-2, SUB1B, SUB1C*	*SUB1B, SUB1C or SUB1A-2, SUB1B, SUB1C*
Carbohydrate consumption	Limited by *SUB1A-1*	High	High
Fermentation capacity	High	Moderate	N.D.
GA response	Inhibited by *SUB1A-1*	Promoted by *SUB1C*	High

Figure 2

Rice responds via different strategies to submergence. Flood-tolerant rice varieties invoke a quiescence strategy that is governed by the polygenic *Submergence1* (*Sub1*) locus that encodes two or three ethylene-responsive factor proteins (41, 159). *SUB1A* is induced by ethylene under submergence and negatively regulates *SUB1C* mRNA levels. Flood-intolerant varieties avoid submergence via the low oxygen escape syndrome (LOES). To this end *SUB1C* expression is promoted by gibberellic acid (GA) and is associated with rapid depletion of carbohydrate reserves and enhanced elongation of leaves and internodes. The LOES is unsuccessful when flooding is ephemeral and deep. Deepwater rice varieties survive flooding via a LOES, as long as the rise in depth is sufficiently gradual to allow aerial tissue to escape submergence (61). N.D., not determined.

distance from the source increases and tissue porosity decreases. For example, the cortex of nonaerenchymatous maize (*Zea mays* L.) roots exposed to 10% O_2 becomes hypoxic, whereas the internal stele becomes anoxic. Even the apex of aerenchymatous roots encounters severe O_2 deprivation (46). In dense storage organs such as potato (*Solanum tuberosum L.*) tubers and developing plant seeds, exposure to 8% O_2 significantly reduces the endogenous O_2 level. However, the decrease in cellular O_2 is strikingly nonlinear from the exterior to the interior of the organ; cells at the interior of the tuber or endosperm maintain a hypoxic state (44, 136). This has led to the suggestion that an active mechanism may allow cells to avoid anoxia (43). Such a mechanism may include proactive limitation in the consumption of both ATP and O_2. The low K_m for cytochrome *c* oxidase (COX) [140 nM (~0.013%) O_2] should ensure that the activity of COX continues as long as O_2 is available (31, 46). However, a mechanism that inhibits the mtETC at or upstream of COX or inhibits

O_2 consumption by other enzymes may allow cells to sustain hypoxia and avoid death.

Normoxia (e.g., 20.9% O_2 at 20°C): characterized by aerobic metabolism, NAD^+ regeneration primarily via the mitochondrial electron transport chain, and ATP production via mitochondrial oxidative phsophorylation (30–36 mol ATP per mol hexose consumed); cellular ATP content is normal

Low-Oxygen Sensing

In animals the perception of O_2 deficit involves O_2-binding proteins, ROS, and mitochondria. The O_2-consuming prolyl hydroxylases (PHDs) are direct sensors of O_2 availability. Under normoxia, PHDs target the proteosomal degradation of hypoxia inducible factor 1α (HIF1α), a subunit of a heterodimeric transcription factor that regulates acclimation to hypoxia (51). The concomitant drop in PHD activity stimulates an elevation in HIF1α as O_2 declines. A paradox is that the production of ROS at the mitochondrial ubiquinone:cytochrome *c* reductase complex (Complex III) is necessary to initiate O_2 deficit responses (7, 51).

There is limited understanding of the mechanisms by which plant cells sense and initiate signaling in response to O_2 deficit (3, 39, 43). Plants lack a HIF1α ortholog, although PHD mRNAs are strongly induced by O_2 deficit in *Arabidopsis thaliana* and rice (67, 146). Furthermore, significant increases in mRNAs encoding enzymes involved in ROS signaling and amelioration (16, 63, 67, 70, 71) and evidence of ROS production have been reported in several species upon transfer to low O_2 conditions. A challenge in monitoring ROS production during O_2 deficit is that ROS are produced readily upon reoxygenation. However, ethane, a product of membrane peroxidation by ROS, evolves from submerged rice seedlings in a closed system as levels of O_2 fall to as low as 1% (112), providing evidence that ROS form as O_2 levels decline. Blokhina and colleagues (11) demonstrated that in response to anoxia, H_2O_2 accumulates to higher levels in the apoplast of root meristems of hypoxic wheat (*Triticum aestivum*) than in the more anoxia-tolerant rhizomes of *Iris pseudacorus*. In *Arabidopsis* seedlings, H_2O_2 levels increase in response to O_2 deprivation in a ROP GTPase–dependent manner (6). Genotypes that limit ROP signaling under hypoxia display lower levels of H_2O_2 accumulation and altered gene regulation in stressed seedlings. Indications that mitochondria are crucial to low-O_2 sensing in plants comes from the release of Ca^{2+} from mitochondria of cultured maize cells within minutes of transfer to anoxia (127). This release may be activated by mitochondrial ROS production at Complex III of the mtETC (99). A rapid spike in cytosolic Ca^{2+} was also observed in the cotyledons of *Arabidopsis* seedlings upon transfer to anoxia and again at higher amplitude upon reoxygenation (119). These Ca^{2+} transients are required for alterations in gene expression that enhance ethanolic fermentation and ATP management during the stress (3, 66, 88, 119, 126, 128). Further studies are needed to confirm whether mitochondrion-to-nucleus signaling, mediated by ROS production and Ca^{2+} release from mitochondria, contributes to reconfiguration of metabolism under low O_2. Additional players in the acclimation response may be the reduction of ATP content and decline in cytosolic pH as well as change in levels of metabolites such as sucrose and pyruvate (3, 39). mRNAs encoding mitochondrial alternative oxidase, (AOX) are strongly induced by low-O_2 stress (63, 67, 70, 71). AOX diverts ubiquinone from Complex III; if active as O_2 levels decrease, AOX would paradoxically reduce oxygen availability for COX and decrease ATP production. However, if active as O_2 levels rise upon reoxygenation, AOX may limit mitochondrial ROS production (99).

Management of the Energy Crisis

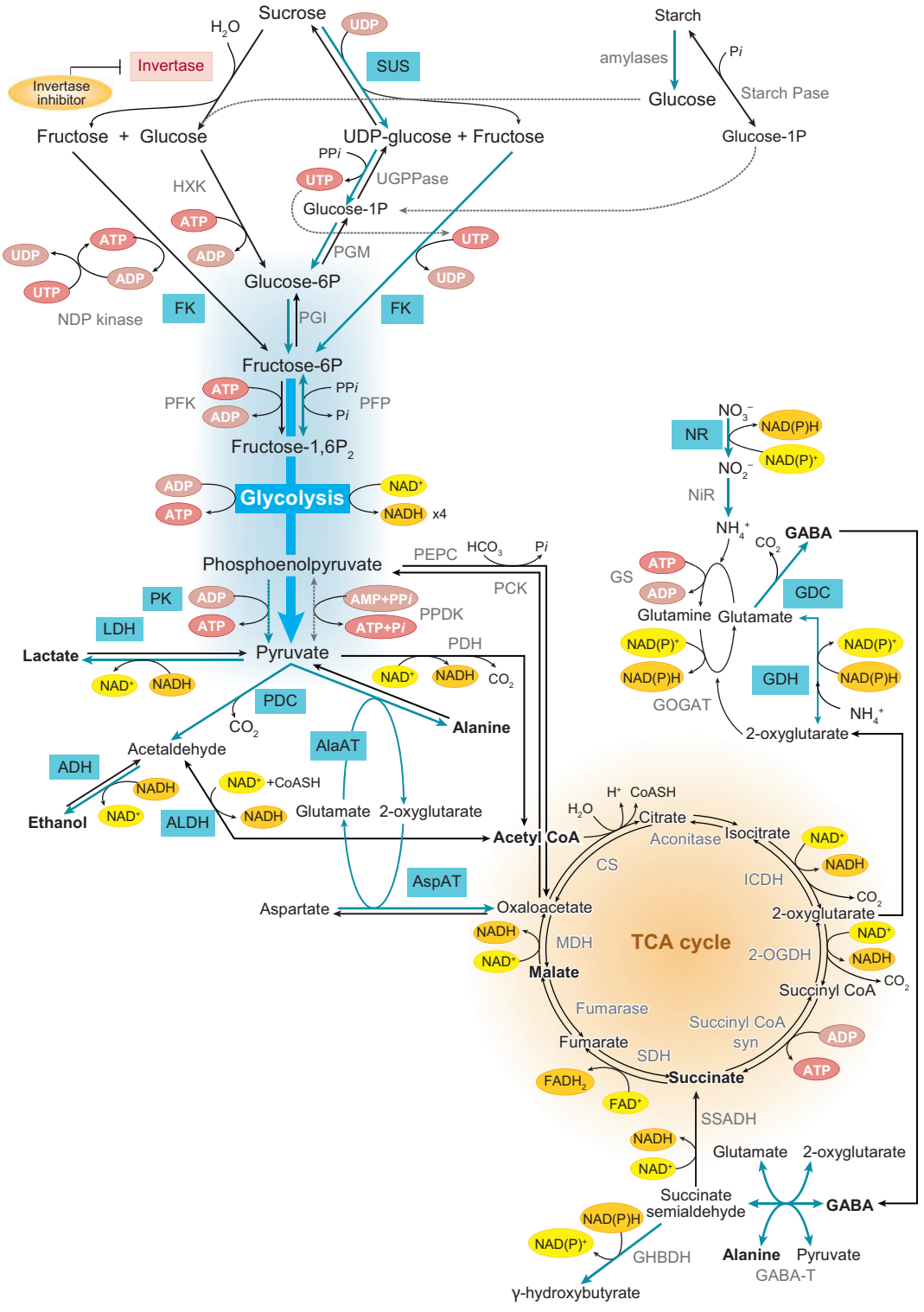
Within minutes of transfer to an O_2-depleted environment, cells reliant on external O_2 limit processes that are highly energy consumptive and alter metabolism to increase anaerobic generation of ATP by cytosolic glycolysis (31). This shift is followed by fermentation of pyruvate to the major end products, ethanol or lactate, yielding NAD^+ to sustain anaerobic metabolism (**Figure 3**). A crisis in ATP availability ensues because glycolysis is inefficient,

yielding 2 to 4 mol ATP per mol hexose as compared with 30 to 36 mol ATP by the mtETC. Evaluation of gene transcripts, enzymes, and metabolites in a variety of species and genotypes demonstrated the production of minor metabolic end products that are also important for NAD^+ and $NAD(P)^+$ regeneration. Although mutant analyses with several species have demonstrated that glycolysis and fermentation are necessary for cell survival under O_2 deprivation, the enhancement of these processes is not well correlated with prolonged endurance of this stress (31, 46).

The anaerobic energy crisis necessitates a blend of optimized ATP production with limited energy consumption. ATP-demanding processes such as DNA synthesis and cell division are curtailed (46), and the production of rRNA is dramatically reduced (36). In *Arabidopsis* and other plants, low-O_2 stress markedly limits protein synthesis but maintains the initiation of translation of a subset of cellular mRNAs, many of which encode enzymes involved in anaerobic metabolism and the amelioration of ROS (16, 36). Therefore, under O_2 deprivation, a mechanism operates that sequesters untranslated mRNAs and lessens ATP expenditure, thereby allowing for the recovery of protein synthesis within minutes of reoxygenation.

Carbohydrate mobilization and sucrose catabolism. The metabolic response to O_2 deprivation is orchestrated by the availability and mobilization of carbohydrates (31, 137). In some plants and tissues, the induction of amylases by low O_2 or flooding promotes the conversion of starch to glucose (**Figure 3**). However, the mobilization of starch during O_2 deprivation is not universal. Both the tubers of potatoes and rhizomes of the flood-tolerant marsh plant *Acorus calamus* L. have considerable carbohydrate reserves, but *Acorus* rhizomes are more capable of mobilizing starch into respirable sugars under anoxia (2). This slow consumption of starch allows the rhizomes to sustain a low level of metabolism that affords survival of long periods of submergence. Seeds of rice, rice weeds (e.g., some *Echinochloa* species), and tubers of *Potamogeton pectinatus* also mobilize starch under anoxia (29, 40, 50). In rice seeds, this starch mobilization requires the depletion of soluble carbohydrates, suggesting regulation by sugar sensing (50, 72). In organs lacking starch reserves or effective starch mobilization, the exhaustion of soluble sugars prior to reoxygenation is likely to result in cell death.

Plants possess two independent routes for the catabolism of sucrose, the bidirectional UDP-dependent sucrose synthase (SUS) and the unidirectional invertase (INV) pathways (**Figure 3**). The net cost for entry into glycolysis is one mol pyrophosphate (PPi) per mol sucrose via the SUS route, if the UTP produced by UDP-glucose pyrophosphorylase (UGPPase) is utilized by fructokinase (FK) in the subsequent conversion of UDP-glucose to glucose-6P or the ATP consumed by FK is recycled by nucleoside diphosphate (NDP) kinase. By contrast, the cost via the INV pathway is two mol ATP per mol sucrose. The SUS route is positively regulated under O_2 deprivation through opposing increases in SUS and the repression of INV gene expression and enzymatic activity (10, 14, 43, 44, 64, 67). The energetic disadvantage of the INV route was confirmed by the inability of transgenic potato tubers with elevated INV activity to maintain ATP levels under 8% O_2 (14). The SUS pathway is enhanced in a variety of species by rapid increases in transcription of *SUS* mRNAs, which is most likely driven by sucrose starvation (64, 71). Other glycolytic reactions may utilize available PPi during O_2 deprivation, thereby improving the net yield of ATP per mol sucrose catabolized. The phosphorylation of fructose-6P to fructose-1,$6P_2$ by the bidirectional PPi-dependent phosphofructokinase (PFP) is favored over the unidirectional ATP-dependent phosphofructokinase (PFK), and a pyruvate Pi dikinase (PPDK) may substitute for cytosolic pyruvate kinase (PK) in O_2-deprived rice seedlings (95).



Metabolic end products. During O_2 deprivation, pyruvate decarboxylase (PDC) converts pyruvate to acetaldehyde, which is metabolized by alcohol dehydrogenase (ADH) to ethanol, with the regeneration of NAD^+ to sustain glycolysis. PDC- and ADH-deficient genotypes confirm the essentiality of ethanolic fermentation in the acclimation to flooding and low-O_2 stress (6, 31, 46, 65). In *Arabidopsis* seedlings, the level of induction of *ADH* is controlled by the activation of a ROP GTPase (5). O_2 deprivation promotes an increase in active ROP, which leads to the elevation of transcripts that encode *ADH* and *ROPGAP4*, a GTPase that inactivates ROP. In a *ropgap4* null mutant, *ADH* mRNA and ROS are significantly elevated under hypoxia, and seedling survival is reduced. This led to the proposal that a ROP rheostat controls the temporal regulation of *ADH* expression under low O_2 (3, 39).

The production of ethanol is benign owing to its rapid diffusion out of cells, whereas the intermediate acetaldehyde is toxic. Acetaldehyde dehydrogenase (ALDH) catalyzes the conversion of acetaldehyde to acetate, with the concomitant reduction of NAD^+ to NADH. A mitochondrial *ALDH* is significantly induced by anoxia in coleoptiles of rice (67, 87), but not in seedlings of *Arabidopsis* (65). ALDH activity correlates with anaerobic germination capability of *Echinochloa crus-galli* under strict anoxia (40). Under O_2-limiting conditions, ALDH consumes NAD^+ and may thereby limit glycolysis, whereas upon reoxygenation acetaldehyde converted to acetate by mitochondrial ALDH enters the tricarboxylic acid (TCA) cycle (**Figure 3**).

In addition to ethanol, lactate is produced in plant cells under O_2 deprivation. The accumulation of lactate under low-O_2 stress has garnered considerable interest (31, 35, 48, 98) ever since the demonstration that its transient appearance precedes that of ethanol in the root tips of maize seedlings (105). The pH of the cytosol of maize root tips declines from 7.5 to a new equilibrium at pH 6.8 following transfer to anoxia. It is posited that the transition from lactic to ethanolic fermentation is controlled by a pH-stat. The ~0.6 unit decrease in cytosolic pH favors the catalytic optimum of PDC and thereby limits lactate and promotes ethanol production. Anoxic ADH-deficient root tips continue to produce lactate and fail to stabilize the cytosolic pH, resulting in rapid cytosolic acidification and cell death (106). Thus, the switch from lactic to ethanolic fermentation is critical for the maintenance of cytosolic pH. An alternative proposal is that this switch, under conditions of O_2 deprivation and in aerobic cells in which ethanol is produced, is driven by a rise in pyruvate rather than the increase in lactate or reduction of cytosolic pH (130). When

Figure 3

Metabolic acclimations under O_2 deprivation. Plants have multiple routes of sucrose catabolism, ATP production, and NAD^+ and $NAD(P)^+$ regeneration. Blue arrows indicate reactions that are promoted during the stress. Metabolites indicated in bold font are major or minor end products of metabolism under hypoxia. Abbreviations are as follows: 2-OGDH, 2-oxyglutarate dehydrogenase; ADH, alcohol dehydrogenase; AlaAT, alanine aminotransferase; ALDH, acetaldehyde dehydrogenase; AspAT, aspartate aminotransferase; CoASH, coenzyme A; CS, citrate synthase; FK, fructokinase; GABA-T, GABA transaminase; GDC, glutamate decarboxylase; GDH, glutamate dehydrogenase; GHBDH, γ-aminobutyrase dehydrogenase; GOGAT, NADPH-dependent glutamine: 2-oxoglutarate aminotransferase; GS, glutamine synthase; HXK, hexokinase; ICDH, isocitrate dehydrogenase; LDH, lactate dehydrogenase; MDH, malate dehydrogenase; NDP kinase, nucleoside diphosphate kinase; NiR, nitrite reductase; NR, nitrate reductase; PCK, phosphenolpyruvate carboxylase kinase; PDC, pyruvate decarboxylase; PDH, pyruvate dehydrogenase; PEPC, phosphenolpyruvate carboxylase; PFK, ATP-dependent phosphofructokinase; PFP, PPi-dependent phosphofructokinase; PGI, phosphoglucoisomerase; PGM, phosphoglucomutase; PK, pyruvate kinase; PPDK, pyruvate Pi dikinase; SDH, succinate dehydrogenase; SSADH, succinate semialdehyde dehydrogenase; Starch Pase, starch phosphorylase; SUS, sucrose synthase; UGPPase, UDP-glucose pyrophosphorylase.

pyruvate levels increase, the low K_m of mitochondrial pyruvate dehydrogenase (PDH) and high K_m of PDC serve to limit carbon entry into the TCA cycle and promote ethanolic fermentation.

Flooding stress is likely to involve a gradual transition from normoxia to hypoxia, allowing cells to initiate processes that favor survival. Plants exposed to a period of hypoxia for 2 to 4 h prior to transfer to an anoxic environment are more capable of avoiding cell death than those that undergo an abrupt anoxic shock (31). The preexposure to 3% or 4% O_2 reduces the severity of ATP depletion, allows the synthesis of stress-induced and normal cellular proteins (19), and activates a lactate efflux mechanism (158). Lactate removal from the cytoplasm may be accomplished by the hypoxia-induced nodulin intrinsic protein (NIP2;1), which was identified in *Arabidopsis* as a plasma membrane–associated protein capable of driving lactate transport in *Xenopus* oocytes (23). Most likely, a decline in cytosolic pH of 0.2 to 0.5 units under O_2 shortfall establishes a new pH set point that influences multiple aspects of metabolism (35, 48, 95). The management of this pH decline involves ethanolic fermentation and is benefited by the availability of a lactate efflux mechanism and proton ATPase activity. However, some species or organ systems, such as the tuber shoots of *Potamogeton pectinatus*, do not show an adjustment in cytosolic pH during O_2 deprivation. The stem elongation in these shoots under anoxia results from cell expansion that occurs in the absence of an adjustment in cytosolic pH and appears to be maintained by tight constraints on ATP production and consumption (29).

Besides the major fermentation end products, lactate and pyruvate, O_2 deficiency is associated with the elevation of alanine, γ-aminobutyric acid (GABA), succinate, and occasionally malate (29, 31, 46, 113, 137, 139). Strong induction of cytosolic and mitochondrial alanine aminotransferase (AlaAT), aspartate aminotransferase, mitochondrial glutamate dehydrogenase (GDH), and mitochondrial Ca^{2+}/calmodulin-regulated glutamate decarboxylase (GDH) mRNA and/or enzymatic activity is consistent with pyruvate conversion to alanine or GABA (**Figure 3**) (63, 67, 70, 71, 100, 139). GABA may be further metabolized via the mitochondrial GABA shunt to γ-hydroxylbutyrate with the regeneration of $NAD(P)^+$ (17). Upon reoxygenation, alanine can be recycled back to pyruvate, and GABA can be converted to succinate. Amino acid oxidation may thereby minimize the decline in cytosolic pH and reduce carbon loss via ethanol or lactate. An appreciation of the relative significance of the major and minor pathways of anaerobic metabolism will require metabolite profiling and flux studies that resolve organ specific and temporal aspects of production in relationship to changes in redox and energy status.

Nitrite, nitric oxide, mitochondria, and hemoglobin. Nitrate and nitrite are also implicated in cellular adjustment to O_2 deprivation. Nitrate is assimilated and reduced to ammonia via nitrate reductase (NR) and nitrite reductase (NiR) (**Figure 3**). *NR* but not *NiR* mRNAs increase significantly in response to hypoxia/anoxia in *Arabidopsis* and rice (67, 70, 71). Even without an increase in NR levels, a reduction of cytosolic pH may increase nitrite production because of the low pH optimum of this enzyme (57). Roots of tobacco plants engineered to have reduced NR levels display several metabolic anomalies under anoxia, including higher levels of soluble hexoses and ATP, enhanced ethanol and lactate production, and increased acidification of the cytosol (125). By contrast, maize seedling roots supplied with nitrate during anoxia maintain a slightly higher cytosolic pH than do control seedlings (69). Notably, the provision of micromolar levels of nitrite to seedling roots had a similar effect on the adjustment of cytosolic pH. This unexpected benefit of low levels of nitrite is unlikely to be due to a direct effect on $NAD(P)^+$ regeneration and may indicate a role of nitrite in a regulatory mechanism that augments homeostasis under low O_2.

A plant-specific association has surfaced between nitrate/nitrite metabolism, mitochondrial ATP synthesis, and a low-O_2-induced nonsymbiotic Class 1 hemoglobin (HB). Plant mitochondria provided with micromolar levels of nitrite under anoxia have the capacity to coordinate the oxidation of NADH and NAD(P)H with low levels of ATP production (124). This nitrite-promoted process involves the evolution of nitric oxide (NO) via a pathway that requires the activity of rotenone-insensitive NAD(P)H dehydrogenases, mtETC Complex III (ubiquinone:cytochrome *c* reductase), and Complex IV (COX). In the proposed pathway (124), NAD(P)H produced during O_2 deficit is oxidized by Ca^{2+}-sensitive NAD(P)H dehydrogenases on the inner mitochondrial membrane surface, providing electrons to the ubiquinone pool. In the absence of O_2, nitrite may serve as an electron acceptor at Complex III or IV, yielding NO, which may activate signal transduction by promoting mitochondrial ROS production and Ca^{2+} release. The cytosolic HB that accumulates under O_2 deprivation, however, scavenges and detoxifies NO in planta by converting it into nitrate in an NAD(P)H-consuming reaction over a broad pH optimum (30, 57). The coupled activities of HB and cytosolic NR regenerate nitrite that may enter the mitochondrion, where it continues the cycle of NO and ATP production (94, 124). A major challenge is to confirm in planta that nitrite conversion to NO functions as a surrogate final electron acceptor. Nonetheless, the scenario is consistent with reports that overexpression of HB in several species decreases rates of ethanolic fermentation, augments ATP maintenance, and fosters NO production under hypoxia. By contrast, the inhibition of HB expression increases NAD(P)H:$NAD(P)^+$ ratios and reduces cytosolic pH (30, 56, 57, 123). Notably, NO inhibits COX activity and thereby reduces ATP production under normoxia. Might NO formed during the transition from normoxia to hypoxia be the factor that dampens O_2 consumption to avoid cellular anoxia (43)? If so, the production of NO prior to the synthesis of HB may allow the cell to transition slowly from normoxia to hypoxia, providing a segue that augments energy management.

THE LOW-OXYGEN ESCAPE SYNDROME

Enhanced Growth Leading to the Emergence of Shoots

Plants forage for limiting resources by adjusting carbon allocation and overall plant architecture such that the capture of resources is consolidated (93, 96). As O_2 and CO_2 become limiting for plants in flood-prone environments, species from widely dispersed families that share the capacity to survive in flood-intense environments initiate signaling pathways that lead to fast extension growth of shoot organs (101, 147). These leaves, when reaching the water surface, function as snorkels to facilitate the entrance of O_2 and the outward ventilation of gases such as ethylene and methane trapped in roots (24, 145). Another benefit of the emergence of leaf blades is a higher rate of carbon gain from aerial photosynthesis (82).

Fast shoot elongation under water is not restricted only to species occurring in environments with periodic floods (e.g., deepwater rice, *Rumex palustris*, *Ranunculus sceleratus*) (61, 148, 150). It persists in true aquatics that develop floating leaves or flowers [e.g., *Nymphoides peltata* (82)] and in species that germinate in anaerobic mud followed by an extension growth phase to reach better-aerated water/air layers (e.g., seedlings of *Oryza sativa*, *Potamogeton pectinatus*, *P. distinctus*) (58, 113, 129). The explored mechanism of shoot elongation in Marsh dock (*R. palustris*) and deepwater rice (92, 115, 149) can be used to shed light on the mechanistic backbone of genetic diversity in flooding-induced shoot elongation.

The shoot elongation response can occur in petioles or internodes, depending on the

developmental stage or predominant growth form of the plant. Interestingly, petiole elongation in rosette plants is accompanied by hyponastic growth that changes the orientation of the petiole from prostrate to erect. This directional growth brings the leaf in such a position that enhanced petiole elongation will result in leaf blade emergence in the shortest possible time. Accordingly, petiole elongation lagged behind hyponastic growth in *R. palustris* rosettes (26).

It is generally accepted that the submergence signal for enhanced shoot elongation is the gaseous phytohormone ethylene (76, 101, 147). Ethylene is biosynthesized via an O_2-dependent pathway, and the endogenous concentration of this hormone is determined predominantly by production rate and outward diffusion. Both aspects are affected by submergence. Several biosynthetic genes [e.g., those encoding ACC synthase (ACS) and ACC oxidase (ACO)] are upregulated by submergence (102, 135, 154), whereas diffusion of ethylene to the outside environment is strongly hampered. As a result, the endogenous concentration rises to a new, higher equilibrium. Ethylene production persists in submerged shoots as O_2 continues to diffuse from the water into the shoot, guaranteeing relatively high endogenous O_2 concentrations in shoot cells even in the dark (80). Submergence or low oxygen also upregulates the expression of ethylene receptor genes, including *RpERS1* in *R. palustris* (155), *OsERL1* in deepwater rice (156), and *ETR2* in *Arabidopsis* (16, 63, 70, 72). An elevation of ethylene receptor levels following submergence is counterintuitive because these molecules are negative regulators of ethylene signaling. However, this increase would allow rapid cessation of ethylene signaling as the plants emerge from the water and vent off the accumulated ethylene.

Ethylene is the input signal for several parallel pathways required for fast elongation under water (**Figure 4**). Under fully submerged conditions the accumulated ethylene downregulates abscisic acid (ABA) levels via an inhibition of 9-*cis*-epoxycarotenoid dioxygenase (NCED) expression, a family of rate-limiting enzymes in ABA biosynthesis that belongs to the carotenoid cleavage dioxygenases, (CCDs) and via an activation of ABA breakdown to phaseic acid (9, 61, 110). The decline of the endogenous ABA concentration in *R. palustris* is required to stimulate the expression of gibberellin (GA) 3-oxidase, an enzyme that catalyzes the conversion to bioactive gibberellin (GA_1) (8), and in deepwater rice to sensitize internodes to GA (61). Downstream of GA, three sets of genes play a role in submergence-induced shoot elongation. The first group encodes proteins involved in cell wall loosening; the second, those involved in the cell cycle; and the third, those involved in starch breakdown. Additional genes with putative regulatory roles in enhanced internode elongation have been identified in flooded deepwater rice (22, 108, 117, 132, 133).

The rigid cell wall constrains the rate and direction of turgor-driven cell growth. Significant increases in acid-induced cell wall extension upon submergence were observed in rice (20), *R. palustris* (152), and *Regnellidium diphyllum* (62). This could be reversed even when *R. palustris* petioles were desubmerged, emphasizing the correlation between extensibility and submergence-induced elongation (152). Cell wall extensibility is thought to be associated with cell-wall-loosening proteins, such as expansins (EXPs) and xyloglucan endotransglycosylase/hydrolases (XTHs) (27). Submergence-induced elongation is strongly correlated with increases in mRNAs encoding expansins A (*EXPA*) and B (*EXPB*), along with EXP protein abundance and activity (21, 62, 68, 89, 152, 153). Interestingly, in some species ethylene directly regulates *EXP* expression (62, 152, 153) (**Figure 4**). In submerged *R. palustris* petioles, ethylene not only enhances *EXP* expression but also stimulates proton efflux into the apoplast (153), which is essential for EXP action.

The second group of GA-regulated genes is involved in cell cycle regulation. In very young petioles of the fringed waterlily (*Nymphoides peltata*) and the youngest

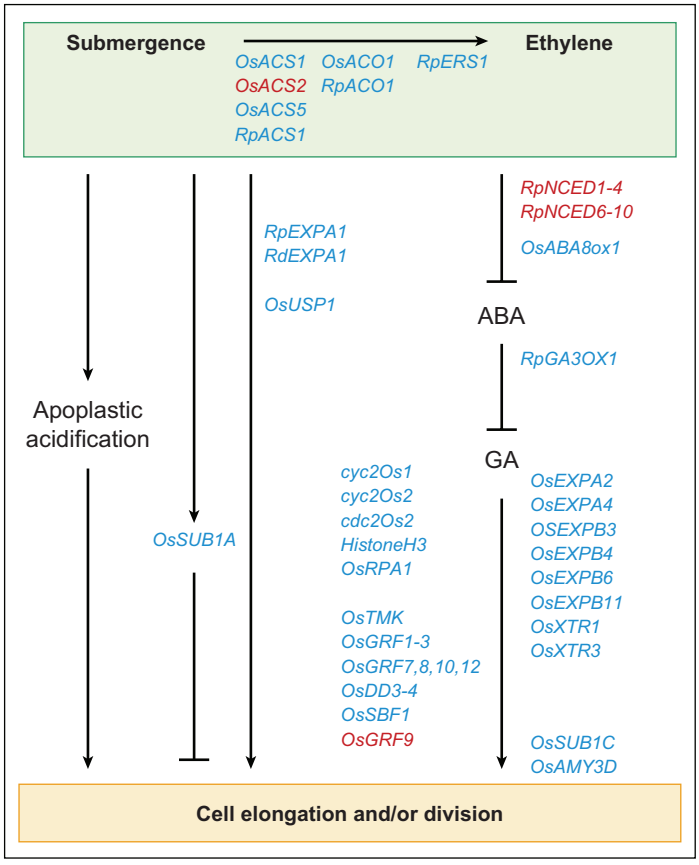


Figure 4

Schematic model of the plant processes, hormones, and genes involved in submergence-induced shoot elongation (*blue* signifies upregulated genes and *red* signifies downregulated genes). Gene abbreviations are as follows: *CYC2Os*, cyclin; *CDC2Os*, cyclin-dependent kinase; *OsACO* and *RpACO*, ACC oxidase; *OsACS* and *RpACS*, ACC synthase; *OsDD*, differentially displayed (61); *OsAMY*, amylase (41); *OsEXP*, *RdEXP*, and *RpEXP*, expansins; *OsGRF*, growth-regulating factor (22); *OsRPA*, replication protein A1; *OsSBF*, sodium/bile acid symporter family (108); *OsSUB1*, submergence1; *OsTMK*, transmembrane protein kinase (133); *OsUSP*, universal stress protein (117); *RpERS1*, ethylene receptor (155); RpNCED, 9-*cis*-epoxycarotenoid dioxygenase; *RpGA3ox*, gibberellin 3-oxidase (8); OsXTR, xyloglucan endotransglucosylase-related (27); OsABA8ox, ABA 8′-hydroxylase (110). Os indicates *Oryza sativa*, Rd indicates *Regnellidium diphyllum*, and Rp indicates *Rumex palustris*.

internode of deepwater rice, ethylene promotes not only cell elongation but also cell division. Consistent with this increase in cell division is the observed upregulation of cyclin (*CYC2Os1*, *CYC2Os2*), cyclin-dependent kinase (*CDC2Os2*), *HistoneH3*, and replication protein A1 (*OsRPA1*) (114, 115, 131).

The third group of GA-regulated genes is involved in starch breakdown. *R. palustris* plants depleted of soluble sugars and starch show a very restricted underwater elongation response (49). Carbohydrates are required to deliver energy and the building blocks for new cell wall synthesis (115, 148). The requirement of carbohydrates can be fulfilled by the translocation of photosynthates and by the degradation of starch reserves via an increase in α-amylase activity (115). Fukao and colleagues (41) reported that α-amylase gene expression (*OsAmy3D*) in leaves of submerged

rice is regulated by SUB1C, an ethylene-responsive factor (ERF)-domain-containing protein of the polygenic *Sub1* locus. This gene is regulated positively by GA and negatively by a related ERF in the *Sub1* locus, *SUB1A-1*, which is present in some rice accessions. These results imply that carbohydrate levels in submerged plants are also under hormonal control.

There is considerable genetic variation between and within species in submergence-induced elongation capacity. The closely related species *Rumex acetosa* and *R. palustris* show inhibition and stimulation of petiole elongation upon exposure to ethylene, respectively. Both accumulate significant amounts of ethylene when submerged (4), but *R. acetosa* lacks ABA downregulation (9), GA upregulation (104), and increased *EXP* expression (153). However, when *R. acetosa* is exposed to elevated GA levels without enhanced ethylene or when ABA levels are reduced with fluridone in submerged plants, petiole elongation is strongly stimulated (9, 104). This demonstrates that signal transduction components required for elongation growth downstream of ABA and GA are present in this species and can be activated. It also shows that in *R. acetosa*, contrary to *R. palustris*, ethylene cannot switch on this cascade. Most likely, elements downstream of ethylene but upstream of ABA/GA explain differences in ethylene-induced elongation between *Rumex* species.

Rice cultivars also show variation in elongation capacity during submergence (28, 41, 120). The *Sub1* locus controls underwater elongation through genetic distinctions in the two to three ERF proteins it encodes (41, 159) (**Figure 2**). *SUB1A-1* is present in the *Sub1* locus only in submergence-tolerant lines and is induced by ethylene. *SUB1C* is present in all rice lines and is induced by GA. The between-cultivar variation in elongation correlates with genotypic variation and expression of ERFs of the *Sub1* locus. The slowly elongating rice varieties of *indica* rapidly and strongly induce *SUB1A-1* upon submergence, whereas all elongating *indica* and *japonica* varieties lack either the *SUB1A* gene or the *SUB1A-1* allele (159). Transformation of an elongating *japonica* variety with a *SUB1A-1* full-length cDNA under the control of the maize *Ubiquitin1* promoter resulted in a significant repression of underwater elongation (159). The expression of *SUB1A-1* coincides with repressed accumulation of transcripts for *EXP*s and reduced expression of *SUB1C* (41), suggesting that SUB1A acts upstream of GA regulation of *EXP*s and *SUB1C*.

Improvement of the Oxygen and Carbohydrate Status in Submerged Plants

At the whole-plant level, complete submergence leads to a dramatic shift in the carbon budget and energy status, potentially resulting in death. Some relief of this problem, with the leaves still submerged, is underwater photosynthesis (83). The significance of this was exposed by studies showing that light availability enhances survival under water in both flood-tolerant and intolerant species (55, 81, 86, 141) and that O_2 levels in submerged plants are affected by light intensity (90). Improved survival of submergence in the light is correlated with a higher carbohydrate status (97) and internal O_2 concentrations (80, 84, 103). However, underwater photosynthesis can be limited by low light and CO_2 availability. Consistent with these findings are studies showing that illumination can maintain sugar transport and leaf ATP content at near-normoxic levels under strict O_2 deprivation in rice and wheat leaves (85).

True aquatics develop specialized leaves characterized by an overall thin leaf and cuticle, a high degree of dissection, and epidermal cell chloroplasts. These traits reduce the diffusion barriers and shorten the diffusion pathways, thus enhancing carbon input per leaf area and unit time (111). Other strategies, developed by true aquatics to enhance carbon gain, are the utilization of HCO_3 as carbon source, C4 or CAM metabolism, or hydrosoil CO_2 consumption

(75, 83). Very little information is available about the occurrence of these last strategies in terrestrial plants from flood-prone environments.

Leaf acclimations to submergence have been characterized for *R. palustris* (82) and other amphibious species (18, 37, 157). Leaves developed under water are 20% thinner with an increased specific leaf area (SLA) ($m^2\ g^{-1}$), indicating a large surface area relative to mass. The higher SLA is related not only to the lower leaf thickness of aquatic leaves but also to their tenfold-lower starch content. Furthermore, aquatic leaves have thinner epidermal cell walls and cuticles, and their chloroplasts lie close to the epidermis rather than toward the intercellular spaces as is typical for aerial leaves (82). These acclimations are consistent with the view that CO_2 directly enters the mesophyll cells of these leaves via diffusion through the epidermis and not via stomata and intercellular gas diffusion. This diffusion pathway under water has a much higher diffusion resistance for gases than does intercellular diffusion. Calculations for *R. palustris* indicate a 15,000-fold-higher resistance to CO_2 diffusion in leaves under submergence than when in air (81). However, the morphological and anatomical changes decrease gas diffusion resistance for CO_2 (38). In *R. palustris* these acclimations result in a dramatic reduction of the diffusion resistance between submerged leaves and leaves in air to a factor of less than 400 (81). Functional consequences of these acclimations in *R. palustris* include higher rates of net underwater assimilation and lower CO_2 compensation points (81). Similar effects are also described for amphibious species (13, 55, 140). The relatively low diffusion resistance in aquatic leaves also permits increased inward diffusion of O_2 from the water layer into the shoot. This results, in the dark, in an internal O_2 concentration of 17% in acclimated petioles of *R. palustris* when submerged in air-saturated water, whereas nonacclimated petioles reach only 9% (80).

These observations demonstrate that the water column can function as an important source of O_2 for terrestrial plants when they are exposed to submergence and that O_2 levels in leaves, stems, and petioles below the critical O_2 pressure (0.8%; 31) are rare and probably restricted to densely packed tissues or to aquatic environments that are extremely stagnant or have low O_2 levels. Although root systems will likely benefit from these shoot acclimations, O_2 pressures in the roots will still be much lower than the values mentioned here for shoots, especially at night, when there is no photosynthesis (90). It is therefore expected that even with LOES acclimations, roots will also rely on the metabolic cellular adjustments to O_2 deprivation for survival.

Plants in frequently flooded environments are expected to display these traits at a higher frequency than do those in rarely flooded areas. Consistently, *Ranunculus repens* populations in temporary lakes are characterized by constitutively dissected leaves. This morphology allows for a relatively large leaf surface and an improved gas exchange and results in relatively high rates of underwater photosynthesis. Plants from more terrestrial populations have less-dissected leaves and relatively low rates of underwater photosynthesis (73, 74). However, a comparative study of nine species, both flooding tolerant and intolerant, showed that gas exchange acclimations under water are not restricted to flood-tolerant species (84). In this study all but one species developed aquatic leaves that were thinner and had thinner outer cell walls and cuticles and a higher SLA. These responses were independent of the species' flooding tolerances. Furthermore, leaf plasticity upon submergence resulted in increased O_2 levels in all species. Therefore, between-species variation in inducible leaf acclimations in terrestrial plants, to optimize gas exchange when submerged, is not related to the variation in flooding tolerance of the species investigated (84). This conclusion hints toward a limited role of submergence signals, such as elevated ethylene, in inducing leaf acclimations that enhance gas exchange under water. Plants that are not exposed to flooding

throughout their life are not expected to use these signals to switch on signaling cascades that lead to altered leaf anatomy and morphology. More likely, signals associated with changed rates of photosynthesis and/or reduced levels of carbohydrates induce these leaf acclimations. This hypothesis is consistent with observations that shade-acclimated plants with reduced rates of photosynthesis develop thinner leaves with higher SLA (78). Consistently, transgenic tobacco plants with substantially reduced Rubisco levels have reduced photosynthesis and increased SLA (34).

Improvement of Internal Gas Diffusion: Aerenchyma

Important traits for survival in flooded environments are those that reduce the resistance for diffusion of O_2 and CO_2 from the environment to the plant. Equally significant, however, is the resistance that hampers gas diffusion within organs. Fast gas diffusion can be accomplished only in a gaseous diffusion medium, over short distances, by limited loss of the gas along the diffusion path, and by restricted tortuosity of the diffusion route. These requirements are met in aerenchymatous tissue, characterized by longitudinally interconnected gas spaces in roots and shoots. Aerenchyma is either constitutively present and/or induced upon flooding (116, 144) and develops in existing tissues or concomitant with the development of new roots (32). Distinct physiological processes are at the basis of aerenchyma formation. This led to the discrimination of two aerenchyma types: (*a*) lysigenous aerenchyma formed by cell death and (*b*) schizogenous aerenchyma in which gas spaces develop through the separation of previously connected cells (23, 33, 60). A third type, termed expansigenous aerenchyma, is characterized by intercellular gas spaces that develop through cell division and cell enlargement, without cell separation or collapse/death (118). Combinations of these aerenchyma types also exist (118), and within one plant species different types can be present in different organs (32, 33).

The mechanism of schizogenous aerenchyma formation is largely unknown as compared with that of lysigenous aerenchyma. Low O_2 and elevated ethylene can induce lysigenous aerenchyma development in roots of maize in a manner that is phenotypically similar to the process promoted by flooding (32). Under flooded conditions, subambient O_2 concentrations stimulate the production of ethylene, which accumulates in roots surrounded by water and induces programmed cell death (PCD) in the cortex tissue (53). Accordingly, hypoxic roots, exposed to inhibitors of ethylene biosynthesis or action, form no gas spaces (53). Downstream components of this regulatory route include protein kinases, protein phosphatases, G proteins, Ca^{2+}, and inositol phospholipids (54). The targets of these signaling routes include proteins associated with cell wall breakdown. The activity of cellulase increases in roots upon exposure to low O_2 or ethylene (52). Furthermore, increases in pectinase and xylanase activity (15) and the induction of *XTH* mRNAs occur in diverse species in response to flooding or hypoxia (67, 70, 109).

Large data collections are available on genetic diversity in traits that contribute to the delivery of O_2 to root tips. Justin & Armstrong (60) compared 91 species from wetland, intermediate, and nonwetland habitats. Nearly all the species from nonwetland environments had low root porosities, whereas high constitutive and increased porosities upon flooding were associated with species from wetland environments. Also, other studies confirmed the strong correlation between high root porosities and occurrence in wet environments (45, 77, 143). Interestingly, a comparative study on 35 wild *Hordeum* accessions from environments that differ in flooding intensity showed that this correlation does not always exist and that aerenchyma development can be constrained by phylogeny (42).

Aerenchyma is also formed in shoot organs, providing a system of interconnected

channels from leaf to root tip. In a study with 14 species divided over seven families, the aerenchyma content of petioles strongly correlated with plant survival during complete submergence. This robust correlation persisted in environmental conditions with (light) and without (dark) underwater photosynthesis (79, 84). These observations suggest that aerenchyma is important not only for survival during partial flooding but also during complete submergence. Petiole aerenchyma likely facilitates the diffusion of O_2 from shoot organs to the roots. The O_2 involved can be photosynthetically derived during the light period or obtained from the water layer by the shoot during the dark.

CONCLUSIONS AND FUTURE PERSPECTIVES

The growing understanding of the molecular basis and genetic diversity in submergence and flooding acclimations provides opportunities to breed and engineer crops tolerant of these conditions that would benefit the world's farmers. The evaluation of diversity exposes plasticity in metabolic and developmental acclimations that enable distinct strategies that increase fitness in a flooded environment. Natural variation in acclimation schemes provides opportunities for development of crops with combinations of submergence tolerance traits that are optimal at specific developmental stages and under particular flooding regimes, which vary substantially worldwide. The first example of this is the use of marker-assisted breeding to introduce the submergence-tolerance conferring *Sub1* genotype to selected rice cultivars (159), which may appreciably benefit rice production in flood-prone lands in the Third World. The further exploration of the molecular basis of genetic diversity in flooding tolerances is critical given the global climate change scenarios that predict heavy precipitation in regions of our planet.

SUMMARY POINTS

1. Evaluation of diversity exposes the remarkable plasticity in metabolic and developmental acclimations that enable increased fitness in a flooded environment.
2. Plants employing an escape strategy develop a suite of traits collectively called the low-oxygen escape syndrome (LOES).
3. A consequence of low-O_2 stress is a requirement for energy conservation that is invoked through adjustments in gene expression, carbohydrate catabolism, $NAD(P)^+$ regeneration, and ATP production.
4. Energy conservation is influenced by a low-O_2-induced nonsymbiotic hemoglobin that regulates cytosolic and mitochondrial processes, including rates of fermentation, NO, and ATP production.
5. Enhanced shoot elongation upon submergence requires the action of at least three hormones (ethylene, ABA, and GA) that regulate processes such as apoplastic acidification, cell wall loosening, cell division, and starch breakdown.
6. Anatomical and biochemical leaf acclimations upon submergence facilitate underwater photosynthesis as well as the inward diffusion of O_2 from the floodwater.
7. Aerenchyma in root and shoot tissue not only is important for survival during partial submergence but also facilitates O_2 diffusion from shoot to root while the plant is completely submerged.

FUTURE ISSUES

1. Plant species differ in their growth response to ethylene during submergence. This is far from understood but probably involves signal transduction components upstream of ABA and GA. The characterization of *SUB1A* is an important finding in this respect. More work is needed because this is probably an important selection point to differentiate survival strategies.
2. A quiescence strategy (carbohydrate conservation) in rice is associated with submergence tolerance. However, not all plants that fail to elongate under water are tolerant. The question arises as to whether cells of these plants are metabolically inactive or simply lack other aspects also needed for tolerance (e.g., the ability to manage ATP, cytosolic pH, or cellular O_2 content; protection against ROS; or aerenchyma development).
3. Characterization of the *Sub1* locus provides an opportunity to breed or engineer submergence-tolerant rice that could benefit farmers in flood-prone areas. Studies are needed to determine if submergence and salt tolerance can be combined because floodwaters can be saline.
4. The development of rice cultivars with improved underwater germination and low-O_2 escape capabilities may reduce herbicide use.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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159. Landmark map-based cloning of a polygenic locus that encodes a determinant of submergence tolerance in rice.

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