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# Life in the balance: a signaling network controlling survival of flooding

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Recent reports on responses to flooding, submergence, and low-oxygen stress have connected components in an essential regulatory network that underlies plasticity in growth and metabolism essential for the survival of distinct flooding regimes. Here, we discuss growth under severe oxygen-limited conditions (anaerobic growth) and less oxygen-deficient underwater conditions (ethylene-driven underwater growth). Low-oxygen stress causes an energy and carbohydrate crisis that must be controlled through regulated consumption of carbohydrates and energy reserves. In rice (*Oryza sativa* L.), low-oxygen stress, energy homeostasis and growth are connected by a calcineurin B-like interacting binding kinase (CIPK) in seeds germinated under water. In shoots, two opposing adaptive strategies to submergence, elongation (escape) and inhibition of elongation (quiescence), are controlled by related ethylene response factor (ERF) DNA binding proteins that act downstream of ethylene and modulate gibberellin-mediated shoot growth. Increased resolution of the flooding signaling network will require more precise investigation of the interactions between oxygen tension and cellular energy status in regulation of anaerobic metabolism and ethylene-driven growth, both essential to survival in variable flooding environments.

## Addresses

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production, particularly rice, in flood-prone regions. Major steps toward breeding tolerant varieties have been made through the characterization of two multigenic loci that control the capacity to endure complete submergence (*SUBMERGENCE 1*, *SUB1*) or rapid outgrowth of adverse partial submergence (*SNORKEL*, *SK*) [2<sup>••</sup>,3<sup>••</sup>,4<sup>••</sup>,5<sup>••</sup>]. New *SUB1* rice varieties, produced by marker-assisted breeding, are high yielding even following two weeks of complete submergence [6<sup>•</sup>,7]. Similarly, better yields of deepwater rice are anticipated, with other improvements to follow [8]. For example, rice and some other species have the remarkable capacity to germinate and elongate coleoptiles and stems under severe oxygen constraints [9,10] (i.e. under anoxia, See glossary). The recognition of rice CIPK15 as a regulator of underwater germination and early shoot elongation [11<sup>••</sup>] provides a critical link between sugar sensing, starch utilization and coleoptile elongation under anoxia. The identification CIPK15 and other genes that regulate underwater seed establishment may enable breeding to reduce the practice of seedling transplantation and overall herbicide use.

These findings in rice complement data from other genera (i.e. *Arabidopsis*, *Lotus*, *Poplar*, *Potamogeton*, and *Rumex*) suggesting there exists a conserved flooding response network in plants that includes ethylene-triggered alterations in gene expression leading to growth and stress-induced catabolism of stored or soluble carbohydrates for energy-efficient production and utilization of ATP. A key challenge is to decipher the interplay between hormones (i.e. ethylene, abscisic acid (ABA), and gibberellic acids (GA)), oxygen availability, and specific metabolites (i.e. ATP, sugars, and pyruvate) that drives a dynamic network balancing growth and quiescence to facilitate survival (Figure 1).

## Ethylene-controlled growth

Recently, a quantitative trait locus was discovered encoding two genes that trigger rapid internode elongation in rice varieties when cultivated under partially submerged 'deepwater' conditions. These genes, *SNORKEL1* (*SK1*) and *SNORKEL2* (*SK2*) encode nuclear-localized DNA binding proteins with a single APETALA2/ethylene response factor (ERF) domain [2<sup>••</sup>,12]. The *SKs* are absent in non-deepwater varieties, including all *japonicas*; however when backcrossed into a *japonica*, 30% of the internode elongation capacity of the deepwater variety was transferred. Moreover, constitutive overexpression of *SK1* or *SK2* in a *japonica* increases the number of elongated internodes, even under non-submerged

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## Introduction

Global warming is associated with increased flooding events adversarial to most plant species and thus affects both crop yield and plant distribution in natural ecosystems [1]. There is an urgent need to increase crop

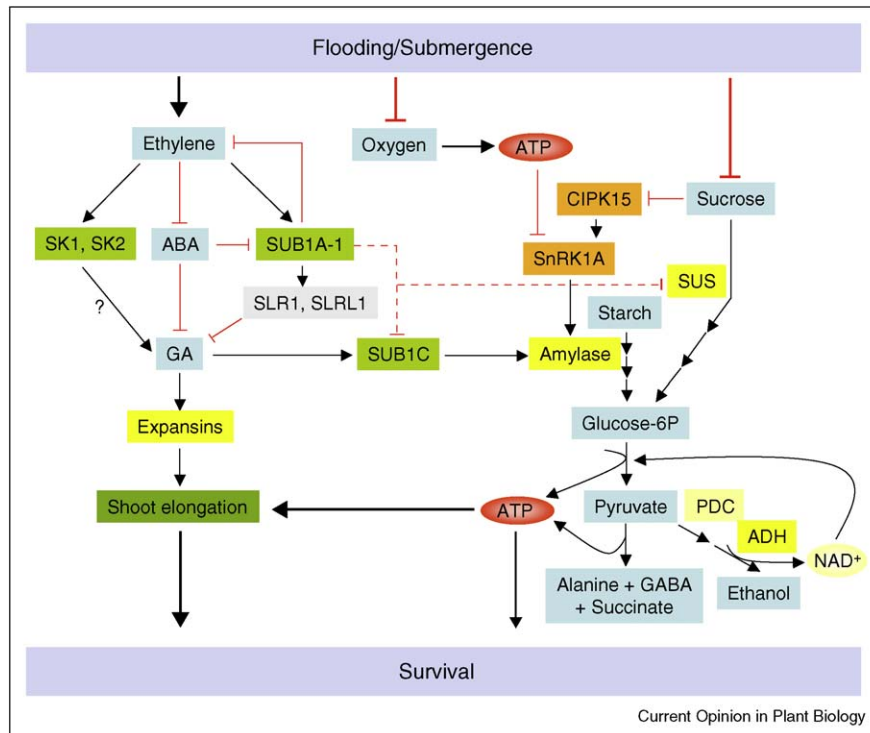
**Glossary**

**anaerobic growth:** growth in the absence of oxygen  
**underwater growth:** growth of tissues under water, often under conditions of reduced oxygen availability  
**normoxia:** oxygen levels at 1 atm, typically 20.6%  
**anoxia:** no oxygen available  
**hypoxia:** oxygen levels below the critical oxygen pressure for mitochondrial oxidative phosphorylation for the cell (or organ)

conditions. The evaluation of the *SK* locus in *Oryza* species with a range of deepwater elongation capabilities, *O. rufipogon*, *O. nivara*, and *O. glumaepatula*, further substantiates the relevance of this locus in underwater elongation. *SK1* and *SK2* are upregulated by the sub-

mergence-induced accumulation of ethylene in internodes, consistent with the essential role of ethylene in GA-stimulated underwater shoot elongation [13]. Although ABA [14] and auxin (i.e. indoleacetic acid) [15] also have regulatory roles in the shoot elongation response, they do not regulate *SK1* or *SK2* transcript levels.

The presence or absence of submergence-induced shoot elongation relates to the selection pressures of different flooding regimes. Long lasting, relatively shallow floods strongly favor enhanced underwater elongation, whereas short, deep floods restrict underwater elongation and thus

**Figure 1**

Overview of the submergence regulatory network in rice. The network involves three key factors: increased cellular ethylene content, depletion of ATP and consumption of readily available sucrose reserves. Submergence of aerial organs results in an accumulation of ethylene that triggers GA-promoted cell elongation. In deepwater rice, ethylene promotes induction of the ERF genes *SK1* and *SK2* and elevation of GA, driving rapid internode elongation and escape of leaves near the water surface [2\*\*]. In lines tolerant of deep submergence, ethylene activates the ERF *SUB1A-1*, which promotes a rise in *SLR1* and *SLRL1*, two transcription factors that directly inhibit GA-mediated activation of gene expression. This enhances survival by a quiescence strategy that limits carbohydrate consumption and elongation growth [3\*\*,5\*\*]. In japonica rice, which has neither *SK1/2* nor *SUB1A-1*, submergence promotes underwater elongation of shoots until energy reserves are exhausted. Ethylene-triggered submergence-induced GA-responsiveness promotes expression of *SUB1C*, which acts upstream of a subset of  $\alpha$ -amylases that convert starch into glucose for ATP production. Submergence includes the deprivation of oxygen, which leads to a deficiency in ATP. Under low-oxygen stress, ATP is produced at the substrate-level via glycolysis coupled with  $NAD^+$  regeneration through ethanolic fermentation [1]. The production of alanine, GABA and succinate also contribute to ATP production [1,41]. In seeds germinated under anoxia or submergence, a signal transduction cascade is initiated by a depletion of sucrose that activates CIPK15, which activates the energy sensor SnRK1A, which turns-on a transcription complex required for synthesis of  $\alpha$ -amylase mRNA [11\*\*]. Genotypes that grow vigorously underwater from seed or as plants, consume starch reserves to generate sufficient ATP for growth at the cost of maintaining cellular homeostasis for a longer period. Dashed lines indicate limited production or partial inhibition. Red lines indicate inhibition, whereas black lines with arrowheads indicate stimulation. Aerenchyma development, underwater photosynthesis and reactive oxygen species management also contribute to underwater growth and survival. Abbreviations: ABA, abscisic acid; ADH, alcohol dehydrogenase; CIPK, calcineurin B-like interacting binding kinase; GA, gibberillic acid; PDC, pyruvate decarboxylase, SK, SNORKEL, an ethylene responsive factor DNA binding protein; SnRK1, sucrose non-fermenting receptor kinase 1A; SUB1A-1, Submergence 1A-1, the product of an allele of the ethylene responsive factor DNA binding protein gene *SUB1A*; SUS, Sucrose synthase.

conserve carbohydrates to facilitate survival under water and re-growth when flood water subsides [16,17\*\*]. A major advance in understanding this growth inhibition was the characterization of the rice *SUB1* [4\*\*]. Depending on the accession, rice contains two or three genes at this locus (*SUB1A*, *SUB1B*, and *SUB1C*) belonging to the same ERF group VII subfamily as the *SKs* [2\*\*,4\*\*,12]. Tolerance of submergence in the absence of an escape response correlates with the presence and pronounced submergence-induced expression of *SUB1A-1*, which confers submergence tolerance when overexpressed in an intolerant *japonica* [4\*\*]. *SUB1A-1* limits ethylene-driven shoot elongation by minimizing the decline in the GA-signaling repressor SLENDER RICE-1 (SLR1) and the related SLR LIKE-1 (SLRL1) proteins in submerged shoots [5\*\*]. Furthermore, *SUB1A-1* reduces submergence-induced synthesis of ethylene, expression of cell wall loosening expansin mRNAs, and starch and sucrose reserve depletion [3\*\*]. Recent transcriptome profiling confirms that *SUB1A-1* regulates multiple pathways associated with growth, metabolism and stress endurance [18\*]. Altogether, this gene's presence in specific rice accessions minimizes energy utilization during submergence to prolong underwater survival.

Natural variation in ethylene-driven submergence-induced shoot elongation is also observed in other semi-wetland plants. In the wild species *Rumex palustris*, this natural variation includes both distinctions in final petiole length following submergence and the timing of elongation [16]. Recently, Manzur *et al.* [19] presented evidence that shoots of *Lotus tenuis* elongate upon partial shoot submergence, but not when the entire shoot is submerged; thus, both antithetical escape and quiescence survival strategies can exist within a single species. This could reflect the requirement for a threshold in ethylene or another metabolite to be reached to trigger quiescence.

Under water shoot elongation acts synergistically with other leaf traits such as aerenchyma development and efficient underwater photosynthesis [14]. Pierik *et al.* [20] showed that partial de-submergence leads to an increase in biomass in *R. palustris*, which displays submergence escape, but not in *R. acetosa* which invokes quiescence. The drawback of extending leaves above water in *R. acetosa* was associated with low petiole porosity, highlighting interdependency between adaptive traits. Another collaboration between traits is observed in shoots that elongate underwater toward better illumination and ultimately, the water surface. Improvements in carbon gain needed to sustain elongation growth are achieved near the water surface where higher light levels act synergistically with traits that improve gas exchange (e.g. thin leaves, thin cuticles, thin cell walls, mesophyll chloroplasts that re-orientate toward the epidermis [21], and leaf surface gas films [22\*\*]).

## Anaerobic germination and early shoot elongation

Rice produces one of the few plant seeds that can germinate under strict anoxia. This so-called anaerobic germination and early growth capacity is accomplished by harnessing reserves to fuel shoot elongation at the expense of root development [10]. This growth strategy ultimately mediates oxygen diffusion via a porous coleoptile/stem to submerged tissues and promotes the transition to autotrophic growth. Work on several species has shown that coleoptile elongation under anoxia or severe hypoxia involves accelerated glycolysis (Pasteur Effect), intensive ethanolic fermentation, limited cytoplasmic acidification, and regulation of cell wall loosening proteins [10,23]. Under anoxia, this elongation growth is not driven by ethylene, which requires oxygen for its biosynthesis [23].

Recently, rice's capacity to germinate and extend its coleoptile under water was shown to involve sensing of cellular energy resources, most likely ATP or soluble sugars [11\*\*]. It requires SnRK1s, the plant's Snf1/AMP kinases, shown to sense and adjust cellular homeostasis in response to limitations in cellular energy imposed by hypoxia in mesophyll protoplasts of *Arabidopsis* [24\*\*] and CIPK15, which phosphorylates SnRK1A, thereby activating the transcriptional activator MYBS1, which initiates production of  $\alpha$ -amylases responsible for starch catabolism to glucose. Seeds with a *cipk15* loss-of-function mutation germinate in air but not underwater unless provided sucrose. This implies that a change in energy homeostasis, most likely depletion of sugars, drives consumption of endosperm reserves for shoot growth. Indeed, rice cultivars with early and vigorous coleoptile underwater display higher amylase activity [25]. Importantly, CIPKs integrate calcium signaling, long considered critical in the low-oxygen-signaling network of plants [26].

The data indicate that the catabolism of starch during anaerobic germination and in submerged shoots of established plants is triggered by distinct mechanisms. In the case of anaerobic germination, an energy deficiency promotes activation of  $\alpha$ -amylase transcription. By contrast in submerged plants, ethylene-promoted GA-responsiveness regulates  $\alpha$ -amylase expression. If the signaling that drives anaerobic germination and early shoot elongation and *SUB1A*-mediated submergence tolerance are indeed independent, then it should be feasible to combine these traits to benefit farmers.

## Adaptive energy management

The reduced efficiency in ATP synthesized per mol of glucose during oxygen deprivation leads to a cellular energy crisis. Plants generally respond by elevating sucrose catabolism, glycolysis and ethanolic fermentation to increase substrate-level production of ATP [1]. Transcriptomic and

metabolomic studies of diverse angiosperms report the elevation of mRNAs and enzymes that enable sucrose catabolism and the fermentation of pyruvate to ethanol as well as lactate, alanine and GABA during oxygen deprivation [18<sup>•</sup>,24<sup>••</sup>,27–34,35<sup>••</sup>,36<sup>••</sup>,37–40]. Anaerobic substrate-level ATP production may be further augmented through alanine metabolism to succinate via a bifurcation of the TCA pathway [41]. Plant cells also limit ATP consumption to cope with the low-oxygen energy crisis, by inducing transcripts encoding enzymes that utilize pyrophosphate (PPi) rather than ATP, particularly in low-oxygen-tolerant species such as rice [42,40], and restricting translation to a minority of cellular mRNAs [32]. Energy is also conserved through reduced expenditure on biosynthetic processes such as ribosome biogenesis and cell wall formation. As in fungi and animals, plants elevate mRNAs encoding proteins involved in metabolite transport, ROS amelioration, and chaperone activity [40], which provide adaptive and protective functions. Together, these studies emphasize that the hallmark response to oxygen deprivation is increased management of ATP production and use.

Oxygen availability varies in organs and cells both during normal development and periods of external oxygen deprivation because of diffusion barriers and distinctions in metabolic activities. Consistent with this, electrode measurements have resolved oxygen gradients across roots, tubers, and stems [43–45]. This heterogeneity within organs raises an important question relative to the report that respiration is dampened in an accelerated manner in pea roots as external oxygen concentration falls below 4% [45]. This is unexpected because it exceeds the oxygen level necessary to saturate mitochondrial cytochrome *c* oxidase (COX), which is estimated to be less than 1% based on the  $K_m$  of COX in isolated soybean mitochondria (140 nM [0.13%] O<sub>2</sub>) [46]. In cells progressively deprived of oxygen, an accelerated decline in respiration at concentrations below 4% would be adaptive, as this would delay cellular anoxia. Such an adaptive mechanism is not unprecedented; the  $V_{max}$  of animal COX decreases by ~50% as a result of progressive oxygen depletion [47]. But these findings emphasize a need to further resolve interactions between mitochondrial respiration, ATP availability and metabolite dynamics in individual cells at oxygen levels that do not limit COX activity. This challenge might be met by the implementation of molecular probes, such as modified green fluorescent proteins, that can report dynamics in redox, specific metabolites, calcium and pH in the cytosol or within mitochondria [48,49]. Combining cell-based probes with genetic mutants should provide the resolution needed to reach a consensus on whether adaptive energy management involves the dampening of oxygen consumption to delay the onset of anoxia.

### Conclusion and perspectives

The depth, duration, frequency and seasonal timing of floods impose distinct selection pressures on adaptive

traits that enable survival of flooding [14]. Recent advances have provided new insights regarding regulation in three interacting networks of response: firstly, ethylene-driven shoot elongation; secondly, anaerobic seed germination and coleoptile growth; and thirdly, maximized conservation of carbohydrates and energy when oxidative phosphorylation is limited. Natural selection favors traits in particular environments when benefits outweigh costs. Shoot elongation is therefore mainly relevant in relatively shallow but prolonged floods, whereas mobilization of seed reserves for rapid shoot elongation aids establishment of seeds buried in anaerobic mud. When either of these two energy-demanding escape strategies is too costly, such as in environments with ephemeral and/or very deep floods, the energy conserving quiescence strategy proves more effective. A general network describing the key submergence-induced pathways in rice is presented in Figure 1. We hypothesize that the essential components — hormones, starch degradation enzymes, fermentation enzymes, and growth machinery — are conserved. It is the flooding regime that determines whether allocation of energy to growth is selected as a survival strategy, as exemplified by evolution within the *Oryza* species of ERF subgroup VII transcription factors that either inhibit (*SUB1A*) or stimulate elongation growth (*SK1,2*) [2<sup>••</sup>,4<sup>••</sup>,50]. Group VII ERFs also contribute to anaerobic gene regulation in *Arabidopsis* [51], leading to the hypothesis that duplication and divergence of group VII ERFs may underlie distinctions in flooding responses in multiple species. Given that the escape versus quiescence strategy of rice varieties reflects alterations in ethylene-driven GA-signaling [5<sup>••</sup>,8], it stands to reason that variation in underwater elongation growth in other species stems from factors that regulate the conserved ethylene, ABA, and GA hierarchy.

Our current challenges include elucidating downstream targets of relevant transcription factors and unraveling essential cell to whole-plant survival strategies. Additionally, it is of utmost importance to precisely link dynamics in oxygen and ATP to metabolic adjustments and survival strategies. The molecular characterization of genetic variation in flooding response strategies is guaranteed to further enable the breeding of crops that can endure or outgrow flooding, as achieved for rice [2<sup>••</sup>,4<sup>••</sup>,6<sup>••</sup>,7]. This is not only essential for crops cultivated in flood-prone farmlands, but is generally relevant because hypoxia exists also in bulky tubers, meristems, and maturing seeds.

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