Trends in Plant Science

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Review

After The Deluge: Plant Revival Post-Flooding

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Increasing flooding events have detrimentally impacted food security amid a growing global population. Complete submergence of plants represents the most severe flooding stress and studies have identified underwater responses to low oxygen and light availability. However, knowledge on plant responses during the post-submergence phase is limited. It is important to consider how plants can resume vegetative growth after enduring submergence and post-submergence stress. This review highlights current knowledge on physiological and molecular adaptations following desubmergence. Interplays of reactive oxygen species (ROS), energy depletion, photoinhibition, desiccation stress, and hormonal signaling have been characterized as components of the post-submergence stress response. Active elucidation of key genes and traits enhancing post-submergence adaptations is highly relevant for the improvement of submergence tolerance and ultimately crop yield.

Highlights

Recovery of submergence is critical to the flooding tolerance of nonaquatic plants.

Post-submergence phase challenges include energy deprivation, oxidative stress, dehydration, photoinhibition, and premature senescence.

There is genetic variation in post-submergence recovery.

Post-submergence stress management strategies include limiting oxidative stress through antioxidant activity and other means, photosynthesis recovery, control of water loss, and other metabolic acclimations.

Hormones coregulate recovery responses but details of their interactions are underexplored.

The Ebb and Flow of the Deluge: Flooding Is a Sequential Stress

Changing global precipitation patterns due to a warming climate include exponential increases in intense rainfall leading to flooding. Flooding events are projected to further rise in the coming decades with calamitous consequences for crop production and food security [1]. Besides rice (*Oryza sativa*), major crops are extremely vulnerable to flooding, resulting in serious yield declines. Floods vary in terms of depth, duration, and type of water (freshwater, saline, or stagnant). This review focuses on transient floods involving complete plant submergence followed by desubmergence.

Submergence of the shoot together with the root compromises aerobic respiration and photosynthesis due to low light availability in murky or deep floodwaters and restricted underwater gas diffusion [2,3]. As oxygen levels drop, metabolic alterations fostering anaerobic ATP production via substrate-level phosphorylation are required to maintain transmembrane potentials for cell survival [4]. This inefficient mode of energy generation coupled with reduced photosynthesis causes an energy crisis due to insufficient carbohydrate reserves. Studies have uncovered a regulatory web of flood-generated signals such as ethylene, hypoxia, **reactive oxygen species** (**ROS**; see Glossary), and transcriptional and hormonal regulators controlling acclimative metabolic responses as well as morphological adaptations increasing the likelihood of submergence survival. The core anaerobic response is largely under the control of the ethylene response factor group VII (ERFVII) transcription factor family [5–7]. Hypoxic stabilization of these transcription factors results in the downstream activation of essential anaerobic response genes via binding to hypoxia response promoter elements [8]. On reoxygenation, degradation of these oxygen-labile transcription factors via the N-end rule pathway of proteolytic degradation switches off the anaerobic response mode.

While the flooding phase is stressful and can be lethal, the post-submergence phase initiated as floodwaters retreat can be equally injurious. Flooding can be regarded as a sequential stress

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with the submergence and post-submergence phases presenting unique stressors. Aerial tissues acclimated to low light and oxygen conditions during prolonged submergence often show further rapid deterioration following desubmergence. Returning to atmospheric oxygen and light conditions can trigger molecular changes damaging plant tissues (reviewed in [9]). Despite widespread observations of post-submergence injury, submergence tolerance has been predominantly linked to survival of the inundation. Tolerance was often allied to performance at the end of a recovery period, making it challenging to untangle the effects of the distinct stress periods. Submergence-tolerant Arabidopsis accessions and rice carrying the *SUBMERGENCE 1A-1 (SUB1A-1)*tolerance allele share a number of post-submergence acclimation strategies (Figure 1). Here we survey post-submergence stress processes and the orchestration of their management that can enhance submergence survival.

After the Deluge: Multiple Challenges of Desubmergence

Reoxygenation Stress

As floodwaters recede, oxygen reenters tissues adjusted to a hypoxic environment. Post-anoxic injury defines damage caused by sudden oxygen re-exposure following prolonged oxygen deprivation and is attributed primarily to excessive ROS formation [10,11]. ROS act in signaling but are also extremely destructive towards macromolecules and cell membranes and often kill affected cells.

ROS are normal byproducts of aerobic metabolism formed by stepwise electron reduction of molecular oxygen [12] mainly produced in organelles with highly oxidizing metabolic activity (i.e., chloroplasts) or high electron flow rates (i.e., mitochondria) (Box 1). Cellular ROS levels are balanced by sophisticated **antioxidant** systems [13] (Box 1). Environmental stresses can skew this balance, leading to '**oxidative stress**'. Prolonged hypoxia during submergence can create low energy-charge values, high reducing-equivalent levels, and saturated electron-transport components. Hypoxia acclimation includes reorganization of the electron-transport chain complexes of the inner mitochondrial membrane so Complex I is separated from Complex III and IV. This configuration is associated with greater ROS production in the mitochondrial matrix in other eukaryotes and is reversed on reaeration in plants [14].

Upon desubmergence of tissues, oxygen reentry provides suitable conditions to generate excessive ROS through multiple pathways. Rapidly reactivated mitochondrial and photosynthetic activities cause electron leakage in electron-transport chains and membrane-associated processes to promote ROS formation [15]. In *Arabidopsis* rosettes, desubmergence induces *RBOHD* transcripts encoding a plasma membrane-localized NADPH oxidase/respiratory burst oxidase producing superoxide in the apoplastic space [16]. A burst of ROS-generating acetaldehyde was observed after desubmergence in the wetland species *Alternanthera philoxeroides* due to ethanolic fermentation [17].

ROS accumulation upon desubmergence has been demonstrated using direct and indirect methods. Direct ROS measurements in *Arabidopsis* leaves confirmed a ROS burst within 3 h after desubmergence [16]. Indirect measurements including increased **malondialdehyde (MDA)** content as a consequence of ROS-mediated lipid peroxidation has been recorded on desubmergence in soybean (*Glycine max*) [18], rice seedlings [19–21], and *Arabidopsis* [16,22]. In *Arabidopsis* and rice, genotypes with lower ROS accumulation on desubmergence display better recovery [16,21]. Excessive ROS accumulation post-submergence is likely to occur due to a combination of reduced scavenging capacity and increased production [23,24].

Glossary

Antioxidants: molecules or enzymes that reduce oxidation.

Fv/Fm: chlorophyll fluorescence measurement parameter representing the probability that the energy of a photon absorbed will be used in the photosynthetic electron-transport chain. An Fv/Fm value of approximately 0.83 in most unstressed plant species indicates full photosynthetic efficiency. Lower values indicate deficiency.

Lp_r: root hydraulic conductance measuring water uptake.

Malondialdehyde (MDA): an organic compound commonly quantified as a proxy for oxidative stress; formed by ROS-mediated degradation of polyunsaturated lipids.

Nonphotochemical energy quenching (NPQ): a process to dissipate excess light excitation energy as heat during sudden reillumination or high light stress.

Oxidative stress: excessive cellular ROS production, resulting from an imbalance between ROS production and ROS detoxification (i.e., through antioxidant availability).

Peroxidase: an enzymatic antioxidant of hydrogen peroxide.

Photoinhibition: inhibition of PSII activity caused by high light intensity.

Reactive oxygen species (ROS): reactive molecules containing oxygen; formed as byproducts of metabolic activity involving oxygen.

Relative water content (RWC): measure of leaf hydration status.

Senescence: a genetically controlled aging process involving the breakdown of cellular structures and macromolecules and mobilization to active, developing tissues.

Stomata: pores found mostly in the leaf and stem epidermis controlling water loss and gas exchange.

Sub1 rice: genotypes bred to contain *SUB1A* encoding an ethylene-responsive transcription factor that regulates metabolic and developmental responses to submergence and desubmergence recovery.

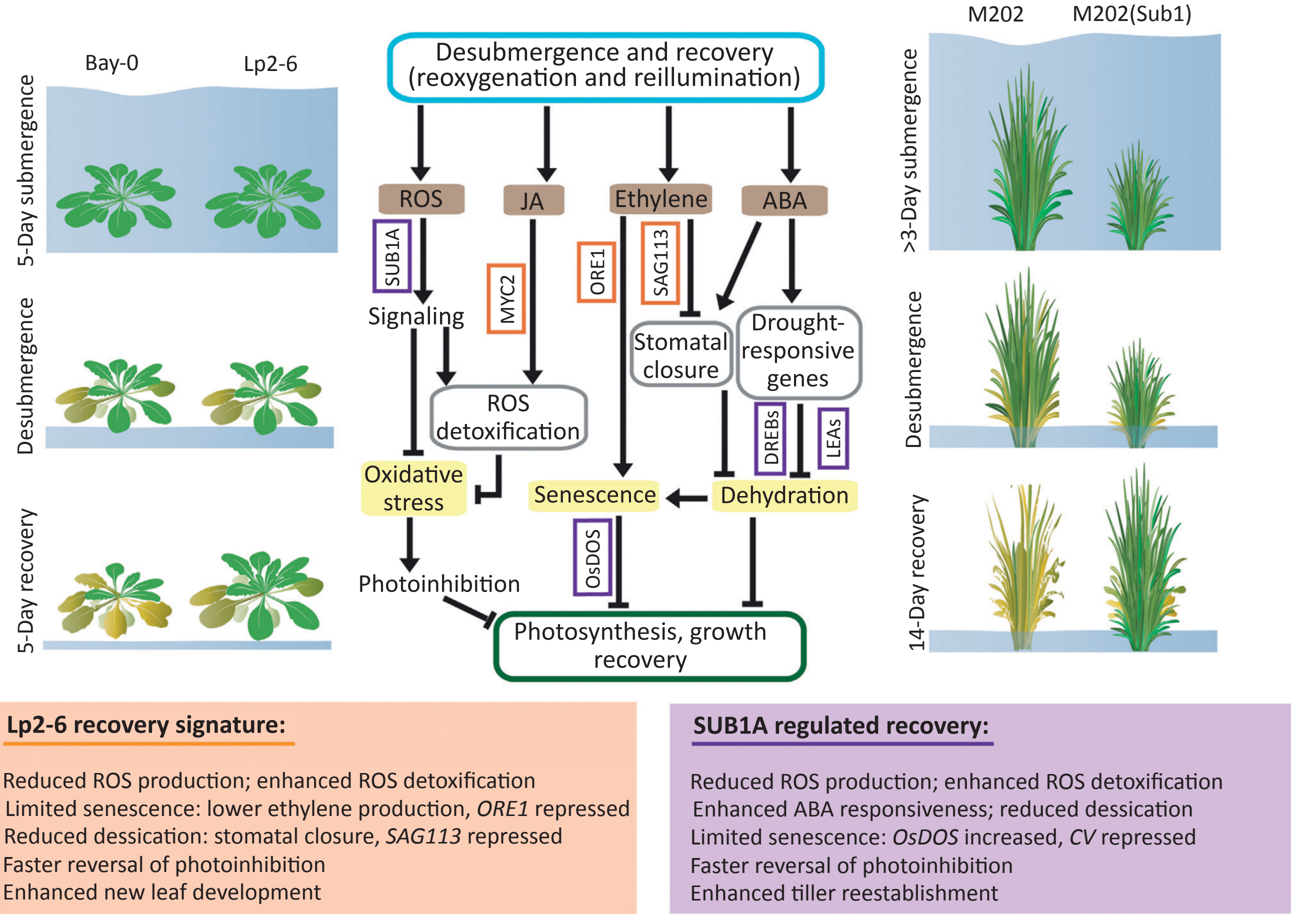

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Figure 1. Post-submergence stress signaling in plants

For a Figure360 author presentation of Figure 1, see the figure legend at https://10.1016/j.tplants.2019.02.007

Following prolonged submergence, shifting to an environment with higher oxygen and intense light triggers post-submergence stress responses mediated through reactive oxygen species (ROS) and jasmonic acid (JA), ethylene, and abscisic acid (ABA) hormonal signaling. Physiological recovery processes are similar across species, but molecular mechanisms can vary between dicots (e.g., submergence-intolerant Bay-0 and -tolerant Lp2-6 *Arabidopsis* accessions) and monocots [e.g., submergence-intolerant M202 and -tolerant M202(Sub1) rice cultivars]. In the schematic, arrows indicate positive regulation while lines represent negative regulation, with *Arabidopsis* genes highlighted in orange and rice genes in purple rectangles. Upregulation of ROS-detoxifying antioxidants for oxidative-stress tolerance is defined by JA-mediated *MYC2* activation in *Arabidopsis* and *SUB1A*-mediated signaling activated by ROS in rice. Prevention of senescence and dehydration in the tolerant *Arabidopsis* accession Lp2-6 depends on restricted ethylene accumulation, therefore repressing ethylene-mediated upregulation of *NAC DOMAIN CONTAINING PROTEIN6/ORESARA1* (*ORE1*) catabolizing chlorophyll and *SENESCENCE ASSOCIATED GENE 113* (*SAG113*) inhibiting stomatal closure. In tolerant M202(Sub1) rice, *SUB1A* promotes ABA responsiveness to prevent dehydration by activating drought-responsive genes, including dehydration-responsive element-binding proteins (DREBs) and late-embryogenesis abundant proteins (LEAs). *SUB1A* also delays senescence in rice by inducing the gene DELAY OF THE ONSET OF SENESCENCE (*OsDOS*) and repressing *CHLOROPLAST VESICULATION* (*CV*) that triggers plastid turnover. Tolerance mechanisms in *Arabidopsis* accelerate new leaf development by reversing photoinhibitory damage. In rice, *SUB1A* restores metabolic homeostasis and effectively reestablishes growth through tiller meristems. Plant templates used in this figure were sourced from the Plant Illustrations repository on figshare [89]. Orange and Purple panels indicate tolerance-related processes leading to superior recovery in Arabidopsis and rice, respectively.

Reillumination Stress

Submergence in deep or turbid floodwaters limits light availability for underwater photosynthesis. Desubmergence can therefore be accompanied by a sudden increase in light intensity, severely damaging the photosynthetic apparatus acclimated to low light underwater. **Photoinhibition** describes this light-induced reduction in photosynthetic capacity. Strong light intensity may accelerate ROS production in photosystem I (PSI) and II (PSII), inactivating

Box 1. ROS Production and Alleviation during Post-Submergence Recovery

Types

ROS include free radicals containing one or more unpaired electrons, such as superoxide ($O_2^{\cdot}$ −), hydroxyl ($^{\cdot}OH$), and peroxide ($O_2^{\cdot}$ −2), or nonradicals formed from two free radicals sharing their unpaired electrons, such as singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2) [83].

Production

ROS can form from excess molecular oxygen in submerged plant tissues returning to an oxygenated and illuminated environment. ROS mainly form in organelles with highly oxidizing metabolic activity. The mitochondria may have electron and proton leakage, possibly due to dissociation of electron-transport chain complexes. Accelerated photosynthesis on desubmergence generates excess electrons from leaky electron-transport chains. Combined with the burst of oxygen availability during reoxygenation, excess electrons and protons interact with oxygen molecules to form free radicals in a stepwise reduction process. ROS can also form enzymatically through the electron donor xanthine oxidase or through the plasma membrane-bound NADPH oxidases/respiratory burst oxidase homologs (RBOHs) that generate superoxide by reduction of extracellular oxygen through electron transfer from NADPH [84]. Superoxide rapidly converts into H_2O_2 through SOD. ROS can leave the site of production to affect other cellular components depending on their half-lives and ability to diffuse across membranes [85].

Alleviation

Antioxidants break down ROS to alleviate oxidative stress. Nonenzymatic low-molecular-mass antioxidants scavenge ROS by serving as electron donors or through a monohydroxy or polyhydroxy phenol group [83,86]. Enzymatic antioxidants are associated with a redox-pair recycling system to regenerate the reduced forms of antioxidants. The ascorbate–glutathione antioxidant cycle detoxifies H_2O_2 and antioxidant enzymes convert the substrates (ascorbate, glutathione, and NADPH) between oxidized and reduced forms [87]. ROS detoxification also occurs directly through secondary ROS-scavenging enzymes, including SOD, catalase, and peroxidases. These enzymatic secondary antioxidants reduce peroxide anion levels and supply NADPH and glutathione for the metabolic activities of primary antioxidants [88]. Balancing the oxidative redox status during post-submergence stress involves a network of antioxidants.

the reaction centers [25]. PSII is particularly sensitive to light damage and production of the ROS singlet oxygen ($^1O_2$) inhibits new D1 protein synthesis required for the repair of photodamaged PSII [26]. Photoinhibition limits photosynthetic apparatus functionality and hampers photosynthetic recovery. Although maximal ATP production via aerobic respiration returns following desubmergence, carbon stores are severely depleted during submergence to meet physiological energy requirements. Restoration of carbon reserves through photosynthate production is limited by reduced light and carbon dioxide (CO_2) diffusion during submergence and may be further hindered by ROS production post-submergence.

PSII photochemistry can be measured by the maximal quantum yield of PSII (**Fv/Fm**) [27]. Significant reduction in Fv/Fm and **nonphotochemical energy quenching (NPQ)** were observed on desubmergence in the riparian species *Hemarthria altissima* and *A. philoxeroides* [28–30] and in rice [19,31]. In *Arabidopsis* accessions recovering from prolonged dark submergence, Fv/Fm dramatically dropped within 5 h of recovery [16]. Recovery following low-oxygen conditions decreased PSII photochemical efficiency in the grass species *Agrostis stolonifera*, *Cynodon dactylon*, and *Zoysia japonica* [32]. PSII photodamage recovery is essential to prevent leaf **senescence** and cell death [33]. In the absence of timely and effective PSII recovery, the inability to replace submergence-depleted energy reserves can increase susceptibility to other post-submergence stressors.

Dehydration Stress

Shoots may experience desiccation stress on desubmergence despite the soil being saturated with water. Roots may be damaged from prolonged submergence and therefore cannot function optimally. Submergence and recovery conditions can decrease root hydraulic conductance (**Lp_r**), which reflects the ability of roots to absorb water via changes in membrane permeability and aquaporin function [34,35]. In a submergence-sensitive rice cultivar, the hydraulic conductivity of the leaf sheath decreased significantly on desubmergence, reflecting low water conductivity in the xylem. Although the reduction in hydraulic conductivity upon desubmergence has not been mechanistically investigated in *Arabidopsis*, hypoxia-mediated Lp_r reduction is attributed to transcriptional or post-translational regulation of aquaporins [35,36]. Both hypoxia and flooding regulate aquaporin transcript abundance in several species [37,38], whereas protein phosphorylation and protonation have been linked to reduced aquaporin function during hypoxia [36,39].

Post-submergence limitations on water absorption can cause shoots to display drought-like symptoms, including leaf rolling, wilting, and decreased **relative water content (RWC)** [16,19,21,40]. Leaves offset transpirational water loss by triggering abscisic acid (ABA) and stomatal closure, as reflected in decreased stomatal conductance during recovery [16,19]. Inability to close the **stomata** leads to severe dehydration symptoms, exemplified in the intolerant *Arabidopsis* accession Bay-0 [16] and rice cultivar M202 [19,21] (Figure 1). Although stomatal closure prevents water loss, limited CO_2 uptake can hinder photosynthesis critical for recovery. Early stomatal closure against water loss must be counterbalanced with sequential reopening to resume photosynthesis. The tolerant *Arabidopsis* accession Lp2-6 and **Sub1 rice** cultivar M202(Sub1) display accelerated recovery of Fv/Fm, correlating submergence tolerance with limited post-submergence stress phenotypes [16,19].

Senescence

Excessive ROS formation triggered by reillumination and reoxygenation, photoinhibition, and water deficit can accelerate leaf senescence in plants recovering from submergence [16,41,42]. Senescence typically occurs in the last phase of leaf development, when nitrogen, carbon, and nutrients are transported to younger developing or storage organs [43]. Senescence can be visually characterized by leaf yellowing through chlorophyll degradation during recovery, as observed in *Arabidopsis* [16,44], rice [19,20], *A. philoxeroides* [45], and soybean seedlings [18]. Oxidative stress can cause chlorophyll breakdown, as shown in methyl viologen-treated rice [21,46]. Early onset of senescence was observed during water-deficit stress and methyl viologen-induced oxidative stress in pea (*Pisum sativum* L.), linked to photosynthetic apparatus degradation and decreased chlorophyll *a*/*b* binding protein abundance [47].

In *Arabidopsis* recovering from prolonged submergence, senescing leaves strongly upregulated senescence and chlorophyll catabolism genes including *NAC DOMAIN CONTAINING PROTEIN6*/*ORESARA1* (*ORE1*) [16], a positive regulator of leaf senescence [48–50]. In rice, chlorophyll breakdown occurs during submergence and is limited when *SUB1A* is present [51]. During submergence and recovery, *SUB1A* limits upregulation of *CHLOROPLAST VESICULATION*, which triggers a pathway of chloroplast degradation in *Arabidopsis* [52]. Control of senescence is implicated in submergence recovery of the forage grass *Melilotus siculus* [53]. Although dying leaves are potential nutrient sources for younger leaves and meristem, chlorophyll retention might benefit photosynthesis and carbohydrate replenishment.

Genetic Variation Underlying Resilience Mechanisms

A Fine-Tuned Balance between ROS Production and Scavenging

Management of post-submergence oxidative stress is important to minimize cellular damage and facilitate faster recovery. In several species, reoxygenation triggers upregulation of transcripts encoding enzymes and molecules associated with ROS detoxification [18,22,54,55]. Submergence-tolerant plants have better antioxidant defense mechanisms to ameliorate harmful ROS effects. Oxidative stress damage following reoxygenation was minimized more effectively in rice containing the *SUB1A* submergence-tolerance gene [21]. Methyl viologen-induced oxidative stress elevated the accumulation of transcripts encoding ROS-detoxifying enzymes such as catalase (CatA and CatB), ascorbate **peroxidase** (APX1 and APX2), and superoxide dismutase (SOD1) in the Sub1 rice genotype M202(Sub1) [21]. Accordingly, M202(Sub1) had lower ROS production and MDA accumulation on desubmergence. The flood-tolerant wetland species *A. philoxeroides* escapes submergence via stem elongation and can rapidly recover photosynthesis and growth [17]. On desubmergence, *A. philoxeroides* leaves effectively scavenged O_2^- and H_2O_2 through increased SOD, CAT, and ascorbic acid activity. In soybean roots and hypocotyls, the activities of peroxidases and haloacid dehalogenase-acid phosphatase were suppressed during submergence but activated on desubmergence to facilitate recovery [56,57]. In *Arabidopsis*, faster recovery was associated with higher glutathione and ascorbate antioxidant levels restricting ROS accumulation [16].

Jasmonic acid (JA) signaling has been implicated in oxidative stress mitigation during submergence recovery. In *Arabidopsis* rosettes recovering from complete submergence, JA levels rose rapidly within a few hours of reoxygenation, peaking at 6 h [22]. JA biosynthesis and signaling mutants displayed increased reoxygenation sensitivity. Accordingly, plants treated with methyl JA prior to submergence had higher biomass and survival after recovery. JA improves oxidative stress tolerance on reoxygenation by activating *MYC2* (a basic helix–loop–helix leucine-zipper transcription factor with a key JA signaling role), which upregulates genes encoding key enzymes in the ascorbate (*VITAMIN C DEFECTIVE*) and glutathione (*GLUTATHIONE SYNTHETASE*) antioxidant pathways [22]. Soybean roots and hypocotyls flooded with JA showed better ROS detoxification and altered nucleotide metabolism due to increased protein abundance and enzymatic activity of antioxidants during submergence recovery [58]. Whereas it restricts oxidative stress, JA also promotes senescence. *SUB1A* overexpression in rice limits JA-promoted upregulation of transcripts associated with chlorophyll catabolism and dramatically elevates transcripts encoding the nucleus-localized CCCH-type zinc-finger protein DELAY OF THE ONSET OF SENESCENCE (*Os*DOS), associated with chlorophyll turnover [58]. Although limiting senescence might be beneficial to recovery, enhanced senescence of older, damaged leaves and associated resource remobilization to younger tissues would support new leaf development.

The balance between ROS production and alleviation must be maintained during post-submergence recovery. While ROS protective mechanisms can prevent excessive ROS accumulation, complete ROS elimination is detrimental for recovery, highlighting a signaling role for ROS in recovery. In rice, methyl viologen treatment significantly upregulated *SUB1A* transcripts [21]. In *Arabidopsis*, suppression of ROS generation on desubmergence by genetic impairment of ROS production or by application of ROS scavengers delayed new leaf generation during recovery [16]. Hence, the desubmergence ROS burst could signal the transition to aerated conditions, triggering acclimative responses.

Survival Is Enhanced by Recovery of Metabolic Homeostasis

Successful management of ROS production on desubmergence might also benefit photosynthesis recovery. This could be mediated by more efficient ROS scavenging in tolerant species and may be linked to effective PSII repair and avoidance of net photoinhibition, thus expediting post-submergence photosynthetic acclimation. The tolerant *Arabidopsis* accession Lp2-6 that limits post-submergence ROS resumed optimal PSII photochemistry within 3 days after desubmergence, correlating with faster starch replenishment than a slower-recovering accession [16]. Interestingly, low Fv/Fm values in Lp2-6 at the end of submergence suggests that low photosynthetic efficiency during the early recovery may be beneficial. Less-active electron transport could prevent leaky electrons from forming ROS.

Replenishment of energy reserves through reactivated photosynthesis during recovery is essential for regrowth. Submergence depleted total soluble sugars and fructans in *A. philoxeroides*, but by the third day of recovery these metabolites were higher than pre-submergence in leaves, stems, and roots [45]. Submergence decreased soluble sugar and starch content in the leaves and roots of *Distylium chinense* (evergreen shrub) seedlings, but their rapid restoration during desubmergence contributed to seedling regrowth [59]. Tolerant species more efficiently replenish energy for regeneration, observed in submergence-tolerant *Polygonum hydropiper* (water pepper) compared with submergence-sensitive *Carex argy* [60]. Nonstructural carbohydrate content decreased in both species under submergence, but *P. hydropiper* replenished more soluble sugar content and starch following desubmergence, correlating with higher biomass and survival rates.

In rice, the post-submergence carbohydrate status of aerial tissues is recognized as a key tolerance criterion [61,62], as insufficient carbohydrate reserves may hinder the rekindling of photosynthesis and reestablishment through tiller meristems, ultimately impacting yield. Submergence dramatically reduces carbohydrate reserves, but flood-tolerant varieties limit overall consumption of photosynthate, as observed in quiescent Sub1 cultivars [20,51,63]. Recent metabolite analyses demonstrated that rice with *SUB1A* has a less extreme nadir in shoot trehalose 6-phosphate content under submergence, consistent with its curbing of energy reserve use sooner than non-Sub1 genotypes [52]. This is likely to be mediated by restricted upregulation of *CALCINEURIN B-LIKE INTERACTING PROTEIN 15* shown to activate SUCROSE NON-FERMENTING KINASE1 to promote starch catabolism in rice seedlings [52,64]. Studies of *indica* and *japonica* cultivars bred to possess the Sub1 region on chromosome 9 indicate that *SUB1A* contributes to metabolic acclimations during submergence that enhance post-submergence recovery. These include better management of ROS and chlorophyll catabolism, both associated with more rapid recovery of photosynthetic rate and survival [51,52,65–68]. Sub1 rice maintained NPQ more effectively and displayed faster Fv/Fm recovery on desubmergence [19]. The combined transcriptome and metabolome analyses of Locke *et al*. [52] indicate that *SUB1A* broadly impacts gene activity during and post-submergence that is associated with accelerated recovery of both sugar and nitrogen metabolic homeostasis over the first 24 h following desubmergence.

Ethylene signaling during reoxygenation is implicated in replenishing tricarboxylic acid (TCA) cycle intermediates under post-anoxic conditions in *Arabidopsis* when energy reserves are low [54,69]. The enzyme *PYRUVATE ORTHOPHOSPHATE DIKINASE* was activated during reoxygenation to regenerate phosphoenolpyruvate from pyruvate, yielding the TCA intermediate oxaloacetate. Glycolysis can also be maintained during reoxygenation in plants through the breakdown of pyruvate and glutamate, thus preventing excess pyruvate accumulation [69,70]. Reoxygenation strongly induced the ETHYLENE INSENSITIVE 3 (EIN3) downstream target glutamate dehydrogenase (GDH) in *Arabidopsis*, which plays a critical role in replenishing TCA cycle substrates [54,69]. GDH converts glutamate into 2-oxoketoglutarate, which further reacts with alanine to replenish pyruvate for TCA cycle reentry. This pathway increases TCA cycle flux during reoxygenation for energy regeneration through EIN3-mediated ethylene signaling. Based on metabolome studies, ethylene-inducible *SUB1A* of rice modulates TCA cycle flux by promoting free amino acid synthesis during submergence [63] that is rapidly reversed on desubmergence [52].

Hormonal Regulation of Water Loss and Senescence

Leaf dehydration is inherent to post-submergence stress and severely limits photosynthesis, but aerial tissue survival can be improved by induction of dehydration-responsive genes associated with osmotic homeostasis, damage limitation, repair, and regulation of stomatal aperture to prevent water loss. Shoot ABA levels and ABA- and drought-responsive transcripts increased post-submergence in *Arabidopsis* [16,22]. In rice, *SUB1A* restricted dehydration stress through increased ABA sensitivity [21]. On desubmergence, a Sub1 genotype exhibited greater upregulation of dehydration-response genes than the near-isogenic line lacking *SUB1A*. In *Arabidopsis*, leaf wilting during recovery was determined by hormonal control of stomatal water loss. The timing of stomatal reopening after desubmergence is critical to ensure a balance between CO_2 uptake for photosynthesis and water loss due to lowered hydraulic conductance of submergence-damaged roots. Despite higher shoot ABA levels during recovery, the poorly recovering accession Bay-0 prematurely opened its stomata resulting in rapid water loss and leaf dehydration [16]. Greater post-submergence ethylene emission in Bay-0 shoots counteracted ABA-mediated stomatal closure through induction of *SENESCENCE-ASSOCIATED GENE 113* (*SAG113*) encoding a protein phosphatase 2C family member implicated in acceleration of water loss during senescence [71]. Post-submergence ethylene enhanced senescence through *ORE1*, which directs chlorophyll breakdown. Accordingly, *sag113* and *ore1* mutants had reduced water loss and higher chlorophyll levels, respectively, compared with corresponding wild-type plants during submergence recovery. Chemical blocking of ethylene perception during recovery achieved a similar result. Post-submergence ethylene induction is thus detrimental to recovery in *Arabidopsis* [16]. Mechanisms regulating post-submergence ethylene production are, however, unclear.

In *Rumex* species found in riparian floodplains, ethylene production was promoted on reoxygenation by oxidative conversion of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) that accumulated in roots and shoots during submergence and was produced *de novo* in shoots on desubmergence [72]. However, increased ethylene production was observed only in species (i.e. *Rumex palustris*) that maintain an escape strategy by elongating leaf petioles, possibly enabling emergent leaves to maintain ethylene-mediated growth and stay above the water surface. Whether ethylene induction is beneficial or harmful to recovery remains to be resolved.

Hormonal crosstalk is likely to fine-tune recovery responses. *MYC2* overexpressors with enhanced JA signaling have limited leaf desiccation stress during submergence recovery, pointing to JA involvement in dehydration regulation. Furthermore, MYC2 physically represses the transcription factors EIN3 and ETHYLENE INSENSITIVE 3-LIKE1 (EIL1), inhibiting their DNA-binding activity and downstream ethylene signaling [73]. This indicates antagonism between ethylene and JA in the regulation of recovery responses. This antagonism may not mean that either hormone is deleterious during reoxygenation, but rather that balanced phytohormone responses are important for cellular homeostasis (Figure 1). JA could modulate EIN3 and EIL1 activity to regulate ethylene-mediated post-submergence responses such as shoot elongation in submergence-escape species like *R. palustris* and *Rumex maritimus* [72]. Conversely, ethylene might fine-tune JA responses like ROS scavenging. Ethylene may be required to limit JA-regulated antioxidant biosynthesis when most ROS have been eliminated so cellular energy can be directed towards other processes.

Considering the pronounced water deficit evoked by desubmergence and the parallels in acclimative responses between these water stresses, some drought-tolerant varieties may have better submergence recovery and vice versa. Sub1 varieties showed superior shoot biomass recovery after water deficit due to reestablishment from tillers [21]. Under both drought and post-submergence recovery, the presence of *SUB1A* reduced water loss by increasing ABA responsiveness and gene expression associated with dehydration acclimation. Overlap between *SUB1A*-mediated responses during submergence recovery and water deficit highlights overlap in signaling under water extremes. Water deficit induces ROS formation, exemplified in rice [74] and *Arabidopsis* [75]. Sunflower (*Helianthus annuus* L.) also demonstrated overlapping water-deficit and submergence recovery responses. The transcription factor *HaWRKY76* was highly upregulated in sunflower seedlings and plantlets after mild water-deficit or post-submergence stress [76]. *Arabidopsis* ectopically overexpressing *HaWRKY76* had greater seed yields following both water deficit and submergence, the latter associated with less leaf water loss achieved through stomatal closure and higher cell-membrane stability. These observations bode well for resilience to both water extremes.

Post-Submergence Recovery and Plant Immunity

Since prolonged submergence depletes plant resources, recovering plants can be extremely vulnerable to biotic stresses. In rice, post-submergence energy availability correlates with the extent of pathogen immunity. Two *SUB1A*-containing varieties (Swarna-Sub1 and Samba Mashuri-Sub1) with restricted carbohydrate consumption during submergence had higher resistance to *Xanthomonas oryzae* pv *oryzae* (bacterial blight) and *Magnaporthe oryzae* (leaf blast) following desubmergence [77,78]. Disease protection is important for seed production, as rice susceptible to bacterial blight had significantly lower grain yield when infected. Submergence may prime plants for biotic stress resilience. Increasing the submergence duration correlated with less severe leaf-blast symptoms for both Sub1 and non-Sub1 genotypes. This is perhaps unsurprising considering the significant enrichment of defense-related genes in the complement of submergence-regulated genes [79,80].

Submerged *Arabidopsis* had higher resistance against *Pseudomonas syringae* pv *tomato* inoculated after desubmergence [81], which was partly linked to WRKY22-mediated transcriptional regulation of post-submergence plant immunity [82]. *WRKY22* was highly induced during submergence and *wrky22* mutants were more vulnerable to infection following desubmergence than wild-type plants [81]. Even during short (2 h) submergence, *Arabidopsis* desubmerged into high humidity developed fewer disease symptoms than nonsubmerged plants, due to WRKY22-dependent innate immunity. While submergence evidently modulates susceptibility to biotic stress, several factors are likely to determine the ultimate resilience of recovering plants. The crosstalk between recovery signaling and defense responses requires further investigation.

Concluding Remarks and Future Perspectives

Molecular, physiological, and developmental processes during submergence recovery are clearly an important aspect of flooding tolerance. The careful evaluation of post-submergence recovery of different genotypes has begun to expose the signals and downstream responses underpinning effective recovery strategies (Figure 1). Ability to adapt to submergence stress may be linked to mechanisms occurring during the post-submergence phase. ROS produced during reoxygenation cause post-flooding injury including photoinhibition, dampened carbohydrate replenishment, desiccation stress, and senescence. Successful recovery requires the overcoming of oxidative and dehydration stress via ROS-scavenging antioxidant activity and appropriate drought-response measures that are likely to be mediated by a hormonal interaction network. However, while primary signals regulating recovery are beginning to be identified, we are far from a full understanding of the molecular mechanisms governing faster and more effective post-submergence recovery. The hierarchy of various recovery signals and their interaction remains unestablished. It is important not only to identify tolerance traits distinguishing vegetative regrowth on desubmergence, but also to gain a mechanistic understanding of the involvement of phytohormones, transcription factors, and other regulatory responses.

Despite the new knowledge highlighted in this review, a more comprehensive view of post-submergence recovery mechanisms is needed to aid the generation of robustly climate-resilient crops (see Outstanding Questions).

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Outstanding Questions

How do roots respond to desubmergence? What signaling pathways regulate root hydraulic conductance that contribute to shoot recovery?

Which environmental and endogenous signals regulate guard-cell movement to control water loss during recovery?

What are the spatial and temporal dynamics of recovery signals and responses? What is the hierarchy and interaction between these signals?

Is ROS signaling during recovery distinct from that during submergence? What downstream molecular components does ROS signaling influence? Does the ROS burst help to 'reset' homeostasis?

Do submergence or desubmergence mechanisms contribute to innate immunity in plants?

What other signals mediate recovery? Involvement of hormones such as cytokinin and regulation via post-translational modifications is likely but underexplored.

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