INRODUCTION

The average land area exposed to flooding on a world scale is currently more than 17 million km$^2$ per year; double the size of the USA. Dramatic floods occur in all continents of our planet and result in an estimated annual damage exceeding 60 billion Euro (www.dartmouth.edu/~floods/Archives/2005sum.htm). An intensification of the global water cycle of 16–24% is predicted in a future 2°C–3°C warmer world. This intensification of rainfall and evaporation will cause dry regions to become drier and wet regions, such as most agricultural zones, to become wetter in response to global warming (Durack et al. 2012). Global climate change is therefore expected to increase the frequency and severity of flooding events in many industrialised and agricultural regions of our planet (Arnell & Liu 2001).

Many terrestrial wild plant species and nearly all crops are intolerant to flooding. Therefore, floods will destroy natural patterns of plant distribution, affect biodiversity (Silvertown et al. 1999) and have a devastating impact on crop growth and thus on food production (Nормile 2008). It is an enormous challenge for the plant science research community to obtain sufficient knowledge of mechanisms that drive flood tolerance in plants, so that stress-tolerant crops can be developed and ecosystem change can be anticipated.

Plant life relies on light energy-driven fixation of CO$_2$ into carbohydrates through photosynthesis. These carbohydrates are used to construct various plant structures and fuel energy production through respiration, which requires a sufficient supply of O$_2$. Cells of flooded terrestrial plants typically suffer from a severe shortage of energy and carbohydrates (Bailey-Serres & Voesenek 2008; Licausi & Perata 2009). This is caused by a physical property of water, which results in extremely slow diffusion of O$_2$ and CO$_2$ into cells of plant organs surrounded by water. Additionally, the light levels reaching submerged plants can vary from almost nil in dark, turbid waters to normal levels in clear water (Vervuren et al. 2003). These varying light levels have a significant impact on the process of underwater photosynthesis and thus on carbohydrate production and the endogenous levels of CO$_2$ and O$_2$ (Mommer & Visser 2005). Two other major changes inside plants during flooding are increases in reactive oxygen species (ROS) formed within plant cells during floods, and particularly upon subsidence of floodwater, as well as the entrapment of the gaseous plant hormone ethylene (ET; Fig. 1). Flood-tolerant plants use changes in ET, ROS, O$_2$ and CO$_2$ as signals to induce adaptive processes.

Our current view sees flooding as a compound stress of interacting changes inside plant cells induced by the floodwater surrounding the plant (Fig. 1). These substances, of which O$_2$, CO$_2$, ROS and ET are of major importance, can occur in various specific cellular concentrations as determined by the plant organ and the flooding regime.

A restricted number of plant species have developed tolerance traits that enable survival during flooding. These plants have developed adaptive strategies that help them to survive the unique flooding regimes in their native habitats. Plant species from habitats with long-lasting, but relatively shallow...
floods have developed traits that enable escape from floodwaters through fast shoot extension (the so-called low-O2 escape strategy). These elongated shoots that emerge out of the water function as 'snorkels', thus restoring gas exchange. However, plants exposed to either deep or very short floods exploit a so-called 'sit-and-wait' or low-O2 quiescence strategy, in which they conserve the use of energy and carbohydrates to prolong survival under water and to quickly restore competitive growth as soon as the floodwater recedes (Bailey-Serres & Voesenek 2008; Colmer & Voesenek 2009).

The most important regulators of adaptive responses to flooding are the internal signals of elevated levels of ET and/or declining O2 concentrations. These changes in ET and O2 have different kinetics and, moreover, the dynamics of both gases differs strongly between plant organs such as roots and shoots (Voesenek & Blom 1999). Consequently, adaptive processes at the root or shoot level might be regulated in different ways. Moreover, such adaptive processes needed at a short time scale may depend on other internal signals than those occurring at longer time scales.

This review will focus on how ET and O2 regulate adaptive morphological and metabolic changes during flooding in an organ-specific way and at different temporal scales.

GASEOUS MODIFICATIONS IN ROOTS, SHOOTS AND THEIR ENVIRONMENTS UPON SUBMERGENCE

This section will give an overview of available information on the concentrations and daily fluctuations in ET and O2 in submerged plants. Moreover, it will shed light on differences between roots and shoots.

The gaseous plant hormone ET is produced by all cells of higher plants (Abeles et al. 1992). The endogenous concentration in cells is mainly determined by the production rate and the fast diffusion of ethylene gas towards the atmosphere (Voesenek & Blom 1999). This latter process is severely hampered as soon as plant organs are surrounded by water due to the very slow diffusion rate of ET in water, a physical characteristic typical for gases (Jackson 1985; Vandenbussche et al. 2012). This enormous restriction of outward diffusion results in rapid accumulation of ET in cells and air spaces inside plant organs.

Various independent methods in different organs demonstrate that ET levels increase substantially in tissues surrounded by water (Table 1). Although the number of analyses in roots is restricted, no substantial differences between internal ET levels upon flooding in shoots and roots have been observed. Only a few studies have included early time points after flooding in

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Table 1. Ethylene concentration in various plant species and organs during drained and submerged conditions.

<table>
<thead>
<tr>
<th>Species</th>
<th>Organ</th>
<th>Time</th>
<th>Method</th>
<th>Drained (µL l⁻¹)</th>
<th>Submerged (µL l⁻¹)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Callitriche platycarpa</td>
<td>Shoot</td>
<td>0–2 day</td>
<td>1</td>
<td>-</td>
<td>1.2 ± 0.2</td>
<td>Musgrave et al. 1972;</td>
</tr>
<tr>
<td>Ranunculus sceleratus</td>
<td>Shoot</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>7.2 ± 2.1</td>
<td>Musgrave &amp; Walters 1974;</td>
</tr>
<tr>
<td>Regnellidium diphyllum</td>
<td>Leaves</td>
<td>12–96 h</td>
<td>1</td>
<td>-</td>
<td>3.1 ± 0.4</td>
<td>Musgrave &amp; Walters 1974;</td>
</tr>
<tr>
<td>Regnellidium diphyllum</td>
<td>Leaves</td>
<td>40 min</td>
<td>2</td>
<td>-</td>
<td>1.0</td>
<td>Malone &amp; Ridge 1983;</td>
</tr>
<tr>
<td>Oryza sativa var.</td>
<td>Internodes</td>
<td>2 day</td>
<td>4</td>
<td>-</td>
<td>1.0</td>
<td>Metraux &amp; Kende 1983;</td>
</tr>
<tr>
<td>Rumex acetosa</td>
<td>Shoot</td>
<td>24 h</td>
<td>2</td>
<td>0.07 ± 0.02</td>
<td>0.25 ± 0.11</td>
<td>Voosenek et al. 1993;</td>
</tr>
<tr>
<td>Rumex acetosa</td>
<td>Shoot</td>
<td>24 h</td>
<td>3</td>
<td>-</td>
<td>5.0</td>
<td>Voosenek et al. 1993;</td>
</tr>
<tr>
<td>Rumex palustris</td>
<td>Shoot</td>
<td>24 h</td>
<td>2</td>
<td>0.05 ± 0.03</td>
<td>0.22 ± 0.03</td>
<td>Voosenek et al. 1993;</td>
</tr>
<tr>
<td>Rumex palustris</td>
<td>Shoot</td>
<td>24 h</td>
<td>3</td>
<td>-</td>
<td>4.4</td>
<td>Voosenek et al. 1993;</td>
</tr>
<tr>
<td>Rumex palustris</td>
<td>Shoot</td>
<td>&lt;1 h</td>
<td>3</td>
<td>-</td>
<td>&gt;1.0</td>
<td>Banga et al. 1996;</td>
</tr>
<tr>
<td>Rumex palustris</td>
<td>Shoot</td>
<td>16 h</td>
<td>3</td>
<td>-</td>
<td>9.0</td>
<td>Banga et al. 1996;</td>
</tr>
<tr>
<td>Rumex acetosella</td>
<td>Shoot</td>
<td>&lt;1 h</td>
<td>3</td>
<td>-</td>
<td>&gt;1.0</td>
<td>Banga et al. 1996;</td>
</tr>
<tr>
<td>Rumex acetosella</td>
<td>Shoot</td>
<td>16 h</td>
<td>3</td>
<td>-</td>
<td>7.5</td>
<td>Banga et al. 1996;</td>
</tr>
<tr>
<td>Rumex palustris</td>
<td>Roots</td>
<td>24 h</td>
<td>3</td>
<td>-</td>
<td>1.8 ± 0.2</td>
<td>Visser et al. 1996a;</td>
</tr>
<tr>
<td>Rumex thrysiflorus</td>
<td>Roots</td>
<td>24 h</td>
<td>3</td>
<td>-</td>
<td>9.1 ± 2.5</td>
<td>Visser et al. 1996b</td>
</tr>
</tbody>
</table>

1, Collection of oxygen-rich bubbles; 2, Vacuum method; 3, Photoacoustic spectroscopy; 4, Direct gas extraction from organ with a syringe.

aDeepwater rice variety Habiganj Aman II.

bPlants only partially submerged.
their analyses (Banga et al. 1996). From those, we can conclude that ET accumulates in submerged plants organs within 1 h to levels of around 1 µl l⁻¹. These levels are 20-fold higher than in non-submerged tissues. Based on dose–response experiments with Regnellidium diphyllum and Rumex palustris, a concentration of 1 µl l⁻¹ ET saturates physiological processes, such as enhanced shoot elongation, in these species (Musgrave & Walters 1974; Voesenek et al. 1997). Interestingly, ET concentrations in roots of waterlogged plants vary strongly; species from dry habitats and species low in root porosity have higher internal ET levels (Visser & Pierik 2007).

In none of the studies that included more time points were there indications of diurnal rhythms or other fluctuations in ET levels in submerged organs (Metraux & Kende 1983; Banga et al. 1996). It can be concluded that ET rapidly accumulates inside submerged tissues (within 1 h) and that the levels stay at a response-saturating level for many days following submergence. Most likely no substantial differences in elevated endogenous ET levels are present between submerged roots and shoots.

In terms of O₂, submerged roots and shoots are exposed to fundamentally different environments. Submerged roots are surrounded by soil, characterised by a rapid depletion of O₂ concentrations due to microbiological activity (Laanbroek 1990). A decline in potting soil from 19 to nearly 0 kPa O₂ in 30 h was observed upon submergence in darkness (Vashisht et al. 2011). This is generally similar in field soils in which O₂ disappears within just a few days (Ponnampерuma 1984). Submerged shoots, on the other hand, are surrounded by water that in general has much higher O₂ levels than submerged soils. The final O₂ concentration in the water layer results from the activity of various sinks (microorganisms in water and top soil), the O₂ produced by photosynthesising organisms and diffusion of atmospheric O₂ into the water layer. Field data from flooded rice fields in Thailand demonstrated that the O₂ levels in the surface water layers varies in a diurnal pattern, with high- to super-saturating levels (ca. 25%) at the end of the light period. With depth, the O₂ partial pressure in the water layer declines to almost 0% at a depth of 1.8 m and the diurnal pattern ceases (Setter et al. 1987). A submergence experiment (under dark conditions) with small Arabidopsis plants that lasted several days, revealed a constant O₂ concentration in the water layer of 15 kPa (Vashisht et al. 2011).

The O₂ concentration in submerged roots depends on the sink strength of the surrounding soil and the leakage of O₂ to that soil via radial O₂ loss, respiration rate of the root cells, distance from the O₂ source (e.g. photosynthesising leaves), concentration of O₂ at the source site and porosity of the tissues between source and sink (Armstrong 1979). The O₂ concentration in roots of Arabidopsis declined upon complete plant submergence in the dark from 5–6% in well-aerated soil conditions (O₂ concentration in soil: 19%) to nearly 0% in 15 min. Re-illumination of these submerged Arabidopsis plants resulted in a slight increase of the internal root O₂ level to 1%, indicating that photosynthetic O₂ diffuses from the leaves to the roots (Lee et al. 2011). Roots demonstrate a diurnal pattern in O₂ levels related to underwater photosynthesis (Sorrell & Dromgoole 1987; Pedersen et al. 1995, 2006; Colmer & Pedersen 2008; Winkel et al. 2011). Within roots, O₂ concentrations vary and are generally low in more densely packed tissues such as the vascular tissues. Furthermore, O₂ levels in roots decline towards the apex, due to continued O₂ loss related to respiration and, in some species, radial oxygen loss to the root environment (Armstrong 1979; Armstrong et al. 2000; Colmer & Greenway 2005).

For submerged shoots, the endogenous O₂ levels are determined by the rate of underwater photosynthesis (depending on light levels and CO₂ availability), leaf morphology, presence of gas films, respiration rate of shoot cells and O₂ concentration in the water layer (Pedersen et al. 2009; Colmer et al. 2011). In contrast to the water layer, a diurnal pattern was not observed in the endogenous O₂ concentration in the internodal lacuna of deepwater rice. The concentrations measured were 21%, 11% and 5% at depths of 2 cm, 0.8 m and 1.8 m, respectively (Setter et al. 1987). As a part of the shoot was above the water level, the constant, relatively high O₂ levels can be explained by inward diffusion of atmospheric O₂ via the leaves to the lacuna. Diurnal patterns in shoot O₂ levels in submerged shoot organs do exist. Moreover, O₂ generated by underwater photosynthesis, was accumulated during the light period and then depleted at night (Mommer & Visser 2005; Pedersen et al. 2006, 2011). Differences in endogenous O₂ exist between leaves of terrestrial plants developed in the terrestrial and the aquatic phase. So-called aquatic leaves of R. palustris typically have internal O₂ levels of 17% in submerged petioles, whereas submerged terrestrial leaves have values of only 9% (Mommer et al. 2004).

In summary, flooded soils are rapidly depleted of O₂, resulting in low O₂ levels up to complete anoxia. In contrast, water layers surrounding submerged shoots rarely become anoxic. In most cases, these water layers contain high amounts of O₂ that often fluctuate in a diurnal pattern, especially near the water surface. Roots of completely submerged plants can be depleted of O₂ within minutes if hardly any O₂ diffuses from the shoot into the roots, whereas in species with high root porosity root O₂ can differ markedly between day and night time. The O₂ levels in roots vary in different tissues and along the length of a root (Colmer 2003b). Shoot internal O₂ levels are in general high, and underwater photosynthesis and emergence of the shoot above the water surface improve the root O₂ status even further.

ETHYLENE SENSING

Ethylene is an important signalling molecule during flooding, and therefore we summarise the available understanding of ET perception and signalling in higher plants. In Arabidopsis, ET is perceived at the membranes of the Golgi and the endoplasmic reticulum (ER) by a family of five receptors. The perception and signalling pathway from ET to its target genes is reviewed in Kendrick & Chang (2008) and Stepanova & Alonso (2009). An overview of this ET signalling route and its points of control will be summarised below.

The ET receptors are negative regulators of ET signalling that share structural similarity with bacterial two-component histidine kinases. ET is bound along with a copper cofactor at the N-terminal membrane-spanning domain of the protein, and the receptors are inactivated upon ET binding, thus allowing ET action. The receptors physically interact with a negative (CTR1) and a positive (EIN2) regulator of ET signalling. It is thought that in the absence of ET, CTR1 is associated with the receptors in such a way that signalling to the more downstream EIN2 is prevented. Upon ET binding, it is hypothesised that
molecular mechanism for direct O\textsubscript{2} sensing. This involves et al. O\textsubscript{2}-dependent post-translational regulation of specific TFs responsible for the activation of ET target genes. Importantly, only 10\% of the genes belonging to the ET family are regulated through ET (Nakano et al. 2006). ET signalling can be regulated at the levels of the receptors, EIN2 and EIN3.

The protein RTE1 co-localises with ETR1, one of the ET receptors, in the Golgi and ER (Dong et al. 2008). RTE1 interacts with ETR1 and modulates its activity. Loss-of-function mutants are hypersensitive to ET, whereas gain-of-function mutants of RTE1, such as the GREEN RIPE mutant in tomato, lead to ET insensitivity (Barry & Giovannoni 2006; Resnick et al. 2006). Another way to regulate receptor activity is through protein degradation (Chen et al. 2007). Proteasome-dependent degradation of ET receptors in tomato fruit results in early fruit ripening, indicating that this mechanism could be responsible for the timing of fruit maturation via ET sensitisation (Kevany et al. 2008). Finally, three of the five ET receptors in Arabidopsis can be activated transcriptionally via ET.

The EIN2 can also be regulated via protein degradation. Two F-box proteins, ETP1 and ETP2, target EIN2 for proteasome-dependent, ubiquitin-mediated degradation. Interestingly, EIN2 is stabilised in the presence of ET and degraded in its absence (Qiao et al. 2009). EIN3 and EIL1 are localised in the nucleus and have specific targets, such as ERF1 and EBF2 (Solano et al. 1998; Konishi & Yanagisawa 2008). The levels of EIN3 and EIL1 can be regulated in three distinct ways:

1. The stability of EIN3 is promoted by phosphorylation at T174 through a MAP kinase cascade, whereas phosphorylation at another site, T592, via a separate MAP kinase cascade involving CTR1 leads to degradation of EIN3 (Yoo et al. 2008).
2. EIN3 and EIL1 can be degraded by the 26S proteasome-dependent pathway. This process is mediated by two F-box proteins, EBF1 and EBF2 (Guo & Ecker 2003).
3. EIN5/XRN4, identified via the ET-insensitive mutant ein5, negatively affects the transcription of EBF2 and thus regulates the levels of EIN3 (Potuschak et al. 2003, 2006; Olmedo et al. 2006).

**OXYGEN SENSING**

Until recently, the lack of evidence for a molecular sensor/sensing mechanism led to suggestions that plants, unlike their animal counterparts, used indirect sensing to perceive changes in O\textsubscript{2} levels (Bailey-Serres & Chang 2005; Licausi & Perata 2009). This meant that plants detected changes in ambient O\textsubscript{2} concentrations through other consequent changes within the cell (reviewed extensively in Bailey-Serres & Chang 2005). This would include, for example, changes in intracellular levels of ROS and reactive nitrogen species, energy charge, carbohydrates and cytosolic pH and calcium levels.

Recent studies in Arabidopsis (Gibbs et al. 2011; Licausi et al. 2011) now provide convincing proof of the existence of a molecular mechanism for direct O\textsubscript{2} sensing. This involves regulation of hypoxia responsive gene expression through the O\textsubscript{2}-dependent post-translational regulation of specific TFs via the N-end rule pathway for protein degradation (Sasidharan & Mustroph 2011; Bailey-Serres et al. 2012).

The N-end rule is an evolutionarily conserved mechanism for protein degradation that is also found in mammals, yeasts and bacteria. The degradation of a protein via this pathway is dependent on the presence of a characteristic sequence of residues in its N-terminus, termed the N-degron (Tsakal et al. 2012). In plants, O\textsubscript{2}-dependent targeting of proteins for degradation, due to the generation of an N-degron, can occur in proteins containing cysteine (Cys) at the second residue. When the terminal methionine (Met) is constitutively cleaved by a methionine aminopeptidase (MAP), the Cys residue is exposed. If this terminal Cys is oxidised, it becomes a target for an arginyl tRNA transferase (ATE), which then adds an arginine residue to the N-terminus. This arginylation marks the protein as a substrate for ubiquitination by E3 ligases such as PROTEOLYSIS6 (PRT6) and, eventually, degradation by the 26S proteasome (Graciet & Wellmer 2010). The oxidation of the Cys residue, in turn dependent on the ambient intracellular O\textsubscript{2} concentration, is therefore the critical step that seals the fate of the protein for degradation via the proteasome.

The Arabidopsis TFs demonstrated to be O\textsubscript{2}-dependent substrates for the N-end rule pathway of proteolysis are members of the group VII ERF family (Nakano et al. 2006). The group VII ERFs are a subgroup of the plant-specific ERF TF family, where members share a common APETALA2 (AP2) DNA binding domain. Description of the entire ERF family and phylogenetic subdivisions is covered in the review of Nakano et al. (2006). Arabidopsis has five group VII ERFs: HRE1, HRE2, RAP2.12, RAP2.2 and RAP2.3 all of which possess a conserved N-terminal motif starting with the amino acids Met-Cys (Gibbs et al. 2011; Bailey-Serres et al. 2012). The functional importance of these ERFs in mediating hypoxia and anoxia survival is well established, and with the exception of RAP2.3, all these ERFs have been implicated in regulating the transcription of genes related to hypoxia acclimation (Hinz et al. 2010). Furthermore, the lack of a complete absence of hypoxia-responsive gene expression in single and double mutants of different ERFs (Hinz et al. 2010; Licausi et al. 2010) indicates functional redundancy amongst members. Interestingly, the overexpression of HRE1, HRE2 (Licausi et al. 2010), RAP2.2 (Hinz et al. 2010) and RAP2.12 (Papdi et al. 2008; Licausi et al. 2011) modulate hypoxia-responsive gene expression only under conditions of low O\textsubscript{2}, hinting already at an O\textsubscript{2}-dependent post-translational regulatory mechanism.

The conserved N-terminal motif of Arabidopsis ERFs, the constitutive hypoxia-responsive gene expression in N-end rule pathway (pnt6) mutants, and the observation that overexpression effects were condition-specific all led to further investigative studies that culminated in the identification of a mechanism for direct O\textsubscript{2} sensing in plants (Gibbs et al. 2011; Licausi et al. 2011). These studies confirmed that all five Arabidopsis group VII ERFs were N-end rule substrates (Gibbs et al. 2011), and revealed how the O\textsubscript{2}-dependent degradation of constitutively expressed ERFs (as demonstrated for RAP2.12) controls hypoxia responses.

Under normoxic conditions, constitutively expressed ERFs are degraded due to oxidation of the terminal Cys and subsequent proteolysis. However, as demonstrated for RAP2.12, this degradation can be prevented by sequestration of the protein. RAP2.12 is sequestered at the plasma membrane during
normoxia, due to its interaction with the membrane localised Acyl CoA binding proteins (ACBP1 and 2) (Li & Chye 2003; Licausi et al. 2011). Plant ACBPs (Arabidopsis has six members) have been traditionally attributed roles in lipid metabolism, although recent studies demonstrated their functional importance in mediating stress responses as well (Xiao & Chye 2011). Four out of the six Arabidopsis ACBPs also have protein–protein interaction motifs (Xiao & Chye 2011), and previous studies identified the ERF RAP2.3 as binding partner (Li & Chye 2004; Li et al. 2008). Although RAP2.12 has been demonstrated to bind only membrane bound ACBPs (Licausi et al. 2011), it is quite likely that sequestration of RAP2.12 and other ERFs can also occur at other locations, e.g. with cytosolic ACBPs. Although not experimentally proven, it is likely that a change in O2 concentration affects the interaction of ERFs with ACBPs and therefore releases the sequestered proteins (Sasidharan & Mustroph 2011; Bailey-Serres et al. 2012). This could potentially launch a rapid hypoxia response. Sequestration of ERFs during normoxia would server a dual purpose. Not only would it prevent the activation of a hypoxia response during O2 replete conditions, but would also facilitate the existence of an established pool of ERFs that can be recruited as soon as hypoxia sets in, thus ensuring a faster response to hypoxia.

When intracellular O2 levels start to decline, both constitutive and hypoxia-induced ERFs accumulate (shown for RAP2.12-GFP and HRE2-HA), as their degradation would now be limited. These ERFs then translocate to the nucleus to initiate transcription of specific downstream target genes that mediate the acclimation and survival from hypoxia. When O2 levels are restored, the hypoxia response is also rapidly reversed, as oxidised ERFs are targeted to the proteasomal machinery via the N-end rule (Licausi et al. 2011).

**Differential regulation of group VII ERFs by oxygen and ethylene**

While the group VII ERFs mediate O2 sensing, members are themselves differentially transcriptionally regulated by both ET and hypoxia signals. HRE1 and -2, which have partially overlapping roles in the hypoxia response, are both strongly induced upon hypoxic treatment (Mustroph et al. 2009, 2010; Licausi et al. 2010). HRE1 expression is also responsive to ET, which likely boosts the induction caused by low O2 stress (Hess et al. 2011; Yang et al. 2011). RAP2.2 and RAP2.3 transcript levels are also up-regulated by ET (Buttner & Singh 1997; Hinz et al. 2010). As mentioned before, ET accumulation occurs rapidly in submerged plant organs, sometimes preceding the onset of hypoxia. ET-responsive ERFs could be important mediators in relaying the underwater status and priming cells of submerged plant organs for initiating suitable responses when O2 levels do start to decline.

Ethylene-inducible group VII ERFs have also been intensely studied in rice, in relation to their role in mediating adaptive growth responses to flooding. The escape strategy of deepwater rice when faced with a progressive flood is regulated by the SNORKEL locus, which encodes two group VII ERFs, SK1 and SK2 (Hattori et al. 2009). In contrast, lowland rice varieties that face persistent floods adopt a quiescence strategy, where growth is reduced in order to conserve energy. This strategy is regulated through the group VII ERF SUB1A-1, which is present in a subset of flooding-tolerant indica varieties (Xu et al. 2006). SUB1A-1 primarily restricts growth by limiting the induction of genes involved in starch breakdown and cell wall loosening (Fukao et al. 2006), and maintaining levels of two gibberellin signalling repressors (SLR1 and SLR1L; Fukao & Bailey-Serres 2008). It also triggers the transcription of genes associated with ROS amelioration and dehydration (Fukao et al. 2011). This, together with the energy conserving strategy, facilitates growth recovery in the post-submergence phase. Interestingly, the N-termini of both the SKs and SUB1A-1 deviates from the characteristic N-terminal motif of the Arabidopsis group VII ERFs, and this most likely excludes these ERFs as substrates for the N-end rule pathway, as confirmed for SUB1A in *in vitro* assays (Gibbs et al. 2011; Bailey-Serres et al. 2012). The escape of these ERFs from the O2-dependent degradation, coupled with their ET inducibility, also further makes a case for ET as a priming hormone in submergence-mediated responses. ET accumulation upon submergence can initiate the transcription of these TFs and, in the case of deepwater rice, trigger escape responses, thereby avoiding the need to face hypoxia, and in the case of lowland rice, trigger the quiescent strategy where energy management would prolong survival in impending low O2 conditions.

**ETHYLENE- AND LOW OXYGEN-INDUCED ADAPTATIONS**

The various ways in which plants can acclimate to flooding of their environment and the variation between plant species herein are summarised in several review articles (Voesenek et al. 2006; Bailey-Serres & Voesenek 2008; Jackson 2008; Colmer & Voesenek 2009; Licausi & Perata 2009). Two distinct types of adaptive traits can be distinguished: (i) those that aim to improve gas exchange between plant organs and between the environment and plant organs, both with the ultimate aim to improve the energy and carbohydrate status of the flooded plant; and (ii) those that metabolically adjust the plant’s energy and carbohydrate management to cope with conditions of severely limited O2 access. These two suites of traits will be discussed briefly, with emphasis on the signals that regulate these traits.

**Improved gas exchange**

The extremely slow diffusion rate of gases in water and the obligate requirement of O2 and CO2 for plant life induced the evolution of plant traits that enhance gas exchange upon flooding. Most important in this respect is the development of a gas-filled diffusion continuum (aerenchyma), morphological and anatomical traits that improve underwater photosynthesis and rapid shoot growth resulting in emergence of leaf tips above the floodwater (Bailey-Serres & Voesenek 2008).

Many flood-tolerant plant species have the plasticity to improve internal O2 diffusion through development of longitudinally interconnected gas-filled pores, whereas others have constitutively high levels of this aerenchymatous tissue (Justin & Armstrong 1987; He et al. 1999; Colmer 2003a). Others hardly have or develop aerenchyma and are classified as flood intolerant. Aerenchyma facilitates fast O2 diffusion from organs in contact with the atmosphere (e.g. during partial submergence) to those that are submerged (Armstrong 1979). Alternatively, aerenchyma also stimulates O2 diffusion to roots from...
organs that are completely submerged but generate O₂ by means of underwater photosynthesis or from organs such a submerged shoots that are O₂-enriched due to inward diffusion of O₂ from the water layer (Mommer & Visser 2005; Sand-Jensen et al. 2005; Pedersen et al. 2009). Finally, aerenchyma also facilitates venting of ET accumulating in root tips, and thus alleviates inhibition of root growth (Visser & Pierik 2007). The efficiency of O₂ diffusion and the length of roots in anaerobic soils can be increased when radial loss of O₂ from the root to the soil is prevented (Armstrong 1979). To this end, plants either have a constitutive O₂ impermeable barrier characterised by deposition of new materials in exodermal cell walls (Shiono et al. 2011) or have the capacity to develop such a barrier upon flooding (Colmer 2003a; Garthaïte et al. 2003, 2006). Some plants improve internal aeration through the development of a completely new root system composed of mainly adventitious roots with a much higher porosity than the primary root system (Visser et al. 1996a; Lorbiecke & Sauter 1999).

The rate of photosynthesis under water is strongly reduced compared to aerial photosynthesis due to limited inward CO₂ diffusion. This is caused by an increased boundary layer resistance, non-functional stomata and resistances induced by the cuticle and the epidermal cells (Mommer et al. 2006a; Colmer et al. 2011). However, new leaves in some terrestrial plants that develop under water are thinner, have a higher specific leaf area (SLA), reorient their chloroplasts towards the epidermis side of the leaf, have thinner cuticles and cell walls, develop dissected leaves and sometimes retain gas films (Mommer et al. 2005b; Colmer et al. 2011). All of these traits reduce the diffusion resistance for CO₂ and result in higher rates of underwater photosynthesis compared to non-acclimated leaves (Mommer et al. 2006b). In addition, these traits also facilitate O₂ influx from the surrounding water (Mommer et al. 2004; Pedersen et al. 2009).

Upon submergence, some plant species demonstrate fast upward changes in leaf angles (hyponastic growth) and strongly enhanced elongation rates of petioles and/or stems (Ridge 1987; Voesenek & Blom 1989). Both growth responses can lead to emergence of leaf tips during shallow submergence, thus restoring influx of O₂ to diffuse via aerenchyma to O₂-depleted organs (Voesenek et al. 2004; Pierik et al. 2009).

In order to develop gas-filled pores in certain plant species, specific existing cells have to die. This type of aerenchyma is called lysigenous aerenchyma, and the primary signal that induces its development is accumulated ET, which, via a largely unknown route, triggers programmed cell death in particular cells (Drew et al. 2000). ET is most likely also the primary signal in the induction of adventitious roots. Further downstream, formation of adventitious roots is mediated by the plant hormone auxin and by H₂O₂ (Visser et al. 1996b; Steffens & Sauter 2009).

Interestingly, in the formation of the barrier in roots that prevents excessive O₂ loss to the anaerobic soil, ET, low O₂, elevated CO₂ or their combinations are not signals that induce barriers in rice (Colmer et al. 2006). The signal responsible for the formation of the barrier remains to be elucidated, but based on barrier induction in response to phytotoxins in rice (Armstrong & Armstrong 2001, 2005), it has been suggested that accumulation of root exudates or cellular degradation products might be important in barrier induction (Garthaïte et al. 2008).

Underwater photosynthesis is strongly stimulated in leaves developed under water due to the aforementioned anatomical and morphological leaf traits. Interestingly, these changes in leaf traits upon submergence were also observed in flood-intolerant species and result in increased gas exchange in these species expressed as internal O₂ concentration (Mommer et al. 2007). This observation suggests that these acclimations are more general and not submergence-specific. Increases in SLA are also observed when plants are grown under shade conditions (Poorter et al. 2009). Both low light environments and submergence environments lead to lower rates of photosynthesis, suggesting that this is directly or indirectly a signal that induces an increase in SLA. This hypothesis is strengthened through studies of plants with reduced rates of photosynthesis that developed elevated SLAs (Evans et al. 1994). Shade probably does not induce the complete suite of traits associated with improved gas exchange under water, as indicated by the lack of improved underwater photosynthesis in plants that were shade pretreated (Mommer et al. 2005a). As research data on the signalling of anatomical and morphological leaf acclimations upon submergence are very scarce, we cannot completely rule out a role for ET in these acclimation processes.

Hyponastic leaf growth and stimulated petiole/stem elongation are primarily regulated via enhanced ET levels in submerged shoots. Further downstream, nuclear-localised TFs belonging to the ERF, such as SNORKEL1 and SNORKEL2, are in deepwater rice important for shoot elongation. Additionally, three plant hormones, gibberellic acid, abscisic acid and auxin, seem to be indispensable for the full elongation response. Ultimately, these components activate target genes such as the cell wall loosening proteins ( expansins) resulting in turgo-driven cell elongation (Vreeburg et al. 2005; Benschop et al. 2006; Hattori et al. 2009).

In four very important inducible acclimations to flooding – aerenchyma, adventitious roots, hyponastic growth and enhanced petiole/stem elongation – accumulation of ET is the primary signal that triggers a signalling pathway resulting in improved gas exchange. ET is produced by almost every cell as long as some O₂ is available, accumulates rapidly in submerged tissues to physiologically relevant concentrations and the levels are fairly constant over time as long as the plant organ is submerged. In summary, ET is a very reliable component to trigger flood-adaptive signalling in submerged plants except under conditions in which the O₂ levels are completely depleted, such as during true anoxia that can develop in roots that are low in aerenchyma and other dense and/or bulky tissue types such as meristems, seeds and tubers. Two adaptive acclimations, the formation of a barrier to prevent excessive O₂ loss from submerged roots and leaf traits that improve gas exchange and thus underwater photosynthesis, are most likely not triggered by ET. It is therefore tempting to speculate that these plastic responses are not primarily selected to improve gas exchange during submerged conditions. It is possible that the barrier is primarily a mechanism developed to avoid influx of toxic soil components, which frequently accumulate in flooded soils, into plant roots. In line with this idea is the observation that phytotoxins, although at very high concentrations, can induce the barrier against radial O₂ loss (Armstrong & Armstrong 2001, 2005).

The molecular and physiological regulation of leaf traits that facilitate underwater gas exchange has not been studied.
thoroughly. However, the observation that these traits are equally induced in terrestrial flood-tolerant and -intolerant plant species (Mommer et al. 2005a,b) suggests that they were not selected under a severe flooding selection pressure. It is more likely that traits such as increased SLA and reduced cuticle thickness have their evolutionary origin in shaded and high humidity environments.

**Metabolic acclimations**

The electron transport chain generates ATP efficiently in mitochondria of higher plants as long as O2 concentrations within the mitochondria exceed those needed to saturate cytochrome c oxidase. During flooding, the generation of ATP is slowed down when the O2 concentration falls below the so-called critical oxygen pressure, caused by slow gas exchange between the atmosphere and the cells of flooded plants (Jackson & Drew 1984). This creates an energy crisis in flooded plants and evokes a regime of adaptive energy management. Plants in general respond to flooding with the following metabolic acclimations:

1. **Reduction of energy use.** To this end, translation is restricted to a minority of mRNAs. Energetically expensive processes such as ribosome biogenesis and cell wall formation are severely reduced (reviewed in Bailey-Serres & Voisenek 2010), whereas biochemical pathways that prefer pyrophosphate instead of ATP are prioritised (Huang et al. 2008).

2. **Activation of pathways that generate ATP without oxidative phosphorylation.** This is achieved via elevated sucrose catabolism, glycolysis and fermentation (reviewed in Bailey-Serres & Voisenek 2008).

3. **Induction of protective functions such as amelioration of ROS and chaperone activity (Mustroph et al. 2010).**

A substantial set of genes that mediate these processes are conserved over species and kingdoms (Mustroph et al. 2010), whereas a core set of 49 genes are similarly regulated in various cell types in Arabidopsis thaliana upon O2 deprivation (Mustroph et al. 2009).

Of these core hypoxia genes, 24 are also differentially regulated upon submergence of Arabidopsis (Lee et al. 2011), indicating overlap between submergence and low O2 regulated responses.

Various transcriptomic and metabolic studies in a range of plant species indicate that many of these core genes are induced upon O2 deprivation, suggesting a signalling role for low O2 (Sachs et al. 1980; Klok et al. 2002; Paul et al. 2004; Liu et al. 2005; Loreti et al. 2005; Lasanthi-Kudahettige et al. 2007; Branco-Price et al. 2008; van Dongen et al. 2009; Kreuzwieser et al. 2009; Mustroph et al. 2009; Christianson et al. 2010; Lee et al. 2011; Narasi et al. 2011). Low O2 also seems to regulate the selective inhibition of translation (Sachs et al. 1980; Branco-Price et al. 2008). ET is not produced under true anaerobic cell conditions and therefore can be excluded as a signal under such very extreme conditions. However, many experiments are done with low O2 levels that do not exclude ET production and therefore ET as a regulator of the core hypoxia genes cannot be ruled out. Surprisingly, experimental manipulation of ET during hypoxia studies of gene expression, by means of chemical inhibitors or specific mutants, is almost completely lacking. The importance of these experiments is stressed with work of Peng et al. (2001), in which the induction of alcohol dehydrogenase (ADH), one of the core hypoxia genes, can be inhibited by an inhibitor of ET biosynthesis and in ET sensing and signalling mutants. Interestingly, in this work it was shown that ET alone cannot induce ADH expression.

It is known that RAP2.12, a member of the group VII ERF family of transcription factors, regulates the expression of ADH1 under hypoxia (Papdi et al. 2008). It is therefore interesting to understand how this gene and other members of group VII ERFs are regulated. The five group VII ERF members in Arabidopsis are regulated by hypoxia, ET and darkness in various combinations and with differences between roots and shoots (Hinz et al. 2010; Licaisi et al. 2010). This illustrates that the interplay between low O2, ET and probably carbohydrate deprivation (induced by darkness) must give plants ample opportunities to fine-tune their acclimative responses. A hypothesis could be that ET-induced ERFs are stabilised during hypoxia/anoxia (as a consequence of the N-end rule), resulting in faster and higher expression of some hypoxia genes.

In summary, low O2 is the most important signal that induces metabolic acclimations to flooding stress. It is the most reliable signal under anaerobic conditions in which ET production is limited and appropriate responses are required. The dominant role of low O2 signalling also makes sense if metabolic adjustments are not needed under conditions with high ET levels and normal O2 levels, which frequently occur in submerged shoots. Intriguing is the exact role of ET in metabolic adjustments during flooded conditions. There is evidence that at least one hypoxia core gene (ADH1) is (co)regulated by ET and that a subset of the Arabidopsis group VII ERFs, potentially operating as transcription factors for the core hypoxia genes, are ET-regulated. It is therefore tempting to speculate that ET might prime plant cells to future anoxic conditions in such a way that faster and/or higher induction of hypoxia-responsive genes is facilitated.

**CONCLUSIONS**

Adventitious roots, aerenchyma, hypoxic growth and elongation growth, plant traits that improve gas exchange between submerged plant organs and organs still above water, are mainly regulated by ET. This gaseous hormone is a very reliable proxy for submerged conditions as long as some O2 is present, which is needed for the continuation of ET production. There are, however, some indications that the presence of both ET and low O2 enhance hypoxic growth and elongation growth in Rumex species, suggesting an interaction between these two gases in stimulating growth of shoot organs (Voisenek & Blom 1999). It is tempting to speculate that the faster elongation rate when O2 and ET are combined is related to increased stress of the shoot and the high need to reach the water surface. More mechanistically it would be interesting to study the role of proteins that are stabilised in response to low O2 (ERFs) in this sensitisation to ET during hypoxic conditions. Growth, as in adventitious root initiation and outgrowth, hypoxic growth and overall shoot elongation, is costly in terms of energy use, and these processes are generally not initiated and continued when O2 is absent. This strongly argues for a dominant role of ET in the regulation of these growth processes as presence of some O2 is guaranteed. A well-described exception is enhanced elongation growth in stems of Potamogeton pectinatus and
coleoptiles of rice seedlings. In contrast to ET-driven elongation, these stems and coleoptiles are situated in anaerobic mud layers that completely lack O$_2$ and are stimulated to grow faster by the anoxic environmental conditions (Pearce & Jackson 1991; Summers & Jackson 1994).

The formation of lysigenous aerenchyma is triggered through ET. This facilitates aerenchyma in shoot organs that rarely become anoxic and ensures that aerenchyma formations in roots is initiated as quickly as possible, independent of the rate of O$_2$ depletion. This guarantees that aerenchyma is well developed by the time that some root cells are completely depleted of O$_2$.

The formation of barriers to prevent radial O$_2$ loss and the various leaf traits that improve exchange of CO$_2$ are likely not regulated either by ET or by low O$_2$. This suggests that these traits are selected for by other submergence-associated environmental conditions, such as low light levels, high humidity and high levels of toxic soil components. We hypothesise that the observed advantage of specific leaf traits and a barrier to prevent radial O$_2$ loss during flooded conditions is more the result of coincidence than directed selection to relieve the O$_2$ problem in flooded plants.

Metabolic adjustments, such as the reduction of energy use and the activation of pathways that generate energy without O$_2$ and protect cells from threatening chemical compounds generated during flooding, are predominantly regulated via declining O$_2$ levels and anoxia. However, understanding of the role of ET in regulating anaerobic metabolism is still in its infancy.

REFERENCES


