



## Tansley insight

# The role of ethylene in metabolic acclimations to low oxygen

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## Summary

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**Key words:** anaerobic metabolism, autophagy, ERFVII, ethylene, hypoxia, oxygen sensing, reactive oxygen species (ROS), submergence.

Submerged plants ultimately suffer from shortage in cellular oxygen availability (hypoxia) as a result of impaired gas diffusion underwater. The gaseous plant hormone ethylene is rapidly entrapped in submerged plant tissues and is an established regulator of morphological and anatomical flood-adaptive responses. Multiple recent discoveries suggest that ethylene also plays a crucial role in hypoxia anticipation and metabolic acclimation during plant submergence. Ethylene was shown to accelerate and enhance the hypoxic response through enhanced stability of specific transcription factors (group VII ethylene response factors). Moreover, we suggest that ethylene could play an important role in the induction of autophagy and promote reactive oxygen species amelioration, thereby contributing to enhanced survival during flooding, hypoxia, and reoxygenation stress.

## I. Introduction

During flooding, submerged plants are exposed to an excess of water in their direct environment. Compared with an aerated environment, gas diffusion underwater is decreased approximately  $10^4$ -fold as described in Fick's law (Fick, 1855), which restricts oxygen ( $O_2$ ) and  $CO_2$  exchange between submerged plant tissues and the environment (Box 1). Moreover, reduced light penetration through typically turbid floodwaters can further limit photosynthesis and subsequent carbohydrate and  $O_2$  production (Pedersen *et al.*, 2018). Consequently, submerged terrestrial plants ultimately suffer from severe  $O_2$  deprivation (hypoxia), terminating mitochondrial respiration and ATP production. The resulting

carbohydrate and energy crisis seriously reduces plant growth and survival. Moreover, the endogenous levels of the gaseous plant hormone ethylene, produced by all cells in nearly all higher plants, significantly increase upon submergence (Voesenek & Sasidharan, 2013). This fast cellular accumulation of ethylene in submerged plants enables this hormone to act as a reliable part of the submergence sensing mechanism and initiator of a cascade of adaptive responses (Sasidharan *et al.*, 2018). Indeed, ethylene entrapment is at the basis of lysigenous aerenchyma formation (Drew *et al.*, 1979), the induction and outgrowth of adventitious roots (Drew *et al.*, 1979; Visser *et al.*, 1996), enhancement of shoot elongation, and a more erect leaf position (hyponasty; Ku *et al.*, 1970; Banga *et al.*, 1997). These morphological and anatomical

features all improve the aeration status of (partially) submerged plants, thus avoiding O<sub>2</sub> depletion.

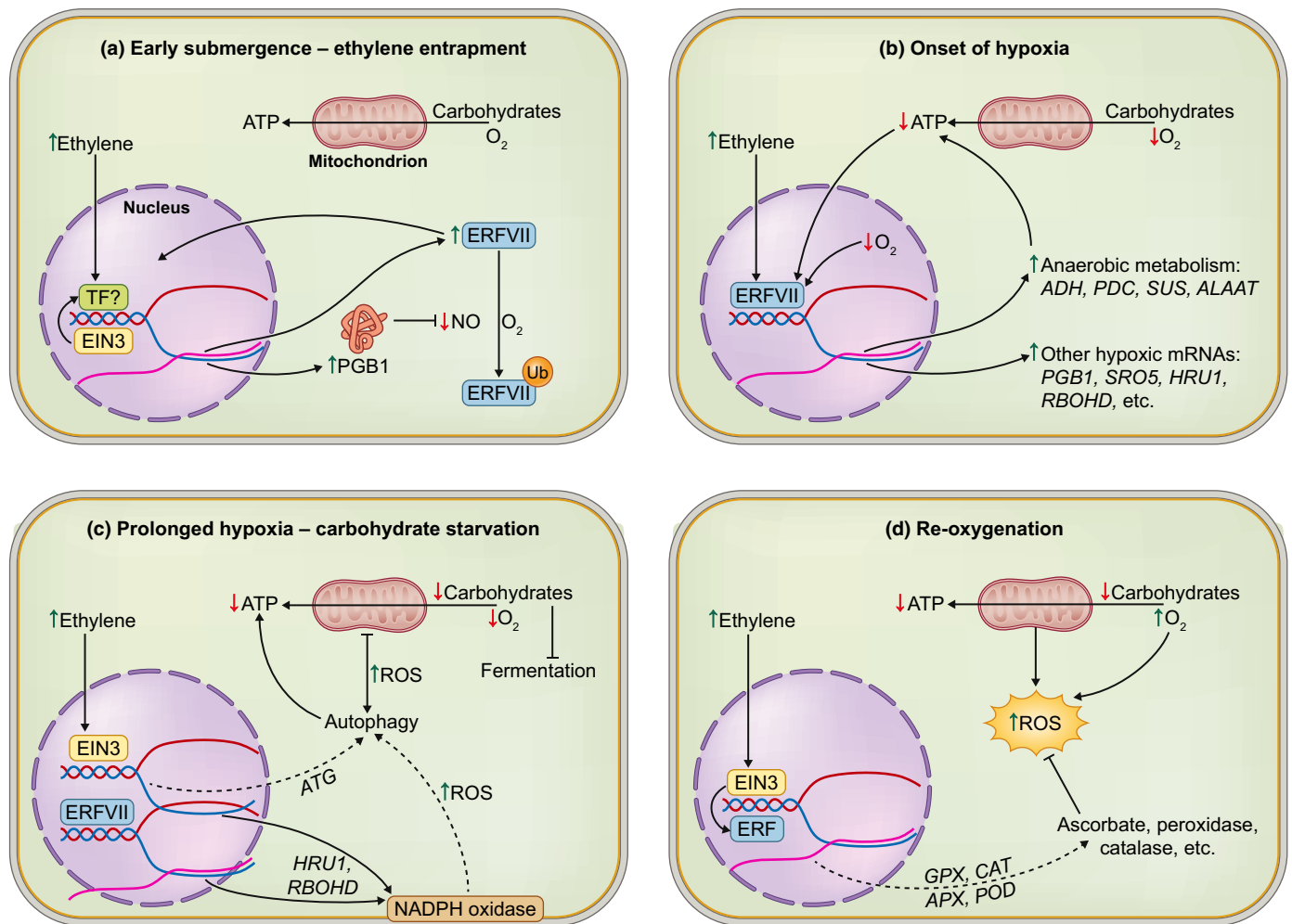
Alternatively, plants can sustain flooding-induced hypoxia through metabolic changes that ultimately result in energy maintenance and oxidative stress management. Indeed, when O<sub>2</sub> levels decline, mitochondrial respiration and ATP synthesis are restricted and hypoxic cells subsequently rely on glycolysis for substrate-level ATP production (Geigenberger, 2003; Bailey-Serres *et al.*, 2012). To maintain the glycolytic flux, fermentation pathways are initiated to regenerate NAD<sup>+</sup> (Fig. 1b). As fermentation quickly depletes the plant's carbohydrates and contributes to cytosolic acidification, alanine synthesis is enhanced to limit this carbon loss and a  $\gamma$ -aminobutyric acid shunt is initiated that assists in stabilization of the cytosolic pH (Ricoult *et al.*, 2006; Miyashita & Good, 2008). In addition, energy-consuming processes such as protein synthesis are reduced (Branco-Price *et al.*, 2008), whereas sucrose and starch metabolism are altered in several plant species/tissues to meet the increased carbohydrate demand during hypoxia (Guglielminetti *et al.*, 1995; Loreti *et al.*, 2018). Moreover, some reports suggest that mitochondrial respiration may be actively downregulated in response to hypoxia (Geigenberger, 2003; Zabalza *et al.*, 2008; Ramírez-Aguilar *et al.*, 2011). Finally, plant cells induce multiple pathways to limit the oxidative stress damage that occurs during hypoxia and subsequent reoxygenation (Fig. 1c, d; Gonzali *et al.*, 2015; Yeung *et al.*, 2018). Interestingly, these hypoxia-induced acclimations are not restricted to flooding stress but seem to be an inherent part of plant development, as hypoxia also occurs in developing seeds, fruits, meristems, vascular bundles, tubers, crown gall tumours, and lateral roots (Kerpen *et al.*, 2019; Shukla *et al.*, 2019; Weits *et al.*, 2019). In recent times, our understanding of adaptive processes during flooding developed significantly, and an important role for ethylene was also established in the initiation and regulation of metabolic modifications during hypoxia. Here, we discuss these ethylene-induced metabolic adjustments that prolong survival during flooding/low O<sub>2</sub> stress.

## II. Early ethylene signalling regulates hypoxia acclimation and anaerobic metabolism

Recent research has shown that ethylene not only accumulates rapidly in submerged plant tissues (Banga *et al.*, 1996), but also leads to rapid ethylene-dependent signalling. Indeed, nuclear accumulation of Ethylene Insensitive 3 (EIN3), the principal transcriptional regulator of ethylene signalling, increased within 1 h of submergence in Arabidopsis root tips (Hartman *et al.*, 2019). This rapid induction of ethylene signalling enables plants to anticipate upcoming hypoxia (Box 1) and was shown as crucial for improved hypoxia tolerance through enhanced expression of a core hypoxia gene set when O<sub>2</sub> levels declined (Hartman *et al.*, 2019). Induction of core hypoxia genes is thought to be adaptive to survive prolonged hypoxia. This core set includes genes encoding proteins involved in fermentation, energy maintenance, oxidative stress, nitric oxide (NO) scavenging, O<sub>2</sub> sensing, and ethylene perception and biosynthesis (Mustroph *et al.*, 2010).

A potential role of ethylene in the induction of fermentation genes has previously been investigated. Morrell and Greenway (1989) described that both alcohol dehydrogenase (ADH) and pyruvate decarboxylase (PDC) activities in maize (*Zea mays*) and rice (*Oryza sativa*) were not significantly increased upon ethylene exposure alone. Furthermore, inhibition of the ethylene receptor ETR1 by means of silver nitrate did not reduce the enhanced activity of these two enzymes during low O<sub>2</sub> conditions (McDaniel & Binder, 2012). However, another study revealed that the hypoxic induction of ADH in Arabidopsis could be partially inhibited by aminooxy acetic acid, an inhibitor of ethylene biosynthesis (Peng, 2001). This inhibition could be rescued by 1-aminocyclopropane-1-carboxylic acid, the direct precursor of ethylene. Furthermore, two ethylene-insensitive mutants showed a strong reduction in ADH transcripts upon hypoxia. Similar to the Morrell and Greenway (1989) study, ethylene alone was unable to induce ADH transcription under normoxic conditions in Arabidopsis. Peng (2001), therefore, concluded that ethylene is needed, but not sufficient, for the induction of the core hypoxia gene ADH during later stages of hypoxia. In the wetland plant *Rumex palustris*, 4 h of ethylene treatment resulted in a small, but significant, induction of the core hypoxia genes ADH, PHYTOGLOBIN1 (PGB1), and ACR7 (van Veen *et al.*, 2013). When the ethylene treatment was followed by hypoxia there was a stronger increase in several core hypoxia gene transcripts, compared with controls that did not receive ethylene pretreatment. These results showed that ethylene is an essential signal preparing *R. palustris* for future hypoxia through a sensitized transcriptional hypoxia response.

Recent evidence demonstrated a similar beneficial effect of ethylene in Arabidopsis and also elucidated a mechanism by which ethylene augments both the transcriptional response to hypoxia and subsequent survival (Hartman *et al.*, 2019). This mechanism, as described subsequently, integrates ethylene signalling with the plant's O<sub>2</sub>-sensing machinery. During normoxia, ethylene response factor proteins belonging to the class VII (ERFVII) are degraded through the arginylation branch of the proteolysis 6 (PRT6) N-degron pathway. These ERFVII are broken down following the oxidation of the N-terminal amino acid cysteine by plant cysteine oxidases in the presence of O<sub>2</sub> (Gibbs *et al.*, 2011; Licausi *et al.*, 2011; Weits *et al.*, 2014). A decline in either O<sub>2</sub> or NO stabilizes ERFVII and other cysteine-initiating proteins, leading to expression of core hypoxia genes and hypoxia acclimation (Gibbs *et al.*, 2011, 2014, 2018; Weits *et al.*, 2019). Hartman *et al.* (2019) demonstrated that ethylene could also impair ERFVII proteolysis, adding a third gas to the regulation of ERFVII stability. Ethylene-induced ERFVII stability is initiated by a rapid increase of PGB1 messenger RNA and protein abundance. PGB1 is a powerful scavenger of NO (Hebelstrup *et al.*, 2012), resulting in a substantial decline of NO and consequential stabilization of ERFVII (Fig. 1a). Interestingly, the resulting nuclear accumulation of ERFVII does not induce the transcription of core hypoxia genes until an additional hypoxia signal (including a drop in ATP) is introduced (Schmidt *et al.*, 2018). This corroborates older observations that ethylene alone cannot induce expression of the full core hypoxia gene set under normoxic conditions (Morrell & Greenway, 1989; Peng, 2001; van Veen *et al.*, 2013). However, as



**Fig. 1** The role of ethylene in known and proposed metabolic adjustments during flooding-induced hypoxia. Key: green arrows, increase; red arrows, decrease; dashed lines, hypothesized and requires experimental confirmation. (a) In *Arabidopsis*, entrapped ethylene upon submergence enhances messenger RNA (mRNA) and protein levels of nitric oxide (NO)-scavenger PHYTOGLOBIN1 (PGB1), limiting NO-dependent class VII ethylene response factor (ERFVII) proteolysis and enhancing ERFVII accumulation in the nucleus. In addition, ERFVII transcription is under direct control of ethylene signalling in *Arabidopsis* and rice. (b) When oxygen ( $O_2$ ) levels decline, oxidative phosphorylation is reduced and limits ATP production. This drop in  $O_2$  (and ATP) activates the ethylene-enhanced ERFVII pool and initiates hypoxia-adaptive gene expression and stimulates anaerobic metabolism through processes like ethanolic fermentation. (c) When carbohydrates are depleted, a feedback loop downregulates fermentation genes downstream of ERFVII action. Under prolonged hypoxia and carbohydrate starvation, autophagy can be induced to supply the cell with alternative energy-rich substrates. Ethylene is suggested to mediate autophagy through the induction of *ATG* genes and altered reactive oxygen species (ROS) levels, but only under carbohydrates-limited conditions. (d) Upon reoxygenation, high light and  $O_2$  levels lead to excess ROS levels. Ethylene was shown to strongly reduce ROS levels and confer tolerance under a variety of abiotic stresses through direct production of ROS scavenging compounds, but a role for ethylene in ROS detoxification under flooding stress remains to be revealed. ADH, alcohol dehydrogenase; ALAAT, alanine aminotransferase; APX, ascorbate peroxidase; CAT, catalase; EIN3, Ethylene Insensitive 3; GPX, glutathione peroxidase; HRU1, Hypoxia Responsive Universal Stress Protein 1; PDC, pyruvate decarboxylase; POD, peroxidase; RBOHD, Respiratory Burst Oxidase Homologue Protein D; SRO5, SIMILAR TO RCD ONE5; SUS, sucrose synthase; TF, transcription factor; Ub, ubiquitin.

soon as  $O_2$  levels decline, the transcription of core hypoxia genes is faster and stronger than in controls without prior ethylene treatment (Fig. 1b). Taken together, these results show that ethylene entrapment can prime aerobic plant tissues for potentially impending severe hypoxia (Box 1) for as long as submergence lasts or sufficient carbohydrates are available to maintain fermentation pathways. Moreover, it provides a molecular mechanism for how ethylene integrates with  $O_2$  sensing and facilitates the appropriate induction of genes involved in fermentation, carbohydrate metabolism, and oxidative stress management during submergence in *Arabidopsis* (Fig. 1a,b).

### III. Ethylene mediates metabolic adjustments facilitating flooding survival strategies

Ethylene initiates both the flooding ‘escape’ and ‘quiescence’ survival strategies in several plant species (Ku *et al.*, 1970; Xu *et al.*, 2006; van Veen *et al.*, 2013). For example, ethylene activates the ERFVII transcription factors SNORKEL1 and 2 in deepwater rice, which in turn enhance internode elongation to escape hypoxia through restoration of above-water gas exchange (Hattori *et al.*, 2009). Conversely, ethylene also actively represses shoot elongation in lowland rice varieties, through induction of

**Box 1** Gas diffusion in submerged plant tissues. 1.1

As a consequence of Fick's first law of diffusion (Fick, 1855), the gas diffusion coefficient in gaseous phases is  $c. 10^4$  times faster than in liquid phases. Therefore, during flooding, there is a much slower exchange of gases such as oxygen ( $O_2$ ),  $CO_2$ , and ethylene between submerged plants and their aerial environment.

$O_2$  solubility in water is relatively low, and its levels decrease rapidly in flooded respiring ( $O_2$ -consuming) tissues when light levels (night/turbid water) and subsequent photosynthetically derived  $O_2$  production are limited (Colmer & Pedersen, 2008). However, in highly illuminated flooded tissues,  $O_2$  levels increase and can even become hyperoxic (Pedersen *et al.*, 2018).

$CO_2$  levels generally increase strongly under reduced light conditions in  $C_3$  plants, but may decrease in illuminated flooded tissues (further limiting  $O_2$  production from photosynthesis; Pedersen *et al.*, 2018).  $CO_2$  can dissolve in water, and increased levels can lead to elevated bicarbonate formation and acidification of the cytosol and the plant's aquatic surroundings (Felle, 2005). Interestingly, the reverse is also possible: some (semi)aquatic species retrieve bicarbonate from the aquatic environment to fuel photosynthesis (Rascio *et al.*, 1999).

**Ethylene** is produced in nearly all plant cells, dissolves poorly in water, and quickly and invariably accumulates to high levels in submerged plant tissues, until floodwaters recede (Banga *et al.*, 1996; Voisenek & Sasidharan, 2013). Ethylene can be detected using a variety of methods (Cristescu *et al.*, 2013). In *Arabidopsis thaliana* and *Rumex palustris*, ethylene is used as a signal to anticipate and acclimate to an impending  $O_2$  decline during plant submergence (van Veen *et al.*, 2013; Hartman *et al.*, 2019). Moreover, ethylene enhances metabolic acclimation responses during hypoxia and reoxygenation (Peng, 2001; Tsai *et al.*, 2014).

the ERFVII SUBMERGENCE 1A (SUB1A; Xu *et al.*, 2006). Interestingly, in rice, some of these ERFVII orthologues are thought to be shielded from the PRT6 N-degron pathway, placing their protein levels under direct control of ethylene signalling (Fig. 1a; Lin *et al.*, 2019). In addition to controlling flood-adaptive growth responses, these ethylene-mediated rice ERFVIIs may also contribute to metabolic acclimations that allow these contrasting adaptive growth responses to occur. For instance, the elongating stems of fully submerged deepwater rice showed strong induction of fermentation and glycolysis under hypoxia (Mori *et al.*, 2019), in turn fueling the snorkelling escape response. Conversely, in SUB1A rice cultivars where growth is restricted, ethylene-regulated SUB1A limits starch breakdown and carbohydrate metabolism under submergence and post-submergence, while increasing catabolism of several amino acids such as alanine (Barding *et al.*, 2012; Locke *et al.*, 2018). Similarly, ethylene was shown to control replenishment of the citric acid cycle through enhanced glutamate dehydrogenase activity and breakdown of alanine under anoxia and reoxygenation in *Arabidopsis* (Tsai *et al.*, 2016). Finally, both ethylene and SUB1A are also required for enhanced fermentation and the amelioration of reactive oxygen species (ROS) during plant submergence and subsequent reoxygenation (Xu *et al.*, 2006; Fukao *et al.*, 2011). Together, these results illustrate that ethylene co-regulates anaerobic metabolism through altered carbohydrate and amino acid breakdown over the course of a flooding event, but that the output is tailored to the plant's survival strategy and its specific ERFVII regulators.

#### IV. A potential role for ethylene in the induction of autophagy

Though the induction of fermentation is crucial for anaerobic metabolism, it will rapidly deplete the plant's carbohydrate reserves. Indeed, under carbohydrate and light-limited

conditions anaerobic metabolism is ultimately dampened downstream of ERFVII action (Loreti *et al.*, 2018). To maintain the energy balance, plants can release alternative energy-rich substrates (such as amino acids and fatty acids) through autophagy and senescence (Barros *et al.*, 2017). We propose that ethylene could also modulate autophagy during flooding stress. Autophagy was shown to be crucial for survival during submergence in *Arabidopsis* (Chen *et al.*, 2015), and is thought to play a major role in abiotic stress tolerance in plants (Avin-Wittenberg, 2019). Recent reports suggest that ethylene controls the induction of autophagy and autophagy (*ATG*) genes in several plant species, but only once starvation occurs (Okuda *et al.*, 2011; Zhu *et al.*, 2018). This is in accordance with the observation that high glucose downregulates ethylene signalling on the level of EIN3 stability through the plant glucose sensor hexokinase (Yanagisawa *et al.*, 2003). How ethylene exactly mediates autophagy is unclear, but it could require modulation of ROS signalling and/or direct binding of EIN3 to several *ATG* promoters under specific conditions (Okuda *et al.*, 2011; Zhu *et al.*, 2018). Interestingly, in mammalian cells, hypoxia-mediated analogous proteins of the plant's  $O_2$ -sensing ERFVIIs induce autophagy of  $O_2$ -consuming mitochondria and confer anaerobic cell survival (Zhang *et al.*, 2008). Moreover, mitochondrial breakdown under severe hypoxia in plants coincides with the timing ( $c. 12$  h) of the aforementioned downregulation of fermentation genes (Vartapetian *et al.*, 2003; Loreti *et al.*, 2018). We therefore suggest it would be highly interesting to determine if similar  $O_2$ -dependent mechanisms are at play in submerged plants and if they are co-regulated by ethylene. Taken together, these reports suggest that ethylene-induced *ATG* genes and autophagy could contribute to the maintenance of energy production during long-term hypoxia through both selective autophagy of energy-consuming compounds and generation of alternative energy-rich substrates (Fig. 1c).

## V. Ethylene ameliorates excessive reactive oxygen species damage

Once flood waters recede, reoxygenation coincides with increased ethylene production and excessive ROS formation damaging plant cells (Yeung *et al.*, 2019). Accordingly, enhanced control of ROS detoxification was shown to be crucial for hypoxia, reoxygenation, and flooding tolerance (Gonzali *et al.*, 2015; Yeung *et al.*, 2018). Although ethylene is known to control ROS production through increased NADPH oxidase levels and activity (Desikan *et al.*, 2006), a functional link with ethylene in reducing and ameliorating ROS damage has hardly been investigated during flooding stress. However, evidence in the literature shows that ethylene regulates ROS-scavenging compounds, and we therefore propose that ethylene is also essential for ROS amelioration during flooding stress. First, one study suggests that ethylene mediates ROS detoxification during reoxygenation in *Arabidopsis* (Tsai *et al.*, 2014). Moreover, ethylene-regulated oxidative stress tolerance has been extensively studied in several plant species for a plethora of other abiotic stresses, including drought, heat, freezing, and salt stress (Wu *et al.*, 2008; Peng *et al.*, 2014). Interestingly, heat, drought, and salt stress tolerance were also shown to be dependent on enhanced ERFVII stability (Vicente *et al.*, 2017), and ERFVIIs regulate several genes that confer oxidative stress tolerance (Gonzali *et al.*, 2015; Papdi *et al.*, 2015). Finally, it was shown that ethylene, EIN3, and EIN3-target transcription factors directly control multiple genes that code for proteins involved in ROS amelioration across multiple plant species, such as carbonic anhydrase, catalase, peroxidase, and ascorbic acid biosynthesis (Wu *et al.*, 2008; Peng *et al.*, 2014; Zhang *et al.*, 2016). Together, these observations support that ethylene could also play an important role in ROS detoxification and oxidative stress tolerance during flooding and reoxygenation stress (Fig. 1d).



## VI. Conclusions and future perspectives

For decades, ethylene has been known as the regulator of flood-adaptive growth responses that avoid or delay hypoxia, but was not considered to mediate hypoxia acclimation in plants. Recent findings show that ethylene entrapment during flooding also plays a crucial role in hypoxia anticipation and acclimation. In addition, ethylene appears to play an important role in metabolic reconfiguration during hypoxia and reoxygenation through the induction and regulation of ERFVII transcription factors, hypoxia-adaptive gene expression, autophagy, and ROS detoxification in plants (Fig. 1). Interestingly, whereas some non-flood-adapted terrestrial plant species, such as *Arabidopsis*, use ethylene to acclimate to hypoxia, other species, like the wild plant *Rumex acetosa* and the cultivated *Solanum lycopersicum*, do not (van Veen *et al.*, 2013; Hartman *et al.*, 2019). In the future it would be worthwhile to uncover what molecular mechanisms uncouple ethylene signalling from hypoxia acclimation responses in these species, with the ultimate aim to (re)introduce adaptive mechanisms in important, but flood-sensitive crop species.

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## References

- Avin-Wittenberg T. 2019. Autophagy and its role in plant abiotic stress management. *Plant, Cell & Environment* 42: 1045–1053.
- Bailey-Serres J, Fukao T, Gibbs DJ, Holdsworth MJ, Lee SC, Licausi F, Perata P, Voeselek LACJ, Van Dongen JT. 2012. Making sense of low oxygen sensing. *Trends in Plant Science* 17: 129–138.
- Banga M, Bögemann GM, Blom CWPM, Voeselek LACJ. 1997. Flooding resistance of *Rumex* species strongly depends on their response to ethylene: rapid shoot elongation or foliar senescence. *Physiologia Plantarum* 99: 415–422.
- Banga M, Slaa EJ, Blom CWPM, Voeselek LACJ. 1996. Ethylene biosynthesis and accumulation under drained and submerged conditions (a comparative study of two *Rumex* species). *Plant Physiology* 112: 229–237.
- Barding GA, Fukao T, Béni S, Bailey-Serres J, Larive CK. 2012. Differential metabolic regulation governed by the rice *SUB1A* gene during submergence stress and identification of alanyl-glycine by <sup>1</sup>H NMR spectroscopy. *Journal of Proteome Research* 11: 320–330.
- Barros JAS, Cavalcanti JHF, Medeiros DB, Nunes-Nesi A, Avin-Wittenberg T, Fernie AR, Araújo WL. 2017. Autophagy deficiency compromises alternative pathways of respiration following energy deprivation in *Arabidopsis thaliana*. *Plant Physiology* 175: 62–76.
- Branco-Price C, Kaiser KA, Jang CJH, Larive CK, Bailey-Serres J. 2008. Selective mRNA translation coordinates energetic and metabolic adjustments to cellular oxygen deprivation and reoxygenation in *Arabidopsis thaliana*. *The Plant Journal* 56: 743–755.
- Chen L, Liao B, Qi H, Xie L-J, Huang L, Tan W-J, Zhai N, Yuan L-B, Zhou Y, Yu L-J *et al.* 2015. Autophagy contributes to regulation of the hypoxia response during submergence in *Arabidopsis thaliana*. *Autophagy* 11: 2233–2246.
- Colmer TD, Pedersen O. 2008. Oxygen dynamics in submerged rice (*Oryza sativa*). *New Phytologist* 178: 326–334.
- Cristescu SM, Mandon J, Arslanov D, De Pessemier J, Hermans C, Harren FJM. 2013. Current methods for detecting ethylene in plants. *Annals of Botany* 111: 347–360.
- Desikan R, Last K, Harrett-Williams R, Tagliavia C, Harter K, Hooley R, Hancock JT, Neill SJ. 2006. Ethylene-induced stomatal closure in *Arabidopsis* occurs via AtrbohF-mediated hydrogen peroxide synthesis. *The Plant Journal* 47: 907–916.
- Drew MC, Jackson MB, Giffard S. 1979. Ethylene-promoted adventitious rooting and development of cortical air spaces (aerenchyma) in roots may be adaptive responses to flooding in *Zea mays* L. *Planta* 147: 83–88.
- Felle HH. 2005. pH regulation in anoxic plants. *Annals of Botany* 96: 519–532.
- Fick A. 1855. Ueber diffusion. *Annalen der Physik und Chemie* 170: 59–86.
- Fukao T, Yeung E, Bailey-Serres J. 2011. The submergence tolerance regulator SUB1A mediates crosstalk between submergence and drought tolerance in rice. *Plant Cell* 23: 412–427.
- Geigenberger P. 2003. Response of plant metabolism to too little oxygen. *Current Opinion in Plant Biology* 6: 247–256.
- Gibbs DJ, Lee SC, Md Isa N, Gramuglia S, Fukao T, Bassel GW, Correia CS, Corbineau F, Theodoulou FL, Bailey-Serres J *et al.* 2011. Homeostatic response to hypoxia is regulated by the N-end rule pathway in plants. *Nature* 479: 415–418.

- Gibbs DJ, Md Isa N, Movahedi M, Lozano-Juste J, Mendiondo GM, Berckhan S, Marin-de la Rosa N, Vicente Conde J, Sousa Correia C, Pearce SP *et al.* 2014. Nitric oxide sensing in plants is mediated by proteolytic control of group VII ERF transcription factors. *Molecular Cell* 53: 369–379.
- Gibbs DJ, Tedds HM, Labandera A-M, Bailey M, White MD, Hartman S, Sprigg C, Mogg SL, Osborne R, Dambire C *et al.* 2018. Oxygen-dependent proteolysis regulates the stability of angiosperm polycomb repressive complex 2 subunit VERNALIZATION 2. *Nature Communications* 9: e5438.
- Gonzali S, Loreti E, Cardarelli F, Novi G, Parlanti S, Pucciariello C, Bassolino L, Banti V, Licausi F, Perata P. 2015. Universal stress protein HRU1 mediates ROS homeostasis under anoxia. *Nature Plants* 1: e15151.
- Guglielminetti L, Perata P, Alpi A. 1995. Effect of anoxia on carbohydrate metabolism in rice seedlings. *Plant Physiology* 108: 735–741.
- Hartman S, Liu Z, van Veen H, Vicente J, Reinen E, Martopawiro S, Zhang H, van Dongen N, Bosman F, Bassel GW *et al.* 2019. Ethylene-mediated nitric oxide depletion pre-adapts plants to hypoxia stress. *Nature Communications* 10: e4020.
- Hattori Y, Nagai K, Furukawa S, Song X-J, Kawano R, Sakakibara H, Wu J, Matsumoto T, Yoshimura A, Kitano H *et al.* 2009. The ethylene response factors SNORKEL1 and SNORKEL2 allow rice to adapt to deep water. *Nature* 460: 1026–1030.
- Hebelstrup KH, van Zanten M, Mandon J, Voesenek LACJ, Harren FJM, Cristescu SM, Møller IM, Mur LAJ. 2012. Haemoglobin modulates NO emission and hyponasty under hypoxia-related stress in *Arabidopsis thaliana*. *Journal of Experimental Botany* 63: 5581–5591.
- Kerpen L, Niccolini L, Licausi F, van Dongen JT, Weits DA. 2019. Hypoxic conditions in crown galls induce plant anaerobic responses that support tumor proliferation. *Frontiers in Plant Science* 10: e56.
- Ku HS, Suge H, Rappaport L, Pratt HK. 1970. Stimulation of rice coleoptile growth by ethylene. *Planta* 90: 333–339.
- Licausi F, Kosmacz M, Weits DA, Giuntoli B, Giorgi FM, Voesenek LACJ, Perata P, van Dongen JT. 2011. Oxygen sensing in plants is mediated by an N-end rule pathway for protein destabilization. *Nature* 479: 419–422.
- Lin C-C, Chao Y-T, Chen W-C, Ho H-Y, Chou M-Y, Li Y-R, Wu Y-L, Yang H-A, Hsieh H, Lin C-S *et al.* 2019. Regulatory cascade involving transcriptional and N-end rule pathways in rice under submergence. *Proceedings of the National Academy of Sciences, USA* 116: 3300–3309.
- Locke AM, Barding GA, Sathnur S, Larive CK, Bailey-Serres J. 2018. Rice *SUB1A* constrains remodelling of the transcriptome and metabolome during submergence to facilitate post-submergence recovery. *Plant, Cell & Environment* 41: 721–736.
- Loreti E, Valeri MC, Novi G, Perata P. 2018. Gene regulation and survival under hypoxia requires starch availability and metabolism. *Plant Physiology* 176: 1286–1298.
- McDaniel BK, Binder BM. 2012. Ethylene Receptor 1 (ETR1) is sufficient and has the predominant role in mediating inhibition of ethylene responses by silver in *Arabidopsis thaliana*. *Journal of Biological Chemistry* 287: 26094–26103.
- Miyashita Y, Good AG. 2008. Contribution of the GABA shunt to hypoxia-induced alanine accumulation in roots of *Arabidopsis thaliana*. *Plant and Cell Physiology* 49: 92–102.
- Mori Y, Kurokawa Y, Koike M, Malik AI, Colmer TD, Ashikari M, Pedersen O, Nagai K. 2019. Diel O<sub>2</sub> dynamics in partially and completely submerged deepwater rice: leaf gas films enhance internodal O<sub>2</sub> status, influence gene expression and accelerate stem elongation for 'snorkelling' during submergence. *Plant and Cell Physiology* 60: 973–985.
- Morrell S, Greenway H. 1989. Evidence does not support ethylene as a cue for synthesis of alcohol dehydrogenase and pyruvate decarboxylase during exposure to hypoxia. *Functional Plant Biology* 16: 469–475.
- Mustroph A, Lee SC, Oosumi T, Zanetti ME, Yang H, Ma K, Yaghoubi-Maslihi A, Fukao T, Bailey-Serres J. 2010. Cross-kingdom comparison of transcriptomic adjustments to low-oxygen stress highlights conserved and plant-specific responses. *Plant Physiology* 152: 1484–1500.
- Okuda M, Nang MP, Oshima K, Ishibashi Y, Zheng S-H, Yuasa T, Iwaya-Inoue M. 2011. The ethylene signal mediates induction of *GmATG8i* in soybean plants under starvation stress. *Bioscience, Biotechnology, and Biochemistry* 75: 1408–1412.
- Papdi C, Pérez-Salamó I, Joseph MP, Giuntoli B, Bögre L, Koncz C, Szabados L. 2015. The low oxygen, oxidative and osmotic stress responses synergistically act through the ethylene response factor VII genes *RAP2.12*, *RAP2.2* and *RAP2.3*. *The Plant Journal* 82: 772–784.
- Pedersen O, Colmer TD, Garcia-Robledo E, Revsbech NP. 2018. CO<sub>2</sub> and O<sub>2</sub> dynamics in leaves of aquatic plants with C<sub>3</sub> or CAM photosynthesis – application of a novel CO<sub>2</sub> microsensor. *Annals of Botany* 122: 605–615.
- Peng H-P. 2001. Signaling events in the hypoxic induction of alcohol dehydrogenase gene in *Arabidopsis*. *Plant Physiology* 126: 742–749.
- Peng J, Li Z, Wen X, Li W, Shi H, Yang L, Zhu H, Guo H. 2014. Salt-induced stabilization of EIN3/EIL1 confers salinity tolerance by deterring ROS accumulation in *Arabidopsis*. *PLoS Genetics* 10: e1004664.
- Ramírez-Aguilar SJ, Keuthe M, Rocha M, Fedyav VV, Kramp K, Gupta KJ, Rasmussen AG, Schulze WX, van Dongen JT. 2011. The composition of plant mitochondrial supercomplexes changes with oxygen availability. *Journal of Biological Chemistry* 286: 43045–53.
- Rascio N, Cuccato F, Dalla Vecchia F, La Rocca N, Larcher W. 1999. Structural and functional features of the leaves of *Ranunculus trichophyllus* Chaix., a freshwater submerged macrophyte. *Plant, Cell & Environment* 22: 205–212.
- Ricoult C, Echeverria LO, Cliquet J-B, Limami AM. 2006. Characterization of alanine aminotransferase (*AlaAT*) multigene family and hypoxic response in young seedlings of the model legume *Medicago truncatula*. *Journal of Experimental Botany* 57: 3079–3089.
- Sasidharan R, Hartman S, Liu Z, Martopawiro S, Sajeev N, van Veen H, Yeung E, Voesenek LACJ. 2018. Signal dynamics and interactions during flooding stress. *Plant Physiology* 176: 1106–1117.
- Schmidt RR, Fulda M, Paul MV, Anders M, Plum F, Weits DA, Kosmacz M, Larson TR, Graham IA, Beemster GTS *et al.* 2018. Low-oxygen response is triggered by an ATP-dependent shift in oleoyl-CoA in *Arabidopsis*. *Proceedings of the National Academy of Sciences, USA* 115: E12101–E12110.
- Shukla V, Lombardi L, Iacopino S, Pencik A, Novak O, Perata P, Giuntoli B, Licausi F. 2019. Endogenous hypoxia in lateral root primordia controls root architecture by antagonizing auxin signaling in *Arabidopsis*. *Molecular Plant* 12: 538–551.
- Tsai K-J, Chou S-J, Shih M-C. 2014. Ethylene plays an essential role in the recovery of *Arabidopsis* during post-anaerobiosis reoxygenation. *Plant, Cell & Environment* 37: 2391–2405.
- Tsai K-J, Lin C-Y, Ting C-Y, Shih M-C. 2016. Ethylene-regulated glutamate dehydrogenase fine-tunes metabolism during anoxia–reoxygenation. *Plant Physiology* 172: 1548–1562.
- Vartapetian BB, Andreeva IN, Generozova IP, Polyakova LI, Maslova IP, Dolgikh YI, Stepanova AY. 2003. Functional electron microscopy in studies of plant response and adaptation to anaerobic stress. *Annals of Botany* 91: 155–172.
- van Veen H, Mustroph A, Barding GA, Eijk MV, Welschen-Evertman RAM, Pedersen O, Visser EJW, Larive CK, Pierik R, Bailey-Serres J *et al.* 2013. Two *Rumex* species from contrasting hydrological niches regulate flooding tolerance through distinct mechanisms. *Plant Cell* 25: 4691–4707.
- Vicente J, Mendiondo GM, Movahedi M, Peirats-Llobet M, Juan Y, Shen Y, Dambire C, Smart K, Rodriguez PL, Charny Y *et al.* 2017. The Cys-Arg/N-end rule pathway is a general sensor of abiotic stress in flowering plants. *Current Biology* 27: 3183–3190.e4.
- Visser EJW, Cohen JD, Barendse G, Blom CWPM, Voesenek LACJ. 1996. An ethylene-mediated increase in sensitivity to auxin induces adventitious root formation in flooded *Rumex palustris* Sm. *Plant Physiology* 112: 1687–1692.
- Voesenek LACJ, Sasidharan R. 2013. Ethylene– and oxygen signalling – drive plant survival during flooding. *Plant Biology* 15: 426–435.
- Weits DA, Giuntoli B, Kosmacz M, Parlanti S, Hubberten H-M, Riegler H, Hoefgen R, Perata P, van Dongen JT, Licausi F. 2014. Plant cysteine oxidases control the oxygen-dependent branch of the N-end-rule pathway. *Nature Communications* 5: 3425.
- Weits DA, Kunkowska AB, Kamps NCW, Portz KMS, Packbier NK, Nemeček V, Zaillochet C, Lohmann JU, Pedersen O, van Dongen JT *et al.* 2019. An apical hypoxic niche sets the pace of shoot meristem activity. *Nature* 569: 714–717.
- Wu L, Zhang Z, Zhang H, Wang X-C, Huang R. 2008. Transcriptional modulation of ethylene response factor protein JERF3 in the oxidative stress response enhances tolerance of tobacco seedlings to salt, drought, and freezing. *Plant Physiology* 148: 1953–1963.

- Xu K, Xu X, Fukao T, Canlas P, Maghirang-Rodriguez R, Heuer S, Ismail AM, Bailey-Serres J, Ronald PC, Mackill DJ. 2006. *Sub1A* is an ethylene-response-factor-like gene that confers submergence tolerance to rice. *Nature* 442: 705–708.
- Yanagisawa S, Yoo S-D, Sheen J. 2003. Differential regulation of EIN3 stability by glucose and ethylene signalling in plants. *Nature* 425: 521–525.
- Yeung E, Bailey-Serres J, Sasidharan R. 2019. After the deluge: plant revival post-flooding. *Trends in Plant Science* 24: 443–454.
- Yeung E, van Veen H, Vashisht D, Sobral Paiva AL, Hummel M, Rankenberg T, Steffens B, Steffen-Heins A, Sauter M, de Vries M *et al.* 2018. A stress recovery signaling network for enhanced flooding tolerance in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences, USA* 115: E6085–E6094.
- Zabalza A, van Dongen JT, Froehlich A, Oliver SN, Faix B, Gupta KJ, Schmalzlin E, Igal M, Orcaray L, Royuela M *et al.* 2008. Regulation of respiration and fermentation to control the plant internal oxygen concentration. *Plant Physiology* 149: 1087–1098.
- Zhang H, Bosch-Marce M, Shimoda LA, Tan YS, Baek JH, Wesley JB, Gonzalez FJ, Semenza GL. 2008. Mitochondrial autophagy is an HIF-1-dependent adaptive metabolic response to hypoxia. *Journal of Biological Chemistry* 283: 10892–10903.
- Zhang H, Li A, Zhang Z, Huang Z, Lu P, Zhang D, Liu X, Zhang Z-F, Huang R. 2016. Ethylene response factor TERF1, regulated by ETHYLENE-INSENSITIVE3-like factors, functions in reactive oxygen species (ROS) scavenging in tobacco (*Nicotiana tabacum* L.). *Scientific Reports* 6: e29948.
- Zhu T, Zou L, Li Y, Yao X, Xu F, Deng X, Zhang D, Lin H. 2018. Mitochondrial alternative oxidase-dependent autophagy involved in ethylene-mediated drought tolerance in *Solanum lycopersicum*. *Plant Biotechnology Journal* 16: 2063–2076.



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