selection of agronomic and quality-related traits other than FHB resistance.

Enabling Technologies for Accelerating Genetic Improvement

Using conventional approaches, breeders can now easily transfer *Fhb1* into elite wheat, but it may be difficult to remove linkage drag in certain genetic backgrounds resulting from suppressed recombination in the target region [9]. However, transgenesis can overcome this limitation [12]. Since *Fhb1* could be a gene complex, transformation of a multigene cassette with *His*, *PFT*, and other genes, might confer more stable FHB resistance than the transfer of a single gene. Gene editing is perhaps another option. Indeed, a CRISPR-Cas9-mediated 1-bp insertion in the *His* gene significantly increased FHB resistance in the susceptible cultivar Bobwhite, indicating the effectiveness of this approach in certain genetic backgrounds [6].

Fhb1 bioengineering may be applicable to other crops such as maize, which is affected by ear or stalk rot caused by the same fungus. The intensive maize–wheat rotation in China, the USA, central Europe, and elsewhere is an important reason for increased prevalence of FHB in wheat in recent years. Therefore, control of maize ear and stalk rot through transgenesis or gene editing could reduce the amount of *Fusarium* inoculum, with huge benefits for protection of both crops.

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Spotlight

Ethylene Signaling Controls Fast Oxygen Sensing in Plants

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When plants are submerged by water they suffer from hypoxia. Although it has long been known that ethylene accumulates in submerged plants, its role in plant tolerance to hypoxia remained elusive. Recently, *Hartman et al.* described a mechanism that explains the role of ethylene in oxygen sensing and signaling.

Oxygen Sensing in Plants Requires the Combined Action of PCOs and ERF-VII

Submerged plants, as a consequence of excessive rainfall, experience low oxygen availability (hypoxia) [1]. However, the time required for hypoxia to be established can be variable. In clear water, photosynthesis can continue even in submerged plants, providing oxygen to the leaves and leading to high-oxygen conditions [2]. Furthermore, the presence of aerenchyma can provide oxygen to the submerged organs of a plant if the tips of the leaves maintain contact with air. However, under natural conditions, submergence often occurs in muddy water conditions, thus limiting photosynthesis, and submergence is often complete, making oxygen transport by the aerenchyma impossible [2]. Hypoxia is therefore the inevitable fate of a submerged plant in most environments. When the plant finally becomes hypoxic the low oxygen availability will limit (and eventually block) aerobic respiration, leading to an energy crisis that can ultimately kill

the plant unless metabolism is rapidly adapted to the new conditions [2]. The group VII ETHYLENE RESPONSE FACTORs (ERF-VIIs) play a crucial role in the activation of the response to hypoxia at the transcriptional level [3,4]. In the absence of oxygen, PLANT CYSTEINE OXIDASE (PCO) is unable to destabilize the ERF-VII transcription factors, leading to activation of hypoxia-responsive gene (HRG) transcription [5]. Together, PCOs and ERF-VIIs thus represent the oxygen-sensing machinery in plants. Three of the ERF-VIIs (RAP2.12, RAP2.2, and RAP2.3) are constitutively expressed but are degraded under aerobic conditions. Degradation of ERF-VIIs is mediated by the PCO-dependent oxidation of the N-terminal Cys2 residue to Cys-sulfinic acid, which leads to arginylation of ERF-VII proteins and their subsequent degradation by the 26S proteasome, a step catalyzed by the N-recognin E3 ligase PRT6 [5,6].

How long does it take for this process to occur? The activity of PCOs is repressed at oxygen concentrations as low <3% [7], and it is possible to observe induction of HRGs within 30 minutes of laboratory-induced gaseous anoxia [8]. When hypoxia is induced by submergence the timing is certainly longer, probably a few hours. Does the plant passively wait for the inevitable to happen when submerged? In a recent paper, Hartman *et al.* [9] identified the sensing mechanism that primes the submerged plant for the later-occurring hypoxic conditions. This mechanism relies on ethylene signaling and its ability to increase the stability of ERF-VII proteins.

The Missing Link between Ethylene Entrapment in the Submerged Plant and Oxygen Sensing

Gas diffusion underwater is severely restricted, and this causes a rapid buildup of ethylene in the plant and in the air layer surrounding it [2]. Although it has been known for a long time that ethylene triggers an array of responses in flooded plants, a link between ethylene signaling and oxygen sensing was missing. There is very good reason for such a link. Hypoxia in plants is not only a consequence of flooding but also occurs in dense plant tissues and organs growing under well-drained, aerobic conditions [10]. However, ethylene is only entrapped during flooding, leading to selective activation of its downstream signaling pathway. The convergence of ethylene signaling with oxygen sensing can therefore allow the plant to distinguish between hypoxia in dense tissues and hypoxia generated by flooding. The distinction between hypoxia caused by submergence (stress hypoxia) and that established in tissues and organs of aerobic plants (physiological hypoxia) is relevant in terms of its occurrence and the timing of its establishment. Physiological hypoxia occurs in fruits and seeds in which their compactness limits oxygen diffusion, as well as in niches in the shoot apical meristem [10], but these types of hypoxia are either constitutive or develop very slowly. Stress hypoxia, instead, is unpredictable and may arise very rapidly as a consequence of excessive rainfall.

Hartman *et al.* [9] observed that submergence results in rapid activation of ethylene signaling in arabidopsis (*Arabidopsis thaliana*) roots – within 1–2 h following treatment. This timing is slower than the induction of HRGs by anoxia [8], but is compatible with the expected slower establishment of hypoxia after submergence. Remarkably, exogenous ethylene pretreatment enhanced hypoxia tolerance [9]. To verify if these responses were connected to ERF-VII proteins acting as oxygen sensors, Hartman *et al.* [9] explored the impact of ethylene and ERF-VIIs on plant tolerance to submergence. The positive effects of ethylene on tolerance were lost in several ERF-VII mutants, indicating that ethylene action is upstream of ERF-VII activity. They also found that the stability of RAP2.12 was enhanced in root tips treated with ethylene under aerobic conditions. How can ethylene influence the stability of ERF-VII in an aerobic tissue? The possibility that ethylene itself induces hypoxia was experimentally ruled out, making a PCO-dependent effect unlikely. The authors then focused on a possible effect mediated by NO. In addition to ERF-VII destabilization by the oxygen/PCO mechanism, nitric oxide (NO) also negatively affects ERF-VII stability [11]. This mechanism is probably independent of PCOs because these enzymes do not appear to require NO for their activity [6]. The NO effect is possibly downstream of PCOs and may contribute to the amount of stable ERF-VII in the cell. Interestingly, it was found that removal of NO by an NO scavenger (carboxyphenyl-tetramethylimidazoline-oxyl-oxide, cPTIO) was sufficient, in the absence of ethylene, to trigger enhanced stability of ERF-VIIs [9]. Possibly even more importantly, exogenous NO treatment also abolished the positive effects of ethylene on ERF-VIIs. Hartman *et al.* [9] demonstrated that ethylene can lower the level of NO in root tips: the next question concerned how ethylene regulates NO levels.

Ethylene Induces PHYTOGLOBIN1: An NO Scavenging Enzyme

NO synthesis in arabidopsis requires the action of NITRATE REDUCTASE (NR)-dependent nitrite reduction, whereas NO scavenging requires the activity of nonsymbiotic phytoglobins. Ethylene did not influence NR activity, but had a strong effect on *PHYTOGLOBIN 1* (*PGB1*) mRNA levels, which was then the best candidate for explaining the effects on ethylene on ERF-VII stability.

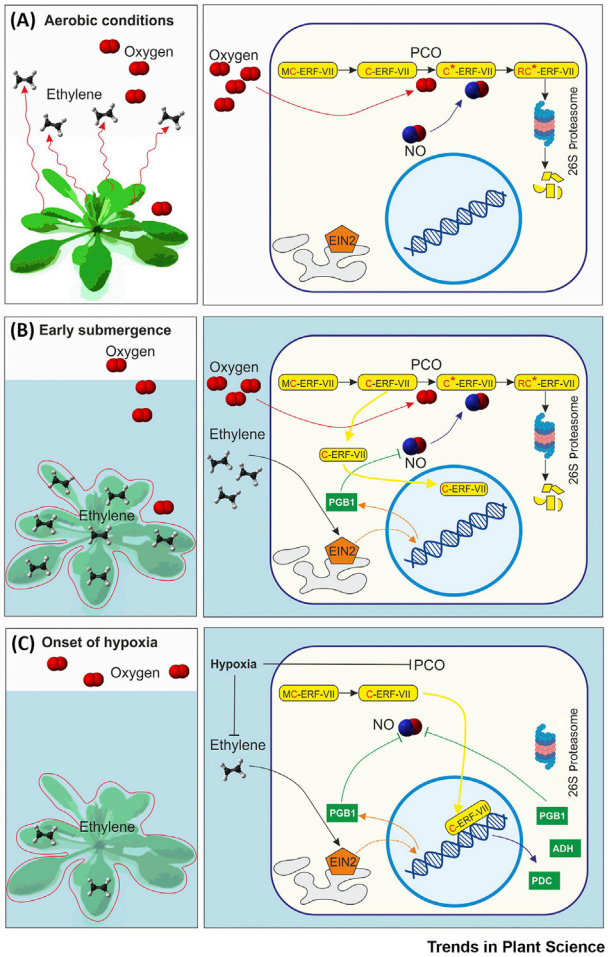

Figure 1. Mechanism Underlying the Role of Ethylene in the Acceleration of Oxygen-Sensing Responses.

(A) Under aerobic conditions group VII ETHYLENE RESPONSE FACTOR (ERF-VII) proteins are constitutively synthesized. At the same time these proteins are degraded through the N-degron pathway. This pathway targets proteins whose semi-terminal cysteine residue is exposed and oxidized by PLANT CYSTEINE OXIDASE (PCO). Nitric oxide (NO) contributes to the destabilization of the ERF-VII proteins. Ethylene, produced by the plant, diffuses in the atmosphere. (B) When submergence is established, ethylene gets entrapped in the plant, because of restriction in gas diffusion underwater. Ethylene concentration increases rapidly and its signaling pathway, requiring EIN2, induces the expression of the NO-scavenging enzyme PGB1. PGB1 removes NO and, despite the presence of some residual oxygen, ERF-VII proteins get stabilized and migrate into the nucleus. However, if oxygen is still present, the ERF-VII proteins fail to induce the expression of hypoxia-responsive genes (*HRG*s). (C) Some hours after submergence started, hypoxia is established, which reduces ethylene synthesis and activates the ERF-VII activity in the nuclei. In the absence of oxygen, PCO activity is hampered and additional ERF-VIII is stabilized and translocated to the nucleus. The synthesis of *HRG*s is activated, encoding for example, the fermentative enzymes ALCOHOL DEHYDROGENASE (ADH) and PYRUVATE DECARBOXYLASE (PDC). Among *HRG*s regulated by ERF-VII is *PGB1*, enabling its synthesis even if hypoxia represses ethylene synthesis. The production of *HRG*s-encoded proteins allows tolerance to submergence. Abbreviations: C, cysteine; C*, oxidized cysteine; M, methionine; R, arginine.

PGB1 is indeed a powerful NO-scavenging enzyme and, interestingly, a PGB1 mutant (*pgb1-1*) was unable to respond to ethylene by lowering local NO levels in root tips. Overexpression of *PGB1* (*35S:PGB1*) resulted instead in enhanced removal of NO, leading to ethylene-independent, constitutive, higher tolerance to hypoxia [9]. Most importantly, the stability of ERF-VII proteins, namely RAP2.3, was clearly ethylene-dependent in the wild-type, but not in the *pgb1* mutant, in which RAP2.3 was almost absent in plants exposed to air, even after ethylene treatment. The authors concluded that ethylene pretreatment influences hypoxia tolerance, as well as the level at which HRGs are induced by subsequent hypoxia, by inducing *PGB1* that removes NO, thus stabilizing ERF-VIIs and increasing their tissue levels. Ethylene pretreatment, however, was unable to induce HRG expression in air, despite the higher level of ERF-VIIs and their nuclear localization in aerobic conditions. The final step in the oxygen signaling cascade therefore requires a drop in oxygen availability.

Concluding Remarks and Future Perspectives

Since the discovery of the oxygen-sensing mechanism in plants in 2011 [3,4], major efforts have been made to unravel the signaling pathway. We now know that oxygen sensing is coupled to NO perception and signaling, that PCOs are the partners of ERF-VII that constitute the actual oxygen sensor, and that other proteins possessing an MC motif at their N-terminus can constitute, together with PCOs, other oxygen-sensing complexes [10]. The work by Hartman *et al.* [9] adds to the picture early events occurring after the establishment of submergence (Figure 1). Ethylene entrapment triggers *PGB1* expression and rapid NO depletion, which stabilizes ERF-VII proteins. This priming ensures efficient induction of HRG expression when hypoxia follows ethylene treatment. However, the ERF-VII proteins that are stabilized by PGB1 activity are unable to activate HRG expression under aerobic

conditions. The ethylene-stabilized ERF-VIIs may localize at the plasma membrane through an interaction with ACBPs [4]. A drop in ATP content, which is typical of hypoxic conditions, is necessary to reduce the activity of LONG-CHAIN ACYL-COA SYNTHETASE (LACS), which eventually affects the composition of the acyl-CoA pool [12]. Increased hypoxia-dependent levels of oleoyl-CoA trigger the release of RAP2.12 from ACBP and consequently activate HRG transcription [12]. Alternatively, assuming that the ERF-VII proteins are stabilized and translocated to the nucleus, post-translational modifications could be essential to activate the proteins as transcriptional activators of HRGs. These questions await an answer in the rapidly expanding field of plant hypoxia. In the context of the increasingly important need to produce plants tolerant to submergence, manipulation of the ethylene responsiveness of *PGB1* genes could be used to develop plants in which passive ethylene entrapment upon flooding rapidly preadapts crops to later-occurring hypoxia.

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Forum

Similar and Yet Different: Oxygen Sensing in Animals and Plants

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The ability to perceive oxygen levels and adapt metabolism on the basis of its availability is vital for most eukaryotic cells. Here, we retrace the key steps that led to the identification of oxygen-sensing mechanisms in animals and plants and compare the essential features of the two strategies.

In 2019, the Nobel Assembly at the Karolinska Institute awarded the Nobel Prize in Physiology or Medicine jointly to William Kaelin, Peter Ratcliffe, and Gregg Semenza 'for their discoveries of how (animal) cells sense and adapt to oxygen availability'. This acknowledgment clearly reflects the relevance of the series of discoveries made by these three scientists to our understanding of animal physiology, including humans. Indeed, as oxygen is essential for energy conversion in the mitochondria via oxidative phosphorylation, its cellular availability deeply affects cell and tissue functioning, maintenance, and development. Thus, oxygen distribution and consumption require tight control and coordination to maintain its homeostasis. Nonetheless, plant and animal cells alike are frequently exposed to changes in oxygen availability, due to variable metabolic rates or alterations in oxygen collection and delivery, which often lead to a condition commonly defined as 'hypoxia'. Thus, evolution has enabled cells to adapt to such a condition by the development of a set of processes that constitute the hypoxic response and include, but are not limited to, the production of new oxygen delivery avenues (angiogenesis and erythropoiesis in animals, aerenchyma in plants) and metabolic adaptations to decrease the demand for oxygen and optimize its usage.

The now detailed picture of the molecular mechanisms that trigger the hypoxic response in animal cells is the product of hundreds of studies, notably the seminal works that were reported by the three 2019 Nobel laureates and their teams over the past 25 years (Figure 1). First, the isolation of the hypoxia responsive element enhancer in the erythropoietin gene allowed the identification of the hypoxia inducible transcription factor (HIF) complex, consisting of two subunits: HIF-1α and HIF-1β [1]. Wang *et al.* [1] also reported that the HIF-1α subunit is ubiquitously and constantly produced in human cells, although degraded by the proteasome under normoxic conditions and stabilized by hypoxia. The identity of the E3