Editorial

Plant performance and food security in a wetter world

As average global temperatures continue to rise, severe droughts in some regions are matched by extreme rainfall and floods in others. Every year, flooding events wreck crop yields, causing substantial economic losses and raising concerns for food security. As more and more global farming regions become flood exposed, it is now more urgent than ever to invest in comprehending the biology of flooding resilience in plants. Understanding how plants sense flooding, escape from it, metabolically adjust to it and ultimately survive these wet conditions is key to selecting and designing flood resilient crop varieties. The compilation of Tansley reviews, Tansley insights and original research papers in this Feature Issue of *New Phytologist,* reflect the progress made by the plant flooding research community in this respect, and the challenges that still remain.

Most terrestrial plants, including nearly all crops, are extremely vulnerable to flooding. The aqueous environment is harmful for plants because it severely impairs gas diffusion and limits the availability of carbon dioxide and oxygen for, respectively, photosynthesis and respiration. The ensuing impedance to these life-maintaining processes leads to a carbon and energy crisis ultimately killing plants (Voesenek & Bailey-Serres, 2015). Hindered gas diffusion in flooded plant tissues also causes a build up of the volatile hormone ethylene. This accumulation occurs rapidly (< 1 h) and precedes the onset of hypoxia and associated consequences (Voesenek & Sasidharan, 2013; Sasidharan *et al.*, 2018). Ethylene is therefore a vital and reliable cue for flooded plants and a primary regulator of numerous adaptive traits that improve internal aeration and stave off hypoxia (Sasidharan & Voesenek, 2015).

A prime example of a hypoxia avoidance trait is submergence-induced acceleration of shoot growth that serves to elevate the photosynthetic tissues above the water surface. This 'escape' response has been observed in numerous plants occupying frequently flooded habitats, with the flooding-induced entrapment of ethylene being an indispensable instigator of shoot elongation across a wide range of species (Sasidharan & Voesenek, 2015). A substantial body of work has contributed to the elucidation of the molecular network controlling underwater shoot growth. These studies, primarily in the model species rice and *Rumex*, have unraveled an ethylene-abscisic acid-gibberellic acid (GA)-expansin regulatory module that serves to modulate underwater shoot growth. However, as Müller *et al.* (2021 in this issue) discovered, in some species such as *Nasturtium officinale* (watercress), the growth control machinery is wired differently. Interestingly in watercress,

submergence triggered opposing growth responses in the stems and petioles. Using a combination of transcriptomics and physiological experiments, the authors concluded that accelerated stem elongation in watercress was not controlled by ethylene. While they could rule out other prime suspects such as hypoxia and GA, the identification of the primary trigger for stem elongation remained elusive.

Another variant of the escape trait, facilitating hypoxia avoidance in flooded conditions is elongation of the coleoptile in germinating seeds when submerged (Pucciariello, 2020). This ability to accelerate growth of the hollow coleoptile out of the water, serves to stimulate diffusion of air to the endosperm and this delivery of oxygen is key for continued growth and survival during shallow submergence. Investigations using the rice model have elucidated the molecular details underlying anaerobic germination and coleoptile elongation (comprehensively summarized by Yu *et al.*, 2021, in this issue). Nghi *et al.* (2021, in this issue) report on the importance of auxin biosynthesis and transport for coleoptile elongation. Understanding traits promoting anerobic germination and seedling establishment is essential for replacing labor-intensive planting of rice seedlings by direct seeding. Young seedlings can be especially vulnerable to flooded conditions. Lee *et al.* (2021, in this issue) demonstrate that Arabidopsis seedling establishment during waterlogging, requires proper cuticle development mediated by ATP-BINDING CASSETTE TRANSPORTER subfamily G (ABCG) proteins. Waterlogged mutant seedlings (*abcg5*) displayed severe developmental problems including smaller shoot apical meristems and failure to produce true leaves. Notably the increased stress sensitivity of abcg5 mutants was linked to a defective cuticle layer, highlighting the importance of the cuticle for waterlogging resilience (Lee *et al.*, 2021, in this issue).

Flood adapted plants also possess a suite of morphological and anatomical root traits that facilitates internal aeration and thus survival in hypoxic soils (Pedersen *et al.*, 2021b, in this issue). The Tansley insight by Pedersen *et al.* (2021a, in this issue) bring us up to date on the current knowledge regarding the molecular regulation and functional significance of such traits including adventitious root growth, aerenchyma formation and formation of a barrier to radial oxygen loss (ROL). ROL refers to the leakage of oxygen from root aerenchyma to the surrounding hypoxic soil. Roots of several wetland species prevent ROL by forming a barrier in the outer cell layers in mainly subapical regions. In addition, such a barrier is also proposed to block the entry of toxic substances that typically accumulate in waterlogged soils. The regulatory mechanisms underpinning barrier formation and the nature of the barrier itself has received considerable attention (Yamauchi *et al.*, 2018). Pedersen *et al.* (2021b, in this issue) document their investigations on ROL barrier formation in roots of *Zea nicaraguensis*, a waterlogging resilient wild relative of *Zea mays* (maize or corn). The presented evidence shows that, hypoxic conditions trigger ROL barrier formation not just in adventitious roots (AR) but also in the lateral roots (LR). Considering the higher profusion of LR compared to ARs such a barrier would, in combination with aerenchyma considerably enhance internal oxygen levels, and facilitate lateral root growth even in anoxic soils.

This article is an Editorial on: Cho *et al.*, **229**: 57–63; Hartman *et al.*, **229**: 64–70; Labandera *et al.*, **229**: 126–139; Lee & Bailey-Serres, **229**: 71–78; Lee *et al.*, **229**: 156–172; Licausi & Giuntoli, **229**: 50–56; Müller *et al.*, **229**: 140–155; Nghi *et al.*, **229**: 85–93; Pedersen *et al.*, (a) **229**: 42–49; Pedersen *et al.*, (b) **229**: 94–105; Tang *et al.*, **229**: 106–125; Valeri *et al.*, **229**: 173–185; van Veen & Sasidharan, **229**: 79–84; Weits *et al.*, **229**: 24–35; Yu *et al.*, **229**: 36–41.

A recent exciting discovery in the field is that hypoxia arises not only during unfavorable environmental conditions, but also represents a physiologically relevant condition for the functionality of specific plant tissues (Shukla *et al.*, 2019; Weits *et al.*, 2019). The importance of oxygen gradients during plant development is reviewed by Weits *et al.* (2021, in this issue) in their Tansley review. Oxygen sensing mediated by the N-degron pathway plays an important role in defining which proteins need to be degraded unless expressed in a hypoxic niche (van Dongen & Licausi, 2015). The members of the group VII Ethylene Response Factor (ERF-VII) family, play a pivotal role in defining plant responses to flooding and other events leading to environmental hypoxia (Giuntoli & Perata, 2018). They also integrate oxygen dynamics in root development (Shukla *et al.*, 2019) and during seedling establishment (Abbas *et al.*, 2015). Interestingly, a hypoxic niche is established in the shoot apical meristem, where vegetative shoot meristem activity and reproductive transition are associated with the activity of VERNALISATION 2 (VRN2) and LITTLE ZIPPER 2 (ZPR2) (Gibbs *et al.*, 2018; Weits *et al.*, 2019). Remarkably, both these proteins are substrates of the Plant Cysteine Oxidase (PCO)-dependent N-degron pathway and being oxygen labile, are present in an active form only in a hypoxic environment. Weits *et al.* (2021, in this issue) therefore propose that molecular oxygen acts as a signaling molecule not only during flooding, but also in plant development. The mechanism regulating VRN2 stability in an oxygen-dependent manner is explored by Labandera *et al.* (2021, in this issue). VRN2 localized to hypoxic niches of aerial and root tissues, has distinct roles. In the shoot apex, VRN2 modulates flowering time, while its presence in hypoxic root regions affects root architecture. Labandera *et al.* (2021, in this issue) suggest that the oxygen-labile nature of VRN2 confines it to meristems and primordia, where it plays specific developmental roles. However, broader VRN2 accumulation outside of meristems may occur under specific environmental conditions when hypoxic zones expand, leading to other functions. Hypoxia therefore occurs following adverse environmental conditions (flooding), constitutively in specific tissues (developmental hypoxia), but also during plant–pathogen interactions (Kerpen *et al.*, 2019; Vicente *et al.*, 2019). Valeri *et al.* (2021, in this issue) describe how hypoxia is rapidly established in Arabidopsis leaves following infection with the necrotrophic fungus *Botrytis cinerea*. At fungus infection sites, vigorous oxygen consumption induces hypoxia, and stabilizes ERF-VII proteins. This implicates ERFVIIs in a potential dual role, namely enhancing hypoxia tolerance but also influencing the plant's response to the fungus. Hypoxia at the site of pathogen infection, besides stabilizing the ERF-VIIs may also affect the stability of other, yet unknown N-degron-regulated proteins as well as the metabolism of elicitors, which is influenced by the presence of molecular oxygen. In this context, Tang *et al.* (2021, in this issue) demonstrated the interplay between RAP2.2, an ERF-VII protein, and WRKY proteins, which are well known for their involvement in plant resistance to pathogens. Interestingly, adaptation to submergence in Arabidopsis involves up-regulation of RAP2.2 through interactive action of WRKY33 and WRKY12, with RAP2.2 acting downstream of WRKY33/WRKY12. Furthermore, WRKY33 and WRKY12 could bind to and activate RAP2.2 individually (Tang *et al.*, 2021, in this issue).

Defining when and at which level hypoxia occurs in plants is of utmost importance. In this context the development of genetic sensors (Puerta *et al.*, 2019) that can accurately report cellular oxygen levels *in vivo* would give us spatio-temporal resolution of oxygen concentrations in plant tissues and expand our knowledge of hypoxia as a physiological condition in plants. Synthetic biology can provide the tools for this technological breakthrough, as described by Licausi & Giuntoli (2021, in this issue). In their Tansley insight, they also propose that genetic tailoring of oxygen-responsive traits, engineering of plant hypoxic metabolism and oxygen delivery to hypoxic tissues can be achieved by exploiting synthetic biology in the field of plant hypoxia.

A major consequence of flooding-associated cellular hypoxia is the inhibition of mitochondrial ATP production. In addition to this energy crisis, flooded plants are exposed to a plethora of other potentially stressful changes: accumulation of the growth regulating and stress controlling plant hormone ethylene, increase of nitric oxide (NO), carbohydrate starvation and enhanced production of reactive oxygen species (ROS). To make a significant progress in our understanding of flooding tolerance, it is critical to develop an integrated view on the interactions between relevant signaling pathways as flooding events progress in time. Two Tansley insights highlight this perspective: the Tansley insight of Cho *et al.* (2021, in this issue) integrating oxygen sensing, energy sensing and the signaling of carbon starvation and the overview by Hartman *et al.*, (2021, in this issue) on how ethylene signaling, via NO, interacts with oxygen signaling. In order to completely understand the interaction network at play between these signaling routes, the recognition of spatio-temporal dynamics and interactions of signals and stressors during different flooding scenarios is important (Sasidharan *et al.*, 2018). The integration of these two Tansley insights suggests the following time model for metabolic flooding adaptation in plants:

(**1**) Ethylene very rapidly (< 1 h) accumulates. Ethylene signaling results in quick (2–4 h) nuclear ERFVII accumulation through increased *ERFVII* transcription and a reduction in NO-mediated ERFVII proteolysis (driven by an increase in NO-scavenging phytoglobins). Downstream enhanced transcription of the majority of Hypoxia Responsive Genes (HRGs) is, however, inhibited as long as tissues remain normoxic. Ethylene also induces genes associated with ROS signaling and amelioration and thus protect cells from oxidative stress associated with hypoxia and re-oxygenation. Together, this places submerged plant cells in a readied state to cope with forthcoming hypoxia.

(**2**) Once light levels are limited and photosynthesis is reduced, O_2 and energy levels decline seriously, resulting in fast ERFVII-dependent transcription of HRGs. This facilitates substrate-level ATP production through processes like fermentation. The hypoxia signal also facilitates further release of ERFVIIs from sequestering proteins such as Acyl CoA Binding Proteins (ACBPs), thus adding them to the ERFVII pool already in the nucleus and further stimulating HRG transcription. Energy depletion also results in SnRK1 activation and translocation of its alpha subunit to the nucleus, preventing energy consuming translation.

(**3**) Glycolysis will ultimately drain the plant's available carbohydrate reserves. The resulting starvation signal leads to transcriptional repression of several HRGs (Cho *et al.*, 2021, in this issue). Accordingly, the costs of transcription and translation are reduced as substrates (sugars) are no longer available. At this stage, literature suggests that ethylene could induce transcription of autophagy genes during starvation. In this way cells can make use of alternative energy-rich substrates such as amino-acids and fatty acids to survive severe, prolonged flooding.

It is an exciting time in the flooding and hypoxia research field with several seminal discoveries in recent years. As we move forward in our quest to generate plants resilient to wet conditions, the flooding research community will need to focus on several challenges. There is a need for high resolution tools and techniques to accurately monitor the real-time dynamics of flooding signals such ethylene, oxygen, ROS and NO *in planta* (Sasidharan *et al.*, 2017). This is critical to understanding the dynamics and relevance of these signals during different flooded conditions and how their amalgamation generates distinct outputs. Simultaneously, we want to analyze across different tiers of gene regulation in a cell-type specific way (Bailey-Serres, 2013; Lee & Bailey-Serres, 2021, in this issue). This will enable us to distinguish between low oxygen as a pre-requisite for normal development and as a stress factor that hampers plant performance. We also need to move beyond established model species and explore the wide diversity of wild plants inhabiting flood-prone areas and representing a reservoir of untapped knowledge on tolerance mechanisms (van Veen & Sasidharan, 2021, in this issue).

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