Recent progress in understanding the cellular and genetic basis of plant responses to low oxygen hold promise for developing flood-resilient crops

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HIGHLIGHT

With flooding events increasing due to climate change, the low oxygen tolerance of plants has become a very important research topic that has evolved thanks to the research effort and recent progress.

Abstract

With recent progress in active research on flooding and hypoxia/anoxia tolerance in native and agricultural crop plants, vast knowledge has been gained on both individual tolerance mechanisms and the general mechanisms of flooding tolerance in plants. Research on carbohydrate consumption, ethanolic and lactic acid fermentation and their regulation under stress conditions has been accompanied by investigations on aerenchyma development and the emergence of the radial oxygen loss barrier in some plant species under flooded conditions. The discovery of the oxygen sensing mechanism in plants and unraveling the intricacies of this mechanism have boosted this very international research effort. Recent studies have highlighted the importance of oxygen availability as a signaling component during plant development. The latest developments in determining actual oxygen concentrations using minute probes and molecular sensors in tissues and even within cells have provided new insights into the intracellular effects of flooding. The information amassed during recent years has been used in the breeding of new flood-tolerant crop cultivars. With the wealth of metabolic, anatomical and genetic information, novel holistic approaches can be used to enhance crop species and their productivity under increasing stress conditions due to climate change and the subsequent changes in the environment.

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Introduction

Water is vitally important for normal functioning of plants, however it can be harmful when it partially or totally covers plants as a result of a flooding event. In this review flooding tolerance is defined as means of survival of conditions that are fatal to most plants coupled with the capacity to recover sufficiently to produce biomass. This is largely due to a lower availability of oxygen (O_2), carbon dioxide (CO_2) and light in water that hamper respiration and photosynthesis. Adverse conditions caused by flooding or complete submergence cause widespread yield losses to crop plants (Food and Agriculture Organization of the United Nations [FAO] 2017 and 2021; Wing *et al.,* 2022). In the US, inundation modelling under the RCP4.5 scenario estimates 26.4% increase in flood risk by 2050 (Wing et al., 2022). Floods during the growing season have especially magnified the severity of crop losses, and at the same time there have been large carbon emissions to the atmosphere from the flooded fields (Perkins, 2020). The massive crop losses have led to an increased interest in finding ways to increase flooding tolerance of especially rice. This was highlighted in a recent review that focused not only on maize, barley, and soybean, but also other crops that are less commonly studied such as wheat and rapeseed, and which are all relatively flooding intolerant (Mustroph, 2018). Showcasing the importance of this research field, several other reviews have been recently published. Zahra *et al.* (2020) reviewed biochemical and molecular approaches aimed at better understanding the complex mechanisms behind tolerance to hypoxic or anoxic conditions. Xie *et al*. (2021) highlighted that lipids and their metabolites act as important intra-cellular signaling agents in plants under low-oxygen stress. Many aspects of flooding tolerance and sensitivity have been reviewed before, such as growth, survival and catabolism (Gibbs and Greenway, 2003), energy requirements (Greenway and Gibbs, 2003), acclimation and genetic diversity (Bailey-Serres and Voesenek, 2008; Fagerstedt *et al*., 2011), and flooding tolerance in halophytes (Colmer and Flowers, 2008).

In this review we focus on the latest developments in research on plant tolerance to hypoxic conditions, i.e. oxygen concentrations lower than atmospheric 21% (Sasidharan et al., 2017), and more importantly, we speculate on the future directions of research within the field of hypoxia tolerance. We reexamine some of the most important developments in the field such as the exploration of the core hypoxia response genes, (Mustroph *et al.,* 2009) which has led to a wealth

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of new research results. Another finding is the N-end rule pathway (N-degron pathway) for oxygen level sensing in plants (Gibbs *et al.,* 2011; Licausi *et al.,* 2011) which has led to many studies on the intricacies of the sensing system (Paul *et al.,* 2016; Kunkowska *et al.,* 2023). Research also includes the SUB1A allele effects in improving flooding tolerance in rice (Xu *et al*., 2006; Fukao *et al.,* 2006; Takeshi *et al*., 2011), the role of nitric oxide (NO) in the regulation of metabolic events under hypoxia (Gupta *et al.,* 2022), the detection of oxygen levels within tissues or even within cells (Wagner *et al.,* 2019; Iacopino *et al.,* 2019; Pedersen *et al.,* 2021), and the regulation of aerenchyma development (Yamauchi *et al.,* 2019).

One important factor in terms of boosting research on hypoxia in plants and in creating a community of like-minded researchers, is the International Society for Plant Anaerobiosis (ISPA), which was founded in the 1975 and has since had a large influence on the understanding of plant behaviour under flooded, hypoxic or anoxic conditions (Vartapetian *et al.,* 2011). At its 14th international conference ISPA was renamed ISPLORE, the International Society for Plant Low Oxygen Research.

Research within ISPA/ISPLORE has developed dramatically over recent years from descriptive projects on gas-exchange, anaerobic metabolism and anatomical and morphological features to detailed and intricate research efforts on hypoxia tolerance mechanisms and their regulation at the gene level, and more recently holistic views on flooding tolerance and its regulation in crop plants. Much of the resulting knowledge has been put into practice in crop breeding and used to identify interesting genetic resources especially but not only in rice by ISPLORE members (Singh *et al*., 2009; Bailey-Serres *et al*., 2010; Nghi *et al*., 2019; Gomez-Alvarez *et al*., 2023).

Plant diversity in adaptation and acclimation to flooding/hypoxia/anoxia

Plant species have evolved and adapted to flooding in a huge variety of ways. In a study of 91 plant species categorized as wetland, non-wetland and intermediate, root porosity was found to correlate positively with flooding tolerance (Justin and Armstrong, 1987). This latter study also indicated that many other root morphological and anatomical features affect plant behaviour in flooded soils. This information was exploited in a study on waterlogging stress in okra and maize

that were given exogenous ethylene priming which improved root porosity and emergence of adventitious roots (Vwioko *et al*., 2017). It was also used in experiments published in an article on plant strategies in waterlogged environments (Pan *et al.,* 2022). The results indicated that trait decoupling (i.e. separate regulation of different traits) enables plants to overcome the stresses of a complex flooded environment.

Clearly, there is no general strategy that perfectly explains the adaptation of plants to hypoxia. Instead, flood tolerance involves complex strategies, which is highlighted by a combination of traits regarding induction under flooded conditions. This also does not exclude leaf economics and size-related traits, which all contribute to plant survival in flooding events across a wide range of habitats (Pan *et al.,* 2022). Within crop plant species there is large variation in flooding tolerance, especially in rice cultivars.

Acclimation to oxygen shortage in rice

Rice is traditionally cultivated in flooded conditions thanks to acclimative responses including morphological, physiological and molecular traits that limit the oxygen shortage. Rice cultivation can be classified according to three ecological situations: deepwater rice that grows in a water depth of over 50 cm, lowland rice that is suitable for irrigated or rain-fed fields of up to 50 cm depth, and upland rice cultivated in non-flooded conditions (Sauter, 2000). However, fields are frequently submerged completely during rainy seasons and some rice varieties cannot survive prolonged complete submergence (Minami *et al.,* 2018).

The occurrence of different water regimes in cultivated and natural environments has supported the development of ecological adaptations to extreme conditions. Adult plants have two opposing strategies of response to water submergence: low oxygen quiescence strategy (LOQS) and low oxygen escape strategy (LOES) (Bailey-Serres and Voesenek, 2008). These are represented by the capacity to escape the water depth through fast and extreme stem/leaf elongation, thus reaching the water surface and continuing aerobic respiration (LOES) and, in contrast, to enter in a state of quiescence underwater. This latter strategy is aimed at reusing energy resources during the subsequent recovery period (Bailey-Serres and Voesenek, 2008).

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LOES is found in deepwater rice where an exceptional internode elongation (20-25 cm per day) reaching several meters of length is manifested during the increase in water depth under months-long deep flooding (Nagai *et al.,* 2010). The quantitative trait locus (QTL) detected on chromosome 12 of the cv C9285 from Bangladesh is responsible for this adaptation, with the group VII ERF (ethylene response factor) SNORKEL1 (SK1) transcription factor and SK2 being the key components (Hattori *et al.,* 2007, 2008, 2009). The entrapment of ethylene underwater leads to SK1 and SK2 transcription which, through stimulating the production of gibberellic acid (GA), promotes the internode growth of stem, thus both cell proliferation and elongation. Subsequently, a genome wide association study (GWAS) conducted on a panel of deepwater and Asian rice varieties identified a haplotype of the SEMIDWARF gene (SD1), which encodes GA200X2 a gibberellin biosynthesis gene that in deepwater is involved in the increase in GA_1 and GA_4, through the activation of the OsEIL1a ethylene responsive transcription factor (Kuroha *et al.,* 2018). Moreover, recent results identified ACCELERATOR OF INTERNODE ELONGATION 1 (ACE1) and DECELERATOR OF INTERNODE ELONGATION 1 (DEC1) to promote and repress, respectively, the initiation of internode elongation in response to gibberellins (Nagai *et al.,* 2020). This mechanism in combination with SK genes is the key for elongation of internodes underwater. Aquatic adventitious roots have been studied in deepwater rice, using near-isogenic lines for QTLs on chromosomes 1, 3 and 12. QTL12, which harbor SK1 and SK2, promotes the development of two types of adventitious roots during partial submergence (Lin *et al.,* 2023). The first type emerged already after 3 days of submergence directly from the nodal tissue, whereas the second type is emerging 3-4 days later as these have to penetrate the leaf sheath. The two types of roots are proposed to have different functions during recurrent flood events, i.e., the first type only emerges once during the first flood event, whereas the second type can emerge multiple times from preformed primordia (Lin *et al.,* 2023).

LOQS was initially observed in in the Flooding Resistant 13 (FR13) rice landrace, which can survive under a quiescent state for up to 14 days (Mackill *et al.,* 1996). Mapping of QTL culminated with identification of the SUB1 locus on chromosome 9 which is responsible for this extreme phenotype (Xu *et al.,* 2006). This locus includes two to three closely-related ethylene response

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factors named SUBMERGENCE 1A (SUB1A), SUB1B and SUB1C, with SUB1A indicated as the flooding tolerance key gene (Fukao *et al.,* 2006; Xu *et al.,* 2006).

The presence of the SUB1A-1 allele in some rice accessions mediates flooding tolerance and is regulated by an increase in ethylene under submergence (Xu *et al.,* 2006). Through a feedback mechanism SUB1A-1 activation reduces ethylene perception, thus suppressing the GA-mediated activation of cell elongation and carbohydrate metabolism-related genes, inducing quiescence. In fact, SUB1A-1 is associated with the accumulation of DELLA proteins SLENDER RICE 1 (SLR19) and non-DELLA protein SLR like 1 (SLRL1) which dampen GA, thus repressing the elongation of the shoot (Fukao and Bailey-Serres, 2008).

SUB1A-1 is also involved in tolerance to drought stress occurring under the post-submergence recovery period, where it enhances the ABA responsiveness and activates genes that are stress-related (Fukao *et al.,* 2011). The transfer of SUB1 QTL through marker-assisted backcrossing into highly productive varieties has led to the production of SUB1 megavarieties which are able to survive flooding and have high yields (Singh *et al.*, 2009; Bailey-Serres *et al.,* 2010). The ERF-VII SUB1A gene is not a substrate of the N-end pathway for proteolysis, suggesting other mechanisms of regulation likely related to enhanced tolerance (Gibbs *et al.,* 2011). Indeed, structural and protein interaction analysis suggests that in SUB1A-1 C terminus interacts with the N terminus, in this way protecting from N-degron mediated degradation and explaining the enhanced stability (Lin *et al.*, 2019). In the same work, other ERF-VII genes, ERF66 and ERF67, have been found to be substrates of the N-degron and transcriptionally up-regulated by SUB1A-1, likely contributing to promote submergence survival in rice accessions harboring SUB1A-1 (Lin *et al.*, 2019). Finally, MITOGEN ACTIVATED PROTEIN KINASE 3 (MPK3) is involved in a positive loop of regulation with SUB1A-1, where it is activated and subsequently interacts and phosphorylates SUB1A, contributing to acclimation (Singh and Sinha, 2016).

Among cereals, rice has a notable ability to germinate under submergence (Perata *et al.,* 1992; Guglielminetti *et al.,* 1995) and elongate the coleoptile with a snorkel-like function (Narsai *et al.,* 2015; Shiono *et al.,* 2022). Being able to degrade starch under oxygen shortage is critical in order to fuel the organs and their growth (Loreti *et al.,* 2003; Lasanthi-Kudahettige, 2007). Rice harbours α-amylase genes that hydrolyse starch and are sensitive to oxygen shortage and

starvation of sugars which rapidly follow under submergence (Yu *et al.,* 2015). This pathway works independently of the air-dependent activation by GA (Loreti *et al.,* 2003) and is regulated by hypoxia-dependent calcium signal and sugar starvation (Lee *et al.,* 2009). In this pathway, a calcineurin B-like protein (CBL)-interacting protein kinase CIPK15 is the central regulator contributing to decoding the calcium signal. CIPK15 is a positive regulator of the sucrose-non-fermenting-1-related protein kinase 1A (SnRK1A) and a transcriptional activator of MYBS1 (Lu *et al.,* 2002, 2007; Lee *et al.,* 2009). MYBS1 binds directly to α-amylase subfamily 3 promoters, thus positively regulating starch degradation.

The trehalose 6 phosphate phosphatase 7 gene (TPP7), involved in trehalose 6 phosphate conversion to trehalose, is crucial for the enhanced anaerobic germination capacity (Kretzschmar *et al.,* 2015). The availability of TPP7 in some rice genotypes modifies the T6P/sucrose balance in the seed, thus increasing starch mobilisation by α-amylase and the establishment of seedlings under submergence. The TPP7 gene contributes to coleoptile length, as demonstrated by the experiments conducted with the near isogenic lines NIL-AGI1, containing the *TPP7* gene (Kretzschmar *et al.,* 2015). A long coleoptile in *japonica* rice accessions has been related to the capacity to translocate auxin through the enhanced regulation of AUX1 (Nghi *et al.,* 2019, 2021), in order to favor the extension of the cell during elongation. In parallel, the auxin dependent signaling is activated in rice through the submergence-dependent dampening of miR399 expression which reduces the degradation of the transport inhibitor response 1 (TIR1), the regulator of auxin responsive gene expression (Guo *et al.,* 2016).

Acclimation to flooding at the metabolic level

When a plant experiences a shortage of oxygen, metabolic acclimation occurs in order to produce energy for survival, even without the final oxidative phosphorylation at mitochondria (Braun, 2020). Pyruvate availability links mitochondrial respiration to the preceding glycolysis in the cytosol. After carbohydrate metabolism through glycolysis, pyruvate is transported to mitochondria and oxidized along the tricarboxylic acid cycle (TCA) to organic acids with the production of NADH, finally leading to ATP production (Braun *et al*., 2020).

Under oxygen shortage, energy is produced from carbohydrates through acclimation process that includes a shift toward the fermentative metabolism. This leads to energy production but can possibility result in an energy crisis, given that the amount of ATP resulting from glycolysis coupled to fermentation is much lower than that obtained through mitochondrial respiration. When respiration is hampered by oxygen shortage, energy production is limited to the glycolysis which leads to the production of some ATP and pyruvate. The availability of substrates and the regeneration of oxidized NAD are vital for the maintenance of glycolysis (Licausi and Perata, 2009). To maintain the glycolytic flux, the fermentation pathway is activated, consisting of a brief period of lactic acid production and prolonged ethanolic fermentation, which leads to the conversion of NADH back to NAD, allowing glycolysis to continue (Perata and Alpi, 1993).

The end product of glycolysis, pyruvate, is either converted by lactate dehydrogenase (LDH) to lactate with the regeneration of NAD^+ or the pyruvate is decarboxylated into acetaldehyde and then ethanol is produced by pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) with regeneration of NAD^+, respectively. In fact, lactic accumulation can be toxic due to its dissociation which contributes to lowering the cell pH, while membranes are permeable to ethanol which rarely reaches dangerous concentrations (Davies *et al.*, 1974; Davies, 1980; Hanhijärvi and Fagerstedt, 1995). Acetaldehyde accumulation can also be toxic and can also be converted to acetate by aldehyde dehydrogenase (Perata and Alpi, 1991). This reaction, however, depletes the NAD^+ pool (Kürsteiner *et al.,* 2003). *PDC* and *ADH* gene transcripts are induced under low oxygen and are targets of the ERF-VII transcription factors involved in oxygen sensing (Gibbs *et al.,* 2011; Licausi *et al.,* 2011). In this context, recent results reported that the energy sensor TARGET OF RAPAMYCIN (TOR) contributes to the transcriptional activity of RAP2.12 through phosphorylation, demonstrating the convergence of oxygen and energy sensing (Kunkowska *et al.*, 2023).

Acidification induced by lactate adjusts the pH to an optimal value for PDC activity with the activation of the ethanolic pathway and subsequent regeneration of NAD^+ which is required for glycolysis (Davies, 1980). Lactate dehydrogenase activity stimulates ethanolic fermentation, suggesting the need for lactic fermentation in order for ethanolic fermentation to take place (Dolferus *et al.,* 2008). Lactic fermentation is a prerequisite for plant tolerance to oxygen

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shortage since *ldh1* Arabidopsis mutants show reduced survival under hypoxia (Dolferus *et al.,* 2008).

A further hypothesis explaining the favoring of ethanolic fermentation under oxygen shortage accounts for the activity of PDC in comparison to pyruvate dehydrogenase (PDH) which converts pyruvate to acetyl CoA. This suggests that an accumulation of pyruvate leads preferentially to the activation of ethanolic fermentation (Tadege *et al.,* 1999). In fact, PDC is a crucial enzyme during fermentation. Arabidopsis *PDC1* and *PDC2* overexpressing plants have shown increased tolerance to low oxygen and increased ethanol production (Ismond *et al*., 2003), while *pdc1* mutant is highly sensitive to anoxia (Kürsteiner *et al.,* 2003). Arabidopsis *ADH* overexpressing plants confer anoxia tolerance but no effect is visible on flooding survival, while *adh1* mutants show an enhanced sensitivity to low oxygen, suggesting the crucial role for ADH in plant tolerance (Ismond *et al.,* 2003).

The rice *reduced adh activity* (*rad*) mutant shows a reduced coleoptile length under submergence (Saika *et al.,* 2006), while the maize *adh1* mutant is very sensitive to anoxia (Johnson *et al.,* 1994). The phenotype of Arabidopsis mutants lacking functional PDC (*pdc1pdc2* double mutant) and ADH (*adh1* mutant), have impaired growth in air, suggesting the importance of the fermentative metabolism in plant growth. These results indicate the importance of the ATP supply by fermentative pathways in hypoxic niches irrespective of the surrounding aerobic environment (Ventura *et al.,* 2020).

Activation of the glycolytic flux followed by fermentation enables ATP production to be maintained for a limited length of time. During fermentation, the amount of energy production is 2-4 mol of ATP per mol of hexose, in comparison to 30-36 mol of ATP produced under normoxia (Greenway and Gibbs, 2003). Early works reported an increase in glycolytic flux under complete anoxia, defined as the "Pasteur effect", in order to produce a higher amount of ATP (Gibbs *et al.,* 2000; Neal and Girton, 1955; Vartapetian, 1982; Bailey-Serres *et al*., 2012; Banti *et al*., 2013; Jethva *et al*., 2022). Subsequent results reported an adaptation of respiration to hypoxia in *Pisum sativum* when the oxygen availability decreases in order to save oxygen, thus suggesting the existence of a regulatory mechanism that works in a concentration-dependent manner (Zabalza *et al.,* 2009).

Ethanolic fermentation has been analyzed in land plant species (Bui *et al.,* 2019), and the results suggest that distant phyla maintain the ability to produce ethanol under hypoxia, while the transcriptional regulation of anaerobic genes is not conserved (Bui *et al.,* 2019). In addition, Arabidopsis *adh1* mutants maintain the capacity to produce ethanol under hypoxia, suggesting that additional, yet unidentified enzymatic pathways are possibly involved (Bui *et al.,* 2019).

The process of glycolysis and thus the survival of the plant require a sugar supply from either sucrose degradation or starch breakdown. Sucrose provides hexose-6-phosphates through two distinct pathways. The sucrose synthase (SUS) pathway firstly produces fructose and UDP-glucose and is bi-directional, but the catabolic direction is favored. UDP-glucose is then converted into glucose-1-P, through the activity of UDP-glucose pyrophosphorylase (UGPPase) which is PPi dependent, and it is subsequently converted into glucose-6-P by phosphoglucomutase (PGM). Fructose is then converted into fructose-6-P through the activity of fructokinase (FK), which is ATP dependent (Huber and Akazawa, 1986; Stitt, 1998).

The invertase (INV) pathway is unidirectional and catalyses the sucrose hydrolysis into fructose and glucose, which are subsequently phosphorylated to fructose-6-P and glucose-6-P by hexokinase (HK) and fructokinase (FK), respectively, using ATP or UDP as energy donors (Renz *et al.,* 1993). Since sucrose degradation to phosphorylated exoses via the INV route requires two molecules of ATP, it is generally assumed that the SUS route is preferred during an oxygen shortage, given it produces F6P and UDP-Glc, the latter converted to G1P. However, Arabidopsis plants defective in *SUS1* and *SUS4*, which are highly induced by oxygen depletion, have been shown to be as tolerant to low oxygen as wild type plants (Santaniello *et al.,* 2014). Only when the plants were under sugar starvation, was the requirement of SUS for the production of ethanol evident, although compensated partially by the activity of invertase. This thus suggests that both the SUS and INV route contribute to sucrose metabolism (Santaniello *et al.,* 2014).

The availability of soluble carbohydrate can be limited under hypoxia when it is prolonged. Leaf transitory starch can instead be a reliable source at night (Smith *et al.,* 2005) or when plants experience hypoxia in darkness (Loreti *et al.*, 2018). In fact, starchless mutants *pgm* (mutated into the plastidial form of phosphoglucomutase), which are unable to produce starch during the day, and *sex1* (defective in glucan water dikinase)*,* which is unable to degrade starch at night, are

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highly sensitive to low oxygen conditions, suggesting that survival requires transitory starch (Loreti *et al.*, 2018).

Other products involved in anaerobic metabolism are alanine and gamma-aminobutyric acid (GABA). Alanine is produced by transferring an amino group from glutamate to pyruvate through Ala-aminotransferase (AlaAT) activity with the reversible generation of 2-oxoglutarate (Streeter and Thompson, 1972; Reggiani *et al.,* 1988). Accumulation is likely involved in reducing the pyruvate level to prevent the inhibition of glycolysis (Rocha *et al.,* 2010). GABA has also been found to increase under low oxygen as the further conversion of glutamate through glutamate decarboxylase (GAD) (Reggiani *et al.,* 1999). Both Ala and GABA production may prevent acidification of the cell and reduce carbon/nitrogen loss that can instead be used during the recovery period (Mustroph *et al.,* 2014). There is thus a rapid reconversion of Ala to pyruvate during the recovery period after oxygen shortage in Arabidopsis plants (Miyashita *et al.,* 2007)

Hypoxia is constitutive in some plant tissues where it plays a role in regulation. Hypoxia has been detected in bulky organs such as potato tubers (Geigeberger *et al.,* 2000), fruits (Ho *et al.,* 2010a, 2010b) and seeds (Borisjuk and Rolletschek, 2009), and a low oxygen state has been detected in the phloem (Van Dongen *et al.,* 2003). A constitutive hypoxia state was also recently shown in roots of Arabidopsis despite the potting medium being well aerated, and proved by the high accumulation of PDC and ADH proteins (Ventura *et al.,* 2020). Hypoxia regulates development in the shoot apical meristem (SAM) (Weits *et al.,* 2019) and in the lateral root primordia (LRP) (Shukla *et al.,* 2019) where fermentation related transcripts of *ADH* and *PDC* are highly expressed. This thus suggests that the energy demand of these hypoxic niches is met by glycolysis plus fermentation.

The fascinating oxygen-sensing story

As described in the chapter on previous rice tolerance to submergence, the discovery of SUB1A in rice was a breakthrough in our understanding of how rice plants tolerate submergence. In 1996, a group of researchers led by David Mackill at the International Rice Research Institute (IRRI) identified a major QTL on chromosome 9 that was associated with submergence tolerance in rice, which they named QTL SUB1 (SUBmergence 1). It took ten years for the molecular identity

of SUB1 to be revealed. SUB1 is a cluster of three genes that encode for ERFs, more specifically those belonging to Group VII.

The discovery of SUB1A as the rice submergence tolerance gene boosted research on the members of ERF-VII in Arabidopsis. In 2008, Papdi *et al*. (2008) screened for the transcriptional activation of the ADH1-LUC reporter gene. They identified the ERF-VII transcription factor RAP2.12 as being able to sustain a high level of ADH1-LUC bioluminescence as well as an enhanced ADH1 transcription rate (Papdi *et al*., 2008).

In 2010, RAP2.2, HRE1 and HRE2 (HRE stands for Hypoxia Inducible ERF-VII), three of the five members of the ERF-VII family in Arabidopsis were identified as being key for the plant's ability to withstand hypoxia (Licausi *et al.,* 2010; Hinz *et al.,* 2010). RAP2.2 is expressed constitutively at high levels in the roots and is induced in shoots by ethylene. Overexpression of RAP2.2 improved plant survival to hypoxia, whereas mutant lines of the gene had poorer survival rates than the wild type (Hinz *et al*., 2010). HRE1 and HRE2 are also involved in hypoxia acclimation, given that they exert a role in low oxygen signaling and improve the tolerance of the plant to low oxygen by enhancing anaerobic gene expression and ethanolic fermentation (Licausi *et al.,* 2011).

The turning point for research on hypoxia in plants occurred the following year, in 2011. Two research groups independently demonstrated the existence of an oxygen-sensing mechanism in plants that was based on the oxygen-dependent destabilization of the ERVII belonging the subgroup that is constitutively expressed in Arabidopsis (Licausi *et al.,* 2011; Gibbs *et al.,* 2011). The mechanism of oxygen sensing, as illustrated in Figure 1, is based on the N-end rule pathway (now known as N-degron pathway), which leads to the degradation of proteins characterized by a specific N-terminal amino acid, the type of amino acid determining whether a protein will be degraded by a proteasome (reviewed in Sasidharan and Mustroph, 2011).

Interestingly, proteins with a Cys residue in the second position, such as the ERF-VII proteins, can be processed by the N-degron pathway in mammals and plants and degraded. Such proteins can become accessible to the protein degradation pathway after oxidation of the N-terminal Cys via a mechanism that was unknown in 2011 (Sasidharan and Mustroph, 2011).

After the identification of ERF-VII as oxygen labile proteins due to their N-terminal Cys (after removal of the terminal Met residue), the question that arose was: is the mechanism of Cys oxidation occurring spontaneously in the presence of molecular oxygen or is it enzymatically driven? In 2009, while reviewing the various hypotheses regarding the possible existence of an oxygen sensing mechanism in plants, Licausi and Perata (2009) revealed, through several microarray analyses of the hypoxic transcriptome, the existence of enzymes requiring oxygen for their activity that are upregulated in the absence of oxygen. Of these, proteins with an unknown function in plants but with a high similarity to cysteamine oxidases (CDOs) in animals were indeed highly induced by hypoxia (Licausi and Perata, 2009). In Arabidopsis, these CDO-like proteins are At5g39890 and At5g15120 (Licausi and Perata, 2009). Interestingly, At5g39890 was identified as a gene whose transcription is very rapidly induced by hypoxia (within 20 minutes after exposure to hypoxia) (Loreti *et al*., 2005).

In 2014, Weits *et al*. discovered that At5g39890 and At5g15120 are indeed enzymes that use oxygen to oxidise the N-terminal Cys in ERF-VII, leading to their destabilization under aerobic conditions. At5g39890 and At5g15120 were then named PLANT CYSTEINE OXIDASE1 and PLANT CYSTEINE OXYDASE2 (PCO1, PCO2; Weits *et al.,* 2014). Conversely, under hypoxia ERF-VII proteins are stable because of PCO inactivity, and able to activate the transcription of hypoxia-responsive genes (HRGs) by binding to the HRPE element present in most HRGs. HRPE was identified as an evolutionarily conserved promoter element that is necessary and sufficient for hypoxic induction of genes through ERF-VIIs RAP2.2 and RAP2.12 (Gasch *et al*., 2016). The oxygen sensor in plants is therefore represented by PCOs (Weits *et al.*, 2014; White *et al.,* 2017) that target ERF-VII in order to trigger the transcriptional upregulation of HRGs. However PCOs may also have other targets showing a terminal Cys residue, such as ZPR2 and VRN2, which are involved in plant development and linked to hypoxic niches in otherwise aerobic plants (Gibbs *et al.,* 2018; Weits *et al.,* 2019).

Very importantly, it has been shown that VERNALIZATION 2 (VRN2) is regulated by the O_2- and NO-sensitive branch of the Arg/N-end rule pathway, thus keeping VRN2 low outside the meristem in the absence of cold or hypoxia (Gibbs *et al*., 2018).

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Notably, ERF-VII proteins also act as nitric oxide sensors (Gibbs *et al.,* 2014), through a PCO-independent mechanism (White *et al.,* 2017). Ethylene is a crucial gaseous hormone which, when it is entrapped between the submerged plant and the surrounding water, provides the plant with a specific submergence-status signal and primes the plants for hypoxia responses. This occurs because ethylene depletes nitric oxide (NO) by increasing the NO-scavenger PHYTOGLOBIN1 (Hartman *et al*., 2019). This leads to enhanced ERF-VII stability prior to hypoxia, which pre-adapts plants for survival under subsequent hypoxia.

Oxygen sensing has recently been revealed to be integrated with several other signaling pathways, including energy sensing via TOR (Kunkowska *et al.,* 2023) and calcium signaling (Fan *et al.,* 2023). Both mechanisms act by activating ERF-VII through phosphorylation, adding a further layer of complexity to oxygen sensing and signaling in plants.

Are differences in oxygen sensing affecting plant's tolerance to submergence? While it appears that the N-degron-based mechanism for oxygen sensing based on PCO/ERF-VII is widespread in the plant kingdom, some evidence suggests that genetic differences in the genes involved in oxygen sensing might define the degree of tolerance to submergence. A recent article reported that in *Arabidopsis thaliana*, allelic changes in the cis-regulatory elements, in the promoter of the ERF-VII gene, RELATED TO AP 2.12 (RAP2.12), are responsible for differentially regulating tolerance to drought and flooding (Lou *et al*., 2022). This implies that there is room for crop improvement by exploring the genetic differences in the genes involved in oxygen sensing, although this might require the discovery of possibly still unknown component of the pathway.

Core hypoxia gene discovery in Arabidopsis

The considerable progress that has been made in dissecting the molecular mechanisms behind the state of environmental hypoxia in plants and described in the previous chapter, took advantage from previous studies of microarray datasets. An initial survey was carried out on Arabidopsis root culture at various time-points after the transfer to low oxygen (Klock *et al.,* 2002) which identified a group of differentially expressed genes. This included proteins that were previously suggested to be involved in the anaerobic metabolism, together with transcription

factors, signal transduction components, and new genes previously not known to be involved. Genes with an analogous expression profile were found to have similar sequence motives. Accordingly, subsequent work on transcription profiling of the Arabidopsis response to hypoxia led to the identification of overrepresented up-regulated genes with promoters of an AtMYB2-binding motif, a G-box-related sequence, and a sugar response element-like motif (Liu *et al.,* 2005).

Subsequently, a transcriptome study on Arabidopsis plants in response to dark anoxia was published in 2005 (Loreti *et al.,* 2005) and the anaerobic transcriptome was analysed both with and without the presence of external sugar. The results showed that, together with a cluster of anaerobic genes involved in sucrose metabolism and alcoholic fermentation, a further group of genes involved in various pathways were positively regulated (Loreti *et al.,* 2005). The application of exogenous sugars showed a lower induction of sucrose synthases and an up-regulation in heat shock protein genes, which were subsequently hypothesized as being the result of the early activation of a ROS-related pathway (Banti *et al.,* 2010; Pucciariello *et al.,* 2012a, 2012b). The reduction in sucrose synthase suggested the presence of a sugar starvation signal under anoxia. Anoxia was shown to induce HSPs with an overlap with heat shock and with a heat shock transcription factor, which helps promote anoxia tolerance (Banti *et al.,* 2010).

In addition, the modulation of total RNA in parallel to RNA in large polysome complexes was analysed during anoxia (Branco-Price *et al.,* 2005), revealing different translation responses to anoxia with a significant decrease that suggested possible energy conservation. This prompted a comprehensive analysis of Arabidopsis root responses to a time-course of different concentrations of oxygen (van Dongen *et al.,* 2009), checking transcripts and conducting metabolite profiling. The results revealed that downregulated genes were related to proteins involved in processes that are energy-consuming, most probably in order to save ATP.

The responses of specific cell populations to anoxia were investigated using Arabidopsis lines and revealing the translatome (Mustroph *et al.,* 2009). This led to the identification of a core of 49 mRNAs, including genes coding for enzymes involved in the anaerobic metabolism but also in signaling (and some uncharacterized proteins) (Mustroph *et al.,* 2009). Subsequently, a cross-kingdom comparison of transcriptomic adjustment under oxygen shortage was conducted

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considering results from different plants and other organisms (Mustroph *et al.,* 2010) for a total of four kingdoms (Plantae, Animalia, Fungi and Bacteria). The response was broadly conserved for orthologous genes in anaerobic metabolism, but also in metabolite transport, ROS amelioration, chaperon activity, and ribosome biogenesis. In contrast, a group of hypoxia-responsive unknown proteins (HUP) genes were found to be plant specific. Subsequently, several HUPs have been found to be co-regulated by submergence and hypoxia, and mutants of these genes seem to be altered in response to submergence (Lee *et al.,* 2011).

Reactive oxygen species (ROS) play an important role in both oxidative damage and signaling during hypoxic stress (Blokhina and Fagerstedt, 2020). A bioinformatics approach to the whole genome level for the analysis of large sets of global expression data revealed the upregulation of some ROS-producing components and antioxidative defense components in Arabidopsis under anoxia and hypoxia (Blokhina *et al.*, 2014). In addition, the physiological role of induced phytoglobin has been investigated in a transcriptomic assay of Arabidopsis shoots and roots under hypoxia (raw data stored at the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/projects/geo/, GSE72168, Blokhina *et al.*, unpublished). In brief, the work showed that co-induction of a few genes led to the incorporation hypoxic metabolites pyruvate, alanine and GABA shunt constituents glutamate and 2-oxoglutarate, in the TCA cycle under hypoxia.

Determining O_2 in flooded habitats and inside tissues

Due to the gas exchange constraints imposed by flooding, many studies have examined tissue O_2 status in partially or completely submerged plant tissues. A recent meta-analysis of over 500 studies and encompassing 112 species showed that O_2 status ranged from effectively 0 kPa in roots to more than 50 kPa in submerged, photosynthesising shoots (Herzog *et al.,* 2023). When flooded, tissue O_2 status was particularly low in rhizomes, potato tubers and root nodules. Interestingly, light was significantly associated with flooding showing a submergence-induced 44% increase in tissue O_2 in the light, compared with a 42% decline when growing in the dark. The latter demonstrates that submergence can be used as a proxy for hypoxia treatment only in

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complete darkness; when submerged in light, even terrestrial plants can attain hyperoxic conditions in the shoots (e.g., Pedersen *et al.,* 2006; Rich *et al.,* 2013).

At the onset of soil flooding, radial O_2 supply from the soil environment to root tissues ceases within days as the soil soon turns anoxic (Ponnamperuma, 1972). Nevertheless, the growing root tip requires molecular O_2 to support mitotic cell division of the apical root meristem (Armstrong and Webb, 1985). Instead, O_2 is supplied internally via longitudinal molecular diffusion via porous cortical tissues (Pedersen *et al.,* 2021), and O_2 supply to the root tip is further facilitated by preventing O_2 loss to the soil via the outer apoplastic barriers (Peralta Ogorek *et al.,* 2023).

Surprisingly, root O_2 status is not influenced by light or darkness as long as the shoot is exposed to air due to the effective O_2 exchange between the shoots and the atmosphere (Herzog *et al.,* 2023). However, measurements using miniaturized Clark-type O_2 sensors (Revsbech, 1989) show that the stele operates at a significantly lower O_2 tension than the cortex even in roots acclimated to soil flooding (Herzog *et al.,* 2023). The difference in tissue O_2 status is due to i) the higher O_2 consumption per tissue mass of the stele (Aguilar *et al.,* 2003), and ii) the lower diffusivity of the stelar tissues (Armstrong, 1979) both resulting in a lower O_2 status of the stele.

Partial or complete shoot submergence produces additional stress on terrestrial plants (Colmer and Voesenek, 2009), whereas aquatic plants possess a range of traits conferring flood tolerance, e.g., reduced or absent leaf cuticle and/or filamentous leaves both facilitating O_2 exchange with the surrounding water (Sculthorpe, 1967). However, superhydrophobic leaf cuticles of terrestrial plants retain a thin gas film upon submergence, and the leaf gas film greatly enhances O_2 exchange with the floodwater (Winkel *et al.,* 2013; Winkel *et al.,* 2011).

In darkness and during submergence, the only source of O_2 is dissolved O_2 in the floodwater, and the leaf gas films facilitate the inward flux of O_2 via open stomata (Verboven *et al.,* 2014). O_2 is then subsequently distributed via porous tissues to the belowground organs (Pedersen *et al.,* 2009). In light, however, underwater photosynthesis is the main source of molecular O_2 supplied to belowground tissues via the porous tissues (Pedersen *et al.,* 2013).

In the above studies, molecular O_2 was determined using miniaturized Clark-type O_2 sensors made from glass capillaries or with O_2 optodes consisting of a glass fibre with fluorophores coated

onto the tip (Pedersen *et al.,* 2020) (Fig. 2). Even the smallest Clark-type O_2 sensor with a tip diameter of only 3 μm is not suitable for obtaining measurements of cellular O_2, but it is an excellent tool for obtaining the O_2 status of the shoot apical meristem of only a few days old Arabidopsis seedlings (Weits *et al.,* 2019). It is often claimed that Clark-type sensors should be avoided due to their O_2 consumption, however the consumption is very low and it would take 36 years to consume the amount of O_2 dissolved in 1 mL of seawater at 20 °C (Gundersen *et al.,* 1998). Nevertheless, glass fibre based O_2 optodes do not consume O_2 at all, but the tiny tapered glass fibre needed to construct a sensing tip of less than 10 μm is extremely flexible and therefore not suitable for penetrating plant tissues (Pedersen *et al.,* 2020).

There are now sensors that can be used for cellular O_2 measurements. These sensors consist of indolequinone, and depending on the different pKa of the dye (DDAO, resorufin or Me-Tokyo green) and substitution on the methylene group of the indolequinone compounds, the sensors are activated at O_2 concentrations ranging from ≤6, ≤4, ≤2, and ≤1 kPa (Wallabregue *et al.,* 2023). Although the indolequinone molecule is relatively large, it is readily taken up by liver cells, and hypoxia has been observed using confocal microscopy. Unfortunately, the reaction is irreversible and once the molecule has been reduced, it does not revert back to its oxidized state even if O_2 is re-introduced. However, the technique is a significant step towards sub-cellular O_2 measurements, and genetically encoded oxygen biosensors can be a very useful tool for measurements in plant tissues. The technique has also been optimized with a hypoxia-signaling reporter, the five-times repeated hypoxia-responsive promoter element (HRPE) driving the expression of different reporter proteins (Panicucci *et al.,* 2020). Similarly, fluorescent protein-based systems have been used in Arabidopsis to simultaneously monitor many intracellular parameters in leaves under hypoxia (Wagner *et al*., 2019).

Morphological and anatomical means of escaping low oxygen conditions

Various morphological and anatomical modifications offer plants an escape from hypoxic or anoxic environments, which is referred to as the Low Oxygen Escape Syndrome (LOES, Bailey-Serres and Voesenek, 2008). It is well known that aerenchyma greatly enhances the diffusion of gases to rhizomes and roots, but to what distances? Diffusion alone cannot supply enough oxygen for root cell respiration at any great distance, for example in peas only 7-8 cm of the roots

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can be supplied based on diffusion (Armstrong *et al.,* 1983). In larger plants a convective flow can take place through humidity-induced pressurization (Armstrong *et al.,* 1996a), thermal osmosis (Grosse, 1996), and venturi-induced convection (Armstrong *et al.,* 1996b). The venturi-induced convection is explained in stems by the reduction in gas pressure and increased speed that results when gas flows through a constricted section i.e. capillaries in aerenchyma.

To assess the variety of changes in root anatomical features across wetland, non-wetland and intermediate plants, a total of 91 plant species were studied by Justin and Armstrong (1987) in control and flooded experimental setups. The variation was large, but the general conclusion was that aerenchyma development greatly enhanced fractional root porosity and enabled root penetration into the flooded soil.

Aerenchyma development can be either constitutive or induced (Visser *et al.,* 1997; Yamauchi *et al.,* 2019). Its development is vital for O_2 and CO_2 gas exchange under waterlogged conditions. The diffusion of O_2 in gases (2.1×10^{-5} cm^2/s) is 10,000 times faster than diffusion in water (0.2 cm^2/s) (Weast, 1989), which permits a reasonably straight diffusion pathway, and prevents the loss of O_2 along the way, especially if the radial-oxygen-loss barrier, ROL, is formed (Colmer, 2003; Colmer *et al.,* 1998). As oxygen demand of the root tip quiescent center cells is low, they are presumably the last cells to die under oxygen deprivation.

Aerenchyma can develop in three ways: lysigenously, where cells die through programmed cell death and large cavities remain in their place; schizogenously, where intercellular spaces enlarge through the splitting of the cell wall middle lamellae, or expansigenously i.e. through cell division and enlargement (Seago *et al.,* 2005). Increases in pectinase and xylanase activity (Branco-Price *et al.,* 2005) as well as in xyloglucan endo-transglucosylase, XET (Saab and Sacks, 1996) take place under oxygen deprivation, and these may participate in the formation of schizogenous aerenchyma.

Although there is data on the regulation and formation of lysigenous aerenchyma where ethylene, ROS signaling, protein kinases and phosphatases, G-proteins, Ca^{2+}, and inositol phospholipids play an important role (He *et al.,* 1996; Steffens *et al.,* 2013; Yamauchi *et al.,* 2016; Mustroph *et al.,* 2018; Yamauchi *et al.,* 2018), regulation of the constitutive aerenchyma

formation is still not fully understood. Auxin and auxin response factors (ARFs) seem to play an important role in constitutive aerenchyma formation (Yamauchi *et al.,* 2019). The latter authors found that in rice *iaa13* mutant constitutive aerenchyma formation was lower than with the WT. This was confirmed in IAA and auxin transport inhibitor experiments.

A study on the IAA13 interactor showed that ARF19 had the highest signal activity and, furthermore, a similar transcript accumulation pattern in the tissues to IAA13. To identify genes regulated by AUX/IAA-mediated signaling, a further study by the same authors revealed that two LBD (LATERAL ORGAN BOUNDARIES) genes LBD1-8 and LBD5-3 are transcriptionally regulated through IAA13- and ARF19-dependent signaling in the cortex, while LBD5-3 also plays a role in the formation of lateral roots (Yamauchi *et al.,* 2019). Yamauchi *et al*. (2017) also showed that ROS-producing RBOHH activation is stimulated by kinases and is vital for the formation of aerenchyma in rice roots under hypoxia. With maize, information of QTLs for constitutive aerenchyma formation in *Zea nicaraguense* has been used in producing introgression lines in cultivated maize and this has led to increased root tolerance to low oxygen conditions (Gong et al., 2019).

Certain morphological features, in addition to anatomical means such as aerenchyma, can help plants to obtain sufficient oxygen. Hydrophobic hairs in the leaf sheaths of rice plants seem to contribute to the oxygen supply (Kurokawa *et al.,* 2018). Surprisingly, it was found that a single gene, Leaf Gas Film 1(LGF1), is required for determining leaf gas films. LGF1 regulates C30 primary alcohol synthesis, which is vital for the formation of epicuticular wax platelets, resulting in leaf hydrophobicity and gas films on submerged leaves.

Interestingly, related plant species can have very different strategies for survival under flooded conditions, depending on the characteristics of their ecological niches. Certain *Rumex*-species, *Rumex palustris* and *R. acetosa* use different strategies for survival. While in *R. acetosa* petiole growth is suppressed under flooding, in *R. palustris* petiole growth is greatly enhanced and leaves reach out into the air (van Veen *et al.,* 2013). At the same time both species reconfigure their primary metabolic pathways to overcome the stress. Regulation of the petiole elongation involves ethylene and abscisic acid (Benschop *et al.,* 2005), gibberellin (Benschop *et al.,* 2006), expansins, and xyloglucan endotransglucosidases and hydrolases (Vriezen *et al.,* 2000).

Under compound stresses, such as those in salt marshes, plants adapt by exploiting a large fine root biomass (Redelstein *et al.,* 2018), and even in these harsh conditions root competition promotes plant species distribution (Levine *et al.,* 1998).

Future research and practical applications

Due to climate change, plant life is suffering from drought and elevated temperatures, and the increased variations in precipitation will have a large impact in a near future (Wing *et al.*, 2022). Plants may be subjected to severe flooding in some seasons, followed by long droughts. As proposed in an ISPLORE conference in Bamberg, Germany (2022), research should focus not only on the impact on agricultural crops of increased flooding or drought tolerance separately, but simultaneously. In the following section we thus focus on the simultaneous breeding of many stress tolerance mechanisms which stem from large-scale or holistic information on genetics and genomics (Fig. 3).

Examples of large-scale changes achieved in plant functions through a holistic understanding of developmental events and their regulation

As genetic and genomic knowledge increases, there is increasing information on the systemic tolerance of stress on all levels: genetic, metabolic and anatomical/morphological. How to solve the regulation of whole metabolic pathways and the development of anatomical features has long been an issue, and now we have increasing information on the regulation of the development of plant structures. One good example is the work by Niko Geldner in Switzerland on the development and functioning of the Casparian strip in the endodermis of roots (Anderson *et al*., 2015; Doblas *et al*., 2017). The root endodermis is the innermost cortical cell layer surrounding the vasculature. The endodermal cell walls contain Casparian strips, which are ring-like hydrophobic cell wall impregnations (lignin and suberin) that are involved in the control of transport from the cortex into the xylem elements. In a series of publications, the authors

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described the endodermal differentiation and molecular markers for the membrane domains vital for the development of the Casparian strip (Alassimone *et al*., 2010; Roppolo *et al*., 2014). This work is an excellent example of where a holistic view led to the discovery of an intricate system with a functionally important anatomical structure. The development of the endodermis and Casparian strip may provide clues as to how the Radial Oxygen Loss (ROL) barrier is formed in the root hypodermis. The development of the ROL has been described as an adaptive mechanism in rice roots under stagnant flooded conditions (Peralta Ogorek *et al*., 2023).

Another example of recent advances is the regulation of cambial activity and the differentiation between xylem and phloem elements in roots by Ari Pekka Mähönen and his group in Finland (Smetana *et al*., 2019; Zhang *et al*., 2019; Wang *et al*., 2020; Ye *et al*., 2021). With the ingenious use of inducible fluorescent probes, these authors disentangled the hormonal regulation of cambial divisions and the subsequent differentiation between phloem and xylem elements in the roots. These methods could be used to reveal the regulation of the development of aerenchyma in roots and rhizomes, and thus enable us to be one step ahead in the breeding of flooding tolerant crop plant species (Fig. 3).

The third example is the large international effort in the engineering of nitrogen-fixing nodules in non-nodulating plants (for a review see Pankievicz et al., 2019). These authors are unravelling the complex interactions between nitrogen-fixing bacteria and the plant root, and are exploiting this information in transgenic attempts to create functional root nodules in non-nodulating crop plant species. A similar approach would be beneficial in the study of aerenchyma development.

Our fourth example is Donald Ort's work in Illinois on the regulation of photorespiration in C3-plants (South *et al*., 2018; Cavanagh *et al*., 2021). With the transformation of only a few genes encoding chloroplast enzymes, they managed to create C3-plants with greatly diminished photorespiration and with dramatic increases in biomass production.

These kinds of studies show that through directed breeding programmes and/or genetic transformation, in the future we will be able to regulate the carbohydrate consumption in root and rhizome tissue so that reserves can be optimised under flooded conditions, and exhaustion can be restricted.

Another desirable feature would be the regulation of aerenchyma formation in agricultural crop plant species in the case of flooding, and, on the other hand, the development of water saving features in sudden periods of drought. A holistic approach is suggested for improving root anatomy in a review Yamauchi *et al*. (2021). They have used all the available information on root anatomical traits for modelling and automated analyses to be used to improve crop tolerance to soil flooding. The precise engineering of root features requires detailed knowledge and now we have all the tools for altering and/or adding regulatory mechanisms for metabolic/anatomical/morphological development in many agricultural crop species. Such work requires a panomics approach to improve waterlogging tolerance in plants as advocated in a review by Tyagi *et al*., 2023. As the ISPLORE community has researchers in basic research in molecular as well as ecological sciences and crop breeders, using the various methods stated above including modelling, and combining the results with crop breeding activity, we are in an advantageous position in creating better performing crop cultivars.

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Conflict of interest

The authors have no conflicts to declare.

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Fig. 1. Oxygen sensing in plants. Under normoxia the presence of oxygen activates PCOs, which utilize it to oxidize the N-terminal CY residue of ERFVII proteins (or that of other proteins with a Cys as N-terminal residue, obviously after removal of the MET residue). Not all proteins showing a Cys residue at the N-terminal are substrates for this pathway. At present, besides ERFVII, the proteins VRN2 and ZPR2 appear to be substrates for this pathway (see main text for references). ERFVII with oxidized Cys are substrates for the N-degron pathway. Nitric oxide is also an important player in the pathway, although the exact site of action is yet to be identified. Under submergence, the lack of oxygen strongly reduces the activity of PCOs or even makes it impossible. As a consequence, ERFVII are stable, migrate to the cell nucleus, where they bind to genes showing the HRPE element (see main text for the details and references), thus activating their transcription. The activation of hypoxia responsive genes leads to the production of proteins and enzymes which contribute to adapting the plant's metabolism to hypoxia and eventually leading to the plant's tolerance to submergence.

Fig. 2. Use of an O_2 microsensor to obtain tissue O_2 status of submerged tissues. A, experimental set-up with submerged *Brassica napus* seedling, where a Clark-type O_2 microsensor is inserted into the petiole and with an O_2 optode used to monitor the floodwater O_2 status. B, time trace of O_2 in the petiole depicted in A, first during darkness and then with the light on. C, quasi steady-state data of five replicate plants diagnosed as shown in B. Data modified from Wittig *et al.* (2021).

Fig. 3. As information on many aspects of low oxygen tolerance and avoidance is gathering, it is becoming even more important to have a holistic view on the many mechanisms enhancing plant production in adverse conditions. This would lead to plant breeding for better biomass production. The outermost boxes present the different methods to be used to solve the research questions in the middle boxes. The narrow arrows suggest combining the methods for even larger scale understanding of resilience mechanisms. The references given are examples and more can be found in the article. The figure has been created using biorender.com.
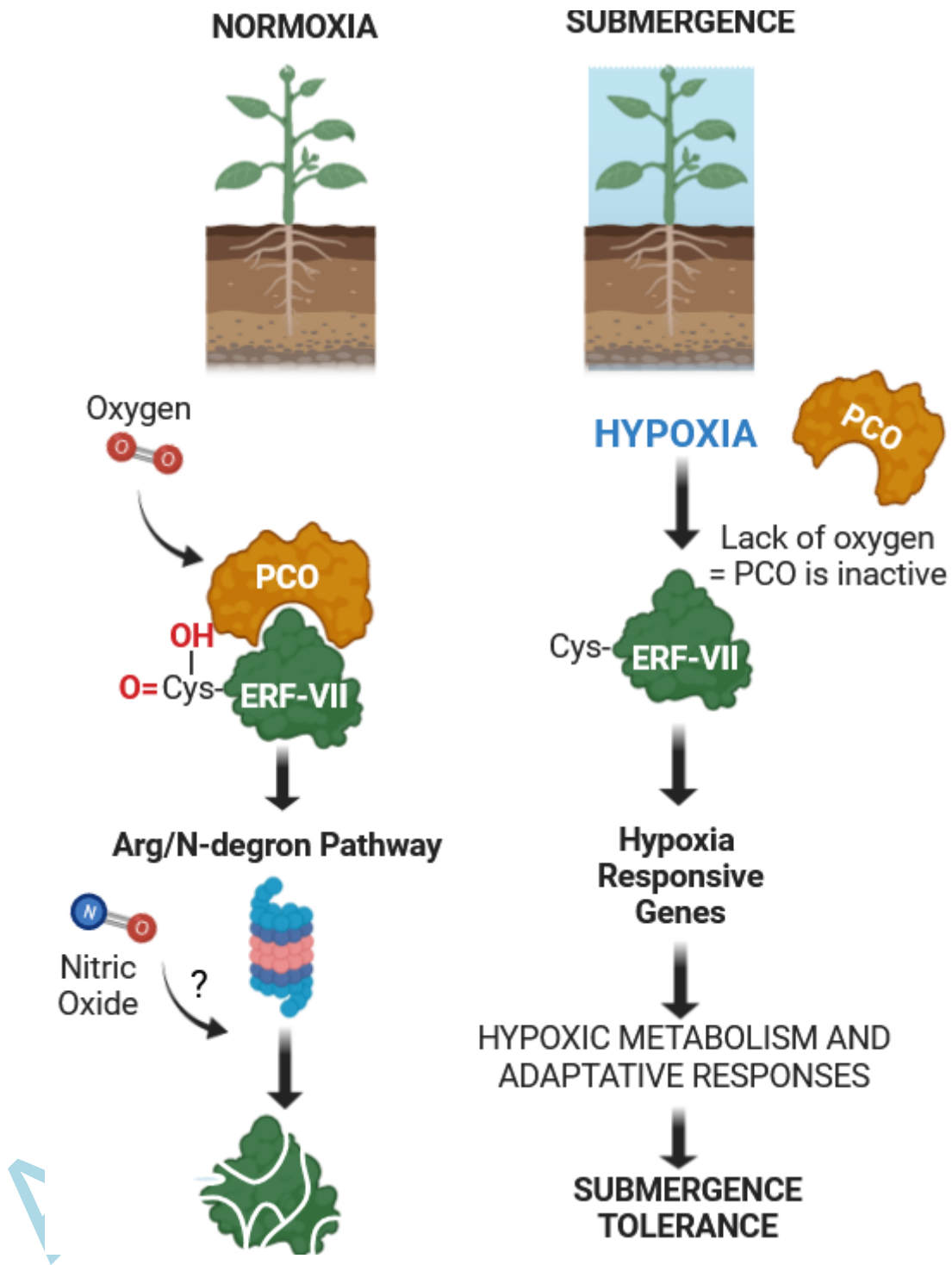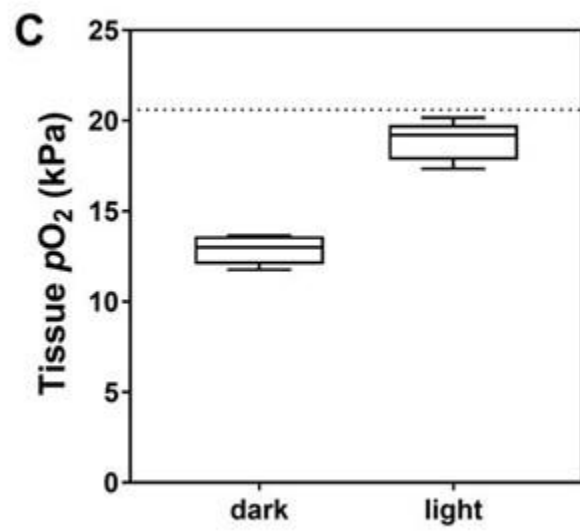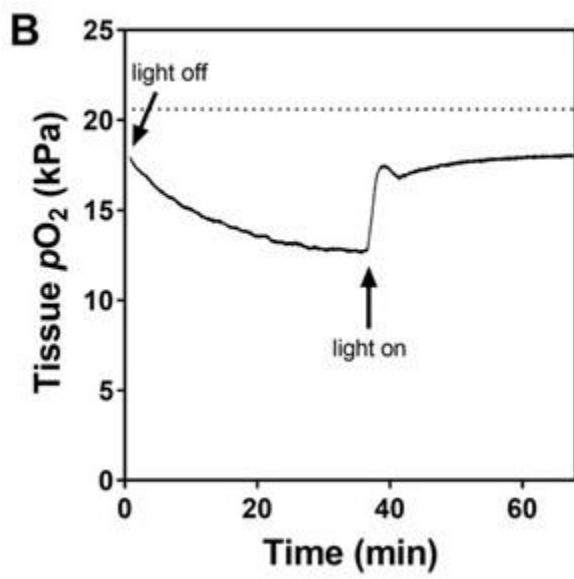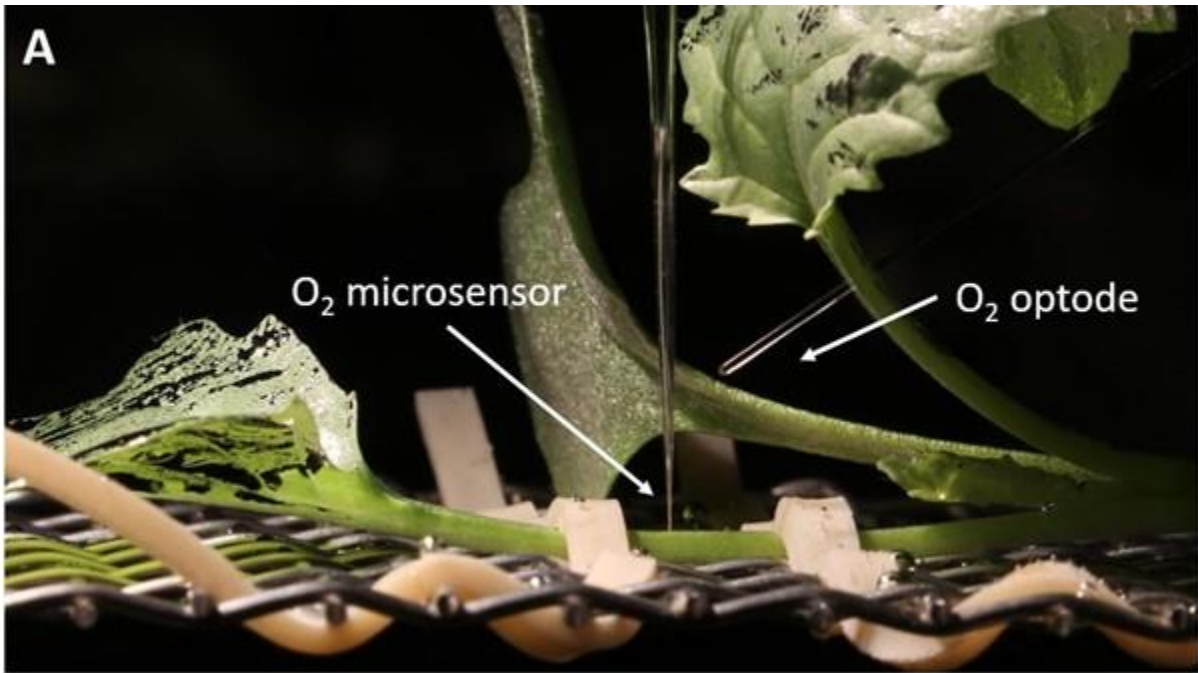
Figure 1

Figure 2

Figure 3