

Flooding stress and responses to hypoxia in plants

Juan de la Cruz Jiménez^{A,*} , Angelika Mustroph^B , Ole Pedersen^{A,C} , Daan A. Weits^D  and Romy Schmidt-Schippers^{E,F} 

For full list of author affiliations and declarations see end of paper

***Correspondence to:**

Juan de la Cruz Jiménez
Department of Biology, University of
Copenhagen, Universitetsparken 4,
Copenhagen 2100, Denmark
Email: juan.jimenezserna@bio.ku.dk

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ABSTRACT

In recent years, research on flooding stress and hypoxic responses in plants has gathered increasing attention due to climate change and the important role of O₂ in metabolism and signalling. This Collection of *Functional Plant Biology* on 'Flooding stress and responses to hypoxia in plants' presents key contributions aimed at progressing our current understanding on how plants respond to low-O₂ conditions, flooding stress and a combination of stresses commonly found in flooded areas. The Collection emphasises the characterisation of diverse plant responses across different developmental stages, from seed germination to fully developed plants, and under different water stress conditions ranging from waterlogging to complete submergence, or simply low-O₂ conditions resulting from limited O₂ diffusivity in bulky tissues. Additionally, this Collection highlights diverse approaches, including eco-physiological characterisation of plant responses, detailed descriptions of root anatomical characteristics and their surrounding microenvironments, evaluation of the seed microbiota under flooding stress, the modification of gene expression, and evaluations of diverse germplasm collections.

Keywords: apoplastic barriers, complete submergence, flooding tolerance, low oxygen, partial submergence, radial oxygen loss, saline flooding, underwater germination.

Introduction

Plant growth in extensive agricultural and natural ecosystems is frequently hampered by flooding or a combination of diverse stress conditions, including saline flooding. Flooding encompasses two different situations: (1) waterlogging where only the roots are affected; and (2) partial or complete submergence also affecting the shoots (Fig. 1). During flooding, the gas-filled porous spaces in the soil are replaced by water, creating a hypoxic environment that limits root respiration and plant growth. In addition to the stress resulting from a lack of O₂, an excess of salts in saline floods can further impair plant growth in such conditions. The adverse consequences of flooding and low-O₂ stress increase in severity, from soil waterlogging to partial or complete submergence of plants (Fig. 1). These conditions can affect all stages of the plant cycle, from seed germination underwater to vegetative growth or grain production. Floods may be temporary, resulting from heavy rains, or cyclic, occurring due to tidal movements in coastal areas. Additionally, extreme conditions can also occur where heavy rains are followed by prolonged droughts, or vice versa. The temporal variability or simultaneous occurrence of multiple stresses during specific plant growth stages can significantly influence their damage and shape their adaptive responses.

The pertinent importance of studying multiple stresses

Large areas of flooded agricultural land or those in proximity to coastal zones are often saline, containing phytotoxic concentrations of Na²⁺ and Cl⁻ (Barrett-Lennard 2003). Common strategies of salt tolerance in plants include salt exclusion from sensitive tissues, vacuolar storage, or the restriction of salt entry into roots (Flowers and Colmer 2008).

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Collection: Flooding Stress and Responses to Hypoxia in Plants

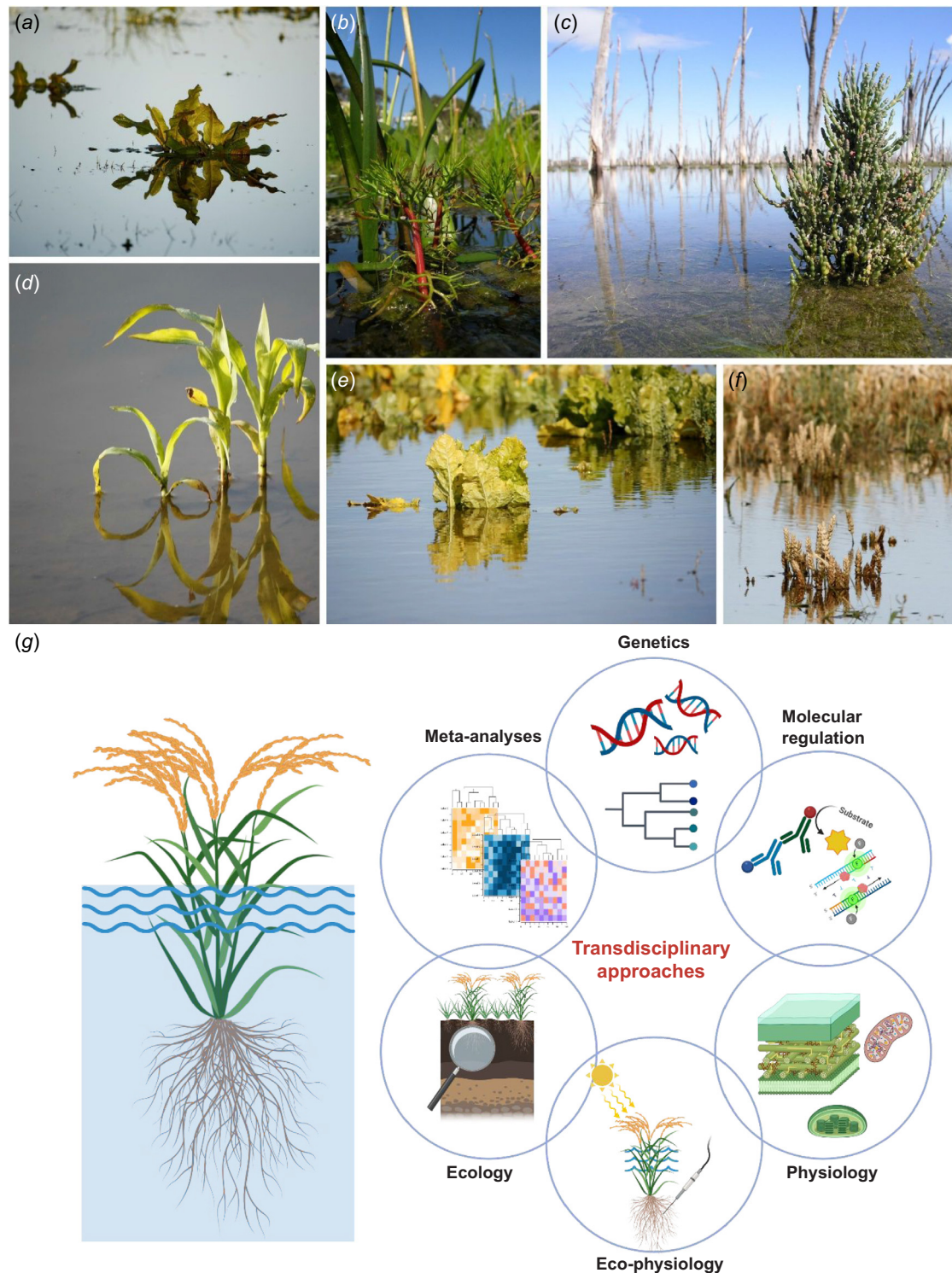


Fig. 1. Flooding of plants leading to low O_2 responses can occur with varying intensity, both in agriculture and in natural environments. Examples include (a) flooded *Rumex crispus*, (b) partially submerged *Meionectes brownii*, (c) *Tecticornia pergranulata* flooded with saline water (note the dead *Melaleuca* trees in the background), (d) flooded maize (*Zea mays*), (e) partially submerged sugar beet (*Beta vulgaris*), and (f) almost completely submerged wheat (*Triticum aestivum*). Flooding and low O_2 responses of plants constitute a compound stress and manifest at multiple levels. Therefore, a transdisciplinary research approach (g) is required to understand stress responses and to utilise this knowledge in breeding attempts aimed at enhancing the flood resilience of our crops. Photos by Ole Pedersen and partly created with BioRender.com.

However, such responses can be hampered when plants are exposed to soil waterlogging with saline water, as O₂ deficiency in roots leads to decline in ion (i.e. K, Na²⁺, Cl⁻) transport capacity and regulation (Kotula *et al.* 2015). Therefore, the combination of flooding and salinity is potentially more deleterious than either of these stresses individually (Fig. 1c). In practice, the characterisation of plant responses to combined stress conditions and the identification of associated traits of tolerance, as well as the molecular signalling related with those mechanisms of adaptation, may appear complicated. In this regard, evaluations of plants exhibiting high tolerance to both flooding and salinity, such as *Salicornia europaea* (Jordine *et al.* 2024), or the evaluation of large populations of *Festuca arundinacea* (Menon-Martínez *et al.* 2024), appear to be excellent resources for traits discovery and selection of outstanding genotypes.

Temporal changes in the water load within soils (for changes in the water table above soil, see section below) can also shape plant responses. In this Collection, Jordine and collaborators found that regular tidal flooding for 5 weeks did not affect growth of *S. europaea*, whereas only 10 days of continuous flooding stopped plant growth (Jordine *et al.* 2024). However, the anatomical plasticity of plant species might play an important role in their acclimation ability. Yamauchi and collaborators found that a larger cortex and aerenchyma formation in roots facilitate internal O₂ diffusion from tissues above water down to the roots of *Phragmites australis* when grown in waterlogged soils (Yamauchi *et al.* 2024). This is in stark contrast to a larger stele tissue with more xylem vessels facilitating water movement in *Phragmites japonica* growing under water deficit conditions. Increased root porosity not only facilitates greater O₂ diffusion in flooded soils but can also improve waterlogging and drought tolerance by reducing metabolic costs in the root resulting from a reduced number of root cells (Chimungu *et al.* 2014). On the contrary, a bulky tissue geometry of low porosity can generate hypoxic conditions due to both increased resistance to O₂ diffusion and cellular O₂ consumption, even under aerated environments (Brunello *et al.* 2024; Jiménez *et al.* 2024).

In addition to changes in root porosity, a barrier to restrict radial O₂ loss (ROL) is formed in roots of flood-tolerant plants when these grow in waterlogged soils. Such barriers are characterised by depositions of lignin and/or suberin in cell walls of the sclerenchyma, exodermis, or epidermis and appear as a key root trait for tolerance to multiple stresses (Peralta Ogorek *et al.* 2024). The formation of apoplastic barriers in the outer part of the root facilitates internal O₂ diffusion by impeding ROL from roots to the rhizosphere. This barrier also restricts water loss from roots (Peralta Ogorek *et al.* 2023) and the entry of soil phytotoxins in the opposite direction (from soils to the roots; i.e. NaCl, Ranathunge *et al.* 2011; reduced iron, Jiménez *et al.* 2021; hydrogen sulfide, Peralta Ogorek *et al.* 2023). Consequently, the outer apoplastic barriers play an important role in scenarios

involving multiple stress conditions, as the barriers can impede O₂ and water loss from roots, while also preventing the entry of salts or other phytotoxins from rhizosphere to roots. However, more research is needed to evaluate the potential drawbacks of such barriers especially for nutrient and/or water uptake.

Type of stress – intensity of the stress condition

In lowland regions or during periods of heavy rainfall, water levels can rise in poorly drained soils, causing partial or even complete submergence of plants, thereby further hampering plant growth. The longer the diffusional path between the atmosphere and the submerged tissues; i.e. the deeper the flood, the lower the net O₂ flux; therefore, deeper water tables above the plant imply less O₂ availability for plant growth. Complete submergence not only affects O₂ diffusion for respiration but also CO₂ diffusion for photosynthesis. As such, plant responses to hypoxic conditions, including molecular signalling, upregulation of hypoxic genes, anatomical changes and overall plant responses to such conditions vary widely between different water levels. These responses are detailed by Lin and colleagues in this issue. In waterlogged soils, aerenchyma formation and the development of barriers to impede ROL facilitate internal O₂ diffusion from shoots above water down to submerged roots (Lin *et al.* 2024; Peralta Ogorek *et al.* 2024). In situations of partial submergence, the plant may escape submergence by elongating the shoots to enhance contact with air or, alternatively, may become quiescent by stopping growth to conserve resources (Hattori *et al.* 2009). Additionally, an increased formation of aquatic adventitious roots developed from submerged stem internodes can enhance O₂ uptake from O₂ dissolved in the floodwater (Lin *et al.* 2021). In the extreme case of complete submergence, the formation of gas films in leaves allow diffusion of molecular O₂ and CO₂ dissolved in water into the leaf (Colmer and Pedersen 2008) and therefore these gas films might play an important role in internal aeration under such conditions.

Evaluations of plant responses during continuous periods of waterlogging, partial or complete submergence, compared to non-stress controls, represent the basis of our current understanding of how plants respond to such conditions. However, plant responses during the recovery phase after the flood has receded have often been overlooked (Striker 2012). Stunted growth during flooding does not necessarily indicate poor performance under stress, nor does enhanced growth during stress indicate tolerance. In fact, the latter can lead to depletion of resources, hampering further growth in the recovery phase. In this Collection, Buraschi *et al.* (2024) found that some genotypes of the forage legume *Lotus* spp. exhibited better growth under partial or complete submergence, whereas other *Lotus* genotypes grew better during the recovery phase. Furthermore, the same study revealed that there was no apparent correlation between the intrinsic

growth ability under non-stress conditions and growth under submergence stress. In contrast, Menon-Martínez and colleagues (this Collection 2024) evaluated 39 tall fescue (*Festuca* spp.) genotypes and indicated that more productive accessions under non-stress conditions also showed greater stress tolerance (Menon-Martínez *et al.* 2024). These differences in response to stress compared to non-stress conditions are highly relevant, as breeding efforts should prioritise high productivity under both stressful environments and optimal growth conditions.

Flooding can also affect plant growth at different stages. The impact on productivity differs among crops; field peas are severely affected at any stage, early flooding greatly impacts wheat production, whereas barley (*Hordeum vulgare*) and rapeseed (*Brassica napus*) can still produce significant yield if transient flooding occurs during early rather than at late stages (Ploschuk *et al.* 2018). Flooding during seed germination stages can hamper crop seed germination while favouring the germination of better adapted weed species (Echeverry Holguín *et al.* 2024). Flood-adapted plants like rice can germinate under water, outcompeting weeds, but flooding during germination stages can be deleterious for other non-adapted crops, and underwater germination can benefit weed survival. After successful germination, constitutive aerenchyma formation in populations of black-grass (*Alopecurus myosuroides*) weeds, but not in wheat genotypes, facilitated rapid establishment of the former when challenged by waterlogging (Harrison *et al.* 2024). Several environmental and intrinsic factors including temperature and nutrient conditions (Echeverry Holguín *et al.* 2024), transcriptomic and metabolomic responses (Harrison *et al.* 2024), and the composition of bacterial microbiota inhabiting the seeds (Gómez-Álvarez *et al.* 2024) play important roles in determining the ability of a seed to germinate and/or grow under water or under hypoxic conditions.

Transdisciplinary research

It requires transdisciplinary research efforts in order to identify mechanisms of adaptation, as complex stress condition can vary in intensity and affect different stages of plant development and/or the normal functioning of different plant tissues (Fig. 1g). A set of genes, including some required for fermentative metabolism, redox management or simply those involved in O₂ sensing, have been widely identified in the model plant *Arabidopsis thaliana* in response to hypoxia (Mustroph *et al.* 2009; Weits *et al.* 2014). Some of these hypoxia-responsive genes were found to be strongly induced in the aerial, yet hypoxic roots of *Phalaenopsis* orchids (Brunello *et al.* 2024), or in the shoots of the halophyte *S. europaea* in response to flooding (Jordine *et al.* 2024). This indicates that similar responses to hypoxia stress are conserved across different plant species or tissues, and that the vast molecular information generated in the model plant

Arabidopsis could be used to integrate the molecular responses of crops to flooding conditions. Interestingly, the upregulation of metabolism-based herbicide resistance genes in shoots in response to root waterlogging (Harrison *et al.* 2024), or the higher expression of hypoxia-related genes followed by salinity treatments (Jordine *et al.* 2024), suggest a parallel stress response to different stress conditions that deserves further investigation.

Root anatomical traits, including aerenchyma development and the formation of barriers to ROL are common adaptive responses of plants to waterlogging, droughts, and saline conditions (Peralta Ogorek *et al.* 2023; Yamauchi *et al.* 2024). The known effect of ethylene in inducing aerenchyma but not a barrier to ROL (Colmer *et al.* 2006), and conversely, the induction of the barrier to ROL following applications of ABA but with no concomitant effect on aerenchyma formation (Shiono *et al.* 2022), indicate that these acclimations are differentially regulated. While the molecular mechanisms of O₂ sensing in hypoxic conditions are well-described in *Arabidopsis* (e.g. reviewed in León *et al.* 2021; Loreti and Perata 2023; Renziehausen *et al.* 2024), further research is needed on the specific signalling pathways and the molecular regulation of traits conferring hypoxia tolerance, as well as their functional characterisation and translation of such resources into flooding-sensitive crops. Excitingly, excellent examples of the use of molecular tools characterising genes responsive to hypoxia, tissue metabolic responses to hypoxia, tissue O₂ status, anatomical changes facilitating effective O₂ diffusion, and whole-plant responses to waterlogging, partial or complete submergence, or a combination of flooding with other stress conditions are well described in this Collection on 'Flooding stress and responses to hypoxia in plants'. These examples represent the diverse efforts of the research community to understand how plants respond to low-O₂ conditions. The evaluation of undomesticated target genotypes or large populations of crops are key strategies to develop genotypes with outstanding adaptation to such stressful conditions, and we believe that transdisciplinary approaches are essential to tackle the deleterious effect of flooding in crops.

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Author affiliations

^ADepartment of Biology, University of Copenhagen, Universitetsparken 4, Copenhagen 2100, Denmark.

^BPlant Physiology, University Bayreuth, Universitätsstr. 30, Bayreuth 95440, Germany.

^CSchool of Biological Sciences, The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia.

^DExperimental and Computational Plant Development, Institute of Environment Biology, Utrecht University, Padualaan 8, Utrecht 3584 CH, Netherlands.

^EDepartment of Plant Biotechnology, Faculty of Biology, University of Bielefeld, Bielefeld D-33615, Germany.

^FCenter for Biotechnology, University of Bielefeld, Bielefeld 33615, Germany.