Flooding and low oxygen responses in plants

Ole Pedersen[A,D], *Pierdomenico Perata*[B] *and Laurentius A. C. J. Voesenek*[C]

[A]Department of Biology, The University of Copenhagen, Universitetsparken 4, 3rd floor,
  2100 Copenhagen, Denmark.
[B]PlantLab, Institute of Life Sciences, Scuola Superiore Sant'Anna, Via Mariscoglio 34, Pisa 56124, Italy.
[C]Institute of Environmental Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands.
[D]Corresponding author. Email: opedersen@bio.ku.dk

Abstract. The world is currently experiencing dramatic increases in flood events impacting on natural vegetation and crops. Flooding often results in low O_2 status in root tissues during waterlogging, but sometimes also in shoot tissues when plants become completely submerged. Plants possess a suite of traits enabling tissue aeration and/or adjusted metabolism during hypoxia or even in the absence of O_2. This special issue of *Functional Plant Biology* presents key papers for plant scientists on the quest to further address and improve flood tolerance of terrestrial plants. The papers address low O_2 responses in roots, shoots or whole plants in controlled laboratory conditions or in the field situation using natural wetland plants as models as well as economically important crops, such as rice, wheat and barley. The studies advance our understanding of low O_2 responses in plant tissues as caused by O_2 shortage during flooding. However, in most instances, submergence not only leads to hypoxic or anoxic tissues, but inundation in water also results in accumulation of CO_2 and the important plant hormone ethylene. Thus, carefully designed laboratory studies are often needed to unravel the mechanistic relationships between a combined decline in O_2 followed by increases in CO_2 and ethylene at tissue as well as on the cellular level.

Additional keywords: anaerobiosis, anoxia, climate change, flood tolerance, flooding tolerance, hypoxia, underwater photosynthesis.

Global background

Since 1950, the world has experienced a dramatic increase in flood events that has led to tragic loss of human life, but also to flood disasters impacting on natural vegetation and crops (Fig. 1*a–d*). Based on insurance enumerations from the past 7 years in the USA, mean annual losses of wheat production due to floods amount US$360M. In 2010, 2011 and 2016 losses caused by floods even exceeded those caused by drought or other natural disasters (Fig. 1*b*). The calamities inflicted by floods upon plants call for action, and since the challenges span several disciplines the road map to solutions should be cross-disciplinary as well. The International Society for Plant Anaerobiosis (ISPA) hosts plant scientists representing areas from genetics to macro-ecology and thus ISPA serves as an excellent umbrella to address flood-related issues in crops and natural vegetation. The 12th International ISPA Conference held in Elsinore, Denmark, in 2016 gathered plant scientists from around the world to share ideas and recent discoveries related to low O_2 stress, a common result of floods causing waterlogging or submergence of terrestrial plants. In this special issue of *Functional Plant Biology*, key papers present a great resource for plant scientists on the quest to further address and improve flood tolerance of terrestrial plants.

Flooding-induced low O_2 conditions re-orchestrate plant metabolism to manage energy production and consumption.

In general, plants elevate starch and sucrose catabolism, glycolysis and ethanolic fermentation to increase substrate-level production of ATP (Bailey-Serres and Voesenek 2008), and energy production can be further stimulated through alanine metabolism to succinate (Bailey-Serres and Voesenek 2010). Moreover, many plants are also able to reduce energy consumption and growth upon low O_2 conditions (Bailey-Serres and Voesenek 2010).

Responses of roots to low O_2

Excess water in terrestrial systems initially results in waterlogging, a situation where all gas spaces in a well-ventilated soil are replaced by water. After some time, waterlogging leads to soil anoxia since the slow diffusion of O_2 in water is unable to balance the quick O_2 consumption by soil microorganisms and plant roots (Ponnamperuma 1972, 1984). This is nicely shown in a study by Pellegrini *et al.* (2017) from a saltmarsh where natural tides result in recurrent soil flooding or even complete plant submergence. Soils that are permanently waterlogged remain anoxic whereas soils that are drained at low tide experience times of re-aeration (Pellegrini *et al.* 2017). The vegetation inhabiting the saltmarsh tackles flooding in different ways and high porosity tissues enabling fast gas phase diffusion of O_2 (Armstrong 1979; Colmer 2003) is an important trait for one of the studied halophytes, whereas unidentified bottlenecks in the
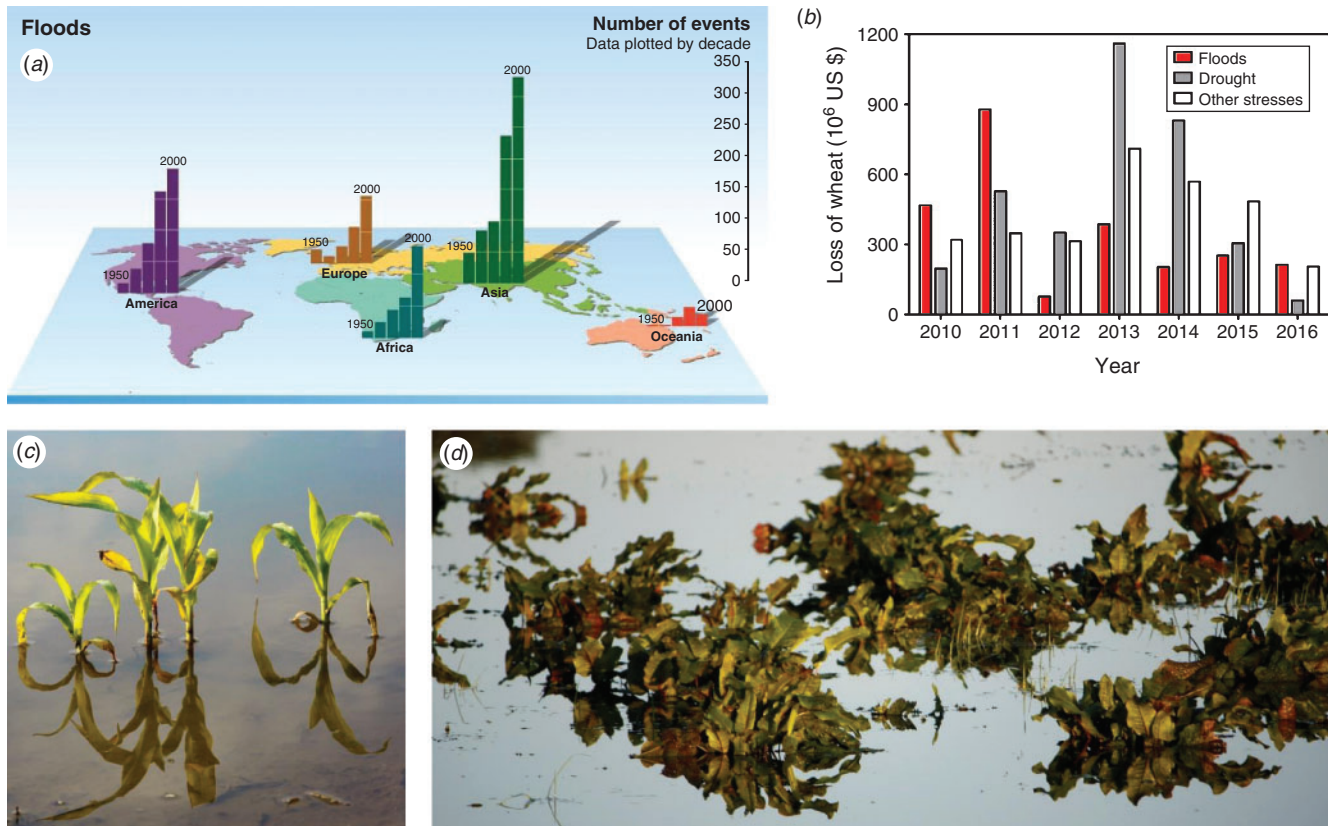
Fig. 1. (*a*) Number of floods per decade since 1950 (source: Millennium Ecosystem Assessment), (*b*) annual loss of wheat production caused by floods and other stressors (source: USA Department of Agriculture Risk Management Agency Cause of Loss Historical Data), (*c*) waterlogged maize (photograph by Ole Pedersen) and (*d*) partially submerged natural population of *Rumex crispus* (photograph by Ole Pedersen).

porous pathway lead to severe tissue hypoxia during submergence in another species (Pellegrini *et al*. 2017). The authors propose that natural wetland plants may serve as important inspiration for plant biologists that work towards improving flood tolerance of crops.

Waterlogging and partial submergence in many cases spark a rich growth of adventitious roots stimulated by the death of the primary root system (Sauter 2013). For example, in *Solanum dulcamara*, numerous adventitious roots are formed during partial submergence whereas few adventitious roots are formed when completely submerged (Zhang *et al*. 2015). Interestingly, high external O_2 concentrations in the floodwater could not compensate for the lack of shoot contact to the atmosphere (Zhang *et al*. 2017). Moreover, carbohydrate depletion before flooding also reduced adventitious root formation unless high light conditions were provided (Zhang *et al*. 2017).

Adventitious roots of many wetland plants form a barrier to radial O_2 loss (ROL) in order to enable supply of O_2 to the growing root tip and to maintain a longer root length (Armstrong 1979; Colmer 2003). The barrier is formed by cell wall depositions of lignin and/or suberin, but in the case of *Hordeum marinum*, only components of suberin differed significantly between waterlogging tolerant accessions (with a strong barrier to ROL and up to 38% root porosity) and

intolerant (with only a weak barrier to ROL and up to 26% root porosity) (Kotula *et al*. 2017).

Responses of shoots to low O_2

Recent studies show that some terrestrial plants possess superhydrophobic leaf cuticles, which retain a thin gas film when submerged. The trait confers flood tolerance because these films enhance gas exchange with the floodwater and thus stimulate O_2 uptake in the darkness and CO_2 uptake – and thus carbohydrate and O_2 production – in the light (Colmer and Pedersen 2008; Pedersen *et al*. 2009). Also the dryland crop, wheat, possesses superhydrophobic leaf cuticles (Raskin and Kende 1983) and initially a gas film is retained when submerged, but the superhydrophobic properties are lost in time and the gas film subsequently vanishes (Winkel *et al*. 2014, 2016). Wheat loses the superhydrophobic properties after only 2–3 days of submergence, whereas a natural wetland grass (*Glyceria fluitans*) used as a trait benchmark retained the gas film for more than 4 days of submergence (Konnerup *et al*. 2017). Despite the fast loss of the gas film in wheat the gas films result in 3 days of extended survival as compared with wheat where the leaf gas films had been experimentally removed before submergence (Winkel *et al*. 2017).

Complete submergence not only results in reduced O_2 levels in soils and roots, but also in densely packed shoot tissues, such as meristems, especially when the flood water is turbid and hampers light penetration (van Dongen and Licausi 2015). However, the impact of hypoxia on shoot tissues is rarely studied. Abadie *et al*. (2017) studied metabolic shifts upon low O_2 in illuminated leaves and found that low O_2 had a negative effect on photosynthesis and, furthermore, it clearly showed signs of the typical hypoxia responses.

Among crops, rice is well known to be exceptionally tolerant to submergence. Rice can germinate even in complete absence of O_2 and rice varieties possessing the *Sub1A* or *Snorkel* genes can adopt survival strategies also at the adult stage (Bailey-Serres and Voesenek 2008). At the germination stage, rice differs from other cereals in its ability to degrade starch, thus providing the germinating embryo with soluble carbohydrates that are channelled through glycolysis to the fermentative pathway (Perata *et al*. 1993). One specific α-amylase gene, *RAMY3D*, plays a special role in rice germination under submergence. Different from other α-amylase genes, *RAMY3D* is not regulated by gibberellins, whose synthesis is hampered under submergence, but rather on sugar availability (Umemura *et al*. 1998). The rapid consumption of soluble carbohydrates during the early phases of anaerobic germination in rice seeds triggers sugar starvation that activates *RAMY3D*. Ho *et al*. (2017) describes a Calcineurin B-like protein, namely CBL4, that interacts with CIPK15, a key player in the mechanism by which sugar starvation and low O_2 converge in the activation of *RAMY3D* (Lee *et al*. 2009). CBL proteins directly sense Ca^{2+} and consequently interact with CIPK15, which transduces the signal, thus linking calcium sensing to the sugar-dependent regulation of *RAMY3D*. Together with the recent identification of trehalose-6-phosphate phosphatase (*OsTPP7*) as a key player for rice germination under submergence (Kretzschmar *et al*. 2015), the work by Ho *et al*. (2017) contributes to drawing a more complete picture of the events enabling rice to germinate under hypoxia.

Shoot elongation is a common flood response of many wetland plants and crops, and the elongation serves to restore contact with the atmosphere so that internal aeration is sustained (Colmer and Voesenek 2009). Similarly to *Rumex palustris* (Chen *et al*. 2011), the tropical forage grass, *Chloris gayana*, benefits from this escape strategy only during prolonged flooding (Striker *et al*. 2017). When exposed to 2 weeks of continuous complete submergence, restoration of aerial contact by shoot elongation resulted in 2.9-fold higher dry mass accumulation compared with repeated submergence of shorter duration (1 week) but still with 2 weeks of total submergence (Striker *et al*. 2017).

Flooding may also occur in combination with other stressors such as salinity (Colmer and Flowers 2008). It is well described that flooding in combination with salinity can result in a severe impact on plant performance since dysfunctional roots caused by waterlogging result in dramatic increases first in roots (Kotula *et al*. 2015) and later in the shoot Na^+ and Cl^- concentration when compared with exposure to salinity stress only (Colmer and Flowers 2008). Falakboland *et al*. (2017) exposed a range barley (*Hordeum vulgare*) cultivars to waterlogging, salt stress

or the combination of both stressors. The combined stress had more severe impact on these cultivars than individual stressors. Interestingly, a much stronger correlation was found between the combined stress and waterlogging than with salinity, indicating that waterlogging tolerance had a large contribution to the tolerance of combined stress. Most likely this is related to severe energy limitation induced by waterlogging (Falakboland *et al*. 2017).

In rice grown in the field, application of K^+ alleviated the stress caused by complete submergence so that survival was enhanced and chlorophyll retained at a higher level when compared with plots with no additional K^+ added (Dwivedi *et al*. 2017).

The studies briefly summarised above advance our understanding of low O_2 responses in plant tissues as caused by O_2 shortage during flooding. However, in most instances, submergence not only leads to hypoxic or anoxic tissues, but inundation in water also results in accumulation of CO_2 and ethylene. Thus, carefully designed laboratory studies are often needed to unravel the mechanistic relationships between a combined decline in O_2 followed by increases in CO_2 and ethylene at tissue as well as on the cellular level.

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