Commentary

Plants and flooding stress

Floods early this year in Queensland, Australia, received a great deal of attention in the media because they affected a land area the size of Germany and France combined. However, on a world scale this is not exceptional as in some years the land area exposed to flooding is > 17 million km^2, equal to twice the size of the USA. These dramatic floods occur in all continents of our planet and result in annual damage costs of > $80 billion (http://floodobservatory. colorado.edu/).

Many wild plant species and nearly all crops are intolerant to these floods and thus excessive water will affect the natural patterns of plant distribution and biodiversity (Silvertown et al., 1999) and have a devastating impact on crop growth and survival and thus on food production (Normile, 2008).

Flooding is a compound stress composed of interacting changes inside plant cells induced by the flood water surrounding the plant. The concentrations of oxygen (O_2), CO_2, reactive oxygen species (ROS) and ethylene change upon flooding and can occur in various combinations, as determined by the flooding regime.

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Plants are either intolerant to flooding and therefore are excluded from flood-prone habitats, or they are tolerant. The latter group can be divided into plants that: exploit a so-called escape strategy based on a suite of (inducible) morphological and anatomical traits allowing re-aeration of flooded tissues; or those that adopt a quiescence strategy composed of traits that conserve the use of energy and carbohydrates to prolong underwater survival (Bailey-Serres & Voesenek, 2008; Colmer & Voesenek, 2009).

The International Society for Plant Anaerobiosis (ISPA; http://www.is-pa.org/) organizes a conference every 3 yr to bring together scientists with expertise in flooding research. The 10th ISPA Conference was held in Volterra, Italy, from 20 to 25 June 2010, with > 100 participants from 15 countries in attendance. Much progress has been made in recent years, as was evidenced by the lively discussion and debate at the conference and now also in published form, in this Special Issue of *New Phytologist*. In this Commentary we introduce and outline the collection of reviews, forum articles and original research papers that make up the Special Issue.

Genetic variation and plant responses to submergence

It is a grand challenge to unravel the molecular mechanisms and the physiological processes that regulate complex flood-adaptive networks. However, this is fundamental to enable us to understand the patterns of plant distribution and abundance in natural flood-prone communities and to improve flood tolerance in economically important crops. For the first time in flooding research, Vashisht et al. (pp. 299–310) exploited the natural variation in flooding tolerance of *Arabidopsis thaliana*. To this end, 86 accessions were submerged in complete darkness and the results demonstrated considerable genetic variation in flooding tolerance. Interestingly, flooding tolerance in Arabidopsis was negatively correlated to petiole growth under water, but was not related to the initial amounts of starch and soluble sugars.

Anatomical adaptations to submergence

Plants are obligate aerobic organisms and thus depend on O_2 to grow and compete successfully with neighbouring plants in natural environments. For those plant species which live in environments that restrict direct uptake of O_2 from the environment (e.g. wetlands) enlarged gas-filled air spaces (aerenchyma), which are longitudinally interconnected, facilitate internal gas diffusion. Aerenchyma not only improve gas diffusion between and inside plant organs, they also conserve oxygen by reducing respiratory demand per unit volume. Furthermore, aerenchyma facilitate the escape of CO_2, ethylene and other potentially harmful volatile products.

In fully or partially submerged plants, aerenchyma, whilst still essential, may not alone be sufficient to ensure the necessary O_2 supplies because O_2 transfer from water to shoot and CO_2 acquisition for underwater photosynthesis can be greatly impeded by the low solubility of O_2 and the slow diffusion of these gases in water compared with diffusion in air. Three papers in this issue embrace the supplementary role that underwater photosynthesis contributes to O_2 and/or respiratory substrate supply. In part, the carbon source for this photosynthesis may be sediment and root system CO_2 transported upwards via the aerenchyma. The O_2 generated, because it cannot escape readily into water,

raises internal concentrations and enhances the supply to roots and to the rhizosphere. Rich *et al.* (pp. 311–319) demonstrate, for the first time, that photosynthetic activity within free-floating flood-induced aquatic roots may confer substantial benefits to plants by providing significant carbon fixation and O_2 evolution during daylight. This will conserve substrate and, together with the O_2 generated, might aid survival of some sediment-borne roots. Sediment-sourced CO_2 is the major carbon supply for some submerged isoetid-type plants, and the O_2 generated is the major source for root and rhizosphere aeration, However, for *Lobelia dortmanna*, Møller & Sand-Jensen (pp. 320–331) reveal this to be a delicate balance sustained only in oligotrophic conditions. They show that even mild eutrophication of the sediments can intensify and prolong night-time anoxia in the roots, leading to plant death and ecosystem degeneration. Higher than atmospheric concentrations of O_2 in the leaves are also characteristic of submerged isoetid plants in the light, and although beneficial for root and rhizosphere aeration, this can reduce photosynthetic efficiency by increased photorespiration. Pedersen *et al.* (pp. 332–339) elegantly show, for the first time, how crassulacean acid metabolism (CAM) in *Isoetes australis* enables higher rates of underwater net photosynthesis over wide ranges of O_2 and CO_2 concentrations in floodwaters, via increased CO_2 fixation and suppression of photorespiration.

The lack of secondary aerenchyma formation is a major reason for flood intolerance among 'woody dicots' (Justin & Armstrong, 1987). Teakle *et al.* (pp. 340–350) report on rapid development of aerenchymatous phellem in the hypocotyls and roots of the salt-tolerant forage species *Melilotus siculus* and confirm it as a functioning path for root aeration. This, and studies on soybean (Thomas *et al.*, 2005; Shimamura *et al.*, 2010), open up the exciting possibility of breeding flood tolerance into other economically important legumes.

Rajhi *et al.* (pp. 351–368) studied aerenchyma formation-associated genes expressed in maize roots by laser micro-dissection coupled with microarray analysis. The authors used 1-methylcyclopropene (1-MCP), an ethylene perception inhibitor, to manipulate ethylene signalling, known to play a crucial role in aerenchyma formation. The genes identified as differentially expressed during aerenchyma formation included genes related to calcium signalling, cell wall loosening and degradation, and for generating or scavenging ROS. Reactive oxygen species may actually be important players in aerenchyma formation. In their article, Steffens *et al.* (pp. 369–378) demonstrated that the ethylene-releasing compound, ethephon, promotes ROS production and that hydrogen peroxide (H_2O_2) promotes aerenchyma formation. Genetic down-regulation of an H_2O_2 scavenger enhanced aerenchyma formation and, altogether, these data led the authors to conclude that ROS

mediate aerenchyma formation in rice (Steffens *et al.*, pp. 369–378).

Some wetland plants facilitate below-ground ventilation by pressurized gas-flow. This can supply O_2 and vent other gases much more rapidly than diffusion and confer a competitive advantage. Reports in this issue extend our knowledge of the range of species that function in this way. Konnerup *et al.* (pp. 379–386) provide evidence of such flows in a range of tropical species. Armstrong & Armstrong (pp. 387–397) detected significant and sometimes high flow rates in four out of nine *Equisetum* species studied, and identify the reasons why flows are or are not generated within the genus.

The anaerobic nature of sediments and carbon inputs from roots and decaying organic matter make some wetlands major emitters of methane (CH_4). Much of the CH_4 passes through the plants before escaping to the atmosphere; however, radial O_2 loss from roots to sediment can support methanotrophy. Net CH_4 efflux reflects the balance among sediment methanogenesis, diffusive resistances in the system and methanotrophy. A detailed study by Fritz *et al.* (pp. 398–408) reveals that, in at least one ecosystem, the high-level Patagonian bogs, deep-rooting cushion plants can severely curtail CH_4 emission by thorough soil and CH_4 oxidation.

Escape from submergence

Aerenchyma formation is an important plant trait for improving mainly waterlogging tolerance. However, it interacts strongly with another trait, which becomes relevant when entire plants are covered with water. Under these extreme submergence conditions, escape through fast shoot elongation becomes a fitness-improving trait that allows leaves to restore gas exchange with the atmosphere (Voesenek *et al.*, 2004). However, emergence through fast shoot elongation requires functional aerenchyma to aerate organs that are still submerged when leaf tips hit the water surface (Pierik *et al.*, 2009). The paper of Chen *et al.* (pp. 409–420) describes the results of a study on the fitness consequences of variation in flooding-induced shoot elongation in *Rumex palustris*. To this end, plants from populations with fast and slow shoot-elongation rates were exposed to different flooding regimes. Emergence under prolonged flooding improved fitness expressed as biomass, whereas slow elongation rates under water during frequent, short-lasting floods resulted in a higher biomass, indicating that costs are involved in underwater shoot elongation. Buried turions of *Potamogeton distinctus* have the remarkable capacity to germinate and elongate stems in the complete absence of O_2. This underwater elongation response does not depend on ethylene and requires active glycolysis and fermentation to fuel the required cell elongation. Koizumi *et al.* (pp. 421–430) demonstrate that upon anoxia the activity of

plasma membrane H^+-ATPase is increased in stems of *P. disctinctus*. The stimulated extrusion of protons induced by anoxia results in acidification of the apoplast, often seen as a prerequisite for cell wall loosening, and it helps to prevent cytoplasmic acidosis.

Physiological and molecular aspects of plant anaerobiosis

Recent suggestions of a sensor-controlled down-regulation of respiration in plant tissues at O_2 concentrations orders of magnitude higher than the critical O_2 pressure for isolated protoplasts and mitochondria, and based on observations of a steady concentration-dependent decline in the respiration of root segments below 240 µM O_2 in respirometer media, are challenged by Armstrong & Beckett (2011a; pp. 431–441). They show that stelar tissues can be already severely hypoxic in an air-saturated respirometer medium. Subsequent respiratory decline is attributed to the radial spread of severe hypoxia with declining respirometer [O_2].

The mechanism(s) by which stress induced by low [O_2] is perceived in plants is still largely unknown (Licausi & Perata, 2009). Hypoxia-responsive transcription factors (TFs) represent a starting point with which to investigate the regulation of the hypoxic response, and Licausi *et al.* (pp. 442–456) provide a comprehensive analysis and identification of hypoxia-modulated transcripts coding for TFs in hypoxic roots of Arabidopsis. Interestingly, it appears that under anoxia an additional set of TFs is induced, suggesting that partly distinct signalling pathways operate under either hypoxia or anoxia.

In the context of transcriptomic adjustments in response to submergence in Arabidopsis roots and shoots, Lee *et al.* (pp. 457–471) identified a set of genes that were ubiquitously co-regulated by submergence and O_2 deprivation. The biological functions of these included signalling, transcription and anaerobic energy metabolism, but *HYPOXIA RESPONSIVE UNKNOWN PROTEIN* (*HUP*) genes comprised many of the co-regulated transcripts. Interestingly, seven HUP mutants were significantly altered in submergence tolerance, suggesting that poorly characterized proteins may provide new opportunities for phenotype manipulation under hypoxic conditions.

Plant tolerance to low [O_2] varies dramatically between species, suggesting the existence of distinct, species-specific molecular mechanisms of plant adaptation to hypoxia. Narsai *et al.* (pp. 472–487) analysed the versatility of primary metabolism in response to hypoxia by comparing nine previously published metabolome profiling studies. Activation of the fermentative metabolism, together with the accumulation of alanine and succinate, were observed in all species, suggesting that this occurs independently of the degree of tolerance. Transcriptional regulation of these metabolic pathways, however, varied, suggesting that this may

account for the different species tolerance. Alternatively, tolerance may rely on genes that have no orthologues or were not affected in the different species (Narsai *et al.*, pp. 472–487).

Anaerobiosis in algae

Besides plants, the green alga *Chlamydomonas reinhardtii* was a protagonist at the 10th ISPA Conference. This model organism attracts increasing interest because of its ability to produce hydrogen when grown under anoxia. Its ability to synthesize hydrogen, if a way were found to harness it, could potentially revolutionize the renewable energies market (Lee *et al.*, 2010). The fact that anoxia is required to induce hydrogen production in *Chlamydomonas* results in increased interest in the fermentative metabolism of this organism. In their review article, Grossman *et al.* (pp. 279–288) describe the fermentation pathways operating in *Chlamydomonas*, its ability to produce molecular hydrogen under anoxic conditions through the activity of hydrogenases, and the molecular flexibility associated with fermentative metabolism. A peculiar day : night fluctuation in the expression of genes that are believed to be induced by anoxia was discovered by Whitney *et al.* (pp. 488–498). They concluded that the regulation of the anaerobic gene expression in *Chlamydomonas* is only partly explained by responses to low [O_2], and that the cell cycle and light : dark cycles are equally important elements in the regulatory network regulating anaerobic gene expression.

Crops and flooding

Many crops are sensitive to waterlogging and complete submergence. Just a few days of flooding can damage plants and will result in significant agricultural losses. It is therefore highly relevant to understand the traits that improve flooding tolerance, and the genes and proteins underlying these traits. Owing to the global nature of flooding and the serious threat that floods will occur more often in the near future, it is to be expected that more knowledge on flooding tolerance will facilitate strongly the development of flood-tolerant crop varieties that can grow and yield on marginal, flood-prone land. The paper of Malik *et al.* (pp. 499–508) shows that hybridization of wheat with *Hordeum marinum*, a waterlogging-tolerant wild relative, improves waterlogging tolerance of wheat. These amphiploids, in contrast to wheat, have more dry mass, a higher porosity in adventitious roots and develop a barrier to prevent radial O_2 loss of roots upon waterlogging.

The review paper of Shabala (pp. 289–298) argues that the traditional approach of plant breeding for waterlogging tolerance, based on field assessment of a range of agronomic and morphical traits, should be redirected towards a physiological approach that targets specific cellular mechanisms.

This may include mechanisms, such as the activity of plasma membrane transporters, which make plants tolerant to phytotoxins.

Concluding remarks and future challenges

A great variety of topics is discussed in this Special Issue, ranging from natural variation and molecular regulation of flooding tolerance, to in-depth studies on the physiology of specific adaptive features, such as aerenchyma, underwater photosynthesis and underwater escape. Furthermore, several papers dealt with the importance of certain plant traits in breeding flood-tolerant crops, and novel contributions were presented on the green alga *C. reinhardtii*. This broad range of topics illustrates the vivid character of the field of flooding research, as highlighted by the lively debates at the conference in Volterra and in the literature on the dampening of respiration rates at an $[O_2]$ higher than the critical O_2 pressure (see Armstrong & Beckett, 2011b; pp. 276–278 and pp. 431–441, and Nikoloski & van Dongen, pp. 273–276).

Recently, the hypothesis was launched that flood-adaptive traits are probably not regulated in isolation, but are part of a network with conserved components such as hormones, starch-degradation enzymes, fermentation enzymes, growth machinery and scavenging mechanisms for ROS. It is the specificity of the flooding regime that selects the most appropriate balance in such a network (Bailey-Serres & Voesenek, 2010). It is a challenge for the future to extend this network knowledge to realistic outdoor conditions, because, to date, most of the experiments have been conducted under controlled laboratory conditions. We should aim to include the ecological complexity in our future flooding experiments to shed more light on the environment–plant interaction in relation to flooding tolerance.

Furthermore, flooding stress should be seen as a compound stress composed of several underlying changes in substances such as ethylene, CO_2, O_2, ROS and phytotoxins inside plants and from without. The changes in these substances are strongly controlled by the type of flooding (e.g. turbid vs light) and by the organ (root vs shoot). It is to be expected that natural selection is acting on these underlying components and not on the so-called compound stress. If true this would suggest that we should expose plant organs not only to flooding treatments but also to isolated substances and to specific combinations of substances to understand better how flooding tolerance is regulated.

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Letters

Modeling alternatives for interpreting the change in oxygen-consumption rates during hypoxic conditions

The rate of respiratory oxygen consumption decreases when the amount of oxygen available to plant tissues is reduced. A multitude of scientific papers have described this phenomenon in different tissues, including leaves (Laisk *et al.*, 2007), roots (Mancuso & Boselli, 2002; Maricle & Lee, 2007), seeds (van Dongen *et al.*, 2004) and fruit (Tucker & Laties, 1985; Ho *et al.*, 2010b). Despite these research efforts, it is still debated by which mechanism the oxygen-consumption rate is controlled. Two different hypotheses have been postulated. The first considers that the kinetic parameters of respiratory metabolism (i.e. not only cytochrome *c* oxidase, but also other components of the respiratory pathway, such as glycolysis, the TCA cycle and the mitochondrial electron transport chain) can change depending on the availability of oxygen, whereas the second theory assumes that the relationship between respiratory activity and the environmental oxygen concentration can be adequately described by Michaelis–Menten kinetics linked to diffusive transport of oxygen into the tissue only. Experimental evidence for the first hypothesis exists for yeast