

Evolution and mechanisms of plant tolerance to flooding stress

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• **Background** In recognition of the 200th anniversary of Charles Darwin's birth, this short article on flooding stress acknowledges not only Darwin's great contribution to the concept of evolution but also to the study of plant physiology. In modern biology, Darwin-inspired reductionist physiology continues to shed light on mechanisms that confer competitive advantage in many varied and challenging environments, including those where flooding is prevalent.

• **Scope** Mild flooding is experienced by most land plants but as its severity increases, fewer species are able to grow and survive. At the extreme, a highly exclusive aquatic lifestyle appears to have evolved numerous times over the past 120 million years. Although only 1–2% of angiosperms are aquatics, some of their adaptive characteristics are also seen in those adopting an amphibious lifestyle where flooding is less frequent. Lowland rice, the staple cereal for much of tropical Asia falls into this category. But, even amongst dry-land dwellers, or certain of their sub-populations, modest tolerance to occasional flooding is to be found, for example in wheat. The collection of papers summarized in this article describes advances to the understanding of mechanisms that explain flooding tolerance in aquatic, amphibious and dry-land plants. Work to develop more tolerant crops or manage flood-prone environments more effectively is also included. The experimental approaches range from molecular analyses, through biochemistry and metabolomics to whole-plant physiology, plant breeding and ecology.

Key words: Abiotic stress, adaptation, anoxia, Charles Darwin, environmental stress, evolution, flooding, hypoxia, rice, submergence, wetlands.

INTRODUCTION

Publication of this Special Issue devoted to plant life under flooding stress happily coincides with the 200th anniversary of the birth of Charles Darwin, the great biologist who, together with Alfred Russel Wallace, first put forward evidence for the now widely accepted theory of evolution through natural selection. The theory was given its initial public airing at the Linnean Society in London on 1 July, 1857, just over 150 years ago (<http://www.linnean.org/index.php?id=380>) and was followed approximately one year later by publication of the first edition of *On the Origin of Species* (Darwin, 1859). Less well recognized are Charles Darwin's pioneering studies on the workings of plants, which took advantage of the comparative ease with which they can be examined experimentally compared to animals. This move to direct experimentation reflected Darwin's strong belief in argument based on primary evidence, the sort of evidence that is best obtained through careful testing and observation in the laboratory. He worked closely with his son Francis, a distinguished plant physiologist in his own right (Ayres, 2008). Francis (Sir Francis from 1913) helped

to start *Annals of Botany* in 1887 (Wilson, 1978) and later used the Journal to describe, in engaging terms, his father's extensive botanical publications (Darwin, 1899) of which *The Power of Movement in Plants* (Darwin, 1880) is perhaps best known since the phototropism experiments it describes led the way to the eventual discovery of the plant hormone auxin. Charles Darwin studied plants intensively and saw their physiological characteristics and links between structure and function as components of evolutionary progress – components that, in themselves, are amenable to experimental study. Reductionist research of this kind blossomed in the 20th Century and remains a major and highly progressive strand of present-day plant biology. Darwin's concept of evolution on the land was thus not merely of an awe-inspiring chronology of extinctions and divergences spanning millions of years but embraced the concept of underpinning adaptations for which there must be physiological explanations. Such a vision fits well with the central topic of this Special Issue.

EVOLUTION OF FLOODING TOLERANCE

Flooding imposes a severe selection pressure on plants principally because excess water in their surroundings can deprive them of certain basic needs, notably of oxygen and of carbon dioxide and light for photosynthesis. It is one of the major abiotic influences on species' distribution and agricultural productivity world-wide (e.g. <http://www.plantstress.com/Articles/index.asp>). The strong challenge to survival

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that flooding exerts has led to the emergence of a sizeable minority of modern-day taxa with abilities to grow, reproduce and compete strongly in permanently or near-permanently flooded environments. Although fossil evidence indicates that plant life on the land has aquatic origins commencing approx. 460 million years ago (Beerling, 2007), all but the most basic attributes of flooding tolerance (e.g. the anaerobic respiratory pathways) seemingly involved an adaptive *return* to aquatic competence by land-dwelling plants. Sometimes, an entire group such as the order Nymphaeales (the water lilies) is aquatic (i.e. grow and reproduce when submerged or floating in water for much or all of each year), indicating an early evolutionary origin of the aquatic life style. Water lilies comprise one of the oldest-diverging lineages and from the fossil record have existed for ~120 million years, i.e. first appearing in the Lower Cretaceous period (Friis *et al.*, 2001) when angiosperms were first developing. At the other extreme, isolated aquatic species in otherwise terrestrial groups indicate a more recent evolutionary origin. For example, *Erigeron heteromorphus* is the only aquatic species out of the ~3000 species comprising the tribe Astereae (Cook, 1999), a group springing from a relatively late divergence event (Angiosperm Phylogeny Group, 2003).

Based on an analysis of morphology and deduced evolutionary history, a return to aquatic competence appears to have taken place over 200 separate times to produce today's taxonomically varied aquatic flora. About 1–2% of extant angiosperms are aquatic but taxonomically highly scattered (Cook, 1999). This implies that a relatively small number of key inheritable characteristics are needed to confer the ability to live almost constantly in water, since their adoption is seen in so many distantly related taxonomic groups. An ability to form replacement adventitious roots, and hypertrophied stems (see Fig. 1) and well-developed aerenchyma come particularly to mind. Constitutive aerenchyma formation is an ancient characteristic that predates emergence of angiosperms (e.g. carboniferous tree ferns; Eble *et al.*, 2003). Its development by differential expansion of lysisigeny has been described in roots of *Acorus calamus*, a basal monocot (Soukup *et al.*, 2005), and in a wide variety of other flooding-tolerant species of lineages that diverged more recently (Justin and Armstrong, 1987). Other commonly occurring characteristics of the aquatic community include the capacity for accelerated underwater elongation that restores submerged shoots to aerial contact before asphyxiation sets-in (Jackson, 2008) and specialized forms for submerged leaves that may enhance gas exchange underwater (see Sculthorpe, 1967, chapters 3–5 for a comprehensive account). However, although Cook (1999) comments on how simple it was for him to breed plants that were terrestrial or aquatic from crosses between amphibious plants of *Ranunculus* subgenus *Batrachium*, the genetical and physiological bases for aquatic or amphibious resilience these and other key features of aquatic species still remain unresolved. This suggests most are more complex than phylogenetic appearances may suggest.

The fully aquatic life style represents only one extreme in the spread of flooding tolerance amongst the angiosperms. In between this and one of a predominately arid existence lies what might usefully be considered the amphibian life style (Braendle and Crawford, 1999), where plants have come to



FIG. 1. *Sesbania javanica* growing successfully in deep water illustrates the contribution of stem hypertrophy and adventitious root formation to flooding tolerance. Both swollen stem and roots are rich in internal gas-filled spaces (aerenchyma), a key feature of the many aquatic and amphibious species (monochrome image previously published in Jackson, 2006).

bear temporary flooding in regular or irregular pulses and also the subsequent return to drier conditions. This highly diverse group includes a sizeable assemblage of growth habits, physiological attributes and degrees of flooding tolerance arising as constitutive features and/or as the outcome of phenotypic plasticity revealed through adaptive plant/environment interactions. This fascinating myriad of solutions to different degrees of flooding stress (e.g. seasonally fluctuating water tables, spasmodic flooding, riverine flooding, standing, stagnant or flowing water) is at the centre of much of the work discussed in this Special Issue. It is based on papers presented at the 9th Conference of the International Society for Plant Anaerobiosis, entitled *Molecular, Physiological and Ecological Adaptations to Flooded Conditions by Crops and Native Plants*, and an integrated workshop *Improvement of Plant Performance for Sustainable Agricultural Development of Wetlands*, which was generously supported by the Organization for Economic Co-operation and Development (OECD). The research described is often driven by botanical curiosity generated by interest in the many varied and elegant solutions to plant survival in an excess of water. Such studies, especially into the cell biology of metabolic regulation in oxygen deficient cells also have relevance to mesophytic species (adapted to neither a very dry nor a very wet environment). This arises because the mere assemblage

of cells to form the familiar tissues and organs of vascular plants imposes flooding-like stresses on more centrally positioned parts by virtue of slowed gas exchange brought about by the tightly packed surrounding (water-filled) cells and their propensity for consuming incoming oxygen (Berry and Norris, 1949; Geigenberger, 2003). Research on flooded plants is also fostered by the imperative to improve cereal production [most notably in rice (see Fig. 2) and wheat] and to manage flood-prone ecosystems, such as that of the Amazonian rain forest where certain parts are flooded annually to a depth of several metres for weeks or months.

SUMMARY OF TOPICS CONTAINED IN THIS SPECIAL ISSUE

The Special Issue opens appropriately with a paper on evolutionary events that gave rise to the present-day structures of the *Sub1* locus of chromosome 9 of domesticated rice (*Oryza sativa*; Fukao *et al.*, 2009). This locus is notable because the paralogous genes it contains code for ethylene-response factors (ERFs) that influence the vigour of the elongation response to submergence by various organs of the shoot. Suppressing the vigour of this reaction can confer an agriculturally useful increase in tolerance to short-term submergence by rice by virtue of the energy and respirable resources it saves. Direct DNA sequence comparisons suggest that, to generate plants where underwater shoot extension is suppressed, one of these genes duplicated early in domestication of the *indica* subspecies. Subsequent nucleotide divergence of the regions outside of the DNA binding domain of the new paralog gave rise to an allele that can confer submergence tolerance. Farmers in Orissa (India) and Sri Lanka unwittingly selected for the retention of this mutation while seeking submergence-tolerant types for use in their frequently flooded fields. Septiningsih *et al.* (2009) illustrate how the tolerance-conferring form of the *Sub1* gene may be introduced into modern rice cultivars to increase



FIG. 2. Deepwater rice is widely grown in the low valley of the Prachinburi River, central Thailand. The photograph shows rice growing in approximately 2 m of water at the Prachinburi Rice Research Center, Bureau of Rice Research and Development, Rice Department, Bansang, Prachinburi, Thailand, where the crop is conveniently managed from a small boat.

their tolerance to short-term (days) submergence, thereby making them more suitable for use in flood-prone, rainfed lowlands of Asia. Work in West Africa too indicates that a suppression of underwater elongation, brought about by the mutated form of *Sub1A*, is beneficial for the endurance of complete submergence (Kawano *et al.*, 2009). However, closely related work by Sakagami *et al.* (2009) emphasizes that this trait is not appropriate when selecting and breeding cultivars of *O. sativa* or *O. glaberrima* for resilience to longer-term submergence in West Africa. Under these circumstances, a vigorous ethylene-mediated underwater elongation response by leaves is needed to return leaves to air-contact and full photosynthetic activity.

The agricultural importance of rice and its multifaceted capability for growth in the humid tropics where, from time to time, monsoon rains create near-aquatic environments, has encouraged detailed studies on the biochemistry and physiology of seed germination and early seedling development. Magneschi and Perata (2009) review a large literature on the biochemical attributes that explain the remarkable ability of rice to germinate anaerobically and to elongate its emerging coleoptile in the absence of oxygen. Seed germination in anaerobic flooded nursery beds or in directly-seeded fields is inevitably associated with suppression or inhibition of other aspects of early seedling establishment, such as root and leaf emergence, vigour and survival rate. Ismail *et al.* (2009) report that some cultivars suffer less than others in these regards. These authors highlight features such as a more effective use of starch reserves through faster amylase activity and anaerobic respiration that help overcome the negative impact of a lack of oxygen during germination and early seedling establishment. Once rooted into flooded soil, the rice plant continues to face a number of challenges. These include a severe limitation to the concentrations of readily available, soluble forms of phosphorus (P) in the soil, particularly if the soil undergoes flooding and drainage with resulting changes in redox conditions, as it will do under rainfed conditions. Cultivars with an enhanced capability to take up P under such conditions would therefore be desirable agronomically. Although Huguenin-Elie *et al.* (2009) were unable to identify such cultivars from amongst six appropriately chosen lowland lines, they did find evidence that rice roots extract soil P by means of a solubilizing action on otherwise unavailable sources in the soil. As shown by the extensive studies in India and Australia by Setter and colleagues (Setter *et al.*, 2009), amounts of accessible, soluble elements in flooded soil are also relevant to the damage to wheat (*Triticum aestivum*) caused by soil waterlogging. In this case, the interaction between the chemically highly reducing flooded soil (low redox potential) enhances availability or deficiency of Mn, Fe, Na, Al or B. Such interactions are shown to be overriding determinants of how severely a crop is injured by waterlogging, while highly localized differences in the availability of these elements explains much variability in the amount of damage waterlogging causes between sites. These findings represent a major challenge to the breeding of tolerant cultivars since these will need to embrace the local differences in soil type. One such local circumstance is where waterlogging is combined with salinity. A way forward to developing wheat with improved tolerance of this

combination is to hybridize it to a related wild species (wide hybridization) such as *Hordeum marinum*, which grows naturally in wet saline conditions. Malik *et al.* (2009) have recognized this as a promising approach and report detailed physiological analysis of 17 *H. marinum* accessions. Their work reveals the existence of several lines with outstanding salinity/waterlogging tolerance. The tolerance is associated with a notable capacity to exclude potentially toxic Na^+ and Cl^- from O_2 -deficient saline surroundings, making these lines promising material for future hybridization with wheat.

A major feature of flooding stress is the deprivation of oxygen it imposes, especially for roots and other underground organs. Describing and understanding the metabolic consequences of this and ways in which a degree of tolerance to it is achieved is a long-standing research topic. Cytoplasmic reactions are at the heart of anaerobic survival, not least because enzymes needed to process sugars through glycolysis and fermentation are mostly cytoplasmic. The subsequent collapse of anaerobic cells is often attributed to damaged pH regulation causing lethal acidification of cytoplasm. *In vivo* nuclear magnetic ^{31}P resonance spectroscopy (NMR) is a sensitive and non-destructive means of estimating shifts in cytoplasmic pH, but its usefulness can be limited by lack of P_i signal strength or a poorly resolved signal. A significant technological advance is reported by Couldwell *et al.* (2009) who find that using methyl phosphonate as an NMR probe overcomes these problems to a significant extent. Their tests of the technique included examining the impact of genetic transformations that reduced the levels of lactate dehydrogenase in potato tubers (*Solanum tuberosum*) and increased expression of pyruvate decarboxylase in leaves of tobacco (*Nicotiana tabacum*). Although both enzymes can be expected to influence pH through effects on lactic acid formation, no effects were discernible. These findings have important implications for the well-known pH-stat hypothesis of acid regulation in plant cells. Studies of anaerobic responses have long included studies of mitochondria (e.g. Vartapetian and Andreeva, 1986) since it is here that the steps generating ATP by oxidation of hexose-derived NADH are to be found. The conventional view is that this can only take place if sufficient oxygen is present for the final oxidation. However, Igamberdiev and Hill (2009) put forward evidence that nitrite can substitute for oxygen at the terminal cytochrome oxidase step, thereby allowing electron transport to proceed, to a degree, anaerobically. They suggest that the nitric oxide generated by the reaction is prevented from inhibiting further electron transfers to nitrite by reacting instead with haemoglobin.

It has been known for 20 years that a period of partial oxygen shortage can induce changes to the biochemistry and patterns of gene expression that enhance tolerance of subsequent anaerobiosis, especially in roots (Saglio *et al.*, 1988). The significance of mild hypoxia for adaptation to oxygen deprivation is expanded and developed by van Dongen *et al.* (2009). Their metabolic and transcript analyses emphasize the contribution of a self-regulated slowing of respiratory demand for oxygen that partial oxygen shortage can induce. This effect goes some way to realigning demand for oxygen to a slower inward flux of oxygen into respiring cells of the root apex. It is associated with a rapid decrease in the accumulation of transcripts of numerous genes coding for

energy-consuming processes while favouring increased levels of transcripts from seven other genes (including a haemoglobin gene) thought to help cells function with little or no oxygen.

A major challenge for plant physiologists is to incorporate knowledge of the biochemistry, molecular biology and biophysics of cells and organs to understand the abilities of the whole plant to withstand flooding stresses and associated oxygen shortages in the soil. In this way, new questions requiring a more reductionist approach to their solution can be opened up. W. Armstrong *et al.* (2009) ask the deceptively simple question of how far oxygen concentrations need to fall before aerobic respiration is first slowed. They explore reasons for the large discrepancy between the very small concentrations required to reduce cytochrome oxidase-mediated oxygen consumption in mitochondria and the much larger concentrations external to the root that have this same effect. They find the whole-plant approach especially powerful, minimizing artefacts of wounding and water penetration and allowing O_2 to enter the roots from the shoot via the gas-space system, as would naturally occur with roots in O_2 -deficient soil. Aided by mathematical modelling, they conclude that respiratory decline begins only when mitochondria at the stelar centre, and farthest from the O_2 source, fall below the critical O_2 pressure for cytochrome oxidase activity (approx. 1 kPa). Peña-Fronteras *et al.* (2009) also take a whole-plant approach in seeking characteristics of *Cyperus rotundus* that make its lowland ecotype a troublesome invasive weed of flooded rice fields. They highlight the contribution of the tubers and an ability to generate respirable sugar from their ample carbohydrate stores. The ability of *Tecticornia pergranulata* to tolerate complete submergence in saline water is examined on a whole-plant basis by Colmer *et al.* (2009). Once more, the handling of carbohydrate reserves is highlighted. Respirable reserves are seemingly conserved through a prompt arrest of expansion growth when the conserved energy source is harnessed to exclude sodium ions, thereby maintaining a healthy $\text{K}^+:\text{Na}^+$ ratio. Studies of root-to-shoot communication inevitably require experimental methods that embrace whole plants. Else *et al.* (2009) report on the root-to-shoot signalling that enables roots of waterlogged tomato plants (*Solanum lycopersicum*) to induce prompt stomatal closure. The adaptive significance of this phenomenon is that it reduces the probability of injury to leaves from dehydration at a time when oxygen shortage in the roots increases their resistance to water entry from the soil. Adjustments to the water relations of shoot tissues is also the subject of the paper by Ooume *et al.* (2009), who use elongating azuki bean epicotyls (*Vigna angularis*) to identify whether increased wall constraints or decreased osmotic forces are responsible for the suppression of cell expansion resulting from a few hours submergence. The results point to decreases in ATP restricting the uptake of osmolites. This, in turn, is seen to cause a decline in cellular hydrostatic pressure and a slowing of growth despite a lessening of cell wall rigidity.

Ecologists are concerned to explain the relative success or failure of plant populations in relation to the extent and frequency of flooding and to the additional problems this may bring along, such as pollutants. Such work harnesses and extends the findings of cell biology and whole-plant

physiology to probe the effectiveness of presumed adaptations in conferring flooding tolerance in the real world. The damage that pollution can inflict on tolerance to flooding of otherwise well-adapted species is clearly illustrated by the work of J. Armstrong *et al.* (2009) on *Phragmites australis*. This shows how oil from contaminated flood water can displace surface gas films on submerged parts, penetrate the leaves, leaf sheaths and nodes, and interfere with the internal diffusive and convective gas flows that sustain inundated organs such as roots and rhizomes. Ecologically orientated research can also help predict the impact of planned changes to landscape management, such as re-introducing natural flooding of riverine areas as a means of tempering the severity of flooding further downstream. Banach *et al.* (2009) have embarked on such work in relation to the flora of the Rhine delta in The Netherlands. They concentrate on the impact of total plant submergence of varying durations on 19 species in laboratory tests. As expected from their relatively slow rates of underwater leaf extension and other characteristics, species that frequent areas presently protected from flooding are more severely damaged by submergence than those found in frequently flooded river forelands. Clearly, these species would suffer a severe set-back if widespread flooding were to be re-introduced. A more narrowly directed ecological question would be to ask if there is an experimentally demonstrable causal link between the probability of surviving submergence and the much-studied ethylene-mediated acceleration to petiole elongation that submergence can induce in numerous aquatic and amphibious species. Pierik *et al.* (2009) address this by comparing species of *Rumex* with and without the capacity for accelerated underwater petiole elongation. They find that securing re-emergence by this means is, alone, not sufficient to rescue the plant's ability to gain dry mass. A functional interconnected aerenchyma is thought to be the key additional component that is required. In a wider context, Parolin (2009) assesses the known characteristics of representatives from over 1000 tree species of the Amazonian rain forest that tolerate a range of flooding intensities that recur each year as the river system floods millions of hectares of forest. Remarkably, up to 150 species tolerate >180 d deep flooding per year and many can preserve green and potentially functioning leaves for weeks or months while totally submerged in the dark, despite the warm tropical temperatures. The mechanisms explaining this remarkable tolerance are only just starting to be revealed but this astonishing phenomenon is, in principle, reminiscent of previous reports of the ability of certain arctic populations of herbaceous species such as *Poa alpina* and *Saxifraga tomentosa* to tolerate anoxia-inducing ice encasement. Laboratory tests have shown these plants retain their green leaves and resume growing after up to 3 weeks in the dark without oxygen at warm temperatures (Crawford *et al.*, 1994). Only arctic populations of these species exhibit this trait since plants collected from more southerly sites are not anoxia tolerant. Thus, it is not only inter-species differences that confer adaptive traits; intra-species differences are also effective, suggesting recent evolutionary origins. This may well apply to populations of white clover (*Trifolium repens*) commonly found in temperate grassland of northern Europe. Huber *et al.* (2009) report the presence of considerable intra-species variation in flooding

tolerance in white clover. They link these differences in tolerance to morphological characters commonly thought to improve flooding tolerance, such as inducible secondary root formation, aerenchyma development and fast petiole extension. They also assess whether their constitutive expression or their inducible expression relates better to flooding tolerance. Their analysis points to the importance of constitutively expressed characteristics.

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