

Flooding tolerance: suites of plant traits in variable environments

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This paper is part of an ongoing series: 'The Evolution of Plant Functions'.

Abstract. Flooding regimes of different depths and durations impose selection pressures for various traits in terrestrial wetland plants. Suites of adaptive traits for different flooding stresses, such as soil waterlogging (short or long duration) and full submergence (short or long duration – shallow or deep), are reviewed. Synergies occur amongst traits for improved internal aeration, and those for anoxia tolerance and recovery, both for roots during soil waterlogging and shoots during submergence. Submergence tolerance of terrestrial species has recently been classified as either the Low Oxygen Quiescence Syndrome (LOQS) or the Low Oxygen Escape Syndrome (LOES), with advantages, respectively, in short duration or long duration (shallow) flood-prone environments. A major feature of species with the LOQS is that shoots do not elongate upon submergence, whereas those with the LOES show rapid shoot extension. In addition, plants faced with long duration deep submergence can demonstrate aspects of both syndromes; shoots do not elongate, but these are not quiescent, as new aquatic-type leaves are formed. Enhanced entries of O₂ and CO₂ from floodwaters into acclimated leaves, minimises O₂ deprivation and improves underwater photosynthesis, respectively. Evolution of 'suites of traits' are evident in wild wetland species and in rice, adapted to particular flooding regimes.

Additional keywords: abiotic stress, adventitious roots, aerenchyma, anoxia tolerance, emergent properties, fermentation, hyponasty, reactive oxygen species, rice, shoot elongation, submergence, synergistic traits, oxidative stress, underwater photosynthesis, waterlogging, wetland plants.

Introduction

Flooding is an environmental stress in many natural and man-made ecosystems worldwide. Anthropogenically-induced global climate change is expected to increase the frequency and severity of flooding events (Arnell and Liu 2001). Most crops and wild plants will not tolerate these floods as the poor gas exchange under water disrupts their energy and carbohydrate economies (Voesenek *et al.* 2006; Bailey-Serres and Voesenek 2008). Many plants suffer severe growth reduction, or even death, when only the root system is surrounded by excess water (i.e. soil waterlogging) (Armstrong 1979; Jackson and Drew 1984). When overland floods occur, and shoots are also either partially or even fully submerged, plants must also cope with the additional adverse effects directly on shoots (Voesenek *et al.* 2006). Knowledge of mechanisms of flooding tolerance in plants will underpin both increases in crop production (Xu *et al.* 2006; Singh *et al.* 2009) and understanding of the distribution of wild species (Blom and Voesenek 1996; Silvertown *et al.* 1999) in flood-prone environments.

Recent research has improved understanding of mechanisms of flooding tolerance in plants, as dependent upon contrasting

flooding regimes in various habitats. Temporary floods differ in seasonal timing, and with much variation in durations, depths and frequencies (Vervuren *et al.* 2003). Disparity in these factors results in a multi-dimensional continuum of flooding regimes in environments inhabited by terrestrial plants. This spectrum of environmental conditions determines species distributions and abundances in flood-prone areas (Armstrong *et al.* 1985; Silvertown *et al.* 1999; Voesenek *et al.* 2004). Such diversity in environments would, as hypothesised by Darwin (1859), impose specific selection pressures for various traits associated with flooding tolerance, based on the assumption that trait benefits outweigh costs (Voesenek *et al.* 2004).

Contrasting flooding regimes are numerous, so only selected examples can be given here. In rice (*Oryza sativa* L.)-growing areas of Asia, hydrological regimes can be broadly classified as deepwater, paddy, rain-fed lowland, and rain-fed upland; these differ in depths (several metres above ground to absence) and durations of overland flooding and soil waterlogging (Grist 1986). In river flood-plains, water levels are highly dynamic, which together with geomorphological characteristics results in deep, short-term floods at river shores, up to shallow floods that

last for several months at some locations on the plains (e.g. Rhine river, Blom and Voesenek 1996). In coastal marshes, tides flood some areas daily and others only monthly or seasonally, with depths varying depending on elevation in the marsh (Armstrong *et al.* 1985; Silvestri *et al.* 2005). A final example is the spectacular seasonal flooding in the Amazon Floodplain in which floodwaters show a mean amplitude of 10 m (range 6–14 m) (Parolin 2009). These floodwaters are also very turbid so that light penetration is poor (Piedade *et al.* 1991), yet several species of trees can survive prolonged submergence (Parolin 2009) and some herbaceous species with leaves that remain in contact with air can be very productive (e.g. $8 \text{ t ha}^{-1} \text{ month}^{-1}$ for the emergent C_4 grass *Echinochloa polystachya* (Kunth) A.S.Hitchc., Piedade *et al.* 1991).

In this review, we first summarise the adverse conditions resulting from soil waterlogging and from floods causing submergence, and discuss the various adaptations in plants to these conditions. The main purpose of our review is to consider the prevailing plant traits associated with flooding tolerance in contrasting types of flood-prone environments, as defined by depths and durations of waterlogging and submergence. Moreover, we highlight that these traits co-occur and act synergistically to enhance fitness. Such suites of traits can be considered as an 'emergent property' (Bhalla and Iyengar 1999) at the organismal level. Our focus is mainly on terrestrial and semi-aquatic plants that occasionally are exposed to higher water levels.

The problem: stresses caused by waterlogging and submergence

During periods of excess rainfall, soil waterlogging can occur so that roots in soil become surrounded by water. During flooding events, the shoots can also be partially or completely submerged. A primary effect of this altered condition is the 10^4 -fold slower diffusion of gases dissolved in water, as compared with in air. Gas diffusion is important for plants; CO_2 influx to chloroplasts is required for photosynthesis and rapid diffusion of O_2 to mitochondria enables respiration. Furthermore, regulation of endogenous concentrations of the volatile hormone ethylene depends strongly on rates of outward diffusion. Fast diffusion of these critical gases is severely hampered when the gas-filled soil pores (waterlogging), or complete shoot space (submergence) become water-filled. The slow outward diffusion in water-saturated soils also results in accumulation of CO_2 and methane (Ponnamperuma 1984). As a consequence, levels of O_2 typically decline, whereas ethylene increases, in submerged tissues (i.e. roots in waterlogged soil; shoots and roots when fully submerged). In the case of CO_2 , levels can increase in roots in waterlogged soils (Greenway *et al.* 2006), whereas when the shoot is also fully submerged, slow entry of CO_2 into leaves typically limits photosynthesis (Mommer and Visser 2005).

In addition to the greatly altered availability of gases, electrochemical changes also occur in water-saturated soils, induced by microorganisms that use oxidised chemicals as electron acceptors (Ponnamperuma 1984; Laanbroek 1990). The soil concentrations of certain potentially-toxic compounds, such as the reduced forms of manganese (Mn^{2+}), iron (Fe^{2+}) and

H_2S and S^{2-} , often increase. These compounds can enter roots, and move to shoots, with adverse effects on both organs (Baba *et al.* 1965; Armstrong and Armstrong 2005). Finally, when completely submerged, an additional stress can be the very low light levels, owing to the often turbid nature of floodwaters. Low light, together with low CO_2 availability, greatly hampers photosynthesis under water (Mommer and Visser 2005).

The flooding-induced changes of the plant environment from air to water, results in several major problems inside the plant body:

- (i) 'Energy crisis'. The majority of ATP for cellular metabolism in plants is generated by oxidative phosphorylation in respiration. This process requires O_2 as the terminal acceptor of electrons. Although the K_m of cytochrome oxidase is low ($0.013\% \text{ O}_2$), indicating a very high affinity to scavenge O_2 , respiration is inhibited in cells, tissues and organs at much higher external O_2 concentrations, owing to diffusion limitations through liquid-phase boundary layers and through the tissues themselves (Berry and Norris 1949; Armstrong *et al.* 2009). When cells become anoxic, oxidative phosphorylation ceases, but some ATP can be produced in glycolysis, provided that NAD^+ is regenerated, for example via conversion of pyruvate to ethanol. Despite this ATP generation, the 'energy crisis' ensues since the production of ATP by glycolysis is much less than compared with oxidative phosphorylation (Gibbs and Greenway 2003). Low ATP availability leads to cellular damage, owing to a deterioration of cellular components, such as membranes (Gibbs and Greenway 2003), and/or cytoplasmic acidosis in sensitive species (Xia and Roberts 1996). Such damage can, however, be avoided (or substantially delayed) in anoxia-tolerant plants (e.g. rice coleoptiles, Kulichikhin *et al.* 2009).
- (ii) 'Carbohydrate crisis'. Soluble sugars and the mobilisation of starch in plants are of importance to sustain glycolysis and thus ATP generation, via either respiration or fermentation, depending on available O_2 . Due to light and CO_2 limitations during submergence, the sugar and starch reserves are not replenished. Ultimately, exhaustion of sugars will result in cell and organ death (Bailey-Serres and Voesenek 2008).
- (iii) *Toxicities*. Reduced soil components such as Mn^{2+} , Fe^{2+} and S^{2-} can accumulate to toxic levels in root tissues (Jackson and Drew 1984). In addition, volatile lower organic acids (e.g. propionic and butyric acids) can accumulate in waterlogged soils and damage roots (Armstrong and Armstrong 1999). These organic acids, as well as high CO_2 , can impose 'acid loads' on cells of roots in waterlogged soils (Greenway *et al.* 2006). Upon re-aeration after a period of O_2 deprivation, ethanol remaining in tissues will be converted into acetaldehyde that can induce post anoxic cell injuries (Bailey-Serres and Voesenek 2008).
- (iv) *Reactive Oxygen Species (ROS)*. Excessive formation of ROS (e.g. superoxide radicals, hydroxyl radicals, hydrogen peroxide, singlet oxygen) during low O_2 conditions and

upon re-aeration is common amongst plants (Blokina *et al.* 2003). An important source of superoxide are the mitochondria in which accumulated electrons at Complex III (ubiquinone : cytochrome *c* reductase) of the electron transport chain are donated to O₂. The production of superoxide radicals is stimulated at low catalysing rates of cytochrome *c* oxidase during low O₂ conditions. Superoxide can quickly dismutate to hydrogen peroxide (Blokina *et al.* 2003; Bailey-Serres and Chang 2005).

- (v) *Water deficits.* Waterlogging can cause wilting of shoot organs in a range of plant species. This response is mediated by a decrease in the hydraulic conductivity of roots (Holbrook and Zwieniecki 2003). Tournaire-Roux *et al.* (2003) demonstrated that upon O₂ deficiency, cytoplasmic acidification in root cells of *Arabidopsis* signals for a permeability decrease of aquaporins in the plasma membrane of these cells.

Although the submerged environment poses many challenges for plant functioning, an aquatic environment also has some advantages over terrestrial life with shoots in air. Water has a much higher density than air and thus provides plants with support and buoyancy. As a consequence, submerged plants can invest less in support structures without losing their upright position to reach for light (Bowes 1987). Furthermore, as hypothesised by Bowes (1987), submerged plant shoots can take up nutrients from the surrounding water (e.g. rice coleoptile, Huang *et al.* 2003; aquatic leaves, Adamec and Kovarova 2006; seagrasses, Lee *et al.* 2007), providing access to nutrients in addition to those in sediments/soils. Uptake of nutrients by shoots of completely submerged plants might be necessary to overcome constraints on root-to-shoot transport imposed by a lack of transpiration, although xylem guttation driven by root-pressure can provide some flow in aquatic species when completely submerged

(Pedersen 1993). Floodwater in a deepwater rice region of Thailand contained higher concentrations of N, P and K than found in oligotrophic lakes, but these concentrations are still considered low compared with those found in most soil solutions (Setter *et al.* 1987).

Plant adaptations to flooding stress: an overview

Terrestrial plants originated from aquatic predecessors. Flood tolerant plants regained their aquatic competence independently over 200 times (Jackson *et al.* 2009), suggesting that a restricted number of mutations can alter flooding tolerance (Voesenek and Pierik 2008). Internal O₂ movement from shoots to roots is crucial for plant tolerance to soil waterlogging and submergence (Armstrong 1979; Jackson and Drew 1984; Colmer 2003a; Voesenek *et al.* 2006). This O₂ movement occurs within aerenchyma, an interconnected series of large gas-filled spaces providing a low-resistance pathway from the shoot to root extremities (Fig. 1). Traits in addition to internal transport of O₂, however, are also essential. Plant adaptations to complete submergence have recently been classified into two main strategies (Bailey-Serres and Voesenek 2008): the Low Oxygen Quiescence Syndrome (LOQS) and the Low Oxygen Escape Syndrome (LOES).

Plants with the LOQS are characterised by traits that enable them to: (i) use ATP economically, (ii) increase the abundance of enzymes necessary to make some ATP without molecular O₂, and (iii) to increase the production of components that counteract harmful cellular changes associated with flooding. A major feature of this syndrome is that when submerged, shoots do not elongate and in extreme cases cease all growth, thus conserving substrates and prolonging survival until waters recede. This 'wait out the stress'-type response was the basis for naming this a 'quiescence syndrome' (Bailey-Serres and

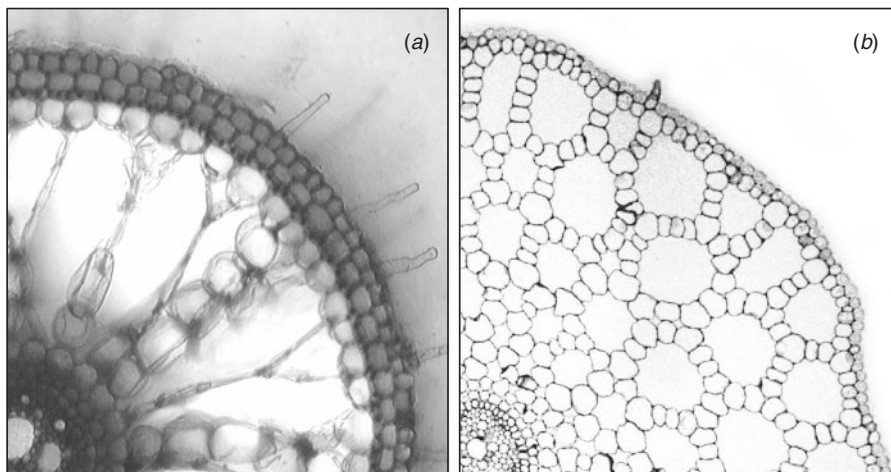


Fig. 1. Examples of aerenchyma in roots. Two main types of aerenchyma are shown: (a) lysigenous aerenchyma (example shown is in rice) and (b) schizogenous aerenchyma (example shown is in *Rumex palustris*). Lysigenous aerenchyma forms due to collapse of radial files of cortical cells; the regulated collapse of cortical cells is considered to occur via programmed cell death. The 'honeycomb-type' schizogenous aerenchyma in *R. palustris* forms due to cells being forced apart owing to oblique divisions by some of the cortical cells in radial rows. Photographs reproduced from Voesenek *et al.* (2006) with permission of Wiley-Blackwell.

Voesenek 2008). The LOQS has been studied in detail in lowland rain-fed rice (described in the section ‘Submergence – short duration’).

Plants with the LOES are characterised by traits that enable them to: (i) re-orientate the growth direction and increase the rate of growth of shoot organs, such as stems and petioles, so as to emerge above floodwaters, (ii) preserve or develop anatomical structures that facilitate internal gas diffusion or pressurised through-flow, and (iii) retain or develop structures that facilitate gas exchange between plants and their submerged environment (Bailey-Serres and Voesenek 2008). The LOES has been studied in detail in *Rumex palustris* Sm. and deepwater rice (described in Shallow submergence – long duration section).

These two syndromes and the traits involved as related to waterlogging and submergence stress are discussed in detail below. The focus of our discussion is on the development of ‘suites of complementary traits’ that act synergistically to enhance fitness of plants in the various flooding environments (Table 1; Fig. 2).

Flooding regimes exert selection pressures leading to distinct suites of adaptive traits

The wide spectrum of environmental conditions in flood-prone environments determines species distributions and abundances (Armstrong *et al.* 1985; Silvertown *et al.* 1999; Voesenek *et al.* 2004). In this section, we discuss the prevailing ‘suites of complementary traits’ that act synergistically to enhance fitness of plants in some of the main types of flooding environments. Table 1 provides a matrix of the environments and traits considered.

The environments and plant traits will be discussed under the two main headings of waterlogging and submergence, and in the order: soil waterlogging, short duration; soil waterlogging, long duration; submergence, short duration; shallow submergence, long duration; deep submergence, long duration. Root traits will be discussed first in the sections on waterlogging. As submergence impacts on shoots as well as roots, shoot traits will be the focus of the sections on submergence, but any consequences for roots in addition to those described for waterlogging will be considered.

Waterlogging

Waterlogging is the situation of excess water in the root zone. Soil pores that normally would be gas-filled become water-filled. Waterlogged soils are usually O₂-deficient and often also contain potentially-toxic reduced soil constituents (Ponnamperuma 1984). Water levels can be dynamic with space and time (e.g. Setter and Waters 2003) and waterlogging can damage dryland species even when below the surface of the soil (Malik *et al.* 2001) or when transient (e.g. days, Malik *et al.* 2002). By contrast, wetland species grow well in waterlogged soil (Justin and Armstrong 1987), owing to several traits discussed below. Examples of species that inhabit environments with short-duration or transient waterlogging events, and have been confirmed to display waterlogging tolerance, are *Lolium multiflorum* Lam. and *Hordeum marinum* Huds. (McDonald *et al.* 2002; Garthwaite *et al.* 2003). Examples of species that inhabit areas with long-duration waterlogging are paddy rice, *Phragmites australis* (Cav.) Steud, various *Typha* spp., *Juncus effuses* L.; with many more examples listed also in Justin and Armstrong (1987).

Table 1. Overview of the hypothesised importance of various traits associated with plant tolerance of soil waterlogging and/or submergence, for five contrasting types of wet environments inhabited by some terrestrial plant species

Evidence in support of these hypothesised combinations of traits is given in the text. *, of little importance; **, of moderate importance; ***, of high importance; –ve*, costs outweigh benefits – such a response can decrease fitness in the specific environment; NA, not applicable; Short duration, <2 weeks; Prolonged duration, >2 weeks, typically >4 weeks; Shallow, <1 m (i.e. water levels that plants are capable of ‘outgrowing’); Deep, >1 m, typically a few metres or more. Note: True aquatic species often display biochemical leaf traits for enhanced underwater photosynthesis. Only biophysical leaf traits are considered here as our focus is on terrestrial species

Traits	Environments				
	Waterlogged – short duration	Waterlogged – long duration	Submerged – short duration	Submerged, shallow – prolonged (emergence possible)	Submerged, deep – prolonged (emergence not possible)
Adventitious roots (sediment)	*	***	*	***	*
Adventitious roots (water)	NA	NA	NA	**	***
Aerenchyma	**	***	**	***	***
Radial O ₂ loss barrier	*	***	*	***	*
Anaerobic energy production	***	*	**	*	***
Energy conservation	**	*	***	*	***
Prevention of reactive oxygen species (ROS) formation/ROS defence system	***	***	***	***	**
Tolerance of toxic soil constituents	NA	***	*	***	***
Nastic movements	*	**	*	***	**
Shoot elongation	NA	NA	–ve*	***	*
Aquatic leaf traits (biophysical)	NA	NA	NA	***	***
Leaf gas films	NA	NA	***	***	**
Convective gas movement	*	***	NA	***	NA

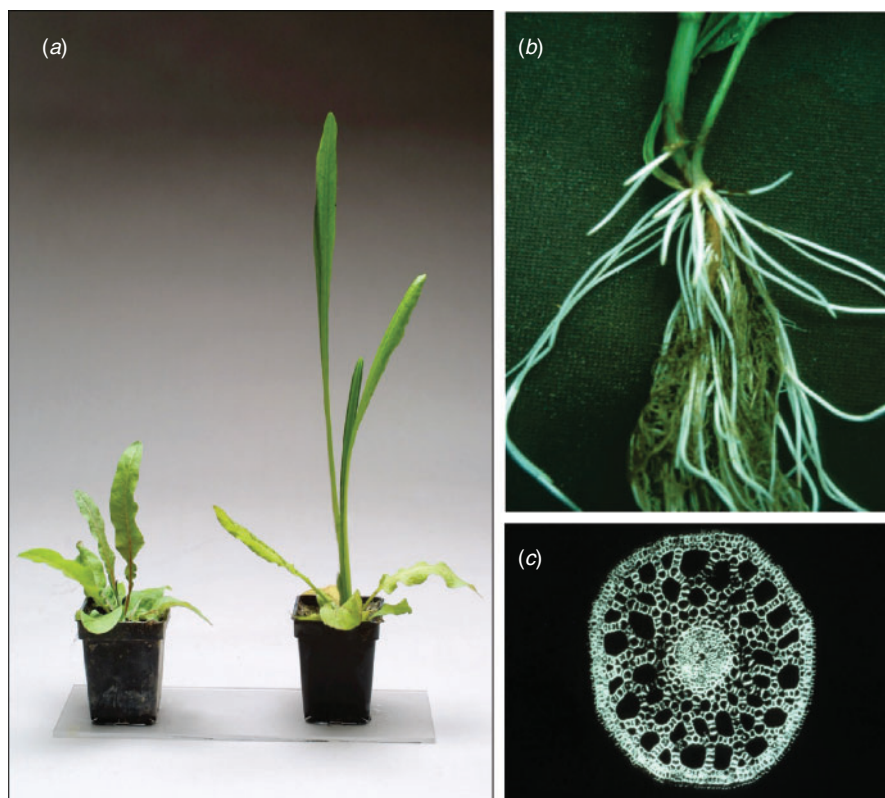


Fig. 2. Terrestrial wetland plants display 'suites of traits' that contribute to flooding tolerance. (a) *Rumex palustris* inhabits soils with long-duration waterlogging and even prolonged, shallow floods (i.e. water level that shoots can emerge above). Shoot elongation response is evident: compare plants grown without (left) or with (right) 14 days of submergence (plants 6 weeks old). Reproduced from Mommer *et al.* (2006a) with permission from Oxford University Press. (b) Numerous adventitious roots emerge from the shoot base and upper tap root when in waterlogged soil. Photograph of *Rumex maritimus* reproduced from Laan *et al.* (1991) with permission of Springer. *R. palustris* shows a similar response (Laan *et al.* 1989). (c) Adventitious roots of *Rumex palustris* contain aerenchyma. Reproduced with permission from Visser (1995). Other traits, such as leaf acclimation for underwater photosynthesis (see Table 1 and Mommer and Visser 2005), are not shown.

Soil waterlogging – short duration

Oxygen deficiency in waterlogged soils results from the consumption of O_2 by plant roots and soil organisms, while the excess water largely prevents O_2 entry into the soil (Armstrong and Drew 2002). During short-term waterlogging, the main stress is likely to be O_2 shortage (hypoxia or anoxia) resulting in an energy crisis in roots.

Metabolic anoxia tolerance is presumably of importance as an adaptive trait for roots of plants during short-term waterlogging, as aerenchyma can take time to develop (e.g. *Zea mays* L., Konings 1982; *Triticum aestivum* L., Thomson *et al.* 1990). Aerenchyma provides a gas-filled pathway for low-resistance movement of O_2 , and other gases, through plant organs. This internal pathway is inter-connected from shoot tissues (stems or sheaths or petioles, and in many cases from leaves – e.g. mid-rib in leaves of rice) to root extremities. Constitutive aerenchyma would be of immediate benefit to roots faced with waterlogging, as O_2 will diffuse into and along the roots. Many wetland species possess roots with constitutive aerenchyma (Justin and Armstrong 1987). Different parts of root systems will likely

experience different O_2 status; surface roots may, for example, have access to O_2 , whereas deeper roots might be without O_2 . So, even in species with constitutive aerenchyma, some tissues will still need to cope with O_2 deficiency.

Central to anoxia tolerance is dealing with the imposed energy crisis; anaerobic catabolism can produce at least some ATP, but apportionment of the scarce energy to essential processes is also needed (Gibbs and Greenway 2003; Greenway and Gibbs 2003). Consistent with the above, glycolytic flux and ethanolic fermentation were stimulated in roots of grey poplar when waterlogged. No changes were observed in leaves, as the shoot remained in contact with air. Various genes involved in biosynthetic pathways such as secondary cell wall formation and other energy-demanding processes, such as transport of nutrients, were downregulated in roots, but again not in leaves (Kreuzwieser *et al.* 2009). Whether roots of wetland species possess greater anoxia tolerance than those of dryland species, however, is still uncertain (Gibbs and Greenway 2003).

Hypoxic tissues and tissues experiencing re-entry of O_2 following anoxia are subject to potential oxidative stress

(Blokchina *et al.* 2003). Oxidative damage can result from increased production of reactive oxygen species (ROS) and/or a reduced capacity to detoxify ROS. In a waterlogging-sensitive species, like wheat, cycles of anoxia and re-entry of O₂ can increase levels of oxidative stress (Goggin and Colmer 2005). Oxidative stress can occur in relatively short time frames following changes in tissue O₂ status, and so likely impacts on roots of some species during transient waterlogging. Whether roots of tolerant and sensitive species differ in oxidative damage during waterlogging is uncertain (Blokchina *et al.* 2003), and only some components of the oxidative defence system have been measured in the few studies available. Upon transfer of intact plants from N₂-flushed to aerobic nutrient solution, the ratio of reduced-to-oxidised glutathione decreased in roots of waterlogging-intolerant species, but not in tolerant species, indicating potentially more oxidative stress in the roots of the less tolerant species (Biemelt *et al.* 1996). However, whether these contrasting responses reflect inherent differences in biochemical root traits, or instead are a consequence of the roots of the more tolerant wetland species possibly containing higher amounts of aerenchyma and so not suffering the same degree of O₂ deprivation, remains unknown. Experiments avoiding or controlling internal O₂ transport to roots are needed so that data on ROS and defence systems in roots can be obtained under well controlled root O₂ status; such as use of excised roots submerged in solutions of known O₂ concentrations or manipulations of shoot O₂ as well as root-medium O₂ so that the supply via aerenchyma is also controlled. Nevertheless, even intact, aerenchymatous roots of some wetland species can suffer oxidative damage upon re-entry of O₂ into the root zone (Chen and Qualls 2003).

Several waterlogging-sensitive species, such as sunflower (*Helianthus annuus* L.), tobacco (*Nicotiana tabacum* L.) and tomato (*Solanum lycopersicum* L.), show epinastic leaf growth (downward leaf movement) in response to waterlogging. This epinastic growth has been suggested to ameliorate the dehydrating effect of a drop in hydraulic conductance of roots in waterlogged soil (Jackson 2002). Waterlogging causes a decrease in root hydraulic conductance (Holbrook and Zwieniecki 2003), which in *Arabidopsis* has been associated with O₂-deficit-induced acidification of root cell cytoplasm causing gating of aquaporins (Tournaire-Roux *et al.* 2003). Such gating of an *Arabidopsis* aquaporin by changes in pH was recently confirmed in studies of proteoliposomes (Verdoucq *et al.* 2008). Vandeleur *et al.* (2005) hypothesised that gating of aquaporins in roots experiencing O₂-deficit might be a mechanism by which water uptake is re-directed to other roots in more favourable regions of the soil (e.g. from O₂-deficient deeper roots, to those in surface layers with access to some O₂).

In summary (Table 1), tolerance of short-duration waterlogging is enhanced by the presence of constitutive aerenchyma, a trait in many wetland species. In addition, tolerance of anoxia in root tissues is also hypothesised to be of importance, especially in roots lacking constitutive aerenchyma. Even when constitutive aerenchyma is present, anoxia tolerance is presumed also to be of importance for some tissues, for example, those most distant from the O₂ source (e.g. root tips) would likely still experience O₂ deprivation. Hypoxic tissue conditions and the re-entry of O₂ following drainage can lead

to formation of ROS in plant cells, so an efficient oxidative defence system is another trait likely to be of importance for plants in environments with transient waterlogging.

Soil waterlogging – long duration

Soil O₂ deficiency occurs soon after waterlogging commences, depending on biological activity. With time, waterlogging also changes other soil factors; CO₂, ethylene (C₂H₄), and reduced compounds, such as Mn²⁺, Fe²⁺, S²⁻ and carboxylic acids can increase (Ponnamperuma 1984). Internal O₂ transport from air, via the shoots, to roots is essential to survival and functioning of roots (Armstrong 1979).

Waterlogging tolerant species form a large adventitious root system when in saturated soils. The newly-formed adventitious roots usually contain aerenchyma (Figs 1 and 2c) and these roots can replace the stress-damaged roots that formed before waterlogging (Jackson and Drew 1984). Waterlogging tolerant species tend to develop larger adventitious root systems than intolerant species, and these newly-formed roots contain more aerenchyma (e.g. *Rumex* species, Laan *et al.* 1989). The initiation and outgrowth of adventitious roots has been studied in deepwater rice and *Rumex* species, respectively. Accumulation of ethylene signals this response, and auxin and H₂O₂ are also involved (Visser *et al.* 1996; Steffens and Sauter 2009).

Aerenchyma in roots either forms constitutively in drained soil conditions and/or is induced upon soil waterlogging. It develops in existing plant organs or concomitant with the outgrowth of new adventitious roots. Induction of lysigenous aerenchyma (Fig. 1a) in roots is promoted by the accumulation of ethylene and subsequently a cascade leading to programmed cell death (Drew *et al.* 2000; Shiono *et al.* 2008). In roots, genes associated with cell wall breakdown show increased expression in response to waterlogging or hypoxia (Saab and Sachs 1996; Liu *et al.* 2005; Lasanthi-Kudahettige *et al.* 2007). Even in situations where only the lower parts of roots are in waterlogged soil, functional aerenchyma can form both in the portion under water and in that above the water-saturated zone (Malik *et al.* 2003).

In wetland species with constitutive aerenchyma, the volume is often further enhanced during soil waterlogging (Justin and Armstrong 1987). Porosity in roots (% gas volume per unit root volume) of some wetland species can increase to as high as 53% (Justin and Armstrong 1987). High porosity greatly reduces resistance to gas-phase diffusion through plant organs and tissues (Armstrong 1979). In addition, many wetland species form a barrier to radial O₂ loss (ROL) in basal zones of roots, a feature that further promotes longitudinal O₂ diffusion down roots, by preventing losses to the surrounding anoxic soil (Fig. 3; Armstrong 1979; Visser *et al.* 2000; Colmer 2003a; Garthwaite *et al.* 2003). In rice, growth in stagnant conditions increased aerenchyma formation and induced a barrier to ROL in basal root zones (Colmer 2003b). An inducible barrier to ROL also occurs in several other species that inhabit wet areas (e.g. *L. multiflorum* Lam., McDonald *et al.* 2002; *H. maritimum* Huds., Garthwaite *et al.* 2003), whereas in other species the barrier appears to be constitutive (e.g. *J. effuses* L., Visser *et al.* 2000). Ethylene enhances the development of the additional aerenchyma but not formation of the barrier to ROL, in roots of rice (Colmer *et al.* 2006). The barrier to

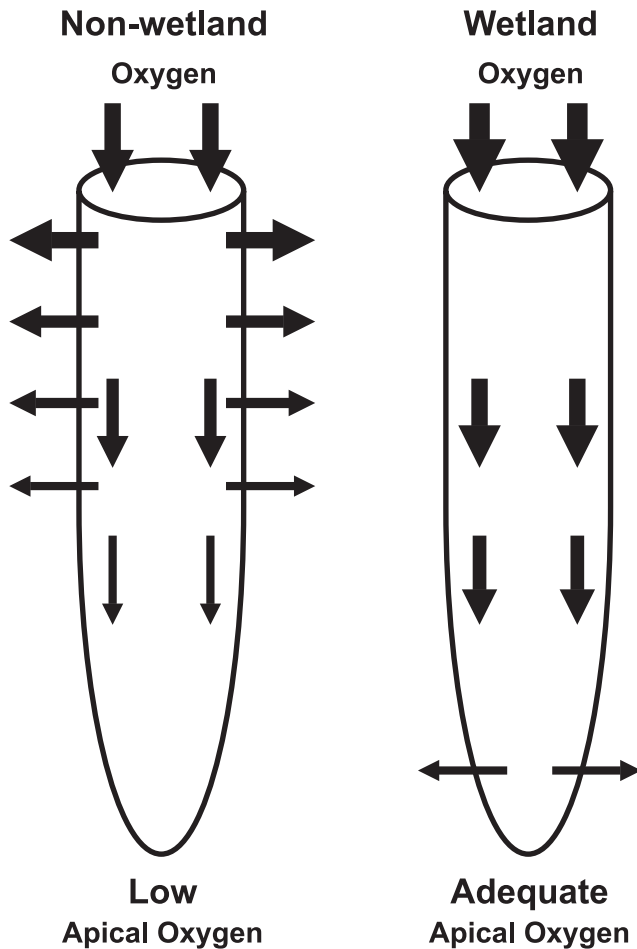


Fig. 3. Diagram showing contrasting patterns of radial oxygen loss (ROL) from roots in waterlogged soils. Many, but not all, wetland species form a barrier to radial O_2 loss (ROL) in basal zones of roots, a feature that in addition to aerenchyma, promotes longitudinal O_2 diffusion down roots by preventing losses to anoxic soils (Armstrong 1979). Enhanced movement of O_2 towards the apex promotes deeper rooting in waterlogged soils. The barrier results from suberin deposition in the exodermis, forming a physical resistance to diminish ROL. The barrier could also impede entry of potentially toxic compounds in highly-reduced soils. Non-wetland species do not form a barrier to ROL and also have less aerenchyma than most wetland species, resulting in less O_2 diffusion to the apex and short roots in anoxic waterlogged soils. Arrow thickness reflects the amount of O_2 available (i.e. thinner arrows = less O_2 diffusion). Figure re-drawn based on Jackson and Drew (1984).

ROL, together with rhizosphere re-oxidation around root tips and laterals, can also diminish entry of potentially toxic compounds in highly-reduced soils (Armstrong 1979; Armstrong *et al.* 1996; Armstrong and Armstrong 2005).

Modelling predicts that the amount of O_2 available within organs is inversely related to diffusion-path lengths (Armstrong 1979). Plants have developed traits to promote diffusion to the most distant destination, the root meristems. Traits that bring the O_2 source closer to roots are formations of lenticels at stem bases and development of vertically-growing roots that reach air, such as pneumatophores (Kozłowski 1984). A specialised trait that also brings an O_2 source closer to roots is convective flow of gases through shoot organs and along rhizomes of some wetland species

(Dacey 1981; Armstrong *et al.* 1992). Such flows elevate the O_2 concentrations in rhizomes, thus enhancing O_2 diffusion into the subtending roots (e.g. *P. australis*, Armstrong *et al.* 1992). Another shoot trait of relevance during soil waterlogging, possessed by several species with rosette-type shoots that inhabit flood-prone environments (Grimoldi *et al.* 1999), is a capacity to change leaf orientation from rather prostrate to almost vertical (hyponastic growth). This acclimation prevents partial submergence when some water ponds on waterlogged soils.

The situation for possible oxidative damage in roots experiencing changes in O_2 regimes was discussed above under short duration waterlogging. Oxidative stress during long-term anoxia has not been studied in roots, as root tissues do not tolerate long-term anoxia (Gibbs and Greenway 2003). Other organs, such as rhizomes, can experience long-term anoxia associated with long duration waterlogging. The classical paper by Monk *et al.* (1987) of oxidative stress during re-aeration of rhizomes of *Iris* species differing in flooding tolerance, showed less lipid peroxidation in the tolerant *Iris pseudacorus* L. compared with intolerant *Iris germanica* L., owing to differences in the oxidative defence systems of these two species.

Roots experience the direct effects of waterlogging, whereas the shoots of plants in waterlogged soils suffer any consequences of root dysfunction. For example, shoots can be damaged by deficiencies of mineral nutrients, particularly nitrogen, and by an influx of reduced soil toxins (e.g. Fe^{2+} and Mn^{2+} toxicities) (Jackson and Drew 1984). Soil element toxicities associated with waterlogging might, in combination with the O_2 -deprivation stress, determine relative performances of different wheat genotypes on different soil types (Setter *et al.* 2009). Even wetland species can suffer from entry of reduced compounds from the soil, depending on soil type and waterlogging duration. Rice grown on some high organic-content soils, for example, can show leaf bronzing and poor growth caused by ingress of Fe^{2+} and S^{2-} , and cultivars differ in susceptibility to development of these symptoms (Baba *et al.* 1965; Tanaka *et al.* 1968). Formation of root barriers to restrict entry of these compounds, as well as having some ROL from key sites (root tips and laterals) to re-oxidise these substances in the rhizosphere, are regarded as important traits for minimising these toxicities (Armstrong 1979; Armstrong and Armstrong 2005). Future research should also assess whether species differ in cellular tolerance of these soil-derived compounds when entry does occur into roots and shoots.

Roots in waterlogged soils might also need to cope with exposure to high CO_2 (Greenway *et al.* 2006). Carbon dioxide accumulated to 30–35 kPa in soil of a waterlogged soybean field (Boru *et al.* 2003). In pots of soil from rice paddy fields, CO_2 ranged 8–40 kPa, depending amongst other factors on initial soil pH (IRRI 2005; Greenway *et al.* 2006). High CO_2 could interfere with root metabolism via an imposed acid load, by general disruption of metabolism (e.g. from high HCO_3^- in cellular fluids), or by direct inhibition of respiratory activity (reviewed in Greenway *et al.* 2006). There is some evidence that species differ in tolerance of high CO_2 in the root zone, with wetland rice reported as more tolerant than dryland soybean (Boru *et al.* 2003), but further work is needed to confirm the reported differences (Greenway *et al.* 2006), and to determine how other wetland species cope with high CO_2 in the root zone.

In summary (Table 1), tolerance of long duration waterlogging requires an efficient system for internal O₂ transport to roots, as well as the continued development of new roots. Traits such as aerenchyma and a barrier against ROL in roots, aerenchyma in shoots, lenticels at stem bases, and in some species pressure-driven flows along rhizomes, are found in various combinations, and these act synergistically to enhance internal aeration. Hyponastic growth of petioles of some rosette-type plants avoids leaves also experiencing submergence from shallow ponding of water. In addition to the O₂-deprivation stress, reduced soil toxins can accumulate during long duration waterlogging, and these can damage root, rhizome and shoot tissues. Formation of root barriers to restrict entry of these compounds, as well as having some ROL from key sites (root tips and laterals) to re-oxidise these substances in the rhizosphere, are important traits.

Submergence

Submergence refers to the situation when floodwaters rise to levels that shoots are completely under water. This prevents direct exchange of gases between the entire plant body and the atmosphere resulting in reduced O₂ and CO₂ levels. Exchange of gases, however, does occur between plant organs and floodwaters, but at slow rates. Furthermore, complete submergence frequently reduces the available light level. Thus, low CO₂ and/or low light hampers rates of underwater photosynthesis.

Three contrasting submergence regimes, based on temporal and depth criteria, have been studied in relation to natural and agricultural systems: short duration floods; long duration, but shallow floods; and long duration, but deep floods (Piedade *et al.* 1991; Jackson and Ram 2003; Voesenek *et al.* 2004). Short duration submergence occurs during flash-flooding events, with variable depths, such as occur frequently in lowland rice areas, and also at various times in most low-lying land areas of the world. Long-lasting submergence can either be shallow or deep. In river systems, the deep floods typically occur on positions that are in direct contact with the river, such as river foregrounds in Europe, and delta areas in Asia supporting deepwater rice cultivation, or in the Amazon. Long-lasting, shallow floods, by contrast, occur in more distal areas of the plains when water flows over embankments that subsequently impede reverse-runoff. In many cases, the flooding durations are further prolonged owing to poor drainage of soils in these areas.

Traits for tolerance of soil waterlogging, discussed in the preceding section, are also present in many plants when completely submerged (Table 1). These traits were, in the main, in roots. During floods, soils are also waterlogged, therefore many of these root traits remain of relevance. The functionality of aerenchyma and a barrier against ROL in adventitious (sediment) roots is dependent upon there being an O₂ source via the shoots (Armstrong 1979). During short-term submergence, if these traits are present in existing roots, then the resultant O₂ movement would enhance root survival. With shoots emergent above long-lasting, shallow floods, these root aeration traits would enhance root growth and functioning, as described previously for waterlogged plants. When floods are prolonged and too deep for shoots to reach the surface, existing roots would

still benefit from these traits enabling some O₂ supply, depending on entry from the water column and/or underwater photosynthesis (Pedersen *et al.* 2006). Availability of O₂ in completely submerged plants shows marked diurnal cycles, as dependent on underwater photosynthesis (Pedersen *et al.* 2006; Colmer and Pedersen 2008a). Interestingly, when some species remain completely submerged, hardly any new adventitious roots emerge into the sediment (Van der Sman *et al.* 1993). By contrast, new adventitious roots emerge into the water column, these have been termed 'aquatic roots' (rice, Lorbiecke and Sauter 1999; *Rumex*, Van der Sman *et al.* 1993). In aquatic roots of some species, chloroplasts develop in cortical cells, thereby enabling photosynthesis in the roots and providing an internal source of O₂ (and sugars) when under water (Rich *et al.* 2008).

In addition, many of the traits described in the preceding section for roots also occur in shoots, such as aerenchyma formation, anaerobic energy production, and protection against ROS (Voesenek *et al.* 2006; Bailey-Serres and Voesenek 2008). Submergence-specific traits of shoots are also evident and their contributions to tolerance in relation to the three contrasting flooding regimes (Table 1) are discussed below.

Submergence – short duration

Plants with the LOQS, characterised by a lack of a shoot extension so as to conserve substrates for enhancement of survival until waters recede, have fitness advantages in environments with short-duration floods (Bailey-Serres and Voesenek 2008). An association of the LOQS with environments prone to short-duration floods, has been demonstrated for rice (Jackson and Ram 2003; Xu *et al.* 2006) and also several species in natural plant communities (Voesenek *et al.* 2004). Non-elongating cultivars of rice are more tolerant of flash-floods, even when these short-duration floods are deep, as carbohydrates are conserved for processes essential for survival (reviewed by Bailey-Serres and Voesenek 2008). The elongation response in lowland rain-fed rice cultivars is controlled by one polygenic locus (*Sub1*) on chromosome 9. A transcription factor within this *Sub1* locus (*Sub1A-1*), belonging to the multi-gene family of AP2/ERF transcription factors, influences submergence tolerance in rice (Fukao *et al.* 2006). The expression of this gene is regulated by ethylene and constrains underwater shoot elongation and carbohydrate catabolism (Xu *et al.* 2006; Perata and Voesenek 2007). Most probably *Sub1A* resulted from gene duplication events that occurred before, and after, rice domestication (Fukao *et al.* 2009). When *Sub1A-1* was introgressed into rain-fed lowland cultivars lacking this gene at the *Sub1* locus, tolerance of submergence lasting 12–17 days was improved (e.g. Fig. 4); the yield advantage under field conditions was up to 3.8 t ha⁻¹ (Singh *et al.* 2009).

In addition to the traits associated with the LOQS as described in Bailey-Serres and Voesenek (2008), another plant trait that improves submergence tolerance of rice is the so-called 'plant plastron' (Raven 2008) or leaf gas films. A thin layer of gas is retained on submerged leaves of some species, depending on leaf surface traits (Colmer and Pedersen 2008b). The gas films enlarge the water-gas interface, thus improving gas exchange between submerged shoots and the surrounding water (Colmer and Pedersen 2008b). Gas films on leaves of rice can enhance

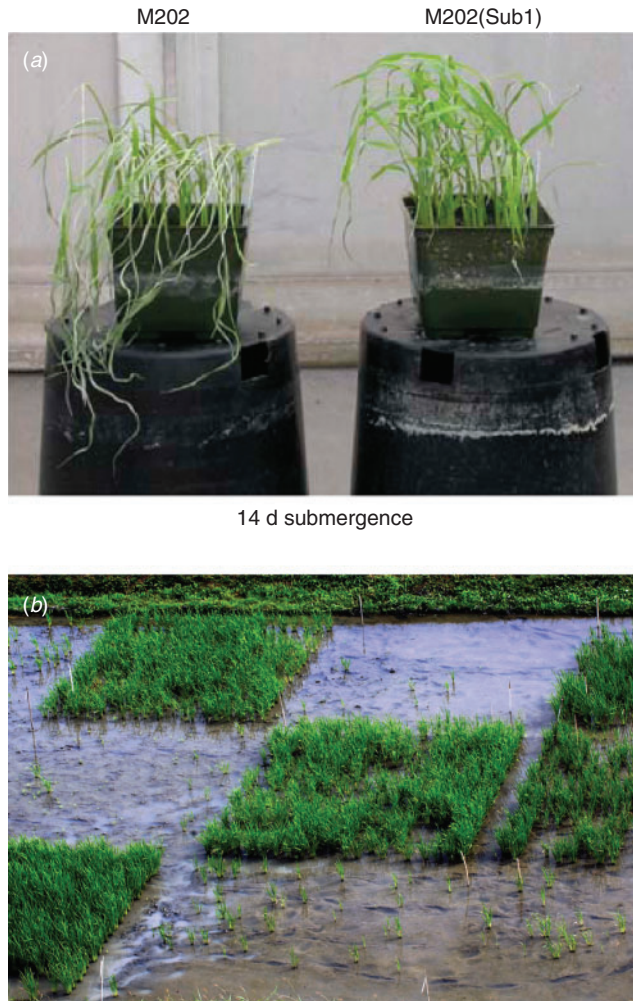


Fig. 4. Influence of the shoot elongation response on submergence tolerance of rice (*Oryza sativa*) (short duration floods, not deepwater environments). Lack of shoot elongation is a major trait within the Low Oxygen Quiescence Syndrome (LOQS) that improves survival during short duration submergence (see Table 1 and Bailey-Serres and Voesenek 2008). (a) Responses to submergence of rice seedlings without (left, line M202) or with (right, line M202(Sub1)) the *Sub1A-1* allele present at the multigenic *Sub1* locus. 14-day-old seedlings were completely submerged for an additional 14 days and the photograph was taken 7 days after de-submergence. Reproduced from Fukao *et al.* (2006) with permission of The American Society of Plant Biologists. (b) Field testing of introgression lines with the *Sub1A-1* allele present in the *Sub1* locus, at IRRI, Los Banos, Philippines. Lines containing *Sub1A-1* tolerate 14 days of complete submergence (dense plots) whereas control lines inundated for the same duration suffer severe damage (sparse plots). The IRRI protocol involves transplanting 14-day-old seedlings into the field, submerge when 28-day-old, de-submerge when 42-day-old, and the photo was taken ~3 weeks after de-submergence (A. M. Ismail, pers. comm.). Image by Ariel Javellana (IRRI PhotoBank. Available at <http://www.ricephotos.org/index.htm> [Verified 16 June 2009]).

underwater photosynthesis to provide additional sugars and O_2 . Even in darkness, O_2 entry from floodwaters was improved, and O_2 that entered shoots moved via the aerenchyma to the roots (Pedersen *et al.* 2009). During short duration floods, O_2 entry from the water column might be adequate to sustain submerged

shoots (e.g. in rice), but distal portions of roots would be expected to become O_2 deficient during nights (Waters *et al.* 1989; Colmer and Pedersen 2008a), so that anaerobic energy production would be of relevance for survival of roots (see sections above on waterlogging and roots).

In addition to cessation of growth during complete submergence, other processes may also be downregulated so as to conserve energy. This information is largely gleaned from molecular studies using model systems. For example, *Arabidopsis* protoplasts that are not well aerated demonstrate large reductions in accumulation of many gene transcripts, especially those coding for energy consuming processes such as ribosome biogenesis and anabolism (Baena-González *et al.* 2007). Furthermore, in *Arabidopsis* seedlings a large portion (70%) of cellular mRNA's become poorly translated upon low O_2 stress (Branco-Price *et al.* 2005, 2008). Even in highly-tolerant species with relatively high rates of ethanolic fermentation supporting anoxic shoot elongation (e.g. rice coleoptile and shoot of *Potamogeton pectinatus* L.), selective protein synthesis conserves energy (Huang *et al.* 2005; Dixon *et al.* 2006). This restriction of the energetically costly protein synthesis significantly reduces the consumption of ATP. A major energy saving is associated with the selective repression of translation of mRNAs encoding the highly abundant ribosomal protein (Branco-Price *et al.* 2008). The downregulation of energy consuming processes is rapidly reversed upon reoxygenation, indicating that cellular sensing of energy status controls the modulation of these processes (Branco-Price *et al.* 2008). Thus, tolerance to anoxia involves both ATP production via glycolysis linked to ethanolic fermentation and apportionment of the limited ATP supply to processes essential for survival (Greenway and Gibbs 2003).

In conditions of hypoxia, an active downregulation of the rate of respiration has been suggested to prolong survival as cells would maintain higher O_2 levels under these conditions (Geigenberger 2003). Further work on this concept is needed, so as to separate effects of physical diffusion limitations (see Armstrong *et al.* 2009) and the proposed downregulation. Recently, Zabalza and co-workers (2009) showed that the pyruvate concentration in pea roots is positively correlated with the respiration rate. This led them to suggest that in hypoxic tissues fermentation not only functions to regenerate NAD^+ for continuation of glycolysis but that it may help to reduce the levels of pyruvate and thus restrict respiration and so reduce consumption of available O_2 during hypoxia. The likely spatial separation of these processes, however, needs also to be considered, as fermentation could mainly occur in an inner 'anoxic core' and respiration in the outer cell layers (reviewed by Gibbs and Greenway 2003).

If severe hypoxia or anoxia occurs in some situations or plant parts, a subset of upregulated genes are associated with regulation of scarce energy resources. Examples are the use of pyrophosphate (PP_i) over ATP as a high-energy donor (Huang *et al.* 2008) and the preferred use of sucrose synthase (SuSy) and UDPglucose pyrophosphorylase for the degradation of sucrose (Geigenberger 2003). More generally, certain mRNAs circumvent translational repression and/or are transcriptionally upregulated thus selectively ensuring production of a specific set of proteins (Branco-Price *et al.* 2005, 2008). These 'anaerobic

proteins' are involved in (i) glycolysis, (ii) conversion of pyruvate to fermentation end products, (iii) sucrose degradation, and (iv) controlling reactive oxygen species (ROS) (Sachs *et al.* 1996; Klok *et al.* 2002; Branco-Price *et al.* 2005; Huang *et al.* 2005; Liu *et al.* 2005; Loreti *et al.* 2005; Lasanthi-Kudahettige *et al.* 2007; Igamberdiev and Hill 2009; Kreuzwieser *et al.* 2009; van Dongen *et al.* 2009). There is evidence that protein kinases belonging to the *SnRK1* group, such as *Arabidopsis KIN10* and *KIN11*, orchestrate transcriptional networks to globally promote catabolism (cell wall, starch, sucrose, amino acid, lipid and protein degradation) to provide alternative sources of energy and metabolites and suppress anabolism during hypoxia-induced carbohydrate starvation and/or energy deprivation (Baena-González *et al.* 2007).

When floodwaters recede, post-submergence damage can also occur. Lodging occurs if shoots have elongated (e.g. rice, Jackson and Ram 2003), and tissues can also be challenged by oxidative stress (Blokhuin *et al.* 2003). Interestingly, oxidative damage in leaves following de-submergence of young rice plants that were under water for 6 days, was greater in an intolerant cultivar (IR42) than in submergence-tolerant FR13A (Ella *et al.* 2003). Oxidative damage was measured as lipid peroxidation products, and in addition to the genotypic differences, oxidative damage was greater under conditions of high light than at lower light (PAR, 1000 and 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively), indicating that photoreduction of O_2 to O_2^- in Photosystem I might have been greater in chloroplasts altered by the submergence treatments (Ella *et al.* 2003). Leaves of the more tolerant FR13A contained higher concentrations of ascorbate and had higher glutathione reductase activity, than those of the intolerant IR42. The lower endogenous ascorbate concentration in the flooding intolerant IR42 was found also in a second study, and when exogenous ascorbate was supplied to discs taken from submerged leaves of IR42, oxidative damage was reduced (Kawano *et al.* 2002). In addition to oxidative damage in leaves of rice upon de-submergence, injury can also result from acetaldehyde toxicity, when ethanol remaining in tissues is converted into acetaldehyde (Mustroph *et al.* 2006). Studies of oxidative stress do not seem to have been conducted for leaves of 'wild' wetland species.

In summary (Table 1), tolerance of short-duration submergence is characterised by the LOQS (Bailey-Serres and Voesenek 2008). Major features of the LOQS are lack of shoot extension to conserve substrates, downregulation of other non-essential processes to further conserve energy, induction of anaerobic energy production in tissues that become anoxic, and upregulation of the ROS defence system to avoid damage upon O_2 re-entry. In addition, leaf gas films on some species (e.g. rice) enhance underwater photosynthesis to provide additional sugars and O_2 (Pedersen *et al.* 2009).

Shallow submergence – long duration

Species from a wide range of families share the capacity to initiate fast extension growth of shoot organs upon submergence (Ridge 1987; Voesenek and Blom 1999). In the river Rhine floodplains, this escape response is prevalent in plants inhabiting environments characterised by long, shallow floods (several weeks and less than 1 m). By contrast, this trait was

generally absent in species from sites with deep floods and sites with short duration floods (Voesenek *et al.* 2004). Shoot extension is only of major benefit for submerged terrestrial species if it leads to emergence (Bailey-Serres and Voesenek 2008; Pierik *et al.* 2009). This shoot extension response is central to plants that respond to flooding events with the LOES, but these plants also possess other traits.

Hyponastic growth of shoot organs upon submergence has been described for several species, such as *R. palustris* (Cox *et al.* 2003), *Ranunculus repens* L., *Caltha palustris* L., (Ridge 1987), *Leontodon taraxacoides* Lam. (Grimoldi *et al.* 1999), *Paspalum dilatatum* Poir. (Insausti *et al.* 2001), and even *Arabidopsis* (Millenaar *et al.* 2005). Hyponastic growth in *R. palustris* occurs mainly in petioles of younger leaves, and results from differential cell elongation across the petiole base (Cox *et al.* 2004). Hyponastic growth alone, is a trait of relevance as vertical re-orientation by itself can bring leaves above the water; an example is *L. taraxacoides* that does not display a shoot elongation response (Grimoldi *et al.* 1999). Even if hyponastic growth occurs and leaves do not reach the water surface, potential benefits might still be improved access to light, shortening of the submergence period, and prevention of sediment/debris covering leaves. Furthermore, hyponastic growth is a prerequisite for submergence-induced petiole elongation in some species (e.g. *R. palustris*, Cox *et al.* 2003).

Both hyponastic growth and petiole/internode elongation bring leaf tips closer to the water surface or ultimately above floodwaters that are not too deep. Shoot emergence is highly beneficial for plants in flooded environments, due to the improved exchange of gases and the re-start of aerial photosynthesis (He *et al.* 1999; Mommer *et al.* 2005; Pierik *et al.* 2009). These adaptive elongation responses are initiated by the accumulation of the volatile hormone ethylene inside submerged plant tissues (Jackson 2008). Subsequently, the interplay of various other plant hormones such as gibberellic acid (GA) and abscisic acid (ABA) and more downstream targets at the cell wall level, such as cell wall acidification and expansins (Vreeburg *et al.* 2005) result in the elongation of shoot organs (reviewed by Bailey-Serres and Voesenek 2008; Jackson 2008).

Deepwater rice also displays a remarkable capacity for stem elongation as floodwaters rise. Deepwater rice grows in areas with water depths of 0.5 m (or more) for at least 1 month, although in some regions water levels can reach several metres (Grist 1986; Catling 1992). Deepwater rice tends to avoid complete submergence as stem elongation proceeds as floodwaters rise, typically keeping a portion of the shoot above water (Catling 1992). When floods recede, the stems show 'kneeing'; the lower nodes root into the sediments whereas the uppermost nodes grow upwards so that floral structures and grains are held in air, rather than being spoilt by damp soil conditions (Catling 1992). As waters rise, internode elongation is stimulated via ethylene-induced reductions in ABA and increased tissue sensitivity to GA (Kende *et al.* 1998). This stem elongation response in deepwater rice, and in a wild relative (*Oryza rufipogon* Griffiths), is determined by three major QTLs common to both species (Hattori *et al.* 2007). When a major QTL on chromosome 12 of deepwater types was introduced into a non-elongating rice genotype (i.e. a paddy type *O. sativa* ssp. *japonica*), the plant demonstrated a deepwater elongation response upon partial

submergence with rising water levels (Fig. 5). Hattori *et al.* (2007) proposed that the gene associated with the QTL might encode a product that senses and switches on the deepwater elongation response, with the downstream machinery already being present in the non-elongating type. Thus, like the Sub1 locus (chromosome 9) for shoot elongation during complete submergence of lowland rain-fed rice (Xu *et al.* 2006), alteration in a major locus (QTL on chromosome 12) can also have a major effect on the response of deepwater rice to rising partial submergence (Hattori *et al.* 2007).

In many cases, shoots of plants with a LOES not only elongate, but also produce new leaves when submerged (e.g. *R. palustris*) (Mommer and Visser 2005). These leaves have a higher specific leaf area (SLA), thinner epidermal cell walls, and cuticles and chloroplasts oriented close to the epidermis (Mommer *et al.* 2005). These traits reduce the diffusion resistance for gases and as a consequence increase the rates of CO₂ entry for underwater photosynthesis (Mommer *et al.* 2006a) and inward O₂ diffusion for respiration (Mommer *et al.* 2004). Leaf gas films are another plant trait that improves gas exchange between submerged shoots and the surrounding water. The gas films enlarge the water–gas interface, thus facilitating entry of CO₂ for photosynthesis when in light, and of O₂ during darkness

(Colmer and Pedersen 2008b). Gas films contribute to the LOES by increased rates of net underwater photosynthesis and improved internal aeration of roots (Pedersen *et al.* 2009). In both cases, leaves produced and then positioned further towards the water surface during elongation growth, would benefit from the higher light in surface layers promoting net photosynthesis.

Thus, the shoot elongation response acts synergistically with other leaf traits enhancing underwater gas exchange. Rapid shoot elongation, however, depletes carbohydrates, ultimately hampering further growth (Groeneveld and Voesenek 2003) and can compromise survival if leaves do not reach air (Setter and Laureles 1996). As shoot elongation occurs, however, these leaves grow into better illuminated water layers. This higher light availability, together with the leaf traits enhancing underwater photosynthesis, results in higher carbon gain to sustain further elongation growth. Moreover, the improved O₂ status, derived from photosynthesis and/or enhanced entry of O₂ from the water column, due to these leaf traits, would also prolong survival during submergence. Maintenance of healthy tissues for as long as possible is advantageous as this enables elongation to be sustained for longer periods, and tissues in a healthy condition would presumably also survive better after recession of floodwaters.

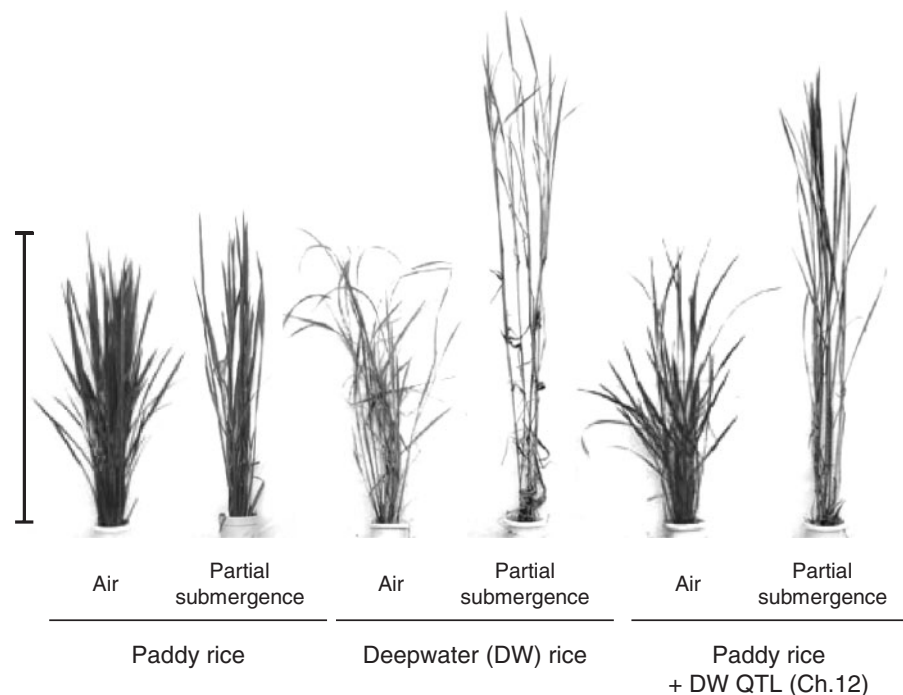


Fig. 5. Deepwater rice (*Oryza sativa*) grows in regions with prolonged, 'shallow' floods (i.e. water levels that plants are capable of 'outgrowing'; up to several metres aboveground). Fast shoot elongation is a major trait within the Low Oxygen Escape Syndrome (LOES) that improves survival in environments with prolonged, shallow flooding (See Table 1 and Bailey-Serres and Voesenek 2008). Thus, in contrast with lowland rain-fed rice and paddy rice, deepwater genotypes show a strong elongation response that is beneficial to survival. Comparison of the responses of paddy- (left) and deepwater (DW) -rice (middle) to rising water levels (rising partial submergence); and introgression of a major deepwater QTL (from chromosome 12 of *Oryza rufipogon*) into paddy rice (right) enables the deepwater elongation response. Each pair of photographs shows one rice genotype when grown with shoot in air or in rising water at 70% of shoot height. Figure kindly compiled by Motoyuki Ashikari using images from Hattori *et al.* (2007); reproduced with permission of the Japanese Society of Breeding.

Restoration of leaf-to-air contact, owing to shoot extension upon submergence, is only functional if shoots contain pathways for low resistance gas diffusion (Mommer *et al.* 2006b; Pierik *et al.* 2009). To this end, the bodies (i.e. shoots and roots) of many wetland plants are highly aerenchymatous. Upon emergence from floodwaters, internal O₂ status is improved as O₂ enters the aerenchyma. In many species (e.g. *R. palustris*, *O. sativa*) the O₂ diffuses throughout the plant body, whereas in some (e.g. *P. australis*, Armstrong *et al.* 1992; waterlily (*Nuphar lutea* (L.) Sm), Dacey 1981) gases are transported through shoots and rhizomes via through-flows generated by pressure gradients. Through-flows can increase by two orders of magnitude the effective aeration distance in plants, as compared with diffusion alone (Armstrong *et al.* 1991). This enhanced O₂ supply enables rhizomes to grow deeper into waters and/or anoxic sediments (Strand 2002). Moreover, higher O₂ within rhizomes enhances the O₂ status in attached roots; ROL was ~4-fold higher from adventitious roots of *P. australis* with through-flows active (Armstrong *et al.* 1992).

In summary (Table 1), tolerance to long duration, shallow, submergence is characterised by the LOES (Bailey-Serres and Voesenek 2008). Major features of the LOES are rapid re-orientation and extension growth of shoots towards the water surface, development of new 'aquatic'-leaves (or possession of leaf gas films) to promote carbon acquisition during the period of extension growth, aerenchyma and other traits for enhanced internal aeration when leaves emerge, as well as defence against ROS. As submergence is prolonged, the capacity to form new roots (sediment and aquatic), and to deal with reduced soil constituents, would also be of importance.

Deep submergence – long duration

In some environments, floods are of such depths that plant organs will never reach the water surface or may take weeks of enhanced shoot elongation to emerge. A shoot elongation response was generally absent in species from sites with deep, long-lasting floods (Voesenek *et al.* 2004). Consistent with the lack of the elongation trait in many species in environments with deep floods, is that woody species in the Amazonian basin also generally do not elongate when deeply submerged (e.g. 10 m) (Parolin 2009). Interestingly, the emergent C₄ grass *E. polystachya* can manage to keep a portion of shoot above deep Amazonian floods, owing to the formation of additional stem nodes; seven new internodes were formed when water levels were rising at ~1.5 m per month (Piedade *et al.* 1991).

Some plants in these environments with long-duration floods develop leaves with traits that significantly improve the exchange of gases with floodwaters (e.g. *R. repens*, Lynn and Waldren 2001). Thus, while the lack of shoot extension in many species that inhabit areas with prolonged and deep floods (Lynn and Waldren 2003; Voesenek *et al.* 2004) is similar to the LOQS, the leaf adaptations (Lynn and Waldren 2003) and acclimations (Mommer *et al.* 2006b; Mommer *et al.* 2007) promote avoidance, rather than escape, of the low O₂ stress. Moreover, new leaves are produced underwater (e.g. Mommer *et al.* 2007), so these plants are not 'quiescent'. Aquatic leaf acclimations (biophysical) upon submergence, such as dissected leaves, increased specific leaf area (i.e. larger surface area relative to

mass), thinner epidermal cell walls and cuticles, and chloroplast re-orientation towards the epidermis, reduce the diffusion resistances for both CO₂ and O₂ and thus improve underwater photosynthesis (Mommer and Visser 2005; Mommer *et al.* 2006a) and respiration (Mommer *et al.* 2004). Such acclimations are relevant during flooding regimes in which light is available to submerged vegetation, such as during deeper floods of low turbidity. Nevertheless, these traits might also be beneficial for plants in deep and/or turbid waters, as the decreased resistance also enhances O₂ entry for respiration (Mommer *et al.* 2004).

Improved O₂ status in shoots of submerged plants, whether from aquatic leaf traits or from gas films, would also benefit the roots, owing to internal O₂ diffusion to roots via the aerenchyma (Waters *et al.* 1989; Pedersen *et al.* 2009). Further evidence for the importance of this shoot-to-root O₂ movement during submergence comes from strong correlations between aerenchyma content in petioles and survival when submerged, both in light and darkness, for diverse species (Mommer *et al.* 2006b).

Submerged plants experience diurnal fluctuations in tissue O₂ status, as dependent on light and thus photosynthetic O₂ production in shoots (Sorrell and Dromgoole 1987; Pedersen *et al.* 2006). O₂ available at a specific location in the plant body is dependent upon resistances (and distance) and O₂ consumption rates along the diffusion pathway (Armstrong 1979). As examples, the O₂ concentration in the root cortex is generally higher than in the adjacent stele (Armstrong *et al.* 1994). These spatial and temporal differences in O₂ supply result in tissue-specific (e.g. stele, Thomson and Greenway 1991) and time-dependent (e.g. rice roots, Waters *et al.* 1989) inductions of anaerobic energy production via glycolysis linked to fermentation. Similarly, submerged rhizomes of waterlily show large fermentation activity when O₂ supply from aerenchyma is restricted, such as when no emergent leaves are present during long, deep winter submergence (Bucher *et al.* 1996). Thus, anaerobic energy production is likely to be of importance during submergence by long and deep floods. Furthermore, the variable O₂ supply might increase ROS, so defence against ROS is also likely to be of importance in tissues with marked dynamics in O₂ status.

Rhizomes and storage organs of marsh species can survive prolonged submergence when buried in anoxic mud over winter. Rhizomes of the marsh species *Acorus calamus* L. survived anoxia for more than 90 days (Crawford and Brändle 1996). Turions of *Potamogeton* species also survive winter in anoxic muds, and the shoot that emerges in spring is very anoxia tolerant; this tolerance is associated with the availability of large reserves of carbohydrates to fuel ethanolic fermentation, as well as regulation of the proteins synthesised during anoxia, so as to re-direct the flow of energy to essential processes (Sato *et al.* 2002; Dixon *et al.* 2006). In spring, shoots grow out of the anoxic sediments and into the water column, and O₂ becomes available from photosynthesis in illuminated water and eventually from air when the shoots reach the atmosphere (Crawford and Brändle 1996). Anaerobic energy production will be a vital trait to energise this escape from anoxic sediments, such as demonstrated also for germinating seeds of rice (e.g. coleoptile elongation, Ismail *et al.* 2009; Magneschi and Perata 2009) and

shoot elongation from turions (e.g. *P. pectinatus* L., Summers *et al.* 2000). Ethanol production rates in these specialist, highly anoxia-tolerant, shoot organs are amongst the fastest reported in plants (e.g. *P. pectinatus*, Summers *et al.* 2000). Yet, even in the coleoptiles of rice (Colmer *et al.* 2001; Huang *et al.* 2005) and in the elongating shoot of *P. pectinatus* (Dixon *et al.* 2006) several processes (e.g. protein synthesis) are downregulated so as to conserve energy, indicating that both energy production and regulation of consumption contribute to anoxia tolerance.

In summary (Table 1), tolerance of long duration, deep, submergence is characterised by some traits from the LOQS, and some from the LOES (Table 1). Plants cannot escape by reaching air, but instead acclimate so as to minimise the detrimental conditions. Thus, although species lack a strong shoot extension response, similar to the LOQS, many do not go into quiescence as new, aquatic-adapted leaves are produced. 'Aquatic' leaf acclimations reduce diffusion limitations against CO₂ and O₂ entry, thus enhancing underwater photosynthesis and respiration, so that the most adverse effects are somewhat 'avoided'. Such plants must also possess aerenchyma so that roots can also receive O₂, although many would be expected to also produce new aquatic roots. Significant diurnal fluctuations in tissue O₂ status, as dependent on light and photosynthetic O₂ production in shoots, mean that anaerobic metabolism for anoxia tolerance, and defence against ROS produced in this variable environment are of importance.

Conclusions and future prospects

Evidence has been presented for 'suites of complementary traits' occurring in specific combinations for plants inhabiting various types of flooding environments (Table 1). Traits contributing to tolerance of short-duration soil waterlogging are anoxia tolerance in root tissues, defence against ROS, and constitutive aerenchyma; and for longer-duration waterlogging the additional traits of induced aerenchyma, a barrier against ROL, and tolerance of reduced soil conditions. When overland floods submerge shoots, plant tolerance is determined by several shoot traits in addition to root traits described for waterlogging. The Low Oxygen Quiescence Syndrome (LOQS) improves survival in environments prone to flash-floods (short duration submergence), whereas the Low Oxygen Escape Syndrome (LOES) improves survival in environments with prolonged, shallow floods (Bailey-Serres and Voesenek 2008). Many species that inhabit areas with prolonged and deep floods also lack a shoot extension response (Voesenek *et al.* 2004), being a major component of the LOQS, but many of these plants produce new leaves adapted to the underwater environment (similarly to some plants displaying the LOES); this 'mixed response' conserves carbohydrates and also promotes avoidance of, rather than escape from, low O₂ stress.

Several examples of beneficial interactions between these wetland plant traits were described. As examples:

- (i) the combination of shoot elongation and large volumes of aerenchyma, to access and enable O₂ movement into the body of plants in areas with prolonged shallow floods. Although such O₂ movement can occur solely by diffusion, some species also possess convective through-flows. Waterlily is one example, but again the effectiveness of

this trait is determined by sub-traits; petiole elongation is a prerequisite for through-flows, as these can only occur when leaves are above water, and high aerenchyma in organs enhances through-flows, as the pathway resistance is less. The through-flows occur along rhizomes, so traits (e.g. large aerenchyma) that enhance O₂ diffusion into the adjoining roots are also required.

- (ii) the positive interaction between enhanced shoot elongation, leaf traits for improved underwater photosynthesis, and aerenchyma. While growing fast under water, leaves grow into better illuminated water layers. These better light conditions together with leaf traits that facilitate gas exchange can greatly enhance underwater photosynthesis. This not only improves the carbohydrate status of the plant thus sustaining elongation towards the water surface, but also improves internal aeration of the entire plant body, thus prolonging survival while still submerged.
- (iii) temporal and spatial differences for the plant body in reliance on suites of traits, such as those enhancing underwater photosynthesis and internal aeration during light periods, but traits for anoxia tolerance during dark periods. Diurnal fluctuations in tissue O₂ status, as dependent on light and photosynthetic O₂ production in shoots, as well as spatial differences depending on location in the plant body, means that both these sets of traits are essential; their contributions to survival depend on temporal and spatial differences in O₂ supply.

Future research on waterlogging and submergence tolerance should evaluate plants from various flooding regimes so as to reveal the synergies and trade-offs amongst traits possessed by plants in variable flooding environments.

Acknowledgements

We thank Rana Munns for suggestions on the final draft of this paper, and for inviting our review as part of the series in *Functional Plant Biology* to honour the 150th anniversary of the publication of Charles Darwin's '*On the Origin of Species by Means of Natural Selection*' (Munns 2009). We thank Julia Bailey-Serres and Ronald Pierik for constructive comments on a draft of this paper, and Eric Visser, Julia Bailey-Serres, Motoyuki Ashikari, and Abdel Ismail for advice/help with the Figures used. The ARC-NZ Research Network for Vegetation Function (WG39 – Wetland Plant Traits) provided us with an opportunity to discuss aspects of this review with colleagues. Waterlogging and flooding research in Colmer's laboratory is supported by GRDC (FF100004) and the Australian Research Council (LP0882350), and in Voesenek's laboratory by NWO and CBSG2012 grants.

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Manuscript received 5 June 2009, accepted 15 June 2009