New Phytologist

Review

Tansley review

How plants cope with complete submergence

Author for correspondence:
L. A. C. J. Voesenek
Tel: +31 30 253 6849
Fax: +31 30 251 8366
Email: L.A.C.J.Voesenek@bio.uu.nl

Received: *17 November 2005*
Accepted: *20 January 2006*

L. A. C. J. Voesenek[1], T. D. Colmer[2], R. Pierik[1], F. F. Millenaar[1] and A. J. M. Peeters[1]

[1]Plant Ecophysiology, Institute of Environmental Biology, Utrecht University, Sorbonnelaan 16, 3584 CA, Utrecht, the Netherlands; [2]School of Plant Biology, Faculty of Natural and Agricultural Sciences, The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia

Contents

Key words: aerenchyma, ethylene, flooding, oxygen, shoot elongation, signal transduction, underwater photosynthesis.

Summary

Flooding is a widespread phenomenon that drastically reduces the growth and survival of terrestrial plants. The dramatic decrease of gas diffusion in water compared with in air is a major problem for terrestrial plants and limits the entry of CO_2 for photosynthesis and of O_2 for respiration. Responses to avoid the adverse effects of submergence are the central theme in this review. These include underwater photosynthesis, aerenchyma formation and enhanced shoot elongation. Aerenchyma facilitates gas diffusion inside plants so that shoot-derived O_2 can diffuse to O_2-deprived plant parts, such as the roots. The underwater gas-exchange capacity of leaves can be greatly enhanced by a thinner cuticle, reorientation of the chloroplasts towards the epidermis and increased specific leaf area (i.e. thinner leaves). At the same time, plants can outgrow the water through increased shoot elongation, which in some species is preceded by an adjustment of leaf angle to a more vertical position. The molecular regulatory networks involved in these responses, including the putative signals to sense submergence, are discussed and suggestions made on how to unravel the mechanistic basis of the induced expression of various adaptations that alleviate O_2 shortage underwater.

New Phytologist (2006) **170**: 213–226

doi: 10.1111/j.1469-8137.2006.01692.x

I. Introduction

Flooding is one of the most common and widespread of all natural disasters. It is detrimental for nearly all terrestrial plants and results in hampered growth and ultimately death of many plant species. Flooding can have catastrophic impacts on the productivity of arable farmland, as most crops are intolerant to excess water (http://www.plantstress.com/Articles/waterlogging_i/waterlog_i.htm).

The negative impact of flooding on terrestrial plant life is a consequence of the slow diffusion rates of gases in water compared with in air and the relatively low solubility of O_2 in water (Jackson, 1985; Armstrong & Drew, 2002). In nonphotosynthesising plant tissues, such as roots, O_2 concentrations decline strongly upon submergence (Gaynard & Armstrong, 1987; Armstrong *et al.*, 1994), whereas CO_2 produced during respiration and fermentation probably accumulates (Crawford, 1992). Submerged green tissues, on the other hand, are characterized by a significant diurnal variation in gas composition (Raskin & Kende, 1984a). During daytime, photosynthesis depletes endogenous CO_2 concentrations, whereas O_2 concentrations can be maintained close to 21%. During the night, however, endogenous O_2 concentrations are reduced as a result of respiration, but complete depletion within shoot tissues should be rare because the surrounding water generally contains O_2 (Mommer *et al.*, 2004). A third, endogenously produced, gas, which changes in concentration when plant tissues are submerged, is the plant hormone ethylene. Physical entrapment, combined with a very low rate of catabolism (Hall, 1991), leads to an increase of ethylene inside the tissues of submerged plants. A physiologically relevant increase in ethylene concentration can occur within minutes after submergence (Vreeburg *et al.*, 2005). Despite these dramatic changes in gas composition and the stress it imposes, some plant species, especially those growing in flood-prone areas, have a remarkable tolerance and can grow vigorously under flooded conditions. However, species from flood-exposed environments are not always flood tolerant. Some avoid the adverse effects of flooding by timing of life cycle events (Blom & Voesenek, 1996). Here, we review how plants regulate morphological adjustments that help to prevent severe O_2 shortages during submergence.

It is generally accepted that energy deficit, caused by a shortage of O_2, and thus inhibition of respiration, is one of the most severe problems encountered by plants when subjected to flooding. Oxygen is the terminal acceptor of electrons in the oxidative phosphorylation that indirectly provides the plant with ATP. Metabolic acclimations that aim to continue energy production in plants during anoxia were reviewed recently by Sauter (2000), Dolferus *et al.* (2001), Geigenberger (2003), Gibbs & Greenway (2003) and Greenway & Gibbs (2003). When O_2 concentrations decline (e.g. ≤ 5%), glycolysis and fermentation pathways are stimulated in *Zea mays* (Sachs *et al.*, 1996) – the classic 'Pasteur' effect. Consistent with this biochemical observation at low conentrations of O_2 (≤ 5%) are the results of several microarray studies with *Arabidopsis* that showed an up-regulation of genes coding for enzymes of sugar metabolism, glycolysis and fermentation upon low O_2 concentrations (Klok *et al.*, 2002; Liu *et al.*, 2005; Loreti *et al.*, 2005). The induction of these pathways allows continuation of at least some energy production when anoxia starts. Low O_2 also induces the expression of nonsymbiotic hemoglobin genes (Dordas *et al.*, 2004). It has been suggested for *Z. mays* that hemoglobin can detoxify NO produced during hypoxia (Dordas *et al.*, 2003) and/or play a role in the regeneration of NAD^+ necessary to maintain glycolysis (Sowa *et al.*, 1998; Igamberdiev *et al.*, 2005). Another group of genes that are up-regulated at low O_2 concentrations are associated with the detoxification of reactive oxygen species (peroxidase, ascorbate peroxidase, monohydroascorbate, gluthatione reductase and superoxide dismutase) (Klok *et al.*, 2002). It is assumed that the products of these genes play roles in the protection against postanoxia injury (Monk *et al.*, 1989).

A second strategy in plants that inhabit areas prone to submergence is functionally associated with the long-term avoidance of low internal O_2 partial pressures. For example, a major adjustment induced by complete submergence is a change in leaf morphology and anatomy. Leaves of *Rumex palustris*, *R. crispus* and *Phalaris arundinacea* developed underwater have a higher specific leaf area (SLA; $m^2\ kg^{-1}$) (Vervuren *et al.*, 1999; Mommer *et al.*, 2005a), and *R. palustris* leaves also have a thinner epidermal cell wall and cuticle, and the chloroplasts are oriented towards the epidermis instead of the intercellular spaces (Mommer *et al.*, 2005b). These acclimations result in higher underwater rates of photosynthesis and lower CO_2 compensation points, ultimately generating higher internal O_2 concentrations in submerged plants (Mommer *et al.*, 2004, 2005a,b). In addition, many species show stimulated elongation of shoot organs; a trait that can restore contact of the leaves with air. Aerenchyma formation is also common in wetland species. The aerenchyma creates longitudinally interconnected pathways of gas spaces that, together with the renewed contact of leaf tips with the atmosphere through enhanced shoot elongation, enable diffusion of O_2 from the air to the root tips. Ethylene is a key regulator of the formation of lysigenous aerenchyma, at least in *Z. mays*, through programmed cell death (Drew *et al.*, 2000), and stimulated shoot elongation mediated by cell-wall loosening in rice and *Rumex* (Voesenek & Blom, 1999; Sauter, 2000; Vreeburg *et al.*, 2005). In *Arabidopsis*, genes involved in the regulation of ethylene synthesis, ethylene signaling, programmed cell death and cell-wall loosening are up-regulated upon low O_2 conditions (Klok *et al.*, 2002; Liu *et al.*, 2005).

The present review summarizes the recent progress in the molecular and physiological regulation of mechanisms that contribute to long-term anoxia avoidance, namely stimulated shoot elongation, aerenchyma formation and underwater photosynthesis, which ultimately enhance the internal O_2 status of plants in flooded environments. In addition, the importance of understanding submergence sensing, and the opportunities for rapid progress in submergence research by the use of *Arabidopsis*, will be highlighted.

II. Sensing of submergence

In order to react appropriately to the flooded environment, plants have to sense the changes that take place. The changes

in endogenous gas composition make O_2, CO_2 and C_2H_4 potential molecules to signal submergence. However, the availability of light complicates the matter with respect to CO_2 and O_2 because their internal concentrations are strongly influenced by both photosynthesis and respiration. For this reason, C_2H_4 is considered to be a far more reliable indicator for complete submergence in green shoot tissues (Voesenek & Blom, 1999). In addition to these gases, reactive oxygen species (ROS), which may accumulate during flooding, have also been proposed to have a regulatory role during submergence (Bailey-Serres & Chang, 2005; Blokhina *et al.*, 2003). Furthermore, we cannot exclude a direct role for light quality and quantity as submergence signals as both can change upon submergence (Holmes & Klein, 1987).

Ethylene

In *R. palustris*, ethylene concentrations increase 20-fold within the first hour of submergence as a result of physical entrapment (Voesenek *et al.*, 1993; Banga *et al.*, 1996). The production rate of ethylene during submergence is maintained at the same level in *Rumex* (Voesenek *et al.*, 1993; Vriezen *et al.*, 1999), or increased, as in the internode tissue of deepwater rice (Kende *et al.*, 1998). Biosynthesis of ethylene is a well-characterized process (Wang *et al.*, 2002) that is dependent on the presence of O_2 (Kende, 1993). In *R. palustris*, it was shown that ethylene plays a pivotal role in initiating responses to submergence, although it is not fully clear whether it is the sensing signal. In flooding-tolerant *Rumex* species, the 1-aminocyclopropane-1-carboxylic acid concentration (ACC, a precurser of ethylene) strongly increased upon submergence, whereas the conversion to ethylene was inhibited. At the gene expression and protein levels (*in vitro* assay), increased expression of ACC synthase genes, and resulting enzyme activity, were observed (Vriezen *et al.*, 1999; Voesenek *et al.*, 2003; Rieu *et al.*, 2005). In *R. palustris*, ACC oxidases are strongly up-regulated during submergence and this correlates with an increase in enzyme activity, measured *in vitro* (Vriezen *et al.*, 1999; Voesenek *et al.*, 2003). The increased concentration of ACC oxidase enzyme during submergence possibly counter-balances the reduced enzyme activity at low O_2 concentrations, thus sustaining ethylene production during submergence (Vriezen *et al.*, 1999).

In plants, ethylene is detected by a set of receptors. These and the subsequent signal-transduction cascade have been elegantly clarified over the last decade in *A. thaliana*, using genetic techniques (Chang, 2003; Alonso & Stepanova, 2004; Guo & Ecker, 2004; Stepanova & Alonso, 2005). An *R. palustris* orthologue of the ethylene response sensor (ERS) ethylene receptor gene was isolated and shown to be strongly up-regulated upon submergence. This desensitizes the tissue towards ethylene (Vriezen *et al.*, 1997), which results from the fact that ethylene receptors actively suppress a downstream regulator of ethylene responses when the receptors have not bound ethylene. This desensitization has probably no effect on the ethylene-induced responses, as ethylene concentrations were found to be saturated in the shoots of submerged plants (Voesenek *et al.*, 1993; Banga *et al.*, 1996).

Carbon dioxide

The role of CO_2 as a sensing molecule in submergence research has received limited attention. High concentrations of CO_2 do not affect petiole elongation in *R. palustris* (Voesenek *et al.*, 1997) but it was shown, in rice, that elevated CO_2 concentrations (6%) could induce elongation of internodes, albeit to a much lesser extent than ethylene (Raskin & Kende, 1984a). Recently, Mommer *et al.* (2005b) showed, in the leaves of submerged plants, reorientation of chloroplasts towards the leaf exterior where CO_2 diffuses in through the cuticle and cell wall. This, and other changes, led to a decreased diffusion resistance for CO_2 to reach Rubisco, resulting in higher underwater assimilation rates at low external CO_2 concentrations. These data suggest that plant cells, in addition to guard cells, are able to sense the CO_2 concentration and respond to it accordingly. Whether these alterations are caused by CO_2 sensing, possibly through a mechanism as in guard cells, or by other mechanisms (perhaps even independent of CO_2), is still to be elucidated.

Much work has been carried out on the sensing of CO_2 in relation to stomatal opening and closure. However, despite the numerous publications on this subject, the CO_2-sensing pathway(s) of guard cells remain poorly understood and may involve cytosolic pH and malate, zeaxanthin, Ca^{2+}, or anion channels in the short-term response to CO_2 (Assmann, 1999; Schroeder *et al.*, 2001).

Elevated concentrations of CO_2 also affect metabolic rates of plant species, as reviewed by (Gonzàles-Meler *et al.*, 1996). Mangrove seedlings demonstrated a marked reduction in the rate of respiration when exposed to low O_2 concentrations in the presence of external CO_2 (Brown *et al.*, 1969). By contrast, exposure of dryland species, such as *Cicer ariefinum*, *Tussilago farfara* and *Elymus repens*, to elevated concentrations of CO_2 up-regulated the glycolytic activity and reduced the viability (Crawford, 1992).

Oxygen

The O_2 concentration in plant tissues decreases upon submergence (Rijnders *et al.*, 2000). Furthermore, it has been shown that low O_2 could sensitize petiole tissues to ethylene, thus suggesting an interaction between ethylene and O_2 (Voesenek *et al.*, 1997). Another indication of the interaction between ethylene and O_2 was found by Peng *et al.* (2001), who showed that ethylene signaling is required for hypoxia-induced gene expression. Furthermore, Kamaluddin & Zwiazek (2002) showed that enhanced ethylene concentrations can overcome the hampered hydraulic conductivity in roots caused by low O_2 concentrations (Tournaire-Roux *et al.*, 2003).

Decreases of O_2, ranging from low concentrations (hypoxia) to complete absence (anoxia), in plants have been associated with changes in the expression of genes related to carbohydrate and lipid metabolism, glycolysis, fermentation pathways, ethylene synthesis, auxin-mediated processes, and calcium- and ROS-mediated signal transduction pathways (Klok *et al.*, 2002; Paul *et al.*, 2003; Branco-Price *et al.*, 2005; Liu *et al.*, 2005; Loreti *et al.*, 2005). Moreover, morphological responses, such as aerenchyma and adventitious root formation, and cell and organ elongation of shoots, are induced in some species. To date, no direct O_2 sensor in plants has been identified (Drew, 1997; Geigenberger, 2003). This is in contrast to animal cells where the hypoxia-inducible heterodimeric transcription factor (HIF, reviewed by Bruick, 2003) subunit, HIF1α, is regulated by an O_2-dependent HIF1α prolyl hydroxylase. It was shown that the activity of this enzyme in *Drosophila melanogaster* and *Caenorhabditis elegans* is proportional to the O_2 availability and, as a consequence, this system might be considered as a direct O_2-sensing system (Bruick, 2003). Another (albeit indirect) mechanism by which O_2 deprivation might be sensed is the rapid acidification of the cytosol upon O_2 deprivation (Roberts *et al.*, 1984). This is a quick response to the energy crisis experienced by cells under anoxia. The lower pH is relatively stable in anoxia-tolerant tissues, but can decline further in less tolerant tissues (Felle, 2005). There has been much debate about the precise role of cytosolic acidification, but it can affect cellular metabolism (Geigenberger, 2003) and water conductivity across membranes (Tournaire-Roux *et al.*, 2003), and could thus play a role in indirect O_2 sensing (Bailey-Serres & Chang, 2005; reviewed in Felle, 2005).

Hydrogen peroxide

It is generally accepted that biotic and abiotic stresses, among these hypoxia and anoxia, lead to a change in H_2O_2 production (Apel & Hirt, 2004; Mittler *et al.*, 2004). In particular, H_2O_2 produced by cytosolic membrane-bound NADPH oxidases has been associated with stress responses (Laloi *et al.*, 2004). As H_2O_2 is potentially damaging, there are cellular antioxidant mechanisms that remove it very efficiently (reviewed in Noctor & Foyer, 1998; Corpas *et al.*, 2001).

Recent reviews by Neill *et al.* (2002), Laloi *et al.* (2004), Apel & Hirt (2004) and Mittler *et al.* (2004) elaborate on the progress made on various aspects in ROS research. More specifically, Bailey-Serres & Chang (2005) discuss the current state-of-the-art about the role of ROS in sensing O_2 deprivation. There is evidence that the change in ROS in the particular cases of hypoxia and anoxia in plants are mediated by a group of compounds called the RHO-like GTPases (ROP). Activation of ROP after treatment with a low concentration of O_2 induces H_2O_2 production and this seems necessary for low O_2 signaling (Baxter-Burrell *et al.*, 2002, 2003; Bailey-Serres & Chang, 2005). Components of the signal-transduction pathway of ROS itself have been revealed by recent studies (reviewed in Apel & Hirt, 2004; Mittler *et al.*, 2004). ROS might be sensed by two-component sensors possessing a histidine kinase, subsequently inducing a mitogen-activated protein kinase (MAPK) signaling pathway, and it has been speculated that ROS may inhibit protein phosphatases, and with some evidence also that ROS can activate transcription factors directly. All of these actions lead to changed gene expression.

Interestingly, it has been shown that ETR1, one of the receptors in the ethylene signaling pathway, mediates stomatal closure in response to H_2O_2 (Desikan *et al.*, 2005). A cysteine at position 65 was essential for this possible interaction between ethylene and H_2O_2 (Desikan *et al.*, 2005). Moreover, it was shown by D'Haeze *et al.* (2003) that ROS production may precede ethylene production during Nodulation factor-stimulated root hair growth, suggesting another interaction between ROS and ethylene.

Light

Light quantity attenuates strongly in water as a result of its absorptive properties and of the absorptive, reflective and scattering properties of suspended material within the water (Holmes & Klein, 1987). Vervuren *et al.* (2003) analysed the light transmission of river Rhine floods and showed that inundations with a depth of 50 cm already have a transmission reduction of 90%, whereas deep floods (> 1.5 m) have light transmissions of < 1%.

Reduced intensities of light and submergence with normal light conditions are both able to induce a similar set of traits in *Rumex* species, such as an increase in petiole length and SLA, and a decline in the underwater compensation point for light (Mommer *et al.*, 2005a). Moreover, both low light and submergence can induce hyponastic growth in *A. thaliana* Col (Millenaar *et al.*, 2005; Pierik *et al.*, 2005). The resemblance in the morphological and biochemical responses to low light and submergence might simply be explained by separate signal-transduction cascades that both affect a common downstream component. Alternatively, it is conceivable that these two pathways operate synergistically or additively when both signals are present at the same time (flooding with a strong attenuation of light). Recently, an additive effect was observed for petiole elongation in *R. palustris*: submerged plants in low light had significantly longer petioles than plants submerged in high light (Mommer *et al.*, 2005a).

In addition to decreases in light quantity, the spectral composition of light changes when it passes through water. Water specifically absorbs the longer wavelength, like far-red (FR), which results in an increase of the red (R) : FR ratio (reviewed in Holmes & Klein, 1987). In turbid water, caused by, for example, silt or clay, the light is scattered and also the shorter wavelengths are absorbed (ultraviolet and blue light). Therefore, in turbid water, the green wavelengths penetrate the furthest (reviewed in Holmes & Klein, 1987).

Elongation of plant organs, such as petioles, is stimulated by low intensities of blue light and a low R : FR ratio (Pierik *et al.*, 2004; Pierik *et al.*, 2005). Therefore, because the R : FR ratio increases in submerged environments, we conclude that submergence-induced elongation is not induced by this change in the R : FR ratio. However, a stimulating contribution of low blue light intensities can occur as this light signal is attenuated in water under turbid field conditions.

Summary of possible submergence signals

Based on the information presented above there is no clear-cut conclusion to be drawn about how submergence is sensed. There is ample evidence that in *R. palustris*, ethylene is responsible for very rapid responses, although the exact role of ethylene needs to be further elucidated. There are indications for possible interactions between ethylene and low O_2, and ethylene signaling and H_2O_2, but these have to be studied further. At this point we do not know whether submergence-induced responses are influenced by direct or indirect sensing of changes in O_2 concentration, or changes in ROS and/or other second messengers, such as NO and Ca^{2+}. The effect of light is probably restricted to conditions in which the floodwater has a high turbidity.

III. Internal aeration and underwater photosynthesis

Internal aeration provides O_2 to submerged organs of plants in flooded environments; enabling aerobic respiration even when the adjacent external environment limits O_2 entrance (Jackson & Armstrong, 1999). In addition, a portion of the O_2 transported to roots is lost radially [radial O_2 loss (ROL)], particularly from laterals and tips of the main axes in the case of many wetland species (Armstrong, 1979; Colmer, 2003). ROL re-oxygenates the rhizosphere (Pedersen *et al.*, 1995; Revsbech *et al.*, 1999), thereby protecting roots from the reduced substances (e.g. Fe^{2+}, Mn^{2+}, S^{2-}, organic acids) commonly present in flooded soils (Ponnamperuma, 1984).

In emergent wetland plants, atmospheric O_2 enters the shoot and moves, via aerenchyma, to the tips of the roots. Gas transport in shoots is mostly diffusive, but can (in some species) occur through pressure-driven flows that force gases along the lacunae in stems or petioles and rhizomes, at least when environmental conditions are suitable (Dacey, 1980; Armstrong & Armstrong, 1991, reviewed in Colmer, 2003). In contrast to throughflow convection, demonstrated elegantly in water lilies (Dacey, 1980) and reed (Brix, 1988; Armstrong & Armstrong, 1991), no significant aeration contribution can be expected from a second type of convective gas flow, the so-called nonthroughflow convection (Beckett *et al.*, 1988).

When plants are completely submerged, however, direct access to atmospheric O_2 is abolished. The sources of O_2 to completely submerged plants are those produced during photosynthesis and present in the floodwaters (Sand-Jensen *et al.*, 2005). Thus, submerged plants can experience marked diurnal changes in tissue O_2 concentrations, the O_2 concentration being highest during the day and lowest during the night (e.g. for rice; Waters *et al.*, 1989). The consequences of distance within the plant body along the diffusion path from an O_2 source, and the combined resistances (physical and O_2 consumption) to diffusion along the pathway, have been reviewed in detail (Armstrong & Drew, 2002; Colmer & Greenway, 2005). The remainder of this section will focus on environmental factors and plant traits, determining: (i) aerenchyma formation; (ii) rates of photosynthesis by submerged plants; and (iii) O_2 exchange between the shoot and floodwater.

Aerenchyma formation

Aerenchyma is tissue containing large gas-filled spaces (lacunae) that interconnect longitudinally to provide a low-resistance pathway for long-distance gas transport along plant organs. The presence of lacunae provides increased porosity (gas volume per unit tissue volume) of tissues to levels much higher than the inherent values resulting from the usual intercellular gas-filled spaces formed as a constitutive part of development. In roots, aerenchyma usually forms in the cortex, whereas in stems (including rhizomes) aerenchyma can occur in the cortex and in the pith cavity (Armstrong, 1979). The volume of aerenchyma formed depends on genotype (species, as well as cultivar/accession) and environmental conditions (Colmer, 2003).

Two basic forms of aerenchyma have been recognized (Sachs, 1882), namely that which develops by the separation of cells ('schizogenous' aerenchyma) or that which develops by the collapse of cells ('lysigenous' aerenchyma) (Fig. 1), although within both of these main 'types', several different processes can form the resulting aerenchyma (Justin & Armstrong, 1987; Longstreth & Borkhsenious, 2000; Seago *et al.*, 2005).

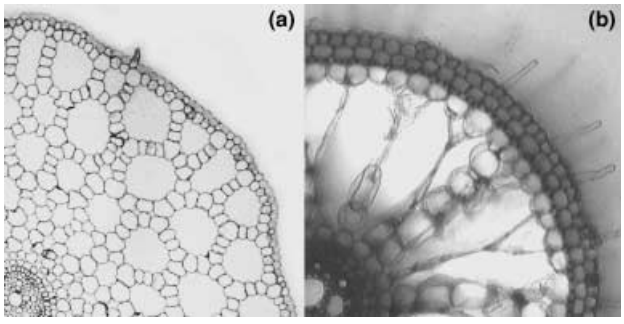

Fig. 1 Two main types of aerenchyma: schizogenous aerenchyma in a *Rumex palustris* adventitious root (a), and lysigenous aerenchyma in a rice adventitious root (b). The 'honeycomb-type' schizogenous aerenchyma in *R. palustris* forms as a result of cells being forced apart because of oblique divisions by some of the cortical cells in radial rows. The lysigenous aerenchyma in rice forms owing to the collapse of radial files of cortical cells.

In many wetland plants, aerenchyma forms constitutively, but is further enhanced in response to flooding. In most nonwetland species, aerenchyma is largely absent until it is induced by environmental conditions, such as flooding (Armstrong, 1979; Colmer, 2003), and also, in some species, by soil compaction or nutrient deficiencies (Drew *et al.*, 1989; He *et al.*, 1996). Information on the regulation of formation of schizogenous types of aerenchyma is scant, whereas the induction of lysigenous aerenchyma has been studied in some detail, at least in roots of *Z. mays*.

The formation of lysigenous aerenchyma involves the removal of files of cortical cells, leaving enlarged gas-filled spaces, although the remnants of cell walls persist (Fig. 1b). Ethylene is the primary signal that initiates programmed cell death in the cortical cells that are removed (Drew *et al.*, 2000). Numerous files of cells die, but some files remain intact and these maintain symplastic connections from the stele to the rhizodermis. The mechanisms by which some radial files respond to the ethylene signal (and die), whilst tangential neighbours do not, is unknown (Evans, 2004). Nevertheless, some aspects of the signaling pathway, details of the ultrastructural events during the programmed cell death, and several biochemical aspects (particularly related to cell-wall degradation), have been described. Drew *et al.* (2000) proposed a hypothetical working model for ethylene signaling, ultimately resulting in the phosphorylation of (unidentified) downstream proteins, which then presumably regulate the required changes in gene expression to control the programmed cell death in these target cells. Stages of the ultrastructural changes in cells destined to die to form lysigenous aerenchyma are described in detail for the roots of *Z. mays* (Gunawardena *et al.* 2001), as are changes in expression of enzymes (cellulases and XET) proposed to have roles in cell-wall degradation during aerenchyma formation (He *et al.*, 1994; Saab & Sachs, 1996). As also indicated in other reviews (Jackson & Armstrong, 1999; Evans, 2004), further studies are needed to elucidate the molecular basis of aerenchyma formation in plants in greater detail.

Underwater photosynthesis

Photosynthesis in submerged plants can commonly be restricted by low availability of light and/or CO_2 (Sand-Jensen, 1989; Maberly & Spence, 1989). Light reaching the leaves of submerged plants is attenuated by water, dissolved organic matter, particles (e.g. silt) and/or phytoplankton suspended in the water column (Bach *et al.*, 1998; Vervuren *et al.*, 2003). It was recently demonstrated for the river Rhine system in the Netherlands that floods with a water depth of 1 m can have a light transmission below 1% (Vervuren *et al.*, 2003). On the other hand, light intensities are not necessarily always low underwater, as demonstrated in a study where a photosynthetically active radiation (PAR) value of > 600 μmol m^{-2} s^{-1} was measured at a flooding depth of 30 cm in an abandoned clay pit in the Netherlands, the typical environment of *R. palustris* (Mommer *et al.*, 2006).

CO_2 concentrations in most water bodies are much lower than the concentrations required to saturate photosynthesis by leaves of vascular plants (under saturating light and well-mixed conditions), and even that by aquatic species (Sand-Jensen, 1989). Diurnal fluctuations in dissolved CO_2 commonly occur in floodwaters, with a build-up of CO_2 during darkness and depletion to concentrations well below those in air-saturated water when light is available for photosynthesis (e.g. to 0.003 mol m^{-3} in flooded rice fields, Setter *et al.*, 1987). Rates of CO_2 uptake by leaves can limit photosynthesis, owing to the already mentioned 10^4-times slower diffusion of gases in water than in air, resulting in the movement of CO_2 across boundary layers adjacent to leaves becoming rate-limiting, even in submergence-acclimated leaves of aquatic species (Smith & Walker, 1980; Maberly & Spence, 1983; Raven *et al.*, 1985). Furthermore, for leaves of terrestrial species that become submerged, the cuticle is a major component of the resistance to CO_2 entry, further impeding underwater photosynthesis (Mommer *et al.*, 2005b). In addition, low concentrations of CO_2, relative to O_2, increase photorespiration in submerged leaves (Mommer & Visser, 2005; Mommer *et al.*, 2005b).

Physiological and morphological adaptations in leaves of aquatic and amphibious macrophytes have been reviewed in detail (Maberly & Spence, 1983, 1989; Raven *et al.*, 1985; Bowes & Salvucci, 1989; Madsen & Sand-Jensen, 1991). CO_2-concentrating mechanisms, the capacity to utilize HCO_3^- and a leaf morphology to reduce boundary layer thickness (e.g. dissected leaves), were identified as important adaptations to access CO_2 in aquatic environments. Thin cutical layers that are relatively permeable to CO_2 are also important (Frost-Christensen *et al.*, 2003; Mommer *et al.*, 2005b). Other strategies to access CO_2 were noted, such as, in many species, the development of floating leaves or aerial leaves to access atmospheric CO_2 (and O_2), or the more specialized strategy by certain plants that exploit CO_2 from the sediment (Raven *et al.*, 1988).

Leaf morphology and anatomy

Diffusion across the cuticle might become the major pathway for CO_2 entry into submerged leaves (Frost-Christensen *et al.*, 2003), even for leaves with stomata (i.e. aerial leaves of amphibious species and leaves of emergent wetland plants); although information on whether the stomata remain open, or close, during submergence appears to be scarce. Most stomata of *R. palustris* are closed when this plant is completely submerged (Mommer *et al.*, 2005b). Resistance to gas movement across the cuticle can be less in water-grown leaves than in air-grown leaves of several species (Frost-Christensen *et al.*, 2003; Mommer *et al.*, 2005b).

In response to long-term (e.g. more than several days) submergence, some plants can produce new acclimated leaves

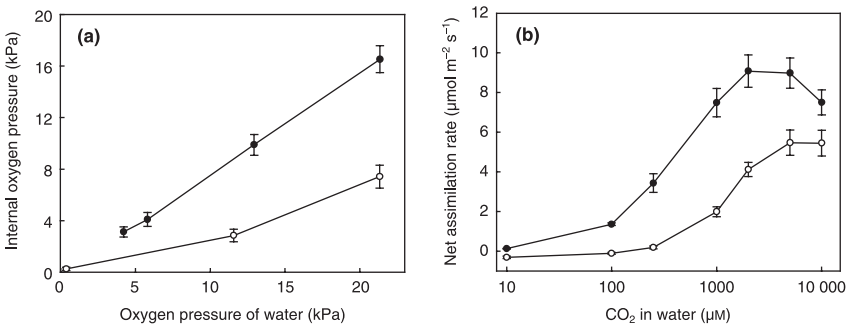

Fig. 2 Acclimation responses to submergence in *Rumex palustris* leaves formed underwater: the new leaves show reduced gas diffusion resistance (a; measured in darkness, data from Mommer *et al*., 2004. © Blackwell Publishing, reprinted with permission) resulting in enhanced rates of underwater photosynthesis (b; data from Mommer *et al*., 2005b. © American Society of Plant Biologists, reprinted with permission). black circles, acclimated leaf; white circles, nonacclimated leaf.

underwater that have an enhanced gas exchange with the water column (Mommer & Visser, 2005). For example, young leaves of *Rumex* species elongate upon submergence, and although most of the elongation growth occurs in the petiole, the lamina also elongates and becomes narrower (e.g. for *R. palustris* see Fig. 2 in Mommer & Visser, 2005). This elongated leaf morphology would be expected to result in smaller boundary layers than present for the broader lamina of nonelongated leaves. Next to becoming narrower, the leaves of submergence-tolerant *R. palustris* and *R. crispus* also become thinner (i.e. increased SLA), which can enhance gas exchange between the leaf and the surrounding water (Vervuren *et al*., 1999; Mommer *et al*., 2005a). Furthermore, submergence-acclimated leaves of *R. palustris* had thinner epidermal cell walls, a thinner cuticle and the chloroplasts were orientated towards the epidermis, all of which would decrease diffusion resistance between the leaf surface and chloroplasts (Mommer *et al*., 2005b). Thus, changes in morphology and anatomy in submergence-acclimated leaves enhance CO_2 entry.

The enhanced capacity for underwater gas exchange by new leaves of *R. palustris* produced following submergence was evidenced by higher internal O_2 concentrations in petioles of these leaves during periods in light, when compared with O_2 concentrations in plants grown in air prior to submergence (Mommer *et al*., 2004). Higher internal O_2 presumably resulted from improved rates of CO_2 entry from the water column and thus enhanced rates of photosynthesis, and this was evident across a range of dissolved CO_2 concentrations (up to ≈ 1 mol m^{-3}) in a turbulent water column (Fig. 2b; Mommer *et al*., 2004; 2005a). Improved uptake of dissolved gases by the acclimated leaves was also demonstrated by manipulations of water column O_2 concentrations in the dark, whilst monitoring O_2 concentrations in the petiole. In darkness, acclimated leaves displayed higher internal O_2 concentrations at any given external O_2 concentration, indicating a greater entry of O_2 (Fig. 2a; Mommer *et al*., 2004).

In summary, leaves of submergence-tolerant plant species (e.g. *R. palustris*) can acclimate to an underwater environment, resulting in enhanced CO_2 uptake for photosynthesis and therefore providing a supply of sugars to the plant. In addition, O_2 status is improved not only during the daytime (O_2 derived from photosynthesis), but also during the night (enhanced entry of O_2 from the water column). An improved O_2 status in the shoots would benefit the roots, as internal O_2 diffusion via the aerenchyma to the roots would increase, not only enabling respiration within the roots, but also oxygenation of the rhizosphere. Despite these recent insights, much remains to be elucidated regarding the physiological and molecular regulation of the acclimations. As suggested by Mommer & Visser (2005), the two hormones ethylene and abscisic acid (ABA) would, however, be good starting points as these are known to be involved in the switch between aquatic and terrestrial leaf type formation (heterophylly) in aquatic plants (Lin & Yang, 1999; Kuwabara *et al*., 2003).

IV. Stimulated shoot elongation

Several plant species from wetland environments demonstrate dramatic changes in shoot anatomy, morphology and growth pattern when submerged partially or completely (Ridge, 1987). Spectacular submergence responses are the altered orientation of petioles from prostrate to vertical (hyponastic growth) and the strongly stimulated petiole and internode elongation (Fig. 3). Not all petioles and internodes within one plant are equally responsive, as demonstrated in a study on *R. palustris* (Groeneveld & Voesenek, 2003). In this study, both the very young and the oldest petioles possess only a very minimal competence to elongate, whereas intermediately aged petioles are characterized by an elongation response of up to 300%, measured over 10 d of submergence. Differentiation in responsiveness was also described for internode elongation in deepwater rice where only the youngest internodes respond to submergence (Sauter, 2000).

Shoot elongation underwater requires energy and carbohydrates for cell divisions and the synthesis of new cell-wall material. Evidence for these costs of elongation growth came from work on rice in which elongation underwater without reaching the water surface occurs at the expense of survival (Setter & Laureles, 1996). Lowland rice cultivars demonstrated a negative correlation between survival and elongation growth. Moreover, enhancing shoot-elongation capacity by the addition of the growth-promoting hormone, gibberellin

Fig. 3 Upon submergence, *Rumex palustris* quickly re-orientates its leaves into a more vertical position (hyponastic growth) followed by strong petiole elongation towards the water surface. Note that petiole elongation without the preceding hyponastic growth would not bring the leaf tips much closer to the water surface.

(GA), decreased survival, whereas an inhibitor (paclobutrazol) of GA biosynthesis, and therefore shoot elongation, enhanced survival. It is suggested that the nonelongating strategy is more suitable for short-lasting and/or deep-flooding events that cannot be outgrown, whereas fast underwater elongation increases fitness mainly under prolonged, but relatively shallow, floods (Setter & Laureles, 1996; Sauter, 2000). This hypothesis was recently confirmed in a study that combined field surveys in floodplains of the river Rhine (the Netherlands) with experiments on the shoot-elongation capacity of 22 selected plant species. Fast-elongating species were exclusively found in flood-prone environments characterized by slow drainage and shallow temporary pools. Under these environmental conditions, the costs of elongation are probably outweighed by benefits such as improved aeration and restored rates of photosynthesis when the leaves emerge. Nonelongating species were only found on rarely flooded sites and in environments with frequent, but short-lasting, floods (Voesenek *et al.*, 2004).

Hyponastic growth interacts with shoot elongation

One of the first phenotypic modifications that takes place upon submergence in some plant species is an increase in the angle, compared with the horizontal, of mainly the younger petioles, thus decreasing the distance between the tips of the leaf blade and the water surface (Banga *et al.*, 1997). This flooding-induced response has been described, to date, for *Ranunculus repens*, *Caltha palustris*, *Leontodon taraxacoides*, *Paspalum dilatatum*, *R. palustris* and *A. thaliana* (Ridge, 1987; Voesenek & Blom, 1989; Grimoldi *et al.*, 1999; Insausti *et al.*, 2001; Millenaar *et al.*, 2005). Interestingly, hyponastic growth responses are also described in response to other environmental cues, such as shading and high temperature (Ballaré, 1999; Pierik *et al.*, 2004; Millenaar *et al.*, 2005). In *R. palustris*, hyponastic growth is caused by differential cell elongation across the petiole base, with epidermal cells on the abaxial surface elongating faster than cells on the adaxial surface (Cox *et al.*, 2004). Furthermore, it was discovered that, in this species, hyponastic growth interacts with nondifferential petiole elongation. A prerequisite for flooding-induced petiole elongation is that a minimal petiole angle of 40–50° (from horizontal) has to be reached. When the initial petiole angle is lower than this threshold, no petiole elongation will take place until hyponastic growth has increased the petiole angle to 40–50° (Cox *et al.*, 2003).

Regulation of submergence-induced shoot elongation: hormone interactions and cell-wall events

Accumulated ethylene is probably the primary signal that triggers the plant to start a cascade of reactions ultimately leading to enhanced cell elongation in submerged shoot organs. A direct effect of this increased ethylene concentration is a fast decrease in the ratio between two other plant hormones: ABA and GA. This is related to a strong and fast reduction of the endogenous ABA concentration (Hoffmann-Benning & Kende, 1992; Benschop *et al.*, 2005) and an increase of the endogenous GA concentration (Rijnders *et al.*, 1997; Raskin & Kende, 1984b). The decrease of ABA

in petioles of *R. palustris* during submergence and treatment with ethylene is regulated via a fast down-regulation of several members of the 9-*cis*-epoxycarotenoid dioxygenase (NCED) gene family, a group of genes that limit ABA biosynthesis in higher plants (Benschop *et al.*, 2005). Furthermore, an increase (of 10–25%) of a product of ABA catabolism, phaseic acid, was observed in the time frame in which ABA decreased by 70% (Benschop *et al.*, 2005), suggesting increased ABA breakdown. Interestingly, application of the ABA biosynthesis inhibitor, fluridone, reduced the lag phase of submergence-induced petiole elongation in *R. palustris* by 50%, resulting in a very rapid elongation response. However, when this fluridone treatment was combined with a 1-MCP pretreatment (inhibitor of ethylene action), elongation growth underwater was completely abolished. This result indicates that a reduction of ABA by itself is insufficient to induce fast petiole elongation; the presence of elevated concentrations of ethylene is required (Benschop *et al.*, 2005).

It is clear that in both *Rumex* and rice, ABA acts as a negative regulator of underwater elongation. This raises the question as to how this is regulated at a physiological level. Recent data indicate that ABA directly interferes with GA biosynthesis. When submerged *R. palustris* plants were additionally treated with 10 μM ABA, no increase was observed in the endogenous GA1 concentration; this result was in contrast to submerged controls. Therefore, ABA probably inhibits the up-regulation of the growth-promoting hormone GA (Benschop *et al.*, 2005). In this respect, it is interesting to note that GA-induced growth stimulation in roots and elongation of shoots in *Arabidopsis* seedlings (Fu & Harberd, 2003; Vriezen *et al.*, 2004) is associated with the destruction of DELLA proteins in the 26S proteasome. These DELLA proteins, localized in the nucleus of plant cells, function as repressors of growth (Alvey & Harberd, 2004). In rice, GA binds to a soluble GA receptor (GID1) present in the nucleus. Upon GA binding, this receptor molecule interacts with DELLA proteins, resulting in the degradation of these growth-repressing DELLA proteins (Ueguchi-Tanaka *et al.*, 2005). It is highly possible that DELLA degradation is a prerequisite for ethylene-induced shoot elongation in submerged plants.

Ultimately, the hormonal changes described above result in cell elongation within the elongating organ (e.g. petioles, internodes). This requires loosening of cell walls, uptake of water and synthesis of cell-wall polysaccharides (Cosgrove, 1999). Elongation of internodal cells in deepwater rice is primarily related to increased cell-wall extensibility, and not to decreases in osmotic potential or increases in hydraulic conductance (Kutschera & Kende, 1988). Cell-wall extensibility is thought to be associated with cell-wall loosening proteins, such as those belonging to the family of expansins (McQueen-Mason *et al.*, 1992). Expansin activity has a pH optimum between 3.5 and 4.5 (McQueen-Mason *et al.*, 1992) and is hypothesized to break H-bonds between hemicelluloses and cellulose microfibrils (McQueen-Mason & Cosgrove, 1994). The expression of expansin genes and the protein activity correlate strongly with submergence-induced elongation in several plant species (Cho & Kende, 1997; Huang *et al.*, 2000; Kim *et al.*, 2000; Vreeburg *et al.*, 2005). Increased expression of expansins in deepwater rice was not only induced by submergence treatments, but also by GA application, indicating that expansin genes are a downstream target of GA action (Cho & Kende, 1997). However, in *R. palustris*, GA application cannot mimic the induction of *RpEXPA1* by submergence. This gene was the only expansin, out of a group of 13 *R. palustris* expansin genes, that was up-regulated in petioles upon submergence (Vreeburg *et al.*, 2005). The increased expression of *RpEXPA1* could be mimicked by ethylene treatment and repressed during submergence when plants were pretreated with the specific ethylene action inhibitor, 1-MCP (Vreeburg *et al.*, 2005). These results suggest that in *Rumex*, ethylene is at the base of at least two parallel operating signal transduction pathways: one leading to a decrease of the ABA: GA ratio and the other resulting in increased expression of the expansin gene and subseqent concentration of expansin protein (Vreeburg *et al.*, 2005). Ethylene also seems to be the triggering agent in expansin gene expression in the semiaquatic fern *Regnellidium diphyllum*, a species characterized by fast underwater elongation of the rachis (Kim *et al.*, 2000).

Moreover, ethylene plays a role in a process leading to cell-wall acidification in submerged shoots. The importance of cell-wall pH for petioles of *R. palustris* was recently illustrated in experiments in which buffers with a low (4.0) and more neutral (6.0) pH were injected into petioles (Vreeburg *et al.*, 2005). The low pH strongly stimulated elongation growth, whereas a temporary inhibition of underwater growth was observed when the neutral buffer was injected. An increased net efflux of H^+ was measured within minutes after starting a submergence treatment; a response that could be abolished completely when the petiole tissue was pretreated with 1-MCP (Vreeburg *et al.*, 2005).

V. Perspectives

The suite of submergence-induced avoidance responses described in this review all are functionally associated with preventing the adverse consequences of the very slow gas diffusion in water. The regulatory mechanisms leading to these responses upon detection of submergence are, however, with the exception of shoot elongation, not well understood. There is, for example, much to be learned about the regulatory mechanisms behind aerenchyma formation and the reduced gas diffusion resistance in leaves formed underwater. Ethylene is not only the key factor in enhanced shoot elongation, but is also a major player in the formation of aquatic leaves in aquatic plants and in lysigenous aerenchyma formation, at least in *Z. mays*. Many physiological and molecular processes interacting with ethylene during submergence have become known from work on stimulated shoot elongation. This

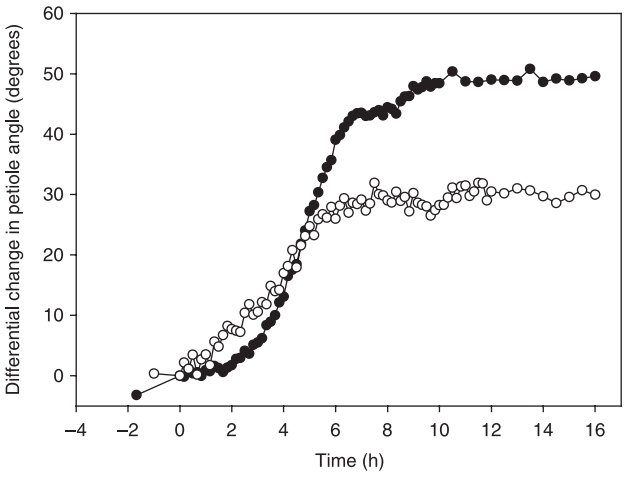

Fig. 4 Kinetics of ethylene-induced hyponastic growth in petioles of *Rumex palustris* and *Arabidopsis thaliana* are highly comparable. Data represent leaf angles in ethylene minus leaf angles of control plants, with values set to zero at $t = 0$. black circles, *Rumex*; white circles, *Arabidopsis*.

information could serve as a starting point for unravelling signaling cascades involved in the submergence-induced regulation of aerenchyma formation and reduced gas-diffusion resistance in leaves.

Although there is extensive information on signal transduction in submergence-induced hyponasty and shoot elongation, considerable progress is to be expected from the recent introduction of the model plant *Arabidopsis* in submergence research (Pierik *et al.*, 2005). The genetic variation in *Arabidopsis* for hyponasty (Millenaar *et al.*, 2005), ethylene sensitivity (Peeters *et al.*, 2002), and tolerance to full submergence (D. Tholen & L.A.C.J. Voesenek, unpublished) and soil flooding (Kolodynska & Pigliucci, 2003), is currently being exploited in quantitative trait loci (QTL) analyses. These analyses should enable the identification of genomic loci, and ultimately the underlying genes (Maloof *et al.*, 2001; El Din El Assal *et al.*, 2001) involved in hyponastic growth (Pierik *et al.*, 2005; B. Snoek & A. J. M. Peeters, unpublished). The kinetics of hyponastic growth in *Arabidopsis*, upon submergence and ethylene exposure, are remarkably similar to those of *R. palustris* (compare Cox *et al.*, 2003 with Millenaar *et al.*, 2005; Fig. 4), making it feasible to translate insights obtained from *Arabidopsis* to flood-tolerant species, such as *R. palustris*. Recent microarray studies on *Arabidopsis* have identified a number of candidate genes involved in hyponastic growth (Pierik *et al.*, 2005; F. F. Millenaar & A. J. M. Peeters, unpublished), and an ongoing mutant screen in our laboratory has already yielded several *Arabidopsis* mutants that fail to show hyponastic growth (M. van Zanten & F. F. Millenaar, unpublished).

Such molecular and genetic research strategies could also identify the regulatory networks that enhance internal aeration during submergence through changes in aerenchyma formation and underwater gas-exchange properties of leaves. These traits may not be well developed in *Arabidopsis* but can probably be investigated in rice, for which extensive genomic information is available and which shows a high degree of genetic variation for submergence tolerance (Jackson & Ram, 2003). Such studies would provide detailed knowledge on the physiological and molecular regulation of the plant responses to submergence, as highlighted in this review, adding to present knowledge on submergence tolerance in this important crop species (Jackson & Ram, 2003; Toojinda *et al.*, 2003). Such mechanistic knowledge is required for direct experimental tests of the costs and benefits of these responses (e.g. Voesenek *et al.*, 2004). This will ultimately elucidate how the different traits are inter-related and how the interactions between these traits determine submergence tolerance.

Acknowledgements

Research in our group is supported by the Dutch Science Foundation, PIONIER grant no. 800.84.470. We thank Dr O. Pedersen for stimulating discussions on underwater photosynthesis in aquatic species and Dr L. Mommer for helpful comments on a draft of this manuscript.

References

Alonso JM, Stepanova AN. 2004. The ethylene signaling pathway. *Science* **306**: 1513–1515.

Alvey L, Harberd NP. 2004. DELLA proteins: integrators of multiple plant growth regulatory inputs? *Physiologia Plantarum* **123**: 153–160.

Apel K, Hirt H. 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annual Review of Plant Biology* **55**: 373–399.

Armstrong W. 1979. Aeration in higher plants. *Advances in Botanical Research* 7: 225–332.

Armstrong J, Armstrong W. 1991. A convective through-flow of gases in *Phragmites australis* (Cav.) Trin. Ex Steud. *Aquatic Botany* **39**: 75–88.

Armstrong W, Drew MC. 2002. Root growth and metabolism under oxygen deficiency. In: Waisel Y, Eshel A, Kafkafi U, eds. *Plant roots: the hidden half.* New York, NY, USA: Marcel Dekker, 729–761.

Armstrong W, Brändle R, Jackson MB. 1994. Mechanisms of flood tolerance in plants. *Acta Botanica Neerlandica* **43**: 307–358.

Assmann SM. 1999. The cellular basis of guard cell sensing of rising CO_2. *Plant, Cell & Environment* **22**: 629–637.

Bach SS, Borum J, Fortes MD, Duarte CM. 1998. Species composition and plant performance of mixed seagrass beds along a siltation gradient at Cape Bolinao, The Philippines. *Marine Ecology Progress Series* **174**: 247–256.

Bailey-Serres J, Chang R. 2005. Sensing and signalling in response to oxygen deprivation in plants and other organisms. *Annals of Botany* **96**: 507–518.

Ballaré CL. 1999. Keeping up with the neighbors: phytochrome sensing and other signaling mechanisms. *Trends in Plant Science* **4**: 97–102.

Banga M, Slaa EJ, Blom CWPM, Voesenek LACJ. 1996. Ethylene biosynthesis and accumulation under drained and submrged conditions: a comparative study of two Rumex species. *Plant Physiology* **112**: 229–237.

Banga M, Bögemann GM, Blom CWPM, Voesenek LACJ. 1997. Flooding resistance of *Rumex* species strongly depends on their response to ethylene: rapid shoot elongation or foliar senescence. *Physiologia Plantarum* **99**: 415–422.

Baxter-Burrell A, Yang Z, Springer PS, Bailey-Serres J. 2002. RopGAP4-dependent Rop GTPase rheostat control of Arabidopsis oxygen deprivation tolerance. *Science* **296**: 2026–2028.

Baxter-Burrell A, Chang R, Springer P, Bailey-Serres J. 2003. Gene and Enhancer Trap transposable elements reveal oxygen deprivation-regulated genes and their complex patterns of expression in Arabidopsis. *Annals of Botany* **91**: 129–141.

Beckett PM, Armstrong W, Justin SHFW, Armstrong J. 1988. On the relative importance of convective and diffusive gas flows in plant aeration. *New Phytologist* **110**: 463–468.

Benschop JJ, Jackson MB, Gühl K, Vreeburg RAM, Croker SJ, Peeters AJM, Voesenek LACJ. 2005. Contrasting interactions between ethylene and abscisic acid in *Rumex* species differing in submergence tolerance. *Plant Journal* **44**: 756–768.

Blokhina O, Virolainen E, Fagerstedt KV. 2003. Antioxidants, oxidative damage and oxygen deprevation stress: a review. *Annals of Botany* **91**: 179–194.

Blom CWPM, Voesenek LACJ. 1996. Flooding: the survival strategies of plants. *Trends in Ecology and Evolution* **11**: 290–295.

Bowes G, Salvucci ME. 1989. Plasticity in the photosynthetic carbon metabolism of submersed aquatic macrophytes. *Aquatic Botany* **34**: 233–266.

Branco-Price C, Kawaguchi R, Ferreira RB, Bailey-Serres J. 2005. Genome-wide analysis of transcript abundance and translation in Arabidopsis seedlings subjected to oxygen deprivation. *Annals of Botany* **96**: 647–660.

Brix H. 1988. Light-dependent variations in the composition of the internal atmosphere of *Phragmites australis* (Cav.) Trin. ex Steud. *Aquatic Botany* **30**: 319–329.

Brown JMA, Outred HA, Hill CF. 1969. Respiratory metabolism in mangrove seedlings. *Plant Physiology* **44**: 287.

Bruick RK. 2003. Oxygen sensing in the hypoxic response pathway: regulation of the hypoxia-inducible transcription factor. *Genes and Development* **17**: 2614–2623.

Chang C. 2003. Ethylene signaling: the MAPK module has finally landed. *Trends in Plant Science* **8**: 365–368.

Cho HT, Kende H. 1997. Expression of expansin genes is correlated with growth in deepwater rice. *Plant Cell* **9**: 1661–1671.

Colmer TD. 2003. Long-distance transport of gases in plants: a perspective on internal aeration and radial oxygen loss from roots. *Plant, Cell & Environment* **26**: 17–36.

Colmer TD, Greenway H. 2005. Oxygen transport, respiration, and anaerobic carbohydrate catabolism in roots in flooded soils. In: Lambers H, Ribas-Carbo M, eds. *Plant Respiration.* Dordrecht, the Netherlands: Springer, 137–158.

Corpas FJ, Barroso JB, del Rio LA. 2001. Peroxisomes as a source of reactive oxygen species and nitric oxide signal molecules in plant cells. *Trends in Plant Science* **6**: 145–150.

Cosgrove DJ. 1999. Enzymes and other agents that enhance cell wall extensibility. *Annual Review of Plant Physiology and Plant Molecular Biology* **50**: 391–417.

Cox MCH, Millenaar FF, de Jong van Berkel YEM, Peeters AJM, Voesenek LACJ. 2003. Plant movement; submergence-induced petiole elongation in *Rumex palustris* depends on hyponastic growth. *Plant Physiology* **132**: 282–291.

Cox MCH, Benschop JJ, Vreeburg RAM, Wagemaker CAM, Moritz T, Peeters AJM, Voesenek LACJ. 2004. The roles of ethylene, auxin, abscisic acid and gibberellin in the hyponastic growth of submerged *Rumex palustris* petioles. *Plant Physiology* **136**: 2948–2960.

Crawford RMM. 1992. Oxygen availability as an ecological limit to plant distribution. *Advances in Ecological Research* **23**: 93–185.

D'Haeze W, de Rycke R, Mathis R, Goormachtig S, Pagnotta S, Verplancke C, Capoen W, Holsters M. 2003. Reactive oxygen species and ethylene play a positive role in lateral root base nodulation of a semiaquatic legume. *Proceedings of the National Academy of Sciences, USA* **100**: 11789–11794.

Dacey JWH. 1980. Internal winds in water lilies: an adaptation for life in anaerobic sediments. *Science* **210**: 1017–1019.

Desikan R, Hancock JT, Bright J, Harrison J, Weir I, Hooley R, Neill SJ. 2005. A role for ETR1 in hydrogen peroxide signaling in stomatal guard cells. *Plant Physiology* **137**: 831–834.

Dolferus R, Klok EJ, Ismond K, Delessert C, Wilson S, Good A, Peacock J, Dennis L. 2001. Molecular basis of the anaerobic response in plants. *International Union of Biochemistry and Molecular Biology Life* **51**: 79–82.

Dordas C, Rivoal J, Hill RD. 2003. Plant haemoglobins, nitric oxide and hypoxia stress. *Annals of Botany* **91**: 173–178.

Dordas C, Hasinoff BB, Rivoal J, Hill RD. 2004. Class-1 hemoglobins, nitrate and NO levels in anoxic maize cell-suspension cultures. *Planta* **219**: 66–72.

Drew MC. 1997. Oxygen deficiency and root metabolism: injury and acclimation under hypoxia and anoxia. *Annual Review of Plant Physiology and Plant Molecular Biology* **48**: 223–250.

Drew MC, He CJ, Morgan PW. 1989. Decreased ethylene biosynthesis, and induction of aerenchyma, by nitrogen- or phosphorus- starvation in adventitious roots of *Zea mays. Plant Physiology* **91**: 266–271.

Drew MC, He CJ, Morgan PW. 2000. Programmed cell death and aerenchyma formation in roots. *Trends in Plant Science* **5**: 123–127.

El-Din El-Assal S, Alonso-Blanco C, Peeters AJM, Raz V, Koornneef M. 2001. A QTL for flowering time in *Arabidopsis* reveals a novel allele of *Cry2. Nature Genetics* **29**: 435–439.

Evans DE. 2004. Aerenchyma formation. *New Phytologist* **161**: 35–49.

Felle HH. 2005. pH regulation in anoxic plants. *Annals of Botany* **96**: 519–532.

Frost-Christensen H, Bolt Jorgensen L, Floto F. 2003. Species specificity of resistance to oxygen diffusion in thin cuticular membranes from amphibious plants. *Plant, Cell & Environment* **26**: 561–569.

Fu X, Harberd NP. 2003. Auxin promotes *Arabidopsis* root growth by modulating gibberellin response. *Nature* **421**: 740–743.

Gaynard TJ, Armstrong W. 1987. Some aspects of internal plant aeration in amphibious habitats. In Crawford RMM, ed. *Plant life in aquatic and amphibious habitats*. Oxford, UK: Blackwell Scientific Publications, 303–320.

Geigenberger P. 2003. Response of plant metabolism to too little oxygen. *Current Opinion in Plant Biology* **6**: 247–256.

Gibbs J, Greenway H. 2003. Mechanisms of anoxia tolerance in plants. I. Growth, survival and anaerobic catabolism. *Functional Plant Biology* **30**: 1–47.

Gonzàles-Meler MA, Drake BG, Azcón-Bieto J. 1996. Rising atmospheric carbon dioxide and plant respiration. In: Breymeyer AI, Hall DO, Melillo JM, Agren GI, eds. *Global change: effects on coniferous forests and grasslands*. Chichester, UK: John Wiley & Sons Ltd, 161–182.

Greenway H, Gibbs J. 2003. Mechanisms of anoxia tolerance in plants. II. Energy requirements for maintenance and energy distribution to essential processes. *Functional Plant Biology* **30**: 999–1036.

Grimoldi AA, Insausti P, Roitman GG, Soriano A. 1999. Responses to flooding intensity in *Leontodon taraxacoides. New Phytologist* **141**: 119–128.

Groeneveld HW, Voesenek LACJ. 2003. Submergence-induced petiole elongation in *Rumex palustris* is controlled by developmental stage and storage compounds. *Plant and Soil* **253**: 115–123.

Gunawardena AHLAN, Pearce DM, Jackson MB, Hawes CR, Evans DE. 2001. Characterisation of programmed cell death during aerenchyma formation induced by ethylene or hypoxia in roots of maize (*Zea mays* L.). *Planta* **212**: 205–214.

Guo H, Ecker JR. 2004. The ethylene signaling pathway: new insights. *Current Opinion in Plant Biology* **7**: 40–49.

Hall MA. 1991. Ethylene metabolism. In: Mattoo AK, Suttle JC, eds. *The Plant Hormone Ethylene*. Boca Raton, FL, USA: CRC Press, 65–80.

He CJ, Drew MC, Morgan PW. 1994. Induction of enzymes associated with lysigenous aerenchyma formation in roots of *Zea mays* during hypoxia or nitrogen starvation. *Plant Physiology* **105**: 861–865.

He CJ, Finlayson SA, Drew MC, Jordan WR, Morgan PW. 1996. Ethylene

biosynthesis during aerenchyma formation in roots of maize subjected to mechanical impedance and hypoxia. *Plant Physiology* **112**: 1679–1685.

Hoffmann-Benning S, Kende H. 1992. On the role of abscisic acid and gibberellin in the regulation of growth in rice. *Plant Physiology* **99**: 1156–1161.

Holmes MG, Klein WH. 1987. The light and temperature environments. In: Crawford RMM, ed. *Plant life in aquatic and amphibious habitats*. Oxford, UK: Blackwell Scientific Publications, 3–22.

Huang J, Takano T, Akita S. 2000. Expression of alpha expansin genes in young seedlings of rice (*Oryza sativa* L.). *Planta* **211**: 467–473.

Igamberdiev AU, Baron K, Manac'h-Little N, Stoimenova M, Hill RD. 2005. The haemoglobin/nitric oxide cycle: involvement in flooding stress and effects on hormone signalling. *Annals of Botany* **96**: 557–564.

Insausti P, Grimoldi AA, Chaneton EJ, Vasellati V. 2001. Flooding induces a suite of adapative plastic responses in the grass *Paspalum dilatatum*. *New Phytologist* **152**: 291–299.

Jackson MB. 1985. Ethylene and the responses of plants to soil waterlogging and submergence. *Annual Review of Plant Physiology* **36**: 145–174.

Jackson MB, Armstrong W. 1999. Formation of aerenchyma and the processes of plant ventilation in relation to soil flooding and submergence. *Plant Biology* **1**: 274–287.

Jackson MB, Ram PC. 2003. Physiological and molecular basis of susceptibility and tolerance of rice plants to complete submergence. *Annals of Botany* **91**: 227–241.

Justin SHFW, Armstrong W. 1987. The anatomical characteristics of roots and plant response to soil flooding. *New Phytologist* **106**: 465–495.

Kamaluddin M, Zwiazek JJ. 2002. Ethylene enhances water transport in hypoxic aspen. *Plant Physiology* **128**: 962–969.

Kende H. 1993. Ethylene biosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* **44**: 283–307.

Kende H, van der Knaap E, Cho HT. 1998. Deepwater rice: a model plant to study stem elongation. *Plant Physiology* **118**: 1105–1110.

Kim JH, Cho HTH, Kende H. 2000. Alpha expansins in the semiaquatic fern *Marsilea quadrifolia* and *Regnellidium diphyllum*: evolutionary aspects and physiological role in rachis elongation. *Planta* **212**: 85–92.

Klok EJ, Wilson IW, Wilson D, Chapman SC, Ewing RM, Somerville SC, Peacock WJ, Dolferus R, Dennis ES. 2002. Expression profile analysis of the low-oxygen response in *Arabidopsis* root cultures. *Plant Cell* **14**: 2481–2494.

Kolodynska A, Pigliucci M. 2003. Multivariate responses to flooding in *Arabidopsis*: an experimental evolutionary investigation. *Functional Ecology* **17**: 131–140.

Kutschera U, Kende H. 1988. The biophysical basis of elongation growth in internodes of deepwater rice. *Plant Physiology* **88**: 361–366.

Kuwabara A, Ikegami K, Koshiba T, Nagata T. 2003. Effects of ethylene and abscisic acid upon heterophylly in *Ludwigia arcuata* (Onagracaea). *Planta* **217**: 880–887.

Laloi C, Apel K, Danon A. 2004. Reactive oxygen signalling: the latest news. *Current Opinion in Plant Biology* **7**: 323–328.

Lin BL, Yang WJ. 1999. Blue light and abscisic acid independently induce heterophyllous switch in *Marsilea quadrifolia*. *Plant Physiology* **119**: 429–434.

Liu F, VanToai T, Moy LP, Bock G, Linford LD, Quackenbush J. 2005. Global transcription profiling reveals comprehensive insights into hypoxia response in Arabidopsis. *Plant Physiology* **137**: 1115–1129.

Longstreth DJ, Borkhsenious ON. 2000. Root cell ultrastructure in developing aerenchyma tissue of three wetland species. *Annals of Botany* **86**: 641–646.

Loreti E, Poggi A, Novi G, Alpi A, Perata P. 2005. A genome-wide analysis of the effects of sucrose on gene expression in Arabidopsis seedlings under anoxia. *Plant Physiology* **137**: 1130–1138.

Maberly SC, Spence DHN. 1983. Photosynthetic inorganic carbon use by freshwater plants. *Journal of Ecology* **71**: 705–724.

Maberly SC, Spence DHN. 1989. Photosynthesis and photorespiration in freshwater organisms: amphibious plants. *Aquatic Botany* **34**: 267–286.

Madsen TV, Sand-Jensen K. 1991. Photosynthetic carbon assimilation in aquatic macrophytes. *Aquatic Botany* **41**: 5–40.

Maloof JN, Borevitz JO, Dabi T, Lutes J, Nehring RB, Redfern JL, Trainer GT, Wilson JM, Asami T, Berry CC, Weigel D, Chory J. 2001. Natural variation in light sensitivity of *Arabidopsis*. *Nature Genetics* **29**: 441–446.

McQueen-Mason SJ, Cosgrove DJ. 1994. Disruption of hydrogen bonding between plant-cell wall polymers by proteins that induce wall extension. *Proceedings of the National Academy of Sciences, USA* **91**: 6574–6578.

McQueen-Mason SJ, Durachko DM, Cosgrove DJ. 1992. Two endogenous proteins that induce cell-wall extension in plants. *Plant Cell* **4**: 1425–1433.

Millenaar FF, Cox MCH, de Jong-van Berkel YEM, Welschen RAM, Pierik R, Voesenek LACJ, Peeters AJM. 2005. Ethylene-induced differential growth of petioles in arabidopsis. analyzing natural variation, response kinetics, and regulation. *Plant Physiology* **137**: 998–1008.

Mittler R, Vanderauwera S, Gollery M, van Breusegem M. 2004. Reactive oxygen gene network of plants. *Trends in Plant Science* **9**: 490–498.

Mommer L, Visser EJW. 2005. Underwater photosynthesis in flooded terrestrial plants: a mater of leaf plasticity. *Annals of Botany* **96**: 581–589.

Mommer L, Pedersen O, Visser EJW. 2004. Acclimation of a terrestrial plant to submergence facilitates gas exchange underwater. *Plant, Cell & Environment* **17**: 1281–1287.

Mommer L, de Kroon H, Pierik R, Bögemann GM, Visser EJW. 2005a. A functional comparison of acclimation to shade and submergence in two terrestrial plant species. *New Phytologist* **167**: 197–206.

Mommer L, Pons TL, Wolters-Arts M, Venema JH, Visser EJW. 2005b. Submergence-induced morphological, anatomical and biochemical responses in a terrestrial species affect gas diffusion resistance and photosynthetic performance. *Plant Physiology* **139**: 497–508.

Mommer L, Pons TL, Visser EJW. 2006. Photosynthetic consequences of phenotypic plasticity in response to submergence: *Rumex palustris* as a case study. *Journal of Experimental Botany* **57**: 283–290.

Monk LS, Fagerstedt KV, Crawford RMM. 1989. Oxygen toxicity and superoxide dismutase as an anti-oxidant in physiological stress. *Physiologia Plantarum* **76**: 456–459.

Neill SJ, Desikan R, Hancock JT. 2002. Hydrogen peroxide signalling. *Current Opinion in Plant Biology* **5**: 388–395.

Noctor G, Foyer CH. 1998. Ascorbate and glutathione: keeping active oxygen under control. *Annual Review of Plant Physiology and Plant Molecular Biology* **49**: 249–279.

Paul AL, Schuerger AC, Popp MP, Manak MS, Ferl RJ. 2003. Hypobaric biology: *Arabidopsis* gene expression at low atmospheric pressure. *Plant Physiology* **134**: 215–223.

Pedersen O, Sand-Jensen K, Revsbech NP. 1995. Diel pulses of O_2 and CO_2 in sandy lake sediments inhabited by *Lobelia dortmanna*. *Ecology* **76**: 1536–1545.

Peeters AJM, Cox MCH, Benschop JJ, Vreeburg RAM, Bou J, Voesenek LACJ. 2002. Submergence research using *Rumex palustris* as a model looking back and going forward. *Journal of Experimental Botany* **53**: 391–398.

Peng HP, Chan CS, Shih MC, Yang SF. 2001. Signaling events in the hypoxic induction of alcohol dehydrogenase gene in Arabidopsis. *Plant Physiology* **126**: 742–749.

Pierik R, Whitelam GC, Voesenek LACJ, de Kroon H, Visser EJW. 2004. Canopy studies on ethylene-insensitive tobacco identify ethylene as a novel element in blue light and plant-plant signaling. *Plant Journal* **38**: 310–319.

Pierik R, Millenaar FF, Peeters AJM, Voesenek LACJ. 2005. New perspectives in flooding research: the use of shade avoidance and *Arabidopsis thaliana*. *Annals of Botany* **96**: 533–540.

Ponnamperuma FN. 1984 Effects of flooding on soils. In: Kozlowski TT, ed. *Flooding and plant growth*. New York, NY, USA: Academic Press, 9–45.

Raskin I, Kende H. 1984a. Regulation of growth in stem sections of deep-water rice. *Planta* **160**: 66–72.

Raskin I, Kende H. 1984b. Role of gibberellin in the growth response of submerged deep water rice. *Plant Physiology* **76**: 947–950.

Raven JA, Osborne BA, Johnston AM. 1985. Uptake of CO_2 by aquatic vegetation. *Plant, Cell & Environment* **8**: 417–425.

Raven JA, Handley LL, MacFarlane JJ, McInroy S, McKenzie L, Richards JH, Samuelsson G. 1988. The role of CO_2 uptake by roots and CAM in acquisition of inorganic C by plants of the isoetid life-form: a review, with new data on *Eriocaulon decangulare* L. *New Phytologist* **108**: 125–148.

Revsbech NP, Pedersen O, Reichardt W, Briones A. 1999. Microsensor analysis of oxygen and pH in the rice rhizosphere under field conditions and laboratory conditions. *Biology and Fertility of Soils* **29**: 379–385.

Ridge I. 1987. Ethylene and growth control in amphibious plants. In: Crawford RMM, ed. *Plant life in aquatic and amphibious habitats*. Oxford, UK: Blackwell Scientific Publications, 53–76.

Rieu I, Cristescu SM, Harren FJM, Huibers W, Voesenek LACJ, Mariani C, Vriezen WH. 2005. RP-ACS1, a flooding-induced 1-aminocyclopropane-1-carboxylate synthase gene of *Rumex palustris*, is involved in rhythmic ethylene production. *Journal of Experimental Botany* **56**: 841–849.

Rijnders JGHM, Yang Y, Kamiya Y, Takahashi N, Barendse GWM, Blom CWPM, Voesenek LACJ. 1997. Ethylene enhances gibberellin levels and petiole sensitivity in flooding-tolerant *Rumex palustris* but not in flooding-intolerant *R. acetosa*. *Planta* **203**: 20–25.

Rijnders JGHM, Armstrong W, Darwent MJ, Blom CWPM, Voesenek LACJ. 2000. The role of oxygen in submergence-induced petiole elongation in *Rumex palustris*: *in situ* measurements of oxygen in petioles of intact plants using micro-electrodes. *New Phytologist* **147**: 497–504.

Roberts JKM, Callis J, Jardetzky O, Walbot V, Freeling M. 1984. Cytoplasmic acidosis as a determinant of flooding intolerance in plants. *Proceedings of the National Academy of Sciences, USA* **81**: 6029–6033.

Saab IN, Sachs MM. 1996. A flooding-induced xyloglucan *endo*-transglycosylase homolog in maize is responsive to ethylene and associated with aerenchyma. *Plant Physiology* 112: 385–391.

Sachs JA. 1882 *A text book of botany*. Oxford, UK: Oxford University Press.

Sachs MM, Subbaiah CC, Saab IN. 1996. Anaerobic gene expression and flooding tolerance in maize. *Journal of Experimental Botany* **47**: 1–15.

Sand-Jensen K. 1989. Environmental variables and their effect on photosynthesis of aquatic plant communities. General features of aquatic photosynthesis. *Aquatic Botany* **34**: 5–25.

Sand-Jensen K, Pedersen O, Binzer T, Borum J. 2005. Contrasting oxygen dynamics in the freshwater isoetid *Lobelia dortmanna* and the marine seagrass *Zostera marina*. *Annals of Botany* **96**: 613–623.

Sauter M. 2000. Rice in deep water: 'How to take heed against a sea of troubles'. *Naturwissenschaften* **87**: 289–303.

Schroeder JI, Allen GJ, Hugouvieux V, Kwak JM, Waner D. 2001. Guard cell signal transduction. *Annual Review of Plant Physiology and Plant Molecular Biology* **52**: 627–658.

Seago JL, Marsh LC, Stevens KJ, Soukup A, Vortubová O, Enstrone DE. 2005. A re-examination of the root cortex in wetland flowering plants with respect to aerenchyma. *Annals of Botany* **96**: 565–579.

Setter TL, Laureles EV. 1996. The beneficial effect of reduced elongation growth on submergence tolerance of rice. *Journal of Experimental Botany* **47**: 1551–1559.

Setter TL, Kupkanchanakul T, Kupkanchanakul K, Bhekasut P, Wiengweera A, Greenway H. 1987. Concentrations of CO_2 and O_2 in floodwater and in internodal lacunae of floating rice growing at 1–2 metre water depth. *Plant, Cell & Environment* **10**: 767–776.

Smith FA, Walker NA. 1980. Photosynthesis by aquatic plants: effects of unstirred layers in relation to assimilation of CO_2 and HCO_3^- and to carbon isotopic discrimination. *New Phytologist* **86**: 245–259.

Sowa AW, Duff SMG, Guy PA, Hill RD. 1998. Altering hemoglobin levels changes energy status in maize cells under hypoxia. *Proceedings of the National Academy of Sciences, USA* **95**: 10317–10321.

Stepanova AN, Alonso JM. 2005. Ethylene signalling and response pathway: a unique signalling cascade with a multitude of inputs and outputs. *Physiologia Plantarum* **123**: 195–206.

Toojinda T, Siangliw M, Tragoonrung S, Vanavichit A. 2003. Molecular genetics of submergence tolerance in rice: QTL analysis of key traits. *Annals of Botany* **91**: 243–253.

Tournaire-Roux C, Sutka M, Javot H, Gout E, Gerbeau P, Luu DT, "Bligny R, Maurel C. 2003. Cytosolic pH regulates root water transport during anoxic stress through gating of aquaporins. *Nature* **425**: 393–397.

Ueguchi-Tanaka M, Ashikari M, Nakajima M, Itoh H, Katoh E, Kobayashi M, Chow T, Hsing YC, Kitano H, Yamaguchi I, Matsuoka M. 2005. *GIBBERELLIN INSENSITIVE DWARF1* encodes a soluble receptor for gibberellin. *Nature* **437**: 693–698.

Vervuren PJA, Beurskens SMJH, Blom CWPM. 1999. Light acclimation, CO_2 response and long-term capacity of underwater photosynthesis in three terrestrial plant species. *Plant, Cell & Environment* **22**: 959–968.

Vervuren PJA, Blom CWPM, de Kroon H. 2003. Extreme flooding events on the rhine and the survival and distribution of riparian plant species. *Journal of Ecology* **91**: 135–146.

Voesenek LACJ, Blom CWPM. 1989. Growth responses of *Rumex* species in relation to submergence and ethylene. *Plant, Cell & Environment* **12**: 433–439.

Voesenek LACJ, Blom CWPM. 1999. Stimulated shoot elongation: a mechanism of semiaquatic plants to avoid submergence stress. In: Lerner HR, ed. *Plant responses to environmental stresses: from phytohormones to genome reorganization*. New York, NY, USA: Marcel Dekker Inc., 431–448.

Voesenek LACJ, Banga M, Their RH, Mudde CM, Harren FJM, Barendse GWM, Blom CWPM. 1993. Submergence-induced ethylene entrapment, and growth of two plant species with contrasting flooding resistances. *Plant Physiology* **103**: 783–791.

Voesenek LACJ, Vriezen WH, Smekens MJE, Huitink FHM, Bogemann GM, Blom CWPM. 1997. Ethylene sensitivity and response sensor expression in petioles of *Rumex* species at low O_2 and high CO_2 concentrations. *Plant Physiology* **114**: 1501–1509.

Voesenek LACJ, Benschop JJ, Bou i Torrent J, Cox MCH, Groeneveld HW, Millenaar FF, Vreeburg RAM, Peeters AJM. 2003. Interactions between plant hormones regulate submergence-induced shoot elongation in the flooding tolerant dicot *Rumex palustris*. *Annals of Botany* **91**: 205–211.

Voesenek LACJ, Rijnders JHGM, Peeters AJM, van de Steeg HM, de Kroon H. 2004. Plant hormones regulate fast shoot elongation underwater: from genes to communities. *Ecology* **85**: 16–27.

Vreeburg RAM, Benschop JJ, Peeters AJM, Colmer TD, Ammerlaan AHM, Staal M, Elzenga TM, Staals RHJ, Darley CP, McQueen-Mason SJ, Voesenek LACJ. 2005. Ethylene regulates fast apoplastic acidification and expansin A transcription during submergence-induced petiole elongation in *Rumex palustris*. *Plant Journal* **43**: 597–610.

Vriezen WH, van Rijn CP, Voesenek LA, Mariani C. 1997. A homolog of the *Arabidopsis thaliana* ERS gene is actively regulated in *Rumex palustris* upon flooding. *Plant Journal* **11**: 1265–1271.

Vriezen WH, Hulzink R, Mariani C, Voesenek LACJ. 1999. 1-Aminocyclopropane-1-carboxylate oxidase activity limits ethylene biosynthesis in *Rumex palustris* during submergence. *Plant Physiology* **121**: 189–196.

Vriezen WHP, Achard NP, Harberd van der Straeten D. 2004. Ethylene-mediated enhancement of apical hook formation in etiolated *Arabidopsis thaliana* seedlings is gibberellin dependent. *Plant Journal* **37**: 671–677.

Wang KL-C, Li H, Ecker JR. 2002. Ethylene biosynthesis and signaling networks. *Plant Cell* **14**: S131–S151.

Waters I, Armstrong W, Thomson CJ, Setter L, Adkins S, Gibbs J, Greenway H. 1989. Diurnal changes in radial oxygen loss and ethanol metabolism in roots of submerged and non-submerged rice seedlings. *New Phytologist* **113**: 439–451.

About *New Phytologist*

- *New Phytologist* is owned by a non-profit-making **charitable trust** dedicated to the promotion of plant science, facilitating projects from symposia to open access for our Tansley reviews. Complete information is available at **www.newphytologist.org**.
- Regular papers, Letters, Research reviews, Rapid reports and both Modelling/Theory and Methods papers are encouraged. We are committed to rapid processing, from online submission through to publication 'as-ready' via *OnlineEarly* – the 2004 average submission to decision time was just 30 days. Online-only colour is **free**, and essential print colour costs will be met if necessary. We also provide 25 offprints as well as a PDF for each article.
- For online summaries and ToC alerts, go to the website and click on 'Journal online'. You can take out a **personal subscription** to the journal for a fraction of the institutional price. Rates start at £109 in Europe/$202 in the USA & Canada for the online edition (click on 'Subscribe' at the website).
- If you have any questions, do get in touch with Central Office (**newphytol@lancaster.ac.uk**; tel +44 1524 594691) or, for a local contact in North America, the US Office (**newphytol@ornl.gov**; tel +1 865 576 5261).

