Learning from nature: the use of non-model species to identify novel acclimations to flooding stress

Abstract. Excess water in the form of waterlogged soil or deeper submergence (generically termed ‘flooding’) influences plant growth, survival and species distribution in many natural ecosystems. It also has a negative impact on crop growth and yield since many agricultural species are flooding intolerant. The often devastating effect of flooding on plant performance is related to its interference with gas exchange between the plant and its environment. This results in energy deficiency and carbohydrate starvation. In the near future, flooding frequency is expected to increase due to global climate change and the human population is expected to increase to \(\approx 9\) billion people by 2050. The need for increased agricultural productivity is self-evident and this will require a better mechanistic understanding of the interaction between plants and abiotic stresses such as flooding. We argue that, in seeking this understanding, we should not restrict the research to model species such as rice (\(Oryza sativa\)) and Arabidopsis (\(Arabidopsis thaliana\)). This is because some stress-tolerance mechanisms are not found in these species. Examples are given of how flooding tolerance is achieved by non-model species of \(Rumex\) and \(Rorippa\) that have evolved to cope with flooding in natural environments. These findings will add usefully to the spread of resources available to plant breeding programmes aimed at improving flooding tolerance in crop plants.

Keywords: Abscisic acid; climate change; ethylene; flooding; learning from nature; \(Rorippa\); \(Rumex\); waterlogging.

Introduction

Flooding in the form of waterlogged soil or deeper submergence of plants occurs in many natural ecosystems such as lakesides, river floodplains and (coastal) marshes. These flooding events vary in seasonal timing, duration, depth and frequency (Colmer and Voesenek 2009). This variation in flooding regime in combination with the variability of flooding tolerance between plant species determines patterns of plant distribution (Silvertown et al. 1999; Voesenek et al. 2004). Excess water can also decrease crop growth and yield since nearly all economically relevant crops are negatively affected by flooding (Voesenek and Bailey-Serres 2013).

Floods hamper plant performance because they interfere with normal gas exchange (oxygen \((O_2)\), carbon dioxide \((CO_2)\) and ethylene) and light availability needed for aerobic respiration and photosynthesis. These stress conditions deprive plants of essential carbohydrates and energy reserves and can, ultimately, kill the plant (Bailey-Serres and Voesenek 2008). However, plant species that...
inhabit permanently or temporarily flooded habitats use specific suites of traits to survive these harsh environments. Such species are, in general, characterized by high tissue porosities (aerenchyma) and restored shoot-atmosphere contact to facilitate internal gas diffusion (escape strategies) (Jackson and Armstrong 1999; Colmer 2003), and large carbohydrate reserves to fuel fermentation during phases of anaerobiosis when aerenchyma cannot conduct O$_2$ effectively to inundated organs (e.g. when the whole plant is under water; Bucher et al. 1996). Other tolerance mechanisms include a dampening of energetically expensive processes such as growth (quiescence strategies; Bailey-Serres and Voesenek 2010) and modes to control cell damage by excessive amounts of reactive oxygen species (ROS) that develop during flooding and upon re-aeration when flood levels decline (Hunter et al. 1983; Monk et al. 1987; Blokhina et al. 2002; Santos et al. 2007). Some species (e.g. Phalaris arundinacea, Phragmites australis, Typha latifolia) even possess gas films on their leaves that improve exchange of CO$_2$ and O$_2$ when leaves are submerged (Colmer and Pedersen 2007).

Despite this variation in adaptive traits in wild plant species, research on flood survival mechanisms has been mostly limited to flood-tolerant rice cultivars and the flood-intolerant model plant Arabidopsis. Over the past few years, work with these species has strongly accelerated the elucidation of molecular mechanisms that contribute to flooding tolerance. A notable example is the identification and functional characterization of the group VII ethylene response factor (ERF) transcription factor family in low O$_2$ sensing, and in survival mechanisms related to escape and quiescence traits (Xu et al. 2006; Hattori et al. 2009; Gibbs et al. 2011; Licausi et al. 2011). However, these major discoveries do not explain why closely related wild plants and crops can vary enormously in flooding tolerance.

Wild plants from flood-prone areas have evolved in a selective environment that favours flood-adaptive traits. Furthermore, the resulting flood-adaptive traits have evolved in locations where neither rice nor Arabidopsis is found naturally. We can therefore assume that these wild species possess many as yet unknown flood tolerance genes and processes. The importance of this ‘Learning from Nature’ approach to understanding flooding tolerance mechanisms and harnessing this knowledge to develop crops with enhanced flood tolerance is illustrated and discussed here.

**Rumex and Rorippa Species: Variation in Flooding Tolerance and Molecular Survival Strategies**

Two species from the genus Rorippa (Ro. sylvestris and Ro. amphibia) and two from the genus Rumex (Ru. acetosa and Ru. palustris) have been investigated extensively in recent studies of flooding tolerance and the underlying explanations. Compared to Arabidopsis and even rice, certain species from either genus are highly tolerant to complete submergence under dark conditions (Sasidharan et al. 2013). Under such demanding conditions, which typically prevent emergence of leaves, these four species can be ranked in the following order (from most to least tolerant): Ro. sylvestris > Ro. amphibia > Ru. palustris = Ru. acetosa. The corresponding average lethal time 50 (LT$_{50}$) values under similar conditions were 32, 24, 20 and 20 days, respectively. When submerged, Ro. sylvestris and Ru. acetosa demonstrated strongly reduced elongation of the youngest leaf, suggesting that they employ a quiescence survival strategy. Rorippa amphibia and Ru. palustris show a mild to very strong elongation response of the youngest petiole, respectively (Akman et al. 2012; van Veen et al. 2013). This demonstrates that Ru. palustris, in particular, can escape submerged conditions via enhanced upward shoot elongation. Adaptive traits related to flooding such as enhanced shoot elongation and shoot porosity appear to act synergistically to enhance plant fitness (Colmer and Voesenek 2009). Consistent with this interpretation are observations that quiescent Rumex and Rorippa species have significantly lower petiole porosities than the escape species (Pierik et al. 2008; Akman et al. 2012).

Despite being highly tolerant, detailed investigations into the underlying molecular mechanisms in wild species such as Rumex and Rorippa have been hindered by the lack of suitable molecular genetic tools. However, recent studies have successfully applied global genome profiling techniques to characterize the molecular basis of flooding tolerance in the four plant species. While the close relatedness to Arabidopsis (both are genera of the Brassicaceae) allowed the use of Arabidopsis ATH1 GeneChip arrays for both Rorippa species, deep sequencing techniques (RNAseq) were used for both Rumex species (Sasidharan et al. 2013; van Veen et al. 2013). In both studies plants were completely submerged, but in Rorippa the transcriptome profile was studied in whole root systems, whereas in Rumex only submerged young petiole tissue was used. Both systems are characterized by an accumulation of ethylene, but have significant differences in O$_2$ concentrations (Voesenek and Sasidharan 2013). Rorippa plants submerged for 24 h have hypoxic to anoxic roots (Sasidharan et al. 2013); submerged Rumex petioles (4–10 h) have endogenous O$_2$ levels between 17 and 21 % (van Veen et al. 2013).

The roots of both Rorippa species were characterized by a downregulation of genes involved in energy-demanding processes. Interestingly, glycolysis and fermentation genes were more strongly upregulated in...
the escaping *Ro. amphibia* compared with the quiescent *Ro. sylvestris* (Sasidharan et al. 2013), suggesting that this species has a stronger Pasteur effect (i.e. faster rates of sugar usage) and thus may produce more ATP per unit time. This would support cell maintenance and specifically in this species also help sustain the costly process of continued underwater shoot growth (Akman et al. 2012). Despite the presumed Pasteur effect, ATP levels declined in submerged *Ro. amphibia* (Sasidharan et al. 2013), indicating that maintenance and growth require more ATP than produced. In contrast, *Ro. sylvestris* employs a quiescence survival strategy during complete submergence. The prolonged survival of this species when completely under water is in line with its stable ATP content during submergence. This demonstrates that ATP production is in balance with the consumption required for maintenance (Sasidharan et al. 2013). Consistent with observations in rice, these results demonstrate that inhibition of energetically expensive elongation processes improves survival under conditions in which the water surface cannot be reached by means of upward shoot extension (Setter and Laureles 1996).

*Rumex acetosa* is characterized by reduced elongation growth upon submergence. That photosynthetically derived O₂ and inward diffusion of O₂ from the water layer can be sufficient to balance respiratory O₂ use is indicated by high O₂ levels found in submerged petioles (van Veen et al. 2013). A notable finding in *Ru. acetosa* was an induction of a trehalose phosphate phosphatase (TPP) during submergence. Trehalose phosphate phosphatase relieves trehalose-6-phosphate (T6P) inhibition on the induction of a trehalose phosphate phosphatase (TPP) during submergence. This demonstrates that ATP production is in balance with the consumption required for maintenance (Sasidharan et al. 2013). Consistent with observations in rice, these results demonstrate that inhibition of energetically expensive elongation processes improves survival under conditions in which the water surface cannot be reached by means of upward shoot extension (Setter and Laureles 1996).

*Ru. acetosa* is characterized by reduced elongation growth upon submergence. That photosynthetically derived O₂ and inward diffusion of O₂ from the water layer can be sufficient to balance respiratory O₂ use is indicated by high O₂ levels found in submerged petioles (van Veen et al. 2013). A notable finding in *Ru. acetosa* was an induction of a trehalose phosphate phosphatase (TPP) during submergence. Trehalose phosphate phosphatase relieves trehalose-6-phosphate (T6P) inhibition on the induction of a trehalose phosphate phosphatase (TPP) during submergence. This demonstrates that ATP production is in balance with the consumption required for maintenance (Sasidharan et al. 2013). Consistent with observations in rice, these results demonstrate that inhibition of energetically expensive elongation processes improves survival under conditions in which the water surface cannot be reached by means of upward shoot extension (Setter and Laureles 1996).

*Rumex acetosa* is characterized by reduced elongation growth upon submergence. That photosynthetically derived O₂ and inward diffusion of O₂ from the water layer can be sufficient to balance respiratory O₂ use is indicated by high O₂ levels found in submerged petioles (van Veen et al. 2013). A notable finding in *Ru. acetosa* was an induction of a trehalose phosphate phosphatase (TPP) during submergence. Trehalose phosphate phosphatase relieves trehalose-6-phosphate (T6P) inhibition on the induction of a trehalose phosphate phosphatase (TPP) during submergence. This demonstrates that ATP production is in balance with the consumption required for maintenance (Sasidharan et al. 2013). Consistent with observations in rice, these results demonstrate that inhibition of energetically expensive elongation processes improves survival under conditions in which the water surface cannot be reached by means of upward shoot extension (Setter and Laureles 1996).

As mentioned earlier, if these escaping plants cannot emerge out of the flood water, their survival is compromised (Setter and Laureles 1996). Surprisingly, our survival data for plants submerged in darkness indicate similar LT₅₀ values (20 days) for both *Rumex* species despite their contrasting elongation strategies (van Veen et al. 2013). Some of these core-hypoxia genes are directly upregulated by elevated levels of ethylene. Additionally, many of the core-hypoxia genes in *Ru. palustris* can be upregulated experimentally by anoxia and the effect is enhanced by an ethylene pretreatment. These observations suggest that ethylene can prime *Ru. palustris* for future anoxia tolerance. Indeed, plants pretreated with ethylene showed higher survival and more vigorous recovery after an anoxia period (van Veen et al. 2013). This mechanism might explain the
relatively long survival under submerged dark conditions of *Ru. palustris* despite expending resources on its strong elongation response. Remarkably, this priming mechanism was absent in *Ru. acetosa* (van Veen et al. 2013).

As mentioned before, in Arabidopsis, group VII ERF transcription factors are involved in O$_2$ sensing. In the presence of O$_2$, members of this transcription factor family are targeted for degradation via the N-end rule pathway of protein degradation (Gibbs et al. 2011; Licausi et al. 2011). Interestingly, mutants (ate1ate2 and prt6) that impair the N-end rule pathway of targeted proteolysis and stabilize the ERFs show constitutive expression of many of the core-hypoxia genes, indicating that these stable ERFs regulate the expression of genes important for surviving anaerobiosis (Gibbs et al. 2011). These observations feed the hypothesis that ethylene-regulated anaerobic priming might work via increased stability of group VII ERFs and therefore enhanced expression of downstream adaptive anaerobic genes during ethylene exposure and even more during subsequent anoxia. This novel role of the volatile hormone ethylene is consistent with the sequence of events that take place during natural flooding events. The rate of O$_2$ decline is often gradual and the rate unpredictable as tissues progress from hypoxia to anoxia over hours or days. In contrast, ethylene accumulates throughout the plant within minutes of submergence, making it a more reliable and timely (early) signal of submergence (Voesenek and Sasidharan 2013).

**Perspectives/Concluding Remarks**

Studies in *Rumex* and *Rorippa* have delivered unprecedented insights into flooding tolerance mechanisms and demonstrate the value of examining wild plant species. From these and earlier performed investigations, it becomes clear that the long-term survival of submergence can only be achieved with a balanced control of energy-demanding and energy-producing processes. In this, activation of catabolism, reduction of elongation growth, and controlled glycolysis and ethanolic fermentation to increase substrate-level production of ATP are key processes. This level of control might also determine variation in waterlogging tolerance in species that cannot avoid low O$_2$ conditions due to very low root porosities.

The unbiased study into the regulation of enhanced shoot elongation in submerged *Ru. palustris* revealed that signalling genes well known in photomorphogenesis and shade avoidance responses are regulated by accumulated ethylene and reduced levels of ABA and are therefore likely to play a role in ethylene-driven shoot elongation. We conclude that *Ru. palustris* uses a conserved signal transduction network that regulates elongation growth to both submergence and changes in the light spectrum (e.g. shade avoidance). This explains the high phenotypic similarity of shade avoidance responses and submergence-stimulated elongation (Pierik et al. 2010).

The observed upregulation of core-hypoxia genes in well-aerated submerged petioles of *Ru. palustris* led to the discovery of a novel ethylene-induced priming mechanism of anoxia tolerance. This mechanism was entirely absent in *Ru. acetosa*, indicating that priming by ethylene is not a general response of plants to flooding but linked to enhanced tolerance. This ‘ethylene training’ resembles the observation that hypoxia-pretreated roots are more anoxia tolerant, have more alcohol dehydrogenase (ADH) activity and maintain higher levels of ATP (Saglio et al. 1988). The significant increase in recovery and survival of plants pretreated with ethylene has high potential to improve the flooding tolerance of agronomically important crops. The next steps will be to identify the underlying regulators.

Improved waterlogging and submergence tolerance of crops is important because more frequent and prolonged flooding events will increasingly hamper crop productivity in the future as a direct consequence of climate change (Kundzewicz et al. 2013). A dramatic example comes from South China where 30 % of the seeds for worldwide rapeseed oil production are grown as a rotation crop in rice paddy fields (Zhang et al. 2008). Young seedlings of this flooding-intolerant plant are frequently exposed to waterlogging, causing an annual yield loss of ~17–42 % (Zhou and Lin 1995). Furthermore, the world human population is expected to increase from the current 7 billion to ~9 billion by 2050, requiring us to increase current agricultural productivity by a similar or greater proportion if widespread hunger and social unrest are to be averted (Tester and Langridge 2010). Elucidation of the molecular mechanisms that regulate flooding tolerance in model plants such as rice and Arabidopsis is important, but this approach should be accompanied by investigations into as yet unknown flood-adaptive traits in wild plant species that have evolved to cope with flooding stress. The *Rorippa* and *Rumex* studies demonstrate that whole-genome transcription profiling technologies can be successfully applied to non-model species to uncover novel tolerance genes and processes.

Important future challenges are the development of crops that can re-allocate their energy and carbon reserves in response to early flooding signals to support maintenance rather than growth and other costly processes. Most flooding damage to crops is related to partial flooding or waterlogging of the soil. Most waterlogging-tolerant wild species have an extensive system of interconnected gas spaces (aerenchyma) that is either stress-inducible or constitutive (Colmer 2003). Aerenchyma development is a complex trait. It involves lysis of specific
cells or schizogeny during root development (Voesenek et al. 2006). Understanding the molecular background of aerenchyma formation will facilitate the development of crops rich in root aerenchyma. Important in this respect is the observed trade-off between root cortical aerenchyma formation and nutrient transport in Zea mays (Hu et al. 2013). This could potentially hamper the productivity of these aerenchyma-rich varieties. A final challenge is exploring the potential of ethylene-induced priming for anoxia tolerance. Identification of the molecular regulators of ethylene-driven priming can be expected to lead to the introduction of this adaptive trait in crops that currently lack it.

Sources of Funding

H.v.V. and R.S. have been financially supported by the Netherlands Organisation for Scientific Research (grants 819.01.006 to H.v.V. and VENI 86312013 to R.S.).

Contributions by the Authors

L.A.C.J.V. and R.S. wrote the paper; R.S. and H.v.V. performed many of the Rumex and Rorippa experiments and analyses.

Conflicts of Interest Statement

None declared.

Acknowledgements

We thank Ankie Ammerlaan, Judith Koerselman-Kooij, Marleen Vermeer-van Eijk and Rob Welschen-Evertman for technical support.

Literature Cited

Akman M, Bhikharie AV, McLean EH, Boonman A, Visser EJW, Schranz ME, van Tienderen PH. 2012. Wait or escape? Contrasting submergence tolerance strategies of Rorippa amphibia, Rorippa sylvestris and their hybrid. *Annals of Botany* **109**:1263–1276.

Baena-González E, Rolland F, Thevelein JM, Sheen J. 2007. A central integrator of transcription networks in plant stress and energy signalling. *Nature* **448**:938–942.

Bailey-Serres J, Voesenek LACJ. 2008. Flooding stress: acclimations and genetic diversity. *Annual Review of Plant Biology* **59**: 313–339.

Bailey-Serres J, Voesenek LACJ. 2010. Life in the balance: a signaling network controlling survival of flooding. *Current Opinion in Plant Biology* **13**:489–494.

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

Benschop JJ, Bou J, Peeters AJM, Wagemaker N, Gühk K, Ward D, Hedden P, Moritz T, Voesenek LACJ. 2006. Long-term submergence-induced elongation in *Rumex palustris* requires abscisic acid-dependent biosynthesis of gibberellin. *Plant Physiology* **141**:1644 – 1652.

Blokhina O, Virolainen E, Fagerstedt KV. 2002. Antioxidants, oxidative damage and oxygen deprivation stress: a review. *Annals of Botany* **91**:179 – 194.

Bucher M, Brandle R, Kuhlemeyer C. 1996. Glycolytic gene expression in amphibious *Acorus calamus* L. under natural conditions. *Plant and Soil* **178**:75–82.

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

Colmer TD, Pedersen O. 2007. Underwater photosynthesis and respiration in leaves of submerged wetland plants: gas films improve CO$_2$ and O$_2$ exchange. *New Phytologist* **177**:918–926.

Colmer TD, Voesenek LACJ. 2009. Flooding tolerance: suites of plant traits in variable environments. *Functional Plant Biology* **36**:665 – 681.

Gibbs DJ, Lee SC, Isa NM, Grammiglia S, Fukuo T, Bassel GW, Correia CS, Corbineau F, Theodoulou FL, Bailey-Serres J, Holdsworth MJ. 2011. Homeostatic response to hypoxia is regulated by the N-end rule pathway in plants. *Nature* **479**:415–418.

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.

Hattori Y, Nagai K, Furukawa S, Song X-J, Kawano R, Sakakibara H, Wu J, Matsumoto T, Yoshimura A, Kitano H, Matsuoka M, Mori H, Ashikari M. 2009. The ethylene response factors SNORKEL1 and SNORKEL2 allow rice to adapt to deep water. *Nature* **460**:1026–1030.

Hu B, Henry A, Brown KM, Lynch JP. 2013. Root cortical aerenchyma inhibits radial nutrient transport in maize (*Zea mays*). *Annals of Botany* **113**:181–189.

Hunter MIS, Hetherington AM, Crawford RMM. 1983. Lipid peroxidation—a factor in anoxia intolerance in *Iris* species? *Phytochemistry* **22**:1145 – 1147.

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.

Jung K-H, Seo Y-S, Walla H, Cao P, Fukao T, Canlas PE, Amonpant F, Bailey-Serres J, Holdsworth MJ. 2010. The submergence tolerance regulator Sub1A mediates stress-responsive expression of AP2/ERF transcription factors. *Plant Physiology* **152**:1674–1692.

Kundzewicz ZW, Kagawa S, Seneviratne SI, Leichenko M, Nicholls N, Arnell N, Glasson J, Handmer J, Mach K, Wettergren M, Otte G, Braconnot P, Charabi Y, Corine Seuzaret M, de Fraiture S, de Vries W, Diaz A, Döll P, Dörr H, Drebs M, El-Sabh A, Entekhabi D, Flenet A, GoodWE, Goodwin L, Klein E, Kironen R, Koirala S, Koster E, Kwon J-K, Leavitt B, Leibundgut C, Liu Y, Meibom G, Mishra RK, Miller-Kucera H, Mohino S, Nicolsky D, Oki T, Ono H, Piao S, Price A, Reuscher S, Salas M, Seckam K, Seneviratne S, Seppälä M, Stahl P, Sun Z, Takahashi K, Tacon D, Vörösmarty CM, Vukovic A, Wada Y, Wu X, Yashiro K, Yeh F, Yuan Q, Zdon K, Zscheischler J, Zsoter M. 2012. The future water balance of the Earth system: a synthesis of recent studies. *Hydrological Sciences Journal* **59**:1 – 28.

Licausi F, Kosmacz M, Weits DA, Giuntoli B, Giorgi FM, Voesenek LACJ, Perata P, van Dongen JT. 2011. Oxygen sensing in plants is mediated by an N-end rule pathway for protein destabilization. *Nature* **479**:419 – 422.

Monk LS, Fagerstedt KV, Crawford RM. 1987. Superoxide dismutase as an anaerobic polypeptide: a key factor in recovery from oxygen deprivation in *Iris pseudacorus*. *Plant Physiology* **85**:1016–1020.

Mustroph A, Zanetti ME, Jang CJH, Holten HE, Repetti PP, Galbraith DW, Giske T, Bailey-Serres J. 2009. Profiling transcripts of discrete cell populations resolves altered cellular priorities during hypoxia in Arabidopsis. *Proceedings of the National Academy of Sciences of the USA* **106**:18843 – 18848.
Mustroph A, Lee SC, Oosumi T, Zanetti ME, Yang H, Ma K, Yaghoubi-Masihi A, Fukao T, Bailey-Serres J. 2010. Cross-kingdom comparison of transcriptomic adjustments to low-oxygen stress highlights conserved and plant-specific responses. *Plant Physiology* **152**:1484–1500.

Pierik R, van Aken JM, Voesenek LACJ. 2008. Is elongation-induced leaf emergence beneficial for submerged *Rumex* species? *Annals of Botany* **103**:353–357.

Pierik R, de Wit M, Voesenek LACJ. 2010. Growth-mediated stress escape: convergence of signal transduction pathways activated upon exposure to two different environmental stresses. *New Phytologist* **189**:122–134.

Saglio PH, Drew MC, Pradet A. 1988. Metabolic acclimation to anoxia induced by low (2–4 kPa partial pressure) oxygen pretreatment (hypoxia) in root tips of *Zea mays*. *Plant Physiology* **86**:61–66.

Santosa IE, RAM PC, Boamfa EI, Laarhoven LJJ, Reuss J, Jackson MB, Harren FJM. 2007. Patterns of peroxidative ethane emission from submerged rice seedlings indicate that damage from reactive oxygen species takes place during submergence and is not necessarily a post-anoxic phenomenon. *Planta* **226**:193–202.

Sasidharan R, Mustroph A, Boonman A, Akman M, Ammerlaan AMH, Breit T, Schranz ME, Voesenek LACJ, van Tienderen PH. 2013. Root transcript profiling of two *Rorippa* species reveals gene clusters associated with extreme submergence tolerance. *Plant Physiology* **163**:1277–1292.

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

Silvertown J, Dodd ME, Gowling DJ, Mountford JO. 1999. Hydrologically defined niches reveal a basis for species richness in plant communities. *Nature* **400**:61–63.

Tester M, Langridge P. 2010. Breeding technologies to increase crop production in a changing world. *Science* **327**:818–822.

van Veen H, Mustroph A, Barding GA, Vergeer-van Eijk M, Welschen-Evertman RAM, Pedersen O, Visser EJW, Larive CK, Pierik R, Bailey-Serres J, Voesenek LACJ, Sasidharan R. 2013. Two *Rumex* species from contrasting hydrological niches regulate flooding tolerance through distinct mechanisms. *The Plant Cell* **25**:4691–4707.

Voesenek LACJ, Bailey-Serres J. 2013. Flooding tolerance: O2 sensing and survival strategies. *Current Opinion in Plant Biology* **16**:647–653.

Voesenek LACJ, Sasidharan R. 2013. Ethylene- and oxygen signalling-drive plant survival during flooding. *Plant Biology* **15**:426–435.

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*. *The Plant Journal* **43**:597–610.

Xu K, Xu X, Fukao T, Canlas P, Maghirang-Rodriguez R, Heuer S, Ismail AM, Bailey-Serres J, Ronald PC, Mackill DJ. 2006. Sub1A is an ethylene-response-factor-like gene that confers submergence tolerance to rice. *Nature* **442**:705–708.

Zhang XK, Chen J, Chen L, Wang HZ, Li JN. 2008. Imbibition behavior and flooding tolerance of rapeseed seed (*Brassica napus* L.) with different testa color. *Genetic Resources and Crop Evolution* **55**:1175–1184.

Zhang Y, Primavesi LF, Jhurreea D, Andralojc PJ, Mitchell RAC, Powers SJ, Schuepmann H, Delatte T, Wingler A, Paul MJ. 2009. Inhibition of SNF1-related protein kinase1 activity and regulation of metabolic pathways by trehalose-6-phosphate. *Plant Physiology* **149**:1860–1871.

Zhou W, Lin X. 1995. Effects of waterlogging at different growth stages on physiological characteristics and seed yield of winter rape (*Brassica napus* L.). *Field Crops Research* **44**:103–110.