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## Program

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<tr>
<td>19:00</td>
<td>Danish Welcome Reception</td>
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<td>ISPA Board meeting</td>
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### Tuesday 6\(^{th}\) September

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<thead>
<tr>
<th>Time</th>
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<tr>
<td>09:00</td>
<td>Welcome</td>
<td>Pedersen</td>
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<td>09:10</td>
<td>Address by the President</td>
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<tr>
<td>09:25</td>
<td>Life at low oxygen</td>
<td>Canfield</td>
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<td>09:55</td>
<td>Plant tissue anoxia</td>
<td>Borum</td>
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<tr>
<td>11:00</td>
<td>Functional traits</td>
<td>Båstrup-Spohr</td>
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<tr>
<td>11:25</td>
<td>Adventitious roots</td>
<td>Visser</td>
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<td>11:50</td>
<td>Submergence of halophytes</td>
<td>Pellegrini</td>
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<td>Lunch break</td>
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<tr>
<th>Time</th>
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<tr>
<td>13:45</td>
<td>Flood tolerance of wheat</td>
<td>Konnerup</td>
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<td>14:10</td>
<td>Internal aeration of deepwater rice</td>
<td>Mori</td>
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<td>14:35</td>
<td>The gas film gene</td>
<td>Kurokawa</td>
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<tr>
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<td>15:30</td>
<td>No escape?</td>
<td>Striker</td>
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<td>15:55</td>
<td>Flood tolerance of pea and lentil</td>
<td>Malik</td>
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<td>Forage grass hybrids</td>
<td>Jiménez</td>
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<td>POSTER SESSION</td>
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<tr>
<td>09:00</td>
<td>Regulation of aerenchyma formation</td>
<td>Nakazono</td>
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<tr>
<td>09:25</td>
<td>Oxygen and root architecture</td>
<td>Shukla</td>
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<td>09:50</td>
<td>Oxygen and root development</td>
<td>Sauter</td>
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<td>10:15</td>
<td>Acyl-CoA and energy status</td>
<td>Schmidt</td>
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<td>11:15</td>
<td>Respiration in bulky organs</td>
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<td>11:40</td>
<td>Oxygen sensing</td>
<td>Geigenberger</td>
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<td>12:05</td>
<td>Hypoxia under sugar starvation</td>
<td>Perata</td>
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<td>Oxygen-dependent regulation</td>
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<tr>
<td>14:00</td>
<td>Flooding and foresight</td>
<td>Sasidharan</td>
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<td>14:25</td>
<td>A hypoxia induced Ca$^{++}$ sensor</td>
<td>Roberts</td>
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<td>Sensing of low O$_2$ by Ca$^{++}$ and K$^+$</td>
<td>Shabala</td>
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<td>15:45</td>
<td>Oxygen sensing technology</td>
<td>Rolletschek</td>
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<tr>
<td>16:10</td>
<td>Sugar and oxygen sensing</td>
<td>Sanclemente</td>
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<tr>
<td>16:35</td>
<td>Phytoglobins and root growth</td>
<td>Hill</td>
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<td>17:00</td>
<td>Phytoglobins and NO scavenging</td>
<td>Hebelstrup</td>
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<td>ISPA General meeting 2016</td>
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<tr>
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<td>Jasmonates and post-submergence</td>
<td>Xiao</td>
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<td>09:25</td>
<td>Post-submergence recovery</td>
<td>Yeung</td>
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<td>09:50</td>
<td>Protein kinases and submergence</td>
<td>Shih</td>
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<td>ATP consumption of mitochondria</td>
<td>Fagerstedt</td>
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<td>11:05</td>
<td>Regulation of energy metabolism</td>
<td>van Dongen</td>
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<td>11:10</td>
<td>Protein kinases and submergence</td>
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<tr>
<td>11:45</td>
<td>Nuclear dynamics of transcription</td>
<td>Lee</td>
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<tr>
<td>13:00</td>
<td>Hypoxia and transcriptional regulation</td>
<td>Mustroph</td>
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<td>13:25</td>
<td>Submergence responses in soybean</td>
<td>Fukao</td>
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<td>Plant cystine oxidases</td>
<td>White</td>
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<td>Plasticity in response to flooding</td>
<td>Bailey-Serres</td>
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<td>ISPA 2016 Awards</td>
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<td>Concluding remarks</td>
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version 1.10 (6. September 2016)
## Poster session program

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<td>Dhananjay Kumar</td>
<td>Anaerobic germination tolerant deep water rice germplasm Kekua Boo displays aberrant genetic perturbation in key anoxia signaling components</td>
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<td>Emese Derzsó</td>
<td>ERF VII transcription factors alter root structure in <em>Arabidopsis</em></td>
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<td>Guillermo Toro</td>
<td>Tolerance of <em>Prunus</em> rootstocks to hypoxia is associated to decreases of energetic costs to protein turnover and maintenance of membrane integrity</td>
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<td>Gustavo Striker</td>
<td>Waterlogging effects on physiological and growth responses in forage grasses with differential tolerance</td>
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<td>5</td>
<td>Jana Müller</td>
<td>Effects of flooding stress on plant responses in selected <em>Brassicaceae</em> wild species</td>
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<td>Judith Bäumler</td>
<td>What is the function of the hypoxia induced transcription factors ABR1 and LBD41?</td>
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<td>Kohtaro Watanabe</td>
<td>A locus controlling formation of a barrier to radial oxygen loss is located on chromosome 3 in <em>Zea nicaraguensis</em></td>
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<td>Max Herzog</td>
<td>Mechanisms of waterlogging tolerance in wheat – a review of root and shoot physiology</td>
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<td>Differential ability to regulate voltage-gated K⁺-permeable channels in mature root epidermis determines genotypic difference in waterlogging stress tolerance in barley</td>
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<td>Nikita Sajeev</td>
<td>Understanding acclimative responses to multiple abiotic stresses in <em>Arabidopsis</em></td>
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<td>Qiaoli Ayi</td>
<td>Flooding impact disentangled: water pressure affects the survival and growth of the terrestrial plant <em>Alternanthera philoxeroides</em> upon complete submergence more than low light or oxygen availability</td>
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<td>12</td>
<td>Shanice F. Martopawiro</td>
<td>Ethylene-mediated hypoxia tolerance: comparing different meristems</td>
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<td>Simon Richter</td>
<td>Characterization of Phosphofructokinases in <em>Oryza sativa</em> and <em>Arabidopsis thaliana</em></td>
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<td>Sjon Hartman</td>
<td>Ethylene-induced hypoxia tolerance: a novel mechanism for flooding tolerance in plants</td>
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<td>Zeguang Liu</td>
<td>Ethylene primes hypoxia and post-hypoxia tolerance in <em>Arabidopsis thaliana</em></td>
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<td>Chen Lin</td>
<td>Growth of adventitious roots in <em>Oryza sativa</em> L.) is regulated by light</td>
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<td>Chiara Pucciariello</td>
<td>Role of CBL/CIPK complex in plants under O₂ shortage</td>
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<td>Daniel Roberts</td>
<td><em>Arabidopsis NIP2;1</em>, a lactic acid transporting aquaporin-like protein induced by low oxygen stress</td>
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<td>Anders Winkel</td>
<td>Internal aeration of paddy field rice during complete submergence – importance of light and floodwater O₂</td>
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<td>Juan Cardoso</td>
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| 23 | Beatrice Giuntoli | Regulation of oxidative stress responses by the ERFVII factors in *Arabidopsis thaliana* |
| 24 | Julia Bailey-Serres | Plasticity in response to submergence: development, genotype and time |
| 25 | Beatrice Giuntoli | HRA1 as a repressor of the anaerobic response: new evidence based on in vivo co-overexpression of RAP2.12 and HRA1 in *Arabidopsis thaliana* plants |
| 26 | Keisuke Nagai | Anatomical analysis of aerenchyma in grass |
Plant tissue anoxia – why, where and when

Jens Borum

Freshwater Biological Laboratory, Department of Biology, University of Copenhagen, Universitetsparken 4, 3rd floor, DK-2100 Copenhagen, Denmark

Plant tissue anoxia occurs when tissue respiration exceeds oxygen supply for sufficient time to deplete internal oxygen pools: \( R > J = D \cdot (C_0 - C_x) / \delta x \) where \( R \) is respiration, \( J \) is the flux or supply, \( D \) is the diffusion coefficient of oxygen, \( (C_0 - C_x) \) is the oxygen gradient from tissue surface to depth \( x \) within the tissue, and \( \delta x \) is diffusion length. Accordingly, oxygen depth penetration can be calculated as \( h = (2DC_0/R)^{1/2} \). \( D \) is highly dependent on the medium, air or water. \( R \), \( D \) and oxygen solubility (i.e. \( C_0 \)) depend on temperature. \( R \) and \( D \) increase with increasing temperature while oxygen solubility declines. \( \delta x \) depends on size of the organisms and \( C_0 \) depends on the oxygen balance of the external medium. In addition, turbulence of the bulk medium may influence \( C_0 \). Hence medium, thallus thickness, ambient oxygen partial pressure and temperature are the most important factors determining the balance between oxygen consumption and supply and thereby the risk of tissue anoxia. Consequently, plant tissues are more prone to anoxia in water or water logged soils, if tissues are thick and have low surface area to volume ratios, at high temperatures, in stagnant environments and at low ambient oxygen availability.

...:: Back to program ::..
The role of root porosity and other functional traits in plant community assembly along flooding gradients

Lars Bastrup-Spohr\textsuperscript{a}, Simone Møller Mortensen\textsuperscript{a}, Hans Henrik Bruun\textsuperscript{b} and Kaj Sand-Jensen\textsuperscript{a}

\textsuperscript{a) Freshwater Biological Section, University of Copenhagen, Universitetsparken 4, 2100 CPH Ø
\textsuperscript{b) Section for Ecology and Evolution, University of Copenhagen, Universitetsparken 15, 2100 CPH Ø

Why do plants grow where they grow? Prediction of species’ occurrence and abundance in relation to the environment is a core aim of ecology and so is understanding the link between environmental stressors and functional traits. Community assembly may be viewed as a sequence of filters, sorting species according to their functional traits. We ask, if root porosity along with other plant functional traits change similarly along different hydrological gradients? If the strength of filtering is stronger some parts of the gradient? And if we can use the information about trait-environment-relationships to predict species´ relative abundance using few, but biologically relevant traits?

Plant community composition and morpho-physiological plant traits were measured along a pronounced hydrological gradients in two sites with highly differentiated species pools. The strength of filtering was quantified using trait environment correlation and a trait dispersion index while the prediction of species´ relative abundance and importance of individual traits was assessed with a maximum entropy model (CATS-modelling).

We show that species are filtered by the hydrological environment through the traits root porosity, specific leaf area and resistance to water loss on drying. For individual traits, the strength of filtering waxes and wanes along the gradient. This strongly suggests that the mechanism, through which species are filtered into communities, acts through different traits as environmental conditions change along the gradient. In general, the traits subject to filtering also were most important in predicting species abundance.

Few plant traits are exposed to environmental filtering across the entire hydrological gradient, and most traits are strongly filtered only in parts of the gradient (e.g., root porosity in wet soils and water loss on drying on thin, dry soils). Evidence for congruence between trait dispersion indices and the CATS model was established, underpinning the importance to plant community assembly of environmental filtering of species through their traits. New functional traits relevant to a specific environmental gradient – and not just some standard traits from a public database – can contribute significantly to resolve how plant communities are assembled.

...: Back to program ::..
Flooding-induced adventitious root formation in *Solanum dulcamara* (Bittersweet) – from regulatory mechanisms to ecological benefits

Eric J.W. Visser\(^a\), Qian Zhang\(^a\), Heidrun Huber\(^a\), Hans de Kroon\(^a\), Thikra Dawood\(^b\), Xingping Yang\(^b\), Ivo Rieu\(^b\), Janny Peters\(^b\), and Celestina Mariani\(^b\)

\(^a\) Department of Experimental Plant Ecology, \(^b\) Department of Plant Science
Institute for Water and Wetland Ecology, Radboud University, Nijmegen, the Netherlands

Development of adventitious roots is a striking acclimation to flooding displayed by many plants, including some crop species. These new roots that form on the stem base replace the original root system, which often rapidly deteriorates in the anoxic flooded soil. Commonly, flooding-induced adventitious roots in species from frequently flooded habitats contain large intercellular gas spaces (aerenchyma), which provide a low-resistance connection of gas diffusion between the root tips and the atmosphere around the shoot.

The wetland species *Solanum dulcamara* (Bittersweet) has numerous dormant root primordia along its stem, which rapidly grow out into adventitious roots when the stem is submerged in water. Although the roots are similar to the nodal roots developing from submerged stem nodes of rice, the dormancy-breaking mechanism in the root primordia differed substantially. We investigated which environmental and hormonal signals were involved in this root formation process, and identified key players in the signal transduction pathway.

**Adventitious root formation on the stem of *S. dulcamara*, less than one week after the onset of flooding.**

*Solanum dulcamara* occurs in a variety of habitats, including wet marshes and reed beds, but also dry coastal primary dunes. We studied a number of populations from such contrasting habitats to determine if the capacity of adventitious root formation was a trait specifically linked to predictable flooding in the habitat of a population. Subsequently, fitness consequences of both flooding and drought were investigated in different populations to determine potential ecotype formation.

Moreover, we manipulated adventitious root formation to quantify the importance of this trait during flooded conditions, and we measured the contribution of these new roots to water and nutrient supply of the plant.

In conclusion, these studies showed that adventitious root formation in *S. dulcamara* resulted from a tightly regulated release of dormancy of pre-formed root primordia on the stem, with a major role for the plant hormones ethylene, auxin and ABA. Inter-population variation in this trait and other plant traits was small, and could not be linked to habitat differentiation. The data suggested a conserved high phenotypic plasticity in all populations, resulting in many adventitious roots in all populations upon partial submergence. The importance of the roots for plant fitness, through their contribution to
water and nutrient uptake, was clear, since manipulation in adventitious root formation substantially affected plant performance.

Publications:

...:: Back to program ::..
Internal tissue aeration in *Limonium narbonense* and *Sarcocornia fruticosa* during partial and complete submergence in saline water

*Elisa Pellegrini*<sup>ab</sup>, *Dennis Konnerup*<sup>b</sup>, *Anders Winke*<sup>b</sup>, *Valentino Casolo*<sup>a</sup> and *Ole Pedersen*<sup>b</sup>

<sup>a</sup>) Department of Agro-Environmental, Food and Animal Sciences, University of Udine, Via delle Scienze 206, 33100 Udine, Italy
<sup>b</sup>) Freshwater Biological Laboratory, Department of Biology, University of Copenhagen, Universitetsparken 4, 2100 Copenhagen, Denmark

*Limonium narbonense* Mill. and *Sarcocornia fruticosa* (L.) A.J. Scott are two perennial saltmarsh species sharing distribution in the Mediterranean coastal region. *L. narbonense* has basal leaves in rosettes and a deep root system connected to a vertical rhizome. *S. fruticosa* is instead a semi-woody plant with modified photosynthetic stems and superficial roots. The distribution of these two species, ranging from elevated saltmarsh sites to low and daily inundated areas, arose the interest around the flooding tolerance mechanisms involved and the possible adaptation to different levels of flooding stress. Field collected plants were conditioned in a controlled tidal system in a greenhouse, imposing two treatments: (1) changing from drained to waterlogged conditions and (2) changing from waterlogged to complete submergence. Internal tissue aeration was monitored using O$_2$ microelectrodes during waterlogging, partial and complete submergence in darkness as well as in light. Underwater photosynthesis and dark respiration were measured by measuring the O$_2$ balance from excised green stems and leaves while porosity measurements were performed in different epigeous and hypogeal plant tissues. O$_2$ dynamics in soil showed a clear and rapid increase of O$_2$ during drainage condition (1) and steady anoxic conditions in the submerged treatment (2), which adversely affected *L. narbonense* leaf production but stimulated the development of new articles in *S. fruticosa*. In both species, internal O$_2$ declined upon partial and complete submergence in darkness: *L. narbonense* showed values closed to zero already during partial submergence, both in petioles (9.1 μmol·l$^{-1}$ in average) and in roots (5.6 μmol·l$^{-1}$), while *S. fruticosa* displayed better root aeration during partial submergence (44.4 μmol·l$^{-1}$). In light, internal O$_2$ concentrations of the articles of *S. fruticosa* and the petioles of *L. narbonense* increased, entailing also a gradual increase in O$_2$ in roots, especially during the re-establishment of waterlogging conditions (over 50 μmol·l$^{-1}$ in both species). At air equilibrium of CO$_2$, rates of underwater photosynthesis and dark respiration differ significantly between treatments only when based on dry mass, highlighting a morphological acclimation in leaf thickness of the plants during submergence (higher SLA). Despite the low photosynthetic rates, intact plants were able to cover the O$_2$ requirement during inundation under light conditions (up to 179.7 and 73.0 μmol·l$^{-1}$ in *S. fruticosa* and *L. narbonense*, respectively). High tissue porosity is a common trait providing a low-resistance internal pathway for O$_2$ diffusion to roots. *S. fruticosa* displayed high tissue porosity both in lignified stems (up to 25%) and in roots (up to 27%) but for *L. narbonense* tissues had even higher porosity (in petioles and leaves up to 63%). These findings suggest that the low internal O$_2$ recorded during submergence of *L. narbonense* must be due to unidentified bottlenecks for O$_2$ diffusion in e.g. root-rhizome junctions or rhizome-petiole junctions, restricting internal aeration. Our findings demonstrate the importance of waterlogging
and submergence tolerance in these two species inhabiting saltmarshes, providing additional knowledge to flooding tolerance of halophytes.

...: Back to program ::...
Leaf gas film retention and underwater photosynthesis of 14 wheat genotypes during submergence

Dennis Konnerup, Anders Winkel, Max Herzog and Ole Pedersen

Freshwater Biological Laboratory, Department of Biology, University of Copenhagen, Universitetsparken 4, 3rd floor, 2100 Copenhagen, Denmark

Gas films on superhydrophobic leaves of terrestrial plants have been shown to enhance gas exchange when the plants are submerged, both in terms of CO₂ uptake for photosynthesis during the day and entry of O₂ for respiration during the night (Colmer and Pedersen 2008; Pedersen et al. 2009). Hence, the gas films contribute to improved internal aeration and survival during temporary submergence, which is highly relevant for some crops. Waterlogging or submergence of winter wheat seedlings after torrential rainfall is becoming an increasing problem in many countries where precipitation patterns are changing due to climate change.

The present study explored gas film retention during submergence of 14 wheat genotypes and compared them to the wild species Catabrosa aquatica. The plants were completely submerged for up to 10 days and gas film thickness was measured and related to the loss of hydrophobicity measured as contact angle (Fig. 1). Underwater photosynthesis measured at 200 μM CO₂ declined with time of submergence and this corresponded to the loss of gas films. The surfaces of the leaves were investigated using Scanning Electron Microscopy (SEM) in order to determine why the leaves lose their hydrophobicity. It is concluded that the loss of gas film is associated with the leaves being covered by an unknown substance.

Fig. 1. Leaf gas film thickness and surface wettability (measured as water droplet contact angle) of three wheat (Triticum aestivum) genotypes (Jensen, Albert and Jackson) and water whorl grass (Catabrosa aquatica) with time of submergence.

References
The role of leaf gas films in oxygen transport during submergence of deepwater rice

Yoshiano Mori\textsuperscript{a}, Keisuke Nagai\textsuperscript{a}, Timothy D. Colmer\textsuperscript{b}, Ole Pedersen\textsuperscript{bc} and Motoyuki Ashikari\textsuperscript{a}

\textsuperscript{a) Bioscience and Biotechnology Center, Nagoya University, Furo-cho, Chikusa, Nagoya, Aichi 464-8601, Japan
\textsuperscript{b) School of Plant Biology, Faculty of Natural and Agricultural Sciences, The University of Western Australia, 35 Stirling Highway, Crawley, WA6009, Australia
\textsuperscript{c) Freshwater Biological Laboratory, Biological Institute, University of Copenhagen, Universitetsparken 4, 3rd floor, 2100 Copenhagen OE, Denmark

Deepwater rice can survive flooded conditions by elongation of its internode and leaves keeping the leaf tip above the water surface. Under complete submergence, uptake of oxygen and carbon dioxide is disturbed because diffusion coefficient of gases is 10000 times less than in air. It is believed that the oxygen produced from photosynthesis in leaf blade is transported through the aerenchyma to other underwater plant parts. Moreover, the gas films on leaf blade facilitate the exchange of gases during submergence. However, the mechanism on how the deepwater rice manages the supply of oxygen under submerged condition is not completely understood. To investigate this, we measured the oxygen partial pressure in aerenchyma of internode using optical oxygen sensor. Our results revealed that: (1) deepwater rice maintained higher oxygen partial pressure under complete submergence than in air condition at day time; (2) oxygen partial pressure decreased when gas film on leaf blade was removed; (3) there was no difference on oxygen partial pressure between the upper internode and lower internode. Taken together, the results suggest that the leaf gas films play an important role to retain and enable the oxygen produced from photosynthesis to be transported to internode during submergence. This mechanism may explain that despite low oxygen environment during complete submergence, the deepwater rice has the ability for rapid internode elongation. In summary, the elongating internode functions as a ‘snorkel’ allowing the transport of oxygen from the leaf blade to the entire deepwater rice plant.

::: Back to program :::
**Leaf Gas Film 1 (LGF1) maintains hydrophobicity of leaves essential for underwater gas exchange**

*Yusuke Kurokawa*, Huan D Phung, Qu Huangqi, Keisuke Nagai, Yosuke Toda, Takeshi Kuroha, Kosuke Shimazaki, Mie Shimojima, Saori Aiga, Junichi Ito, Imran Malik, Timothy D Colmer, Ole Pedersen, and Motoyuki Ashikari

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Among the three world’s major food crops including wheat and maize, rice is the only crop which can survive in a semi-aquatic environment. A rice plant possesses aerenchyma in leaves, stems, and roots which functions as air channels enabling gas exchange between the tissues above and submerged in the water. Moreover, the superhydrophobic layer on the leaves also called as “gas film” is also essential for gas exchange in underwater photosynthesis and respiration. However, despite this importance, the factors regulating the formation of gas film are not yet identified. Here we demonstrate the physiological, biochemical, and genetic bases of gas film formation using the *dripping-wet leaf (drp)* rice mutant, which lacks water repellency of leaves. The submerged wild type rice Kinmaze maintained its gas film for several days while the *drp* mutant, after 1 day of submergence, almost lost the gas film and showed a reduced underwater photosynthesis. Consequently, the *drp* mutant was not able to survive in the irrigated paddy field. Positional cloning identified the *Leaf Gas Film 1 (LGF1)* gene which regulates gas film formation. Complementation test using *LGF1* overexpression lines rescued the phenotype of *drp* mutant. Scanning electron microscopy revealed that the *drp* mutant leaves noticeably lacked wax crystals. Gas chromatography-mass spectrometry confirmed the altered wax composition in *drp* mutant as indicated by accumulation of carbon (C) 30 aldehyde and reduction of C30 primary alcohol. The results suggest that the *LGF1* may be specifically involved in the synthesis of C30 primary alcohol from C30 aldehyde for formation of leaf wax. The *LGF1* defines a regulatory mechanism for wax deposition to maintain the gas film on leaves essential for underwater gas exchange. This study provides new insights on the adaptation mechanism of rice for an effective gas exchange in a semi-aquatic habitat such as the paddy field.

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No escape? Costs and benefits of plant de-submergence in the pasture grass *Chloris gayana* under different submergence regimes

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Rapid leaf/shoot elongation is one of the main plant responses to cope with complete submergence by re-establishing leaf contact with the atmosphere (‘escape’ strategy). This response is supposed to be beneficial under long term submergence rather than under alternated short-term submergence events (e.g. fluctuating environment) as the costs of repeated plant ‘adjustment’ can exceed the initial benefits. Nevertheless, this is an assumption that rarely has been quantitatively addressed. In a first experiment, we examined the tolerance of 3-leaf seedlings of seven cultivars of *Chloris gayana* (Callide, Epica, Fine cut, Katambora, Pioneer, Tolga and Top cut) to complete submergence in clear water along 14 days. This species is a highly productive forage grass of increasing importance in grasslands, prone to flooding, in South America. All cultivars displayed an escape strategy without differences in dry mass (44-53% of drained controls at the end of submergence). However, after water subsides, the cultivars Fine cut, Pioneer, Tolga and Callide resumed their growth (dry mass 24 to 54% of their drained controls at end of recovery); contrary to Top cut, Katambora and Epica, which perished. Among the first group, Fine cut was the most tolerant/promising material. We used this cultivar in a second experiment to quantify the benefit (or cost) of exhibiting (or not allowing) the ‘escape’ strategy, and to assess the effects on grow of two submergence regimes. So, 3-leaf seedlings in 0.4 L pots with soil (3.3% organic carbon) and sand (1 v/v) grew in plastic containers during (i) 4 weeks at well drained conditions (i.e. field capacity), (ii) two cycles of 1-week submergence followed by 1week at well drained conditions (total: 4 weeks; repeated short-term submergence), (iii) 1 cycle of 2-week submergence followed by a 2-week period at well drained conditions (total: 4 weeks; long-term submergence). Water level was 24 cm and initial seedling height ranged between 18 and 20 cm. Plastic nettings were placed 2 cm below water over an additional set of seedlings to prevent leaf de-submergence, under the same submergence regimes commented before. Netting only reduced light by 5.5% (e.g. from 544 ± 5.1 to 514 ± 4.0 μmoles m<sup>−2</sup> s<sup>−1</sup> when underwater) and did not affect seedlings growth in well-drained soil as they attained similar dry mass with and without netting (P=0.64). Impeding leaves from emerging above water through netting did not compromise survival when submergence was 1-week long, but determined death of all seedlings when submergence was extended to 2 weeks. After a second 1-week submergence cycle, survival was also compromised as 50% of seedlings perished, and growth was reduced by 30% among the remaining alive seedlings (compared to those submerged without netting; P<0.05). Growth as affected by submergence regimes (without netting) at the end of experiment revealed that under one event of 2-week submergence, seedlings accumulated a 65% higher dry mass than when they experienced the same submergence duration but in two separate events of 1-week (0.73 vs. 0.25 g/seedling, respectively, P<0.01; controls: 1.73 g/seedling). Therefore, it can be concluded that the ‘escape’ strategy in *C. gayana* by which leaf contact with air is re-established is essential for its survival, and it is more beneficial under long-term submergence than under repeated short-term submergence cycles.
Waterlogging tolerance in lentil and pea associated with geographic origin

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Lentil (*Lens culinaris* subsp. *culinaris* Medik) is grown under rainfed conditions after rice in South Asia. However, production of legumes in rice-based cropping systems is difficult due to their susceptibility to waterlogging and drought. To develop an economically profitable rice-pulse-rice cropping system it is important to find pulse genotypes that can tolerate soil waterlogging at germination to early developmental stage.

Pea (10 advanced lines) and lentil (8 advanced lines) were sown (i.e. broadcasted) into a standing rice in western Bangladesh. Rice was manually harvested two weeks later. Pulses were sown on the standing rice field to expedite maturity and an early harvest to vacate the field for a timely subsequent rice crop. Soils were at reduced state (redox value close to 0 mV) and remained reduced for 2 weeks during establishment of the pulse crop. There was a yield penalty (up to 50%) for both pea and lentil for early sowing in reduced soils. However, significant variation was observed among advanced lines in both peas and lentils.

In a glass house experiment in Western Australia, 127 lentil accessions from 11 countries and 120 pea accessions from 49 countries were evaluated for waterlogging tolerance in a pot soil system at germination. There was significant variation among accessions in response to soil waterlogging, which was associated with geographic origin.

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Non-destructive phenotyping to identify *Brachiaria* hybrids tolerant to waterlogging stress

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*Brachiaria* grasses are one of the most important forage grasses planted in tropical regions around the world. Seasonal waterlogging is a major constraint in humid regions that limit persistence of *Brachiaria* grasses thereby reducing livestock productivity. While some *Brachiaria* cultivars are moderately tolerant to seasonal waterlogging, none of the commercial cultivars combine superior yield potential and nutritional quality with greater level of waterlogging tolerance. The *Brachiaria* breeding program at the International Center for Tropical Agriculture (CIAT), has been using recurrent selection for the past two decades to combine plant vigor with resistance to biotic and abiotic stress factors. A subset of 24 promising *Brachiaria* hybrids were tested under waterlogged conditions in the field with the main objective to identify better adapted hybrids. Both destructive and non-destructive methods of phenotyping were used to test the performance of *Brachiaria* hybrids under waterlogged conditions. Strong correlations between non-destructive measurements (NDVI, canopy cover) and harvested plant biomass were found. Using our experimental methodology, we have identified five hybrids with superior adaptation to waterlogging compared to commercial cultivars. NDVI and canopy cover analyses have potential for selection of superior hybrids tolerant to waterlogging stress. These findings suggest that significant genetic variation for waterlogging tolerance exists within the CIAT *Brachiaria* breeding materials and that current phenotyping methodologies can be used to effectively select for increased tolerance to waterlogging in *Brachiaria*.

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The roles of ethylene and auxin in constitutive lysigenous aerenchyma formation in rice root

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Lysigenous aerenchyma forms in roots as a result of the death and subsequent lysis of cortical cells. In rice roots, lysigenous aerenchyma is constitutively formed even under aerobic conditions, and its formation is induced under oxygen-deficient conditions. The former and latter are respectively designated as “constitutive” and “inducible” aerenchyma formations. Inducible aerenchyma formation is stimulated by a phytohormone, ethylene.

To determine whether ethylene is also involved in constitutive aerenchyma formation in rice roots, rice seedlings were treated by an ethylene perception inhibitor (1-methylcyclopropene (1-MCP)) under aerated conditions. 1-MCP treatment partly inhibited aerenchyma formation, suggesting that constitutive aerenchyma formation in rice roots involves both ethylene-dependent and ethylene-independent pathways.

We found that the ethylene-independent pathway involves auxin, another phytohormone. Auxin is essential for root development, including adventitious root and lateral root formation. Auxin signaling progresses through the degradation of Aux/IAA proteins (IAA proteins) after perception of auxin. In a lateral-root-number-reduced mutant, Osiaa, in which the degradation of OsIAA protein is inhibited, constitutive aerenchyma formation is substantially reduced. Aerenchyma formation was also inhibited by an inhibitor of auxin transport (N-1-naphthylphthalamic acid (NPA)) in wild-type rice grown under aerated conditions. Expression of a gene encoding a transcription factor (LATERAL ORGAN BOUNDARIES (LOB)) was significantly reduced in roots of the Osiaa mutant under aerated conditions, suggesting that LOB expression is controlled by an IAA-mediated auxin signaling pathway. Introducing the LOB gene into the Osiaa mutant partially restored aerenchyma formation. Together, these results suggest that auxin signaling mediated by OsIAA protein is involved in constitutive aerenchyma formation in rice roots.
Regulation of Arabidopsis root architecture by oxygen availability

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Postembryonic development of lateral roots (LRs) in plants is controlled by genetic and environmental factors to optimize nutrient acquisition in a heterogeneous and changing environment. LR formation is controlled by several auxin signaling modules at different stages of development starting from establishment of LR primordia to emergence of LR. Among other environmental cues, oxygen levels potentially direct root architectural variations. A decrease in oxygen availability is known to cause root growth inhibition in Arabidopsis, although this is mainly considered to be associated to reduced metabolic rates. Instead, exploiting transgenic plants impaired in oxygen perception, we gathered evidences that support a proactive strategy of LR repression under hypoxia, independently of the restriction in respiration. Therefore, we now aim at deciphering the hormonal regulation of LR development under low oxygen conditions. Preliminary results hint at a crosstalk between ERF-VII transcription factors and hormone signaling modules to hinder LR emergence when oxygen availability is reduced.

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Developmental adaptations of Arabidopsis roots to low oxygen conditions

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Flooding is a major abiotic stress that is encountered foremost by plant roots. Roots adapt to reduced oxygen availability by altered metabolism and, dependent on the species, formation of aerenchyma and strengthening of the rhizodermis to improve oxygen supply to the root tip. Arabidopsis is a widely used model plant in flooding stress research but developmental adaptation of the root system to limited oxygen availability has not been exhaustively studied in this species. We show that the gravitropic growth response of the main root is impaired at low oxygen conditions causing the primary root to slant. Furthermore, hypoxia inhibits lateral root growth postemergence. Interestingly, both responses are linked to subgroup VII ERF transcription factors suggesting that these ERFs are important to maintain the root structure at low oxygen conditions.

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Regulation of energy metabolism when the oxygen availability decreases

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An early response of plant cells to decreasing oxygen availability is the reprogramming of primary energy metabolism. Energy (ATP) consuming processes are downregulated in favor of more ATP efficient pathways; the rate of respiratory oxygen consumption goes down; substrate level phosphorylation by the glycolytic pathway is upregulated while fermentation is activated. Previously, it was shown that transcriptional regulation of enzymes involved in these adaptive responses is involved in the reprogramming of primary energy metabolism in plant cells upon hypoxia. Examples of this are the expression of enzymes involved in fermentation, the futile Nitric oxide cycle, and alanine and GABA metabolism to modify the TCA-cycle activity. Oxygen dependent destabilization of ERF transcription factors allows a precise control of the transcriptional activity of these constitutively expressed transcription factors. Mutations that affect this control mechanism have detrimental effects on the stress tolerance of plants, indicating the importance of conditional regulation of the metabolic hypoxia response. Recent investigations indicate that not only the oxygen concentration initiates the hypoxic responses, but integration of this signal with a combination of the cellular energy charge and the production of reactive oxygen and nitrogen species is required to guarantee an efficient control of hypoxic gene expression and respiratory metabolism. In this presentation, I will discuss how respiratory metabolism is affected upon hypoxia. Special emphasis will be given on the activity of the futile nitric oxide cycle that controls glycolytic flux with minimal loss of carbon as ethanol or lactate. Furthermore, the importance of integration of the oxygen concentration as signal with the energy charge of the cell will be discussed.
Temperature, a complicating factor of the respiratory down-regulation in bulky plant organs

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The respiration rate of plant tissues decreases when the amount of available O\textsubscript{2} is reduced. There is, however, a debate on whether the respiration rate is controlled either by diffusion limitation of oxygen or through regulatory processes at the level of transcriptome. We used experimental and modelling approaches to demonstrate that both diffusion limitation and metabolic regulation affect the response of respiration of bulky plant organs to O\textsubscript{2} stress.

The underlying concept for the experimental work was based on the fact that, if temperature is reduced, the respiratory activity decreases correspondingly. Any gradients of the O\textsubscript{2} concentration in the fruit would then be reduced, rendering direct experimental control over fruit internal O\textsubscript{2} concentrations by changing the fruit external storage atmosphere, thereby potentially exposing other regulatory mechanisms of the respiration pathway.

The experimental data contradicted simulation results obtained with the gas exchange model incorporating conventional Michaëlis Menten based respiration kinetics suggesting an additional reduction in respiration rate beyond the substrate effects already accounted for. Therefore we modified the respiration kinetics to allow for V\textsubscript{m,O2} to change as a function of the O\textsubscript{2} level rather than keeping it constant. The obtained modelling results matched the measurements well indicating that additional regulatory effects of the respiration pathways are likely to occur. The dynamics of regulatory and signalling pathways in the cell were modelled by reaction kinetics at the transcriptome level. We assumed that an O\textsubscript{2} signal could modulate the biosynthesis of respiratory enzymes in the cell through activation of an O\textsubscript{2} receptor.

Based on the combined experimental and modelling results we could conclude that diffusion limitation highly affects fruit respiration at high temperature, but at low temperature the respiration is reduced through a regulatory process, presumably as a response to a signal generated by a plant oxygen sensor. The response of respiration to O\textsubscript{2} is time dependent and is highly sensitivity, particularly at low O\textsubscript{2} levels in the surrounding atmosphere. Down regulation of the respiration at low temperatures may save internal O\textsubscript{2} and relieve hypoxic conditions in the fruit, particularly under commercial storage conditions.

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RAP2.12 oxygen sensing regulates plant metabolism and performance under both normoxic and hypoxic conditions

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Subgroup-VII-ethylene-response-factor (ERF-VII) transcription factors are involved in the regulation of hypoxic gene expression and regulated by proteasome-mediated proteolysis via the oxygen-dependent branch of the N-end-rule pathway. While research into ERF-VII mainly focused on their role to regulate anoxic gene expression, little is known on the impact of this oxygen-sensing system in regulating plant metabolism and growth. By comparing Arabidopsis plants overexpressing N-endrule sensitive and insensitive forms of the ERF-VII-factor RAP2.12, we provide genetic evidence that RAP2.12 oxygen sensing plays important roles to regulate central metabolic processes to sustain growth, development and anoxic resistance of plants. (i) Under normoxia, overexpression of N-endrule insensitive delta13RAP2.12 led to increased activities of fermentative enzymes and increased accumulation of fermentation products, which were accompanied by decreased adenylate energy states, starch and sugar levels and impaired plant growth, indicating a role of RAP2.12 oxygen sensing to prevent aerobic fermentation. (ii) In delta13RAP2.12 plants, decreased carbohydrate reserves also led to a decrease in anoxic resistance, which was prevented by external sucrose supply. (iii) Overexpression of delta13RAP2.12 led to decreased respiration rates, changes in tricarboxylic acid cycle intermediates and accumulation of a large number of amino acids, including alanine and gamma-amino butyric acid, indicating a role of RAP2.12 oxygen sensing in controlling the flux-modus of the tricarboxylic acid cycle. (iv) The increase in amino acids was accompanied by increased levels of immune-regulatory metabolites. These results indicate RAP2.12 oxygen sensing to be of central importance to sustain metabolic performance, plant growth and development under both normoxic and hypoxic conditions.
Metabolic control of the anaerobic response in Arabidopsis

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Arabidopsis is not highly tolerant to submergence, which would not necessarily make this species an ideal system to unveil adaptive mechanisms regarding survival to low oxygen availability. Nevertheless, the use of Arabidopsis made the discovery of oxygen sensing and signalling mechanisms possible. Arabidopsis shares with other plants the classical response to low oxygen, including the activation of the fermentative pathway coupled to glycolysis to compensate for the lack of mitochondrial ATP production and NADH recycling. Under low oxygen RAP2.12 and the other two constitutively expressed group VII ERFs, RAP2.2, RAP2.3, stable and redundantly activate the core anaerobic response in Arabidopsis. The RAP2.12 dependent activation of the downstream genes is essential to survive submergence, but also needs to be finely tuned. The HYPOXIARESPONSE ATTENUATOR1 (HRA1) is a trihelix transcription factor that represses the action of RAP2.12. It is tempting to speculate that excessive activation of the fermentative pathway by RAP2.12 may deplete sugars to a level that induces severe starvation, hampering long term survival and recovery from hypoxia. HRA1 attenuation of the anaerobic response might thus provide an essential breaking mechanism to achieve optimal fermentative activity.

Here, we describe the integration of oxygen sensing and downstream gene regulation with the metabolic status of the plant. We analyse the importance of starchy reserves for tolerance to submergence and for the induction of anaerobic genes. Mutants defective in starch synthesis or degradation were used to mimic conditions with varied levels of carbon reserves and/or rates of Cutilization.

The results demonstrated that the degree of induction of several anaerobic genes was strictly dependent on the available carbohydrates levels. These results suggest the existence of a cross-talk between the sugar-sensing machinery and the hypoxic signalling pathway. The contribution of RAP2.12 and HRA1 to this regulatory pathway will be discussed.
Oxygen-dependent regulation of meristematic activity in the *Arabidopsis thaliana* shoot apex

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In animals, internal oxygen gradients have been shown to act as essential positional cues to control stem cell pluripotency and cell division. Interestingly, using a combination of molecular oxygen reporter constructs stably expressed in plants, we discovered that the shoot apical meristem (SAM) is enclosed in a hypoxic niche. Alterations of the hypoxic niche compromised stem cell activity, resulting in an altered phyllochron index. Accordingly, we observed that changes in the exogenous oxygen availability affects the expression of typical SAM marker genes. Thus far, hypoxic conditions in plants have been studied mostly as stressful situation due to a disproportion between plant respiratory rates and environmental oxygen availability. To cope with this, the Cys-branch of the N-end rule pathway acts as a mechanism to control responses to low oxygen stress, by promoting the accumulation of ERF-VII transcription factors in the nucleus when oxygen levels decrease. Following this observation, we now focus our attention on proteins characterized by a conserved N-terminal cysteine residue and a potential role in the regulation of meristematic activity in the shoot apex. We are currently testing whether these new candidates behave as true N-end rule substrates and if their stability is controlled by oxygen availability, to ultimately characterize new mechanisms by which oxygen availability controls developmental patterns in addition to metabolic processes.

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Flooding stress survival: the importance of foresight

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The underwater environment results in several changes in flooded plant organs. Depending on the conditions of flooding, these include changes in oxygen and light intensity. There is also accumulation of the volatile gas hormone ethylene. While oxygen levels can differ vastly within submerged plant tissues, ethylene is assumed to accumulate to saturating concentrations in all flooded tissues very rapidly (within an hour). It is therefore considered an early and reliable signal of flooding. We observed that Arabidopsis plants that have been exposed to high concentrations of ethylene have a higher tolerance to subsequent hypoxic conditions. We hypothesized that during submergence, the early ethylene accumulation triggers changes that are advantageous to survival of low oxygen conditions that occur later. We show how this response involves the group VII ethylene response factor family of transcription factors and is also dependent on light levels and plant developmental stage. How differences in hypoxia tolerance between plant organs and meristems could result from differential interactions and dynamics of ethylene, low oxygen and light intensity levels will be discussed.

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Arabidopsis CML38, a hypoxia-induced calcium sensor that associates with stress granule and P-body messenger ribonucleoprotein particles

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Arabidopsis CML38 is a core hypoxia-response gene that encodes a calmodulin-like calcium sensor protein. Phylogenetically, CML38 is structurally related to a subfamily of calmodulin-like proteins known as the "regulator of gene silencing" calmodulins (rgsCaM). rgsCaM was initially discovered as an endogenous regulator of posttranslational gene silencing (PTGS), and as a target for the viral proteins that suppress the small RNA PTGS response. CML38 transcripts are up-regulated >300 fold in roots during the early stages of hypoxia treatment, showing a gradual decline and complete disappearance upon reoxygenation. T-DNA insertional mutants of CML38 show an increased sensitivity to anaerobic stress suggesting that CML38 plays a role in sensing and transducing calcium signals during the anaerobic response. By using YFP translational fusions and recombineering technology, localization analyses of CML38 protein show association with cytosolic granule structures similar in morphology to hypoxia-induced stress granules. Immuno-precipitation of CML38 from the roots of hypoxia-challenged transgenic plants harboring CML38pro::CML38:YFP followed by LC MS/MS analysis reveal the presence of protein targets associated with mRNA ribonucleoprotein (mRNP) complexes including stress granules, which are known to accumulate as mRNA storage and triage centers during hypoxia, as well as P-bodies, which are involved in mRNA destabilization and turnover. These findings are further supported by the co-localization of CML38 with stress granule and P-body specific markers upon co-transfection of Nicotiana benthamiana leaves. Ruthenium red treatment results in loss of CML38 signal in cytosolic granules and localization to the nucleus, suggesting calcium is necessary for stress granule association. The results confirm that CML38 is a core hypoxia response calcium-sensor protein, and suggest that it serves as a potential calcium signaling target within mRNP particles during low oxygen stress responses (supported in part by a subcontract from a subcontract from National Science Foundation (NSF) Award IOS-1029803, and by NSF IOS-1121465).
Revealing the role of plasma membrane calcium and potassium transport systems in plant sensing and adaptation to low oxygen environment

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Oxygen deprivation is a key determinant of root growth and functioning under waterlogging. We measured changes in net K\textsuperscript{+} flux and membrane potential (MP) of root cells from elongation and mature zones of two barley varieties under hypoxia and anoxia in the medium, and as influenced by ability to transport O\textsubscript{2} from the shoot. We show that O\textsubscript{2} deprivation results in an immediate K\textsuperscript{+} loss from roots, in a tissue- and time-specific manner, affecting root K\textsuperscript{+} homeostasis. Both anoxia and hypoxia induced transient membrane depolarization; the extent of this depolarization varied depending on severity of O\textsubscript{2} stress and was less pronounced in a waterlogging-tolerant variety. Intact roots of barley were capable of maintaining H\textsuperscript{+}-pumping activity under hypoxic conditions while disrupting O\textsubscript{2} transport from shoot to root resulted in more pronounced membrane depolarization under O\textsubscript{2}-limited conditions and in anoxia a rapid loss of the cell viability. Pharmacological experiments have revealed that depolarization-activated outward-rectifying K\textsuperscript{+} channels (GORK in Arabidopsis) are the most likely downstream target of low O\textsubscript{2} stress. This hypothesis was further confirmed in direct experiments using a range of K\textsuperscript{+} transport mutants, with the gork mutant lacking a functional K\textsuperscript{+}-efflux channel showing strongest phenotype under low O\textsubscript{2} conditions. Overall, these results suggest that the ability of root cells to maintain MP and cytosolic K\textsuperscript{+} homeostasis is central to plant performance under waterlogging, and efficient O\textsubscript{2} transport from the shoot may enable operation of the plasma membrane H\textsuperscript{+}-ATPase in roots even under conditions of severe O\textsubscript{2} limitation in the soil solution (Zeng et al 2014 Plant Cell Environment; Shabala et al 2016 J Exp Bot).

The crucial role of Ca\textsuperscript{2+} as a second messenger in response to abiotic and biotic stimuli has been widely recognized in plants; this also involves plant sensing of hypoxia stress. However, the physiological and molecular mechanisms of Ca\textsuperscript{2+} distribution within specific cell types in different root zones under hypoxia are poorly understood. To fill this gap in our knowledge, whole-plant physiological and tissue-specific Ca\textsuperscript{2+} changes were studied using several ACA (Ca\textsuperscript{2+}-ATPase) and CAX (Ca\textsuperscript{2+}/proton exchanger) knock-out Arabidopsis mutants subjected to waterlogging treatment. In the wild type (WT) plants, several days of waterlogging decreased the expression of ACA\textsubscript{8}, CAX\textsubscript{4} and CAX\textsubscript{11} by 33 and 50\% compared to the control. The treatments also resulted in an up to 11-fold tissue-dependent increase in Ca\textsuperscript{2+} accumulation in root tissues as revealed by confocal microscopy. The increase was much higher in stelar cells in mature zone of Arabidopsis mutants with loss-of-function for ACA\textsubscript{8}, ACA\textsubscript{11}, CAX\textsubscript{4} and CAX\textsubscript{11}. In addition, significantly increased Ca\textsuperscript{2+} concentration was also found in cytosol of stelar cells in mature zone after hypoxic treatment. Three weeks of waterlogging resulted in a 67\% reduction of shoot dry mass in cax\textsubscript{11} plants, while in WT and other Ca\textsuperscript{2+}-transport mutants this decline was only 14 to 22\%. These results were also consistent with a decline in leaf chlorophyll fluorescence (Fv/Fm) in cax\textsubscript{11} plants. It is suggested that CAX\textsubscript{11} plays a key role in maintaining cytosolic Ca\textsuperscript{2+} homeostasis and/or signalling under hypoxic conditions (Wang et al 2016 J Exp Bot).
An update on seed hypoxia and oxygen sensing technology

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This presentation is directed at defining the “where”, “when” and “why” of hypoxia in the developing seed of our major crops, especially cereal grains. High resolution structural imaging using X-ray/synchrotron radiation revealed that seed’s void spaces are small and poorly inter-connected, strongly impeding gas diffusion (1). The quantification of the effect of oxygen availability on gene expression, enzymatic activity, metabolite pool sizes and metabolic fluxes have demonstrated convincing evidence for the occurrence of hypoxia in vivo, affecting the rate of accumulation of seed biomass and individual storage products. Although possibly coincidental, the spatiotemporal pattern of hypoxia corresponds to those of cell size and programmed cell death in the cereal endosperm. It is demonstrated how in silico modelling approaches can elucidate metabolic compartmentation and dynamic metabolic acclimations to local occurrence of hypoxia inside the seed (2-4). We also present the latest developments in oxygen sensor technology: planar optodes, made to capture the bi-dimensional oxygen distribution at cellular resolution (5). When monitoring oxygen consumption over time, detailed 2D-respiration maps can be deduced. This imaging technology is quantitative, and can be used to identify those regions with most intense oxygen demands (prone to hypoxia/anoxia). Examples are demonstrated for its application on developing and germinating seeds, stems and roots.

(1) Verboven et al., Void space inside the developing seed of Brassica napus and the modelling of its function. New Phytologist 199 (2013).

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A previously-unexplored link between sugar and oxygen effects on metabolism and sensing: *Nucleoside diphosphate kinase 1 (Ndpk1)*

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When O$_2$ is limited, sucrose metabolism shifts from a path that begins with invertase to one mediated by sucrose synthase (SucSynth). Invertase is repressed while SucSynth is up-regulated. Sucrose cleavage through the SucSynth pathway depends on balanced ratios of UDP/UTP. A key player is nucleoside diphosphate kinase (NDPK), which is responsible for recycling nucleoside triphosphates (e.g. UTP) into their corresponding nucleoside diphosphate (e.g. UDP) (Bailey-Serres and Voesenek 2008). In addition, NDPK activity is enhanced in plant organs under low oxygen (Perata et al, 1996; Guglielminetti et al, 1995). Moreover, *Ndpk1* mRNA is highly expressed in tissues with a low-oxygen microenvironment and high energy demands, such as meristems (Dorion et al, 2006) and seed endosperm (Sanclemente et al, 2016).

Recent research indicates that NDPK has the capacity to bind non-canonical forms of DNA such as G4-quadruplexes (G4s) and modulate transcription of respective genes (Kopylov et al, 2014; Postel et al, 1993). In maize, the G4s are disproportionately prevalent in the 5’UTRs of many sugar- and low-oxygen-responsive genes, including those encoding SucSynth (Sh1) and alcohol dehydrogenase (Adh1, Adh2) (Andorf et al, 2015). The NDPK1 is predominantly cytoplasmic, however, previous evidence indicates this protein can migrate to the nucleus in response to abiotic stress. In vitro binding of ZmNDPK1 to G4s in the hexokinase gene (Hxk4) (Kopylov et al., 2014) suggests a potential role for NDPK1 in plant signaling pathways by modulating sugar-and stress-responsive genes. The objective of work reported here was to test hypotheses for physiological roles of NDPK1 in maize root tips responding to changes in sugar status and oxygen availability. Toward this end, we used a cultured-root-tip system that allows precise manipulation of not only the oxygen status, but also key aspects of metabolism while maintaining growth. Here, excised root tips from 3-d old seedlings of a W22 inbred were subjected to two oxygen levels (20% or 0.2%) in combination with two glucose levels (2% or 0.2%). Oxygen supply was controlled in a gas flow system and glucose content adjusted in the Whites media that circulated with root tips during incubations. Time-course analysis with qPCR showed rapid increases in levels of both *ZmNdpk1* and *Hxk4* mRNAs within 3h of treatment with low-oxygen (0.2%) or incubation with physiologically-abundant glucose (2%). The *Adh1* and *Adh2* transcripts (used as indicators of O$_2$ deficiency) were also up-regulated by plentiful glucose regardless of O$_2$ availability, however, increases were greater under low oxygen conditions. A sugar-oxygen overlap is thus evident for *Adh* responses. Maximal accumulation of *Ndpk1* and *Hxk4* mRNAs had taken place by 6h. However, levels of *Ndpk1* remained abundant throughout at least 22h while *Hxk4* decreased. The *Adh* transcripts rose as expected between 6 and 22h. The NDPK1 is a key player in the metabolism of sucrose through the SucSynth path that allows glycolytic and biosynthetic pathways to proceed under low oxygen conditions. The early response of the *Ndpk1* transcript is consistent with proposed roles of *Ndpk1* in metabolism, and also in proposed transcriptional modulation of sugar- and oxygen-responsive genes such as *Hxk4* at the onset of hypoxia. Results are thus consistent with contributions by *Ndpk1* to the core of low oxygen, sugar, and G4 signaling.
Effects of varying phytoglobin expression on maize photosynthesis and root growth during hypoxia

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Transgenic lines of maize have been developed that suppress or overexpress the two Class 1 phytoglobins of maize. The response of the lines relative to the non-transformed parental line were examined under root flooding, either without controlling aqueous oxygen concentrations or under equilibrium with nitrogen gas containing 4\% oxygen. Relative to wild type, flooded plants overexpressing \textit{Pgbs} retained a greater photosynthetic rate and enhanced activity of several antioxidant enzymes. These plants also exhibited high levels of ascorbic acid and reduced ROS staining. This was in contrast to flooded plants down-regulating \textit{Pgbs} which were characterized by the lowest photosynthetic rates and reduced expression and activities of many antioxidant enzymes. Suppression of \textit{ZmPgb1.1} or \textit{ZmPgb1.2} inhibited root growth exposed to 4\% oxygen causing structural abnormalities in the root apical meristems. These effects were accompanied by increasing levels of reactive oxygen species (ROS), possibly through the transcriptional induction of four \textit{Respiratory Burst Oxidase Homologs (RBOHs)}, and TUNEL-positive nuclei in meristematic cells. These cells also accumulated NO and stained heavily for ethylene biosynthetic transcripts and 1-aminocyclopropane-1-carboxylic acid (ACC). A sharp increase in the expression level of several ACC synthase (ZmACS2, 6, and 7), ACC oxidase (ACO15, 20, and 35), and ethylene responsive (\textit{ZmERF2} and \textit{ZmEBF1}) genes was observed in hypoxic \textit{ZmPgb}-suppressing roots. Hypoxic roots overexpressing \textit{ZmPgbs} showed the lowest expression of ethylene biosynthetic and responsive genes, as well as a reduction in ROS staining and TUNEL-positive nuclei in the meristematic cells. These roots retained functional meristems and exhibited the highest growth performance when subjected to hypoxic conditions. Collectively the results suggest a novel function of Pgbs in protecting root apical meristems and photosynthetic capacity by ameliorating the effects of ROS damage.

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Phytoglobins

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Phytoglobins (a.k.a. plant hemoglobins) are prominent scavengers of NO in plant cells and therefore important players in the equilibrium between NO formation and NO removal. In particularly, cell- and tissue-specific expression of phytoglobins serves to control local levels of NO in plant cells. Three classes (1 to 3) of phytoglobins have been identified. Functions of the class 3 type are mostly unknown, whereas the class 1 and 2 phytoglobins have been associated with modulation of NO. For this, transgenic over-expression or silencing of phytoglobins have proven to be excellent tools to modulate levels of NO with the intention of studying biological functions of NO and its crosstalks with other RNS/ROS and with hormones in development and in stresses. Under normoxic conditions the gene expression pattern of phytoglobins is related to cell- and tissue-specific regulation of NO levels in responses to different environments and developmental stages. However, during hypoxic stress conditions class 1 plant phytoglobin gene expression is highly upregulated in all plant tissues, where phytoglobin plays a role in reducing nitrogen loss through NO emission and to maintain energy status. NO is a central molecule in the distinct signaling pathways of the responses towards necrotrophic or biotrophic pathogens, and phytoglobin gene overexpression or gene silencing interferes with progression of infections in very different types of plant-pathogen interactions in a pattern related to modulation of NO levels.

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Essential role of jasmonates in plant response to post-submergence reoxygenation

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Submergence induces hypoxia in plants; exposure to oxygen following submergence, termed reoxygenation, produces a burst of reactive oxygen species, which causes oxidative stress. The mechanisms of hypoxia sensing and signaling in plants have been well studied, but how plants respond to reoxygenation remains unclear. Here, we show that reoxygenation in Arabidopsis thaliana involves rapid accumulation of jasmonates (JAs) and increased transcript levels of JA biosynthesis genes. Application of exogenous methyl jasmonate improved tolerance to reoxygenation in wild-type Arabidopsis; also, mutants deficient in JA biosynthesis and signaling were very sensitive to reoxygenation. Moreover, overexpression of the transcription factor gene MYC2 enhanced tolerance to post-hypoxic stress and myc2 knockout mutants showed increased sensitivity to reoxygenation, indicating that MYC2 functions as a key regulator in the JA-mediated reoxygenation response. MYC2 transcriptionally activates members of the VITAMIN C DEFECTIVE (VTC) and GLUTATHIONE SYNTHETASE (GSH) gene families, which encode rate-limiting enzymes in the ascorbate and glutathione synthesis pathways. Overexpression of VTC1 and GSH1 in the myc2-2 mutants suppressed the post-hypoxic hypersensitive phenotype. The JA-inducible accumulation of antioxidants may alleviate oxidative damage caused by reoxygenation, improving plant survival after submergence. Taken together, our findings demonstrate that JA signaling interacts with the antioxidant pathway to regulate reoxygenation responses in Arabidopsis.

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What are the molecular mechanisms mediating post-submergence recovery in *Arabidopsis*?

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Recovery from submergence stress is dependent not only on the plants’ viability under water, but their post-submergence tolerance as well. Some plants die after the floods recede, thus studying the recovery phase of submergence may provide clues behind the plants’ overall flooding tolerance. Here we compare a submergence-tolerant *Arabidopsis* accession with a submergence-intolerant accession. They are studied due to their differing recovery rates upon de-submergence, even though both show similar submergence-mediated damage. Differing physiological and molecular mechanisms mediating post-submergence recovery was investigated using a Ribo-seq approach. We described the differences between the accessions in the submergence and the recovery phase. Gene Ontology identified categories related to oxidative stress, photosynthetic processes, desiccation stress, and hormonal-related responses. When plants are exposed to air after a long submergence period, a sudden burst of oxygen leads to formation of damaging reactive oxygen species (ROS) molecules. In keeping with our hypothesis that the ability to restrict or recover from ROS production during de-submergence is important for recovery, we identified several oxidative stress-related genes that were differentially regulated between the accessions. Oxidative stress can damage the photosynthetic apparatus and induce downstream desiccation responses thus inhibiting plant regrowth. Studying the post-submergence translatomic responses revealed the plant’s molecular adaptations to submergence stress, identifying candidate genes inducing protective mechanisms during post-submergence recovery.

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The protein kinase SnRK1.1 regulates translation selectivity in Arabidopsis under submergence

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In plants under anaerobiosis the synthesis of a group of anaerobic polypeptides increased, whereas synthesis for the majority of aerobic proteins decreased drastically. Recent studies showed that cellular oxygen deprivation caused by submergence resulted in reprogramming of translation dynamics in plants. However, the mechanism by which plants modulate their translational dynamics in response to oxygen deficiency remains largely unknown. Here we show that the Snf related protein kinase 1 (SnRK1), the AMP-dependent protein kinase (AMPK) homolog in plants, enhances the translation of specific mRNAs through phosphorylating elfiso4G1, which is a plant specific translation initiation factor. We found that SnRK1 directly phosphorylated elfiso4G1 to enhance the polysome loading of specific mRNAs that have a low GC content in their 5' UTR. The phosphorylation residues of elfiso4G1 recognized by SnRK1 are conserved in plants. Although elfiso4G1 knock out line has no visible phenotype in normal conditions, it lost the ability to conserve energy under submergence, and was more sensitive to submergence. Taken together, our results characterized a unique translational regulation in plants under stress. Unlike AMPK in animals, which suppresses the translation under stress, SnRK1 enhances the translation selectivity of specific mRNAs under submergence.
ATP consumption of root mitochondria in anoxia-intolerant wheat (Triticum aestivum L. cv. Leningradka) and tolerant Iris (Iris pseudacorus L.) species

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Plant mitochondria perform an important part in the production of ATP much in demand for cellular maintenance and biosynthesis. Under oxygen deprivation, mitochondria will function, at least in theory, until practically all oxygen has been consumed. This is due to the very high affinity for oxygen of the mitochondrial cytochrome c oxidase. The values vary between species but the \( K_M \) values are in the order of 1-10 \( \mu \text{M} \) \( \text{O}_2 \) (Krab et al. 2011). Below these values mitochondria will not produce ATP, but may actually use ATP and maintain the mitochondrial inner membrane potential (St-Pierre et al. 2000). It is well known that in deep sea turtles and in overwintering frogs, that this ATP consumption, potentially harmful for the cells and tissues, is inhibited. We performed experiments with isolated plant mitochondria of anoxia-intolerant and tolerant plant species and tested their mitochondrial ATP contents and respiration under aerobic control conditions and under anoxia. The results show that in anoxia-intolerant wheat root mitochondria, ATP-levels fall after 30 min anoxia to considerably more lower levels than in anoxia-tolerant Iris species (Table 1). Accordingly, the respiration levels, measured as oxygen consumption, where much higher in mitochondria of the anoxia-intolerant wheat. This phenomenon may partly explain the short-term anoxia intolerance of wheat root tissue.

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Acyl-CoAs integrate the cellular energy status with ERFVII-dependent hypoxia signalling in Arabidopsis

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The induction of genes specific to hypoxic adaptation is under the control of group VII ETHYLENE RESPONSE FACTORS (ERFVII), of which RELATED TO AP2 12 (RAP2.12) has been shown to be crucial for low oxygen adaptation in Arabidopsis. Both the sequestration of RAP2.12 to the plasma membrane in the presence of oxygen and the stabilization of the transcription factor under hypoxia restrict its action to low oxygen conditions. Despite our broad knowledge of RAP2.12’s function and the transcriptional networks it controls, virtually nothing is known about the upstream signal which ultimately results in release of plasma membrane-associated RAP2.12 under low oxygen. Hence, the initiation of hypoxia signalling remains elusive. Here we will show data that reveal this critical step in oxygen sensing, i.e. the translation of changes in the level of molecular oxygen into a cellular signal required for RAP2.12 translocation from the plasma membrane to the nucleus. ACYL-COA-BINDING PROTEIN 1 (ACBP1), the docking site for RAP2.12 at the plasma membrane, is capable of binding long-chain acyl-CoAs. We identified a specific acyl-CoA to promote dissociation of the ACBP1:RAP2.12 protein complex and translocation of RAP2.12 into the nucleus under normoxic conditions. Concomitantly, hypoxic core-specific transcripts were found to be induced upon acyl-CoA application. Consistently, profiling of the acyl-CoA pool under hypoxia revealed an upregulation of the identified acyl-CoA as compared to the control. In agreement with external acyl-CoA application, elevated endogenous acyl-CoA levels in transgenic plants induced hypoxic core genes under aerobic conditions accompanied by an impaired tolerance to low oxygen. Based on initial experiments using chemical inhibitors to decrease mitochondrial ATP production in Arabidopsis, we propose that ATP-dependent dynamic changes of specific acyl-CoAs under hypoxia induce low oxygen adaptation by linking the energy status of the cell with ERFVII-dependent signalling.
Nuclear dynamics of transcriptional regulation in response to hypoxia stress in *Arabidopsis thaliana*

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Submergence and oxygen deprivation (hypoxia/anoxia) lead to rapid changes in transcript accumulation and translation that promote anaerobic metabolism and facilitate stress survival in *Arabidopsis thaliana* and other species. Many genome-wide studies have documented changes in transcriptomes (total mRNA abundance) and highlighted about a set of less than 50 mRNAs that are routinely upregulated in response to these stresses. Although informative, a caveat to the interpretation of changes in steady-state levels of transcript is that it reflects both the synthesis and degradation of mRNAs. But there are numerous other aspects of gene regulation that function in response to hypoxia and subsequent re-oxygenation including the alternative splicing of introns as well as the differential sequestration, degradation and translation of individual mRNAs (Branco-Price et al., 2008; Juntawong et al., 2014; Sorenson et al., 2014). Group VII Ethylene Response Factors (ERF-VII) regulate transcriptional activation of a cohort of genes in response to submergence and hypoxia. In *Arabidopsis thaliana* (Col-0), the three more constitutively synthesized ERF-VII, RAP2.2, RAP2.3 and RAP2.12, transactivate via a Hypoxia-Responsive Promoter Element (HRPE) (Gasch et al., 2015). We have explored whether additional nuclear processes contribute to transcriptional dynamics, including the access of ERF-VII and other transcript factors to their target DNA elements through the positioning of nucleosomes and post-translational modification of specific amino acid residues of histone tails, such as Histone 3 Lysine-4 trimethylation (H3K4me3) and Histone 3 Lysine-27 trimethylation (H3K27me3), known to promote and inhibit transcription initiation, respectively. To monitor transcriptional activity, chromatin accessibility was probed by use of Assay for Transposase Accessible Chromatin (ATAC-seq), histone modifications were monitored by Chromatin Immunopurification sequencing (ChIP-seq), and RNA polymerase II (Pol II) engagement was surveyed by ChIP-seq using an antibody that recognizes the actively elongating complex (phospho Ser2) in seedlings exposed to short-term hypoxic stress. We are exploring if nucleosome repositioning is coincident with ERF-VII binding. These high resolution results demonstrate that hypoxia promotes dynamics in chromatin.

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Transcriptional control of the hypoxia response of Arabidopsis thaliana

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During their lifetime, plant cells may encounter periods of low-oxygen concentrations, for example by limited diffusion within tissues, or by low availability from outside due to flooding events. In recent years, much progress has been made in identifying factors and mechanisms how oxygen concentrations within plant cells are measured, and how this is transmitted into a modification of gene expression. Major components of the signal transduction pathway under hypoxia are group-VII ERF transcription factors, whose stability depends on the oxygen concentration. In our group, we identified a promoter element that is bound by a sub-group of the group-VII ERF transcription factors, namely RAP2.2, RAP2.12, and to a smaller part RAP2.3 (Gasch et al. 2016). This 12 bp-long cis-regulatory motif was named the Hypoxia-Responsive Promoter Element (HRPE). By use of protoplast transactivation assays, Yeast-1-Hybrid analyses and chromatin immunoprecipitation assays we could show that the element is necessary and sufficient for the activation of hypoxia-responsive promoters. A comparative phylogenetic motif search confirmed the HRPE motif and demonstrated that it is evolutionary conserved among plant species. A resemblance with the previously identified Anaerobic Responsive Element (ARE) from the maize ADH1 promoter suggests an ubiquitous binding mechanism in monocot and dicot plant species under hypoxia, but this hypothesis requires further experiments.

Comparative analysis of genome-scale gene expression responses to submergence, drought and recovery from these stresses in leaves and roots of soybean

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Both submergence and drought are major environmental constraints that reduce growth, reproduction and survival in soybean. High and low extremes in precipitation do not take place simultaneously, but these water-related stresses can occur sequentially (submergence followed by drought and vice versa) within a single growing season or independently in the same fields in different years. Therefore, improvement of combined tolerance to submergence and drought would substantially increase soybean productivity and stabilize yield variability. Several transcription factors that enhance adaptability to both submergence and drought have been recognized in Arabidopsis, rice and sunflower. However, the regulatory mechanisms underlying combined tolerance to water extremes at genome-scale remain unknown in soybean and other plants. Here, we compared transcriptomic responses to submergence, drought and recovery from these stresses in leaves and roots of soybean plants. Plants at the V1 stage were exposed to submergence for up to 3 days or drought for up to 6 days under the same growth environments. Plants exposed to the longest stress periods were recovered for 24 h after desubmergence or rehydration. mRNA-Seq analysis identified 15,075 gene transcripts that were significantly up- or down-regulated in response to submergence or drought in leaves or roots. k-mean clustering and GO enrichment analysis detected stress-specific, tissue-specific and conserved pathways associated with signal transduction, metabolism, development and stress responses. Biological significance of overlapped pathways and networks in the adaptation to and recovery from water extremes will be discussed.
Regulation of oxidative stress responses by the ERFVII factors in Arabidopsis thaliana

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In Arabidopsis, transcriptional responses to oxygen deprivation are promoted by Group VII Ethylene-response factors (ERFVII), among which RAP2.12, whose turnover rate and cellular localization are determined by an oxygen-dependent branch of the N-end rule-pathway of proteolysis. To uncover whether, in addition to hypoxic gene expression, RAP2.12 may mediate the regulation of other unexplored responses, we generated \textit{35S:Δ13RAP2.12} plants that expressed a deleted version of RAP2.12 lacking its first conserved 13 amino acids. Transcriptome comparison between \textit{35S:Δ13RAP2.12} and wild-type rosette samples, taken at the adult stage of vegetative development, not only showed constitutive expression of core hypoxia-responsive genes, but also highlighted the up-regulation of a vast proportion of non-hypoxic targets. Gene ontology and transcriptional meta-analyses showed that the genes falling in the second class were mainly associated to defense, oxidative stress and hormone response processes. Overlapping gene expression patterns were observed when comparing either \textit{35S:Δ13RAP2.12} or the N-end rule mutant \textit{prt6} with wild-type seedlings, indicating that, in the post-germinative stage of development, PRT6 is sufficient to control the expression of ROS-responsive genes through the ERFVII. After selection of oxidative stress markers among the newly identified targets of RAP2.12, we were able to assess that the ERFVII act as essential components of the mitochondrial oxidative stress response. The impact of the novel regulatory functions proposed for the ERFVII on plant submergence responses are currently under evaluation.

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Biochemical characterisation of the Plant Cysteine Oxidases in the N-end Rule mediated response to hypoxia

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The plant hypoxic response resulting from submergence is mediated by the group-VII ETHYLENE RESPONSE FACTORS (ERF-VIIs), transcriptional regulators that promote the expression of a number of core anaerobic genes. ERF-VII stability is regulated by the Arg/Cys branch of the N-end rule pathway (NERP), whereby N-terminal cysteine oxidation under normoxic conditions ultimately results in ERF-VII proteasomal degradation, switching off the hypoxic response. The Plant Cysteine Oxidases (PCOs) have been identified as enzymes which catalyse this cysteine oxidation in Arabidopsis thaliana, suggesting, therefore, that they can act as ‘oxygen sensors’ during hypoxia in plants.

Biochemical studies conducted in our laboratory have verified that the PCOs are metal binding proteins belonging to the non-heme iron-dependent oxygenase family, which use molecular oxygen as co-substrate to modify the N-terminal cysteine of ERF-VII derived peptides. They incorporate both O atoms to generate cysteine sulfinic – rather than sulfonic – acid as product, making the PCOs the first identified cysteinyldioxigenases and plant cysteine dioxygenases. Subsequent radiolabelling experiments have established that this PCO catalysed oxidation is sufficient for processing by sequential components of the NERP: oxidised ERF-VII peptides can be arginylated by Arginyl tRNA Transferase 1 (ATE1) \textit{in vitro}, indicating that the PCOs provide a direct link between molecular O\textsubscript{2} availability, protein arginylation and ERF-VII degradation. Accordingly the PCOs may offer a viable intervention point for ERF-VII stabilisation and enhance submergence tolerance in agronomy. Kinetic and structural investigations continue to aid in the rational manipulation of PCO activity for this purpose.

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Plasticity in response to submergence: development, genotype and time

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Rice displays a suite of responses to flooding that are dependent on developmental age, organ and cell type, availability of energy reserves, and genotype. To decipher gene networks regulated by SUBMERGENCE1A (SUB1A) and ANAEROBIC GERMINATION1 (AG1) when present singly or together in the IR64 cultivar, transcriptome analysis or shoot tissue was performed from the seed to seedling stage on continuously submerged plants. Despite evidence of genotype and genotype x time interactions, IR64 (SUB1 AG1) displayed both early coleoptile elongation (anaerobic germination) and submergence tolerance. To explore plasticity of response in specific cell types and regions that underlie developmental adaptations such as the radial oxygen barrier and lateral/secondary root initiation, we retooled INTACT (Isolation of Nuclei TAgregated in specific Cell Types) and TRAP (Translating Ribosome Affinity Purification) for rice. These methods enable monitoring of dynamics in chromatin, nuclear pre-mRNA and ribosome-bound mRNAs. Our first experiment monitored the response of the root apex of young seedlings to short term submergence (hypoxic stress). The data yield new perspectives on gene transcription and mRNA turnover activated by the stress. We are now exploring gene networks in individual cell types/regions (i.e., cortex, endodermis and exodermis, apex) under waterlogged, well-watered and water-deficit. Supported by NSF DBI-1238243.

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Anaerobic germination tolerant deep water rice germplasm Kekua Bao displays aberrant genetic perturbation in key anoxia signaling components

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One of the most serious problems in rice production is the recurrence of devastating floods almost every year which severely affect rice production. Floods occurring right after sowing of seeds result in more than 50% yield loss. Thus there is an urgent need to provide farmers with rice varieties that besides being highly adoptive to local environment, also have the additional trait of tolerance to such flooding condition during early seed establishment. Around 160 indigenous deep water rice landraces (Bao rice) were screened for their tolerance to anoxia during germination and potential lines were compared with KHO, line from IRRI as positive control. Profuse variation was observed in terms of tolerance to anaerobic condition with respect to germination potential. The Kekua bao had potential to germinate and stabilize more than 70% of total seed sown. It took 11 days for the seedling to emerge from water in case of Kekua Bao while it took 9 days in case of KHO. However this delay in emergence was compensated with higher percentage of seedling establishment in Kekua Bao (72%) compared to KHO (45%). The qAG1, a major QTL associated with anoxia tolerance that corresponds to gene TPP7 involved in trehalose metabolism in rice was present in both Kekua Bao and KHO. However, the transcript level for this gene in Kekua Bao was significantly lower compared to KHO and was comparable to that from sensitive germplasm. Similarly, cipk15 gene expression which mediates the signaling in response to sugar starvation and O2 deficiency was also significantly lower in Kekua Bao while transcript level of SnRK1A gene that regulates the downstream starch degradation through αRamy3D, was similar to that in KHO. This is contrary to the generally held view that the TPP7 and cipk15 are transcriptionally up-regulated under anoxia in tolerant plant. Thus, the current understanding on the role of TPP7, cipk15 and SnRK1A may not be universal phenomenon for anoxic survival as showed by its redundancy with respect to Kekua Bao germination. Furthermore, characterization of such genetic resources would lead to identification of novel components associated with anaerobic germination that would eventually lead to development of rice varieties able to withstand anaerobic condition during germination under flooded condition.

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ERF VII transcription factors alter root structure in Arabidopsis

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Flooding is a major abiotic stress that can cause severe damage to plants. Arabidopsis is a widely employed model plant to study the molecular mechanisms that mediate plant adaptation to flooding. AP2/ERF transcription factors of the subgroup VII that include RAP2.2, RAP2.3, RAP2.12, HRE1, and HRE2, have emerged as key regulators of the metabolic low oxygen response. These TFs induce the expression of several hypoxia core genes that encode mostly metabolic enzymes such as alcohol dehydrogenase and pyruvate decarboxylase resulting in increased survival rates upon oxygen deprivation. By contrast, our knowledge on the role of subgroup VII AP2/ERF transcription factors in developmental adaptations to low oxygen conditions is limited. In this study, we focus on developmental reprogramming of root architecture of Arabidopsis seedlings in response to oxygen deprivation. Our results show that hypoxia represses the initiation of lateral root primodia and leads to postemergence lateral root quiescence. Furthermore, hypoxia causes slanting of the primary roots likely as a result of altered auxin accumulation. Using subgroup VII ERF mutants we show involvement of these transcriptional regulators in controlling root development at low oxygen conditions.

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Tolerance of *Prunus* rootstocks to hypoxia is associated to decreases of energetic costs to protein turnover and maintenance of membrane integrity

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The low oxygen concentration is a key determinant of root growth and functioning under waterlogging. Changes in the costs of protein turnover and maintenance of membrane integrity in roots of three *Prunus* rootstocks contrasting in hypoxia tolerance (Marianna 2624 as tolerant, CAB6P as moderately tolerant and Mazzard F12/1 as sensible), both under normoxia and hypoxia conditions were evaluated. To examine the protein turnover, cycloheximide as protein synthesis inhibitor was used and to membrane integrity the electrolyte leakage was evaluated.

We test that in *Prunus* roots less than 20 min following application of 1mM of cycloheximide, root respiration rate was inhibited in the three *Prunus* rootstocks, decreasing in average by 76% compared with control roots (0 mM) at 180 min. The rate of protein accumulation of Mazzard F12/1 in average was 50% higher than Marianna 2624 and CAB6P. In turn, high ATP cost of protein turnover in roots of Mazzard F12/1 was founded over the time. The ATP cost was positively correlated with the root protein content in the three *Prunus* rootstocks. Marianna 2624 was capable of maintaining the metabolism under hypoxia investing in average 18% less energy than CAB6P and Mazzard F12/1. The ATP cost was negatively correlated both NNUR and RGR in roots of three *Prunus* rootstocks. Mazzard F12/1 invested higher ATP to protein related with growth and nitrogen uptake than Marianna 2624 and CAB6P. After 30 d at hypoxia the root membrane integrity was reduced both in CAB6P and Mazzard F12/1. By the contrast, Marianna 2624 achieve maintain lower electrolyte leakage, which allow maintain high membrane integrity. The proportion of maintenance respiration ascribed as protein turnover in Marianna 2624, CAB6P and Mazzard F12 at normoxia was 6%, assuming that the rest of the energy was to maintain cell ion concentration. At hypoxia, the participation of protein turnover increases to 32, 18 and 9% of the maintenance respiration in Marianna 2624, CAB6P and Mazzard F12/1, respectively.

It is concluded that the ability to tolerate hypoxia stress in *Prunus* rootstocks will consist in invest less energy in the protein turnover and maintenance of membrane integrity may enable that another processes as nitrogen uptake can operate, even under conditions of severe oxygen limitation.

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Waterlogging effects on physiological and growth responses in forage grasses with differential tolerance

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Waterlogging tolerance differs among forage species. The grasses \textit{Dactylis glomerata} and \textit{Bromus unioloides} are regarded as relatively sensitive to soil oxygen deficiency while \textit{Festuca arundinacea} and \textit{Phalaris aquatica} are considered tolerant. So far, these reputations are mostly based on dry mass responses at the end of a waterlogging period. However, the physiological performance associated with differential responses among species and the ability to recover from waterlogging after water subsides have not been addressed. To expand our knowledge, during 15 days, 42-day-old plants of these species growing in 0.6L pots with top soil of a natural grassland (3.3% organic carbon) and sand (1:1 v/v) were subjected to: (i) well drained conditions (i.e. field capacity) and (ii) waterlogging (1-2 cm water above soil). After the waterlogging period, plants were allowed to grow for 15 days in well drained conditions to assess the recovery degree. Plant physiological responses, including net photosynthesis, stomatal conductance and transpiration rate of fully expanded leaves of similar age/position were measured at the beginning of the experiment (day 0), and at 2, 7 and 15 days of waterlogging. Plant morphology was characterized by examining the number of tillers per plant and green leaves per tiller at days 0, 5, 12 (during waterlogging), 19 and 26 days (during recovery). Dry mass accumulation was assessed by harvesting plants at the beginning of experiment (day 0) and at the end of waterlogging (day 15) and recovery (day 30) periods. Results showed that, under waterlogging conditions, the species \textit{D. glomerata} and \textit{B. unioloides} showed progressive reductions in net photosynthesis (20-36% respectively) and stomatal conductance (29-32%, respectively) compared with their controls (P<0.05). This contrasts to what occurred with \textit{F. arundinacea} and \textit{P. aquatic} where these parameters remained similar in control and waterlogged plants (P>0.3). Along the experiment, the number of tillers per plant was not affected by waterlogging in any of these grasses (P>0.6), while the number of green leaves per tiller was reduced (23%; P=0.03) by the end of waterlogging only in the sensitive species \textit{D. glomerata}. Plant dry mass accumulation during the waterlogging period was slightly reduced (22-11%; marginally significant with P=0.1) in \textit{D. glomerata}, \textit{B. unioloides} and \textit{F. arundinacea}, mainly due to root growth arrestment, and it was increased by 39% in the waterlogging tolerant \textit{P. aquatic} (P<0.05). Importantly, the injury to roots during waterlogging impacted later on the subsequent growth of the sensitive species \textit{D. glomerata} and \textit{B. unioloides}, which did not show any increase in either shoot or root dry mass during the recovery period. By contrast, \textit{F. arundinacea} and \textit{P. aquatic} continued with shoot and root growth during recovery, attaining a final total dry mass of 76% and 110% of their respective controls. In conclusion, while the physiological performance of sensitive grasses was directly affected during waterlogging, the negative effects on growth were evident only after considering a recovery period after water subsided. Therefore, it is strongly recommended when defining waterlogging tolerance to consider the examination of plant performance during a recovery period to cover potential long term effects caused by this stress.
Effects of flooding stress on plant responses in selected
Brassicaceae wild species

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Due to global climate change, the number and duration of flooding events increased significantly. Two strategies have been developed by plants to cope with flooding. Plants with an escaping strategy show rapid stem or petiole elongation to reach the water surface and restore air contact. The quiescence strategy is defined by the down-regulation of plant growth and metabolism to save energy until the floods recede. For monocots, rice is a well-established flooding-tolerant model plant, whereas for dicots no flooding-tolerant and genetically accessible model plant is available yet. Therefore the goal of our project is to establish a genetically accessible dicot model system for understanding flooding tolerance mechanisms.

Our research is focused on comparing tolerance mechanisms of flooding-tolerant Brassicaceae wild species (\textit{Rorippa, Nasturtium, Cardamine}) to the flooding-sensitive species \textit{Arabidopsis thaliana}. The first flooding treatments indicated that all tested rosette-growing species are quiescent, whereas the only non-rosette growing species displays an escaping strategy. Regarding flooding survival, we could not only see differences between escaping and quiescent species, but also a variation within the quiescent species with some of them being considerably more flooding tolerant than others. The use of RNAseq will provide information to identify the underlying mechanisms responsible for these effects. Besides transcriptomic changes we will also focus on metabolic changes upon submergence. Meanwhile it is necessary to establish a transformation system for these wild species in order to further analyze possible candidate genes.

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What is the function of the hypoxia induced transcription factors ABR1 and LBD41?

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Plants respond to low oxygen stress by a variety of transcriptomic changes. In Arabidopsis thaliana, major regulators of the hypoxic response are the group VII ERF transcription factors: when both oxygen and nitric oxide are available, they are degraded by the N-end rule pathway (NERP), whereas under hypoxic conditions they are stabilized to initiate the transcription of hypoxia-responsive genes. Due to the fact that a large number of these genes are transcription factors (TF), whose functions are still unknown, the hypoxia-induced TFs LOB DOMAIN-CONTAINING PROTEIN 41 (LBD41) and ABA REPRESSOR 1 (ABR1) gained our interest.

LBD41 is part of the 49 core hypoxia responsive genes and is predicted to act as a repressing TF. Mutants of this protein do not show any obvious phenotypes. Therefore, we performed a microarray analysis of protoplasts overexpressing different forms of LBD41 in order to provide an informative basis. Furthermore we tested, if LBD41 influenced the promotor activity of the putative target genes identified above by using a protoplast-based assay.

ABR1, however, seems to be highly induced by hypoxia and other stress conditions. According to its name, a previous study suggested a role in abscisic acid signalling, which could not be confirmed by our data. Interestingly, the protein contains the conserved N-terminal methionine/cysteine (MC) motif, which makes it a possible target for the NERP. In addition, ABR1 overexpression led to multiple phenotypes. Here again, by performing a microarray analysis, we aimed at linking the expression data to the observed phenotypes and stress conditions.

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A locus controlling formation of a barrier to radial oxygen loss is located on chromosome 3 in *Zea nicaraguensis*

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*Zea nicaraguensis*, which is a wild relative of maize (*Z. mays* ssp. *mays*), has higher tolerance of soil waterlogging than maize. We previously found that *Z. nicaraguensis* was superior to maize in transporting oxygen from shoot base to root tip due to the formation of larger aerenchyma and a stronger barrier to radial oxygen loss (ROL), both of which enhance oxygen transport to the tip of roots in waterlogged soil. However, the genetic regulation and anatomical basis of the ROL barrier formation was unknown. To identify the locus controlling ROL barrier formation in *Z. nicaraguensis*, we used a library of introgression lines (ILs), each containing a chromosome segment from *Z. nicaraguensis* in the maize inbred line Mi29. We screened for root ROL barrier formation in deoxygenated stagnant agar, 42 ILs that cover nearly the whole genome of *Z. nicaraguensis*. One IL (IL#11) had the trait of root ROL barrier formation similar to that in *Z. nicaraguensis* (Figure 1), all other ILs exhibited a ROL pattern which resembled that of maize. Further analyses verified that a locus controlling the ROL barrier formation is located on the short arm of chromosome 3 of *Z. nicaraguensis*. We are now fine-mapping and phenotyping to discover the gene(s) controlling ROL barrier formation in this region. During the genetic analysis, we produced the line #468, which is homozygous for the locus controlling ROL barrier formation. We conducted suberin or lignin staining of root cross sections using #468 as well as maize and *Z. nicaraguensis*. We found that the patterns of suberin or lignin staining in #468 were similar to those in maize, but not to *Z. nicaraguensis*. The relationship of suberin and lignin histochemical staining and root ROL barrier formation will be discussed.

**Figure 1. Evaluation of ROL barrier formation in roots by methylene blue staining in an oxygen-free medium when roots are reliant on internal oxygen diffusion.**

Maize (inbred line Mi29), *Z. nicaraguensis* and IL#11 were grown in stagnant deoxygenated solution for 2 weeks. Methylene blue dye (colourless without oxygen, blue when oxygen is present) stained the entire root in maize (i.e. ROL occurred along the entire root), but stained only the tips of the roots of *Z. nicaraguensis* and IL#11 (arrowheads) indicating that a ROL barrier had formed in the subapical region and to the base of roots in *Z. nicaraguensis* and IL#11.

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Mechanisms of waterlogging tolerance in wheat – a review of root and shoot physiology

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We review the detrimental effects of waterlogging on physiology, growth and yield of wheat. We highlight traits contributing to waterlogging tolerance and genetic diversity in wheat. Death of seminal roots and restriction of adventitious root length due to O\textsubscript{2} deficiency result in low root:shoot ratio. Genotypes differ in seminal root anoxia tolerance, but mechanisms remain to be established; ethanol production rates do not explain anoxia tolerance. Root tip survival is short-term, and thereafter, seminal root re-growth upon re-aeration is limited. Genotypes differ in adventitious root numbers and in aerenchyma formation within these roots, resulting in varying waterlogging tolerances. Root extension is restricted by capacity for internal O\textsubscript{2} movement to the apex. Sub-optimal O\textsubscript{2} restricts root N uptake and translocation to the shoots, with N deficiency causing reduced shoot growth and grain yield. Although photosynthesis declines, sugars typically accumulate in shoots of waterlogged plants. Mn or Fe toxicity might occur in shoots of wheat on strongly acidic soils, but probably not more widely. Future breeding for waterlogging tolerance should focus on root internal aeration and better N-use efficiency; exploiting the genetic diversity in wheat for these and other traits should enable improvement of waterlogging tolerance.
Differential ability to regulate voltage-gated K+-permeable channels in mature root epidermis determines genotypic difference in waterlogging stress tolerance in barley

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Waterlogging (WL) is a major constraint that limits plant growth around the globe. Oxygen plays a crucial role in plant survival under WL conditions as oxygen is a terminal electron acceptor and an absolute requirement for ATP production in aerobic conditions (Bailey-Serres & Chang, 2005; Voesenek \textit{et al.}, 2006). As a result, operation of H+-ATPase is compromised, with strong implications for cytosolic pH regulation, membrane potential maintenance, and transport of all nutrients across membranes. The above effects, however, are highly tissue-specific and time-dependent, and the causal link between hypoxia-induced changes to cell’s ionome and plant adaptive responses to hypoxia is not well established. This work aimed to fill the above gap in our knowledge and investigate the effects of oxygen deprivation on K+-signalling and homeostasis in plants, and its regulation under oxygen-deprived conditions. Net fluxes of K- and H- and membrane potential (MP) were measured from functionally different root tissues in barley roots. The significant K-loss was observed in roots exposed to hypoxic conditions; this loss was stronger in the root apex immediately after stress onset but became more pronounced in the root base as the stress progressed. The plants ability to uptake and retain K-in waterlogged soil showed a strong correlation with K-flux under hypoxia measured in laboratory experiments. Hypoxia induced membrane depolarization; the severity of this depolarization was less pronounced in the tolerant group of varieties. Intact barley roots were capable to maintain H-pumping activity while excising coleoptiles (thus preventing oxygen supply from the shoot) resulted in a rapid loss of K- due to reduced H-pumping activity. Tolerant verities showed more ability to maintain cell viability as compared to sensitive group of varieties. Application of vanadate, a known inhibitor of the plasma membrane H+-ATPase, has compromised root K-retention and cell’s viability, suggesting that H+-ATPase plays important role in maintaining K-retention by controlling voltage-gated Shaker-like K-channels. It is concluded that (i) High K-retention and uptake is crucial in plant tolerance; (ii) The ability of plant roots cells to maintain membrane potential is very important for plant survival under waterlogging; and (iii) plants could survive even under severe hypoxia conditions providing the continuous oxygen transport from shoot is not compromised.

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Understanding acclimative responses to multiple abiotic stresses in Arabidopsis

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In the field, most abiotic stresses occur either in combination (such as heat and drought) or in succession (flooding followed by dry periods or hot days followed by cold nights). Episodes of high ambient temperatures, droughts and prolonged flooding have been projected to increase in frequency and severity due to the global climate change. This can be devastating for crop production as most plant traits that are affected by these abiotic environmental stresses are directly or indirectly relevant for agriculture. Plants have evolved integrated responses called ‘mediated response syndromes’ (MRS), consisting of different combinations of physiological, morphological and phenological traits to cope with detrimental abiotic stresses, including flooding events and droughts. Here we present preliminary data defining the MRS in Arabidopsis associated with post submergence drought (submergence followed by drought) using variation in acclimation capacity among natural Arabidopsis accessions from the molecular to the whole-plant level. Ultimately functional structural modelling (FSP) will be used to quantify the relative importance of acclimation in different identified trait combinations for whole-plant performance under multiple abiotic stresses. This will enable us to identify optimal stress acclimation strategies (MRS) and characterize the molecular mechanistic basis of how the identified genes contribute to the expression of the mediated response syndromes underlying stress acclimation in Arabidopsis.

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Flooding impact disentangled: water pressure affects the survival and growth of the terrestrial plant *Alternanthera philoxeroides* upon complete submergence more than low light or oxygen availability

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Flooding imposes stress upon terrestrial plants, and causes the death of intolerant species. The decline in species richness with water depth has been seen in many wetlands and river forelands. The depth range a species occupies is determined by its tolerance to the gradient of factors that vary with water depth, notably light regimes, dissolved oxygen availability and water pressure. Here, we set up an unique full factorial experiment with three factors at two levels: 8 treatments (2 light regimes \(\times\) 2 dissolved oxygen concentrations \(\times\) 2 water pressure levels (i.e. depths)), and used *Alternanthera philoxeroides* (Mart.) Griseb., a submergence-tolerant terrestrial species as a model, to quantify the interacting effects of water pressure, light and dissolved oxygen on the growth and survival of terrestrial plants under complete submergence at different depths. The present study demonstrated that the water pressure has largest influence on the growth and survival of completely submerged plants, the high water pressure decreased the stem elongation and adventitious roots growth, increased the biomass and carbohydrate loss; at the same time, the effect of dissolved oxygen on the adventitious roots growth is also shown, that plants under high dissolved oxygen conditions had more and longer adventitious roots; but the light regime didn’t cause significantly differences between plants under light and shade conditions.

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Ethylene-mediated hypoxia tolerance: comparing different meristems

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Plants are damaged or often even killed by floods due to the restricted gas diffusion underwater. The slow gas diffusion rate causes a dramatic decline of O\textsubscript{2} and CO\textsubscript{2} concentrations, which negatively affect the energy and carbohydrate status of the plant. Furthermore, it leads to rapid accumulation of the plant hormone ethylene inside plant tissues. These increased ethylene levels is considered to be an early flooding signal for plants and can induce adaptive mechanisms that facilitate survival during severe O\textsubscript{2} shortage (hypoxia). Previous studies showed that plants pre-treated with ethylene have an increased survival rate and are thus more tolerant to subsequent hypoxia. However, how ethylene mediates this increased hypoxia tolerance remains unclear. The hypothesis is that ethylene indirectly stabilises transcription factors that facilitate the expression of core hypoxia genes, which subsequently increase hypoxia survival. The aim of this study is to establish the molecular mechanism of ethylene-mediated hypoxia tolerance in the three different meristems systems of Arabidopsis: the shoot, lateral root and primary root meristems. In addition, we will investigate whether there is variation in ethylene-induced hypoxia tolerance among Arabidopsis accessions and how this is related to flooding tolerance.

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Characterization of Phosphofructokinases in *Oryza sativa* and *Arabidopsis thaliana*

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Although rice grows well on waterlogged fields, prolonged submergence leads to irreversible damages. This causes yield losses up to 1 billion Dollar only in Asian countries every year. Due to a lower oxygen diffusion rate in water, submerged plants have to cope with oxygen deficiency. These hypoxic conditions inhibit the mitochondrial respiration so that ATP production mainly depends on glycolysis. Phosphofructokinases (PFKs) are crucial enzymes of glycolysis and catalyse the reaction from fructose-6-phosphate to fructose-1,6-bisphosphate in an ATP-dependent manner. Interestingly, plants possess an alternative enzyme which catalyses the same reaction but uses pyrophosphate instead of ATP, the pyrophosphate: fructose-6-phosphate 1-phosphotransferase (PFP). It has been hypothesized that the usage of pyrophosphate instead of ATP under oxygen deficiency provides an adaptive response to the stress.

*A. thaliana* and *O. sativa* have different isoforms of both, PFK and PFP, and some of them are induced under hypoxic conditions (Mustroph et al. 2007, 2013). Interestingly, while PFP-coding genes are induced by hypoxia in rice, PFP-coding genes from *A. thaliana* are not induced. However, it is still not much known about the functions of the different PFP and PFK isoforms. Our goal is to reveal the roles of selected PFP and PFK isoforms by use of knockout and overexpressor plants in order to get a greater insight into the glycolytic pathway under normoxic as well as hypoxic conditions.


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Ethylene-induced hypoxia tolerance: a novel mechanism for flooding tolerance in plants

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Floods have disastrous effects on agricultural productivity, threatening global food security. Submerged plant tissues ultimately experience oxygen deprivation (hypoxia), challenging their survival. In addition, ethylene rapidly accumulates in submerged plant tissues. Recently, we discovered that early ethylene signalling in flooded plants is essential for maximizing hypoxia tolerance. We identified a novel mechanism that drives this ethylene-induced hypoxia tolerance in Arabidopsis. We suspect that early accumulation of transcription factors that induce hypoxia acclimation is crucial and requires modification of endogenous nitric oxide (NO) levels. However, the mechanism of how ethylene may mediate NO levels is unknown. We hypothesize that ethylene modifies NO levels via an increase in NO scavenging capacity. In addition, substantial variation for flooding tolerance exists between and within plant species. However, the cause of these differences in flooding tolerance is largely unknown. The novel mechanism of ethylene-induced hypoxia tolerance may be instrumental to identify which factors drive flooding tolerance and lead to the diversity observed in plants. The outcome of our research will improve our understanding of molecular mechanisms that drive flooding tolerance and can be pivotal for flood tolerance crop breeding.

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Ethylene primes hypoxia and post-hypoxia tolerance in *Arabidopsis thaliana*


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When flooded, plants typically suffer from restricted gas diffusion in the underwater environment leading to reduced photosynthesis and respiration. In submerged plant tissues, internal ethylene levels rise rapidly due to decreased gas diffusion resulting in physical entrapment. Ethylene is an important early flooding signal for plants and it mediates adaptive responses to this stress in many plant species. Previous research suggested that ethylene accumulation upon flooding triggers molecular processes that mediate survival of subsequent oxygen deficient conditions. Indeed plants pre-exposed to ethylene showed improved survival under subsequent hypoxia conditions. Hypoxia tolerance is dependent on survival of not just the hypoxia phase itself but also the period following hypoxia. When plants are returned to normoxic conditions following hypoxia, re-oxygenation induces the production of high quantities of reactive oxygen species. Our preliminary experiments suggest that ethylene improves survival of both the hypoxic and post-hypoxic phase but the molecular regulatory pathways underlying may be distinct.
Growth of adventitious roots in rice (*Oryza sativa* L.) is regulated by light

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In rice (*Oryza sativa*), adventitious root primordia develop at stem nodes. Their emergence and growth is induced upon submergence and is triggered by the gaseous hormone ethylene. We observed that prolonged darkness shows a similar promoting effect on adventitious root growth that is inhibited in the presence of the ethylene perception inhibitor 1-methylcyclopropene (1-MCP) suggesting that ethylene mediates dark-induced adventitious root growth. However, in the dark, the angle at which adventitious roots grow differs from that in the light. Dark-grown roots show a stronger negative gravitropic response. We demonstrate that blue light inhibits this exaggerated agravitropic growth response in a dose-dependent manner. In summary, submerged rice plants have an altered root architecture that is shaped by ethylene and light.

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Role of CBL/CIPK complex in plants under O$_2$ shortage

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Fluctuation of Ca$^{2+}$ levels serves as a key signal when plants experience external changes, including abiotic stresses. Previous results from different studies indicated the occurrence of an increase in cytosolic Ca$^{2+}$ concentration also under O$_2$ shortage. One of the Ca$^{2+}$ decoding mechanism in plants is the activation of the CIPK/CBL complex. This has been found to play a pivotal role in rice germination and survival under submergence, since the identification of OsCIPK15 as a key regulator of starch degradation. Additional reports highlighted a variation in anaerobic genes expression due to Ca$^{2+}$ modulation, suggesting a possible involvement of Ca$^{2+}$ sensing/signalling in the low O$_2$ sensing mechanism. A direct interconnection between Ca$^{2+}$ signalling and ROS production has also been proved, implying its involvement in defence. In this study, we investigated the OsCIPK15 interaction with a possible OsCBL partner under low O$_2$. We also investigated whether a CBL/CIPK complex is activated in Arabidopsis under O$_2$ shortage, through the characterization of AtCIPK proteins likely involved in tolerance.

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Arabidopsis NIP2;1, a lactic acid transporting aquaporin-like protein induced by low oxygen stress

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Arabidopsis thaliana NIP2;1 is a core hypoxia-induced gene that encodes a member of the nodulin26-like intrinsic protein family of aquaglyceroporin channels. Unlike other family members, AtNIP2;1 is a water impermeable channel that selectively transports protonated lactic acid, and is induced rapidly during anaerobic stress. Based on quantitative-PCR and promoter::GUS experiments, AtNIP2;1 is elevated acutely and rapidly under anaerobic conditions, increasing >1000-fold in roots within 2 hr post hypoxic treatment. NIP2;1 expression is particularly high within the cells localized in the stele of the differentiated zone of roots. Under low oxygen stress, translational fusions of NIP2;1 and YFP show high accumulation within phloem tissue with a significant number of cells showing polarized localization to the tips of phloem cells. Experiments with T-DNA insertional mutants of the AtNIP2;1 gene showed that the reduction of AtNIP2;1 expression correlates with an elevation of lactic acid levels as well as differences in the profiles of a number of metabolites. Under normoxic growth conditions, T-DNA insertional mutants grew normally but showed subtle changes in root morphology and growth showing increased numbers of lateral roots as well as increased primary root length and mass. Surprisingly, these T-DNA insertional mutants showed an enhanced survival rate in response to anaerobic stress compared to wild type plants. The potential role of AtNIP2;1 as a lactic acid specific channel mediating the homeostasis of this fermentation end product will be discussed (supported in part by National Science Foundation Award MCB-1121465).
Internal aeration of paddy field rice during complete submergence – importance of light and floodwater O₂

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Flash floods can submerge paddy field rice (Oryza sativa), with adverse effects on internal aeration, sugar status and survival. Here, we investigated the in situ aeration of roots of rice during complete submergence, and elucidated how underwater photosynthesis and floodwater O₂ influence root aeration in anoxic soil.

In the field, root O₂ was measured using microelectrodes during 2 d of complete submergence. Leaf gas films that formed on the superhydrophobic leaves were left intact, or experimentally removed, to elucidate their effect on internal aeration.

In darkness, root O₂ declined to very low concentrations (0.24 kPa) and was strongly correlated with floodwater O₂. In light, root O₂ was high (14 kPa) and primarily a function of the incident light determining the rates of underwater net photosynthesis. Plants with intact leaf gas films maintained higher underwater net photosynthesis relative to plants without gas films when the submerged shoots were in light.

During complete submergence, internal aeration of rice in the field relies on underwater photosynthesis during the day and entry of O₂ from the floodwater during the night. Leaf gas films enhance photosynthesis during submergence leading to improved O₂ production and sugar status, and therefore contribute to the submergence tolerance of rice.

Fig 1. Root O₂ during darkness in completely submerged rice (Oryza sativa) vs floodwater O₂ in a bunded field at the International Rice Research Institute, the Philippines. Root O₂ within adventitious roots was measured using O₂ microelectrodes inserted c. 200 μm into the root, 10mm below the root–shoot junction, in two plants with intact leaf gas films (Plant 1, closed circles; Plant 2, open circles).

Fig. 2 Partial pressures of O₂ (pO₂) in roots of completely submerged rice (Oryza sativa) vs incident light (a, b) in a bunded field at the International Rice Research Institute, the Philippines, and residuals vs floodwater pO₂ (c, d). Root O₂ within adventitious roots was measured using O₂ microelectrodes inserted c. 200 μm into the root, 10mm below the root–shoot junction, in two plants with intact leaf gas films and in two plants in which the gas films had been removed just before submergence. Roots were buried in the anoxic soil with the root–shoot junction c. 4 cm below the soil surface, so that root O₂ entered from the shoot via aerenchyma.
Improving tolerance to waterlogging in *Brachiaria* forage grasses: progress in phenotyping for associated morpho-anatomical traits

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Perennial *Brachiaria* grasses are the most widely planted forage grasses in tropical America, and their use is increasing in East Africa and South East Asia for milk and meat production. During the rainy season, *Brachiaria* pastures are exposed temporarily to waterlogging conditions that severely limit their productivity. The international Center for Tropical Agriculture (CIAT) has an on-going *Brachiaria* breeding program which aims to develop *Brachiaria* hybrids with greater forage yield, forage quality, adaptation to biotic and abiotic stress factors (including waterlogging tolerance), and seed production than the current commercial cultivars. It is accepted that oxygen limitation is, at first instance, the major constraint for root growth under waterlogging. As such, recent work undertaken at CIAT has been contextualized in terms of the impact of oxygen deficiency to the root system of *Brachiaria* grasses, and morpho-anatomical traits that influence the internal aeration of the root system. Variation in tolerance to waterlogging among *Brachiaria* grasses has been associated with differences in a number of morpho-anatomical traits of roots (e.g., aerenchyma formation, stele size, suberin deposition, root diameter). Since measurement of morpho-anatomical traits influencing root aeration are time consuming, we suggest that rooting depth and the tortuosity of the root system (ratio of length to depth) might serve as proxies for phenotypic evaluation of internal aeration efficiency in large number of apomictic and sexual hybrids developed from the *Brachiaria* breeding program.

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Deregulation of RAP2.12-based low oxygen sensing has major influence on growth and metabolism in *Arabidopsis* plants

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Hypoxic gene expression in plants is triggered by subgroup-VII-ethylene-response-factor (ERF-VII) transcription factors, which are regulated by proteasomal degradation via the oxygen-dependent branch of the N-end-rule pathway (NERP). To investigate the influence of the oxygen-sensing ERF-VII system on plant growth and metabolism in *Arabidopsis* thaliana, two overexpression lines of the ERF-VII transcription factor RAP2.12 were compared, expressing full-length NERP-sensitive or truncated NERP-insensitive forms of RAP2.12. We could show that overexpression of the NERP-insensitive form of RAP2.12 (consistently stabilized) led to strong hypoxic responses under normoxic conditions (21% v/v oxygen), including increased expression of hypoxia responsive genes and activities of fermentative enzymes, aerobic fermentation, decreased adenylate energy states and starch levels, and reduced plant growth. This was accompanied by decreased respiration rates, changes in tricarboxylic acid cycle intermediates and accumulation of amino acids. These metabolic impairments under normoxic conditions led to a decrease in anoxic resistance of plants overexpressing the NERP-insensitive form of RAP2.12, which could be prevented by external sucrose supply.

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HRA1 as a repressor of the anaerobic response: new evidence based on in vivo co-overexpression of RAP2.12 and HRA1 in *Arabidopsis thaliana* plants

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**HYPOXIA RESPONSE ATTENUATOR1** (HRA1) is highly up-regulated by hypoxia and counteracts the induction of core low oxygen-responsive genes (Giuntoli et al., 2015, PLoS Biology, volume 12, issue 9:e1001950, doi: 10.1371/journal.pbio.1001950). After publication of Giuntoli et al. (2015), we realized that a construct used in protoplast-based experiments was different from stated in the methods. Instead of the 35S:RAP2.12₁₄-358 plasmid described, a 35S:GAL4DBD:RAP2.12₁₄-358 construct was used. Differently from what observed for the GAL4DBD:RAP2.12₁₄-358 transcription factor, HRA1 was not able to repress RAP2.12₁₄-358 activity in protoplasts. This did not change the conclusion of Giuntoli et al. (2015), which is the core hypoxia-responsive gene *HRA1* encodes a repressor of the anaerobic response. Based on data generated with the construct that was incorrect, we concluded that HRA1 acts on transcriptional activation of hypoxia-responsive promoters by RAP2.12. To further address this hypothesis *in planta*, we crossed homozygous 35S:RAP2.12₁₄-358 and 35S:HRA1 parental plants and analyzed the F₂ progeny, which, as a segregating population, enabled us to observe the combinatorial effect of the two transgene loci in a uniform genetic background. The expression analysis of a group of hypoxia-responsive genes representing potential targets of RAP2.12 confirmed a significant effect exerted by RAP2.12 expression over the steady state levels of all selected mRNAs. An ANOVA for these data showed a significant interaction between the two variables (RAP2.12 and HRA1 transgene expression) on levels of *LBD41*, *HUP7* and, marginally, on *HB1*, *HRA1* and *PDC1* marker gene transcripts. In four of the five cases where the interaction took place, the effect of HRA1 transgene expression was antagonistic to RAP2.12. A genetic interaction between HRA1 and RAP2.12 was also evident as the distinct abnormalities visible in the rosette phenotypes of 35S:RAP2.12₁₄-358 and 35S:HRA1 were absent when the two transgenes were combined in the F₁, producing a phenotype very similar to the wildtype (Col-0). The evidence we collected points to the ability of HRA1 to restrict RAP2.12 functionality, broadly speaking, although the extent of HRA1 impact might be confined to a range or threshold of RAP2.12 protein abundance.

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Anatomical analysis of aerenchyma in grass

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Oxygen is essential for organisms which maintain biological activity by aerobic respiration. In particular, plants inhabiting the waterlogging or flooding areas are necessary to transport oxygen to the plant tissues under the wet soil or water effectively. Rice has adapted to waterlogging condition by developing aerenchyma in its internodes, leaves and root. Two types of aerenchyma have been reported: schizogenous and lysigenous aerenchyma. Schizogenous aerenchyma develops aerial spaces between cells and lysigenous aerenchyma is formed by the cell death. In lysigenous aerenchyma formation, the rice root forms this aerenchyma in the cortex, whereas the stem forms it in the cortex and the pith. Furthermore, it has been reported that the process of lysigenous aerenchyma formation is associated with accumulation of ethylene and reactive oxygen species. However the channel of oxygen from stem to root is largely unknown because stem lysigenous aerenchymas are divided with septum of node. In this study, we clarified the node structure and cell form in node by light and confocal microscopy. In addition, we compared node structure and cell form between rice and other grasses. Our results demonstrated that the rice and reed (\textit{Phragmites australis}), which inhabits in pond, develop schizogenous aerenchyma by multi-arm cell in node (Figure). On the other hand, barley and wheat didn’t develop schizogenous aerenchyma in the node though they formed pith cavity in the internode. The data provided in this study show that schizogenous aerenchyma formation in node is important for oxygen diffusion in aquatic grass plants.

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</tr>
</tbody>
</table>

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## Practical information

<table>
<thead>
<tr>
<th>Abstract book</th>
<th>The abstract book is available prior to arrival from the website; there will be NO PRINTED version of the abstract book available. You will also receive a USB key with the latest version of abstract book upon registration.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arriving via Copenhagen Airport</td>
<td>Also known as Kastrup Airport with airport code CPH. Take the train from Copenhagen Airport to Helsingør (Elsinore). See next section ‘Arriving by train’</td>
</tr>
<tr>
<td>Arriving by train</td>
<td>Catch a train from Copenhagen Airport (CPH) or Copenhagen Central Station to Helsingør (Elsinore); leaves every 20 min. and the train ride takes 1 h 4 min. Train tickets to Helsingør can be purchased by credit card from ticket machines. There are also counters where tickets can be paid by cash (DKK only). The price (one-way) is DKK 108 (approximately EUR 15). At Helsingør St. (end station of this line) there is an ISPA information kiosk, which is open from 12:00 to 20:00 on Monday the 5th September where transportation to the venue is organized.</td>
</tr>
<tr>
<td>Arriving by car</td>
<td>Set the GPS to Gl. Hellebækvej 70, 3000 Helsingør or use the coordinates 56.048890, 12.584651. Free parking is provided at the venue.</td>
</tr>
<tr>
<td>Bus</td>
<td>You can purchase your ticket on the bus with cash only; bus No. 803 “Hellebo Park” leaves every 30 min. from the train station. Exit the bus at the last stop “Hellebo Park” next to the venue, Konventum.</td>
</tr>
<tr>
<td>Check-in</td>
<td>Your room is available for check-in from 14:00 and onwards on Monday 5th September.</td>
</tr>
<tr>
<td>Check-out</td>
<td>Check-out is before 10:00 on Thursday the 8th Sep. It is <strong>not</strong> possible to book additional nights at the venue.</td>
</tr>
<tr>
<td>Credit cards</td>
<td>All major international credit cards are accepted in shops, supermarkets and taxis; most common is VISA and Mastercard</td>
</tr>
<tr>
<td>Currency</td>
<td>Danish Kroner are used with an exchange rate of 7.4 DKK per Euro; Euro is sometimes accepted at major shops and restaurants in Copenhagen.</td>
</tr>
<tr>
<td>Internet</td>
<td>There is free WI-FI everywhere on the venue including your room. Many cafés and restaurants also provide free WI-FI.</td>
</tr>
<tr>
<td>Meals</td>
<td>All meals and non-alcoholic beverages are included starting Monday night with a welcome snack and drink and ending Thursday afternoon with afternoon tea/coffee.</td>
</tr>
<tr>
<td>Money</td>
<td>Danish Kroner are used with an exchange rate of 7.4 DKK per Euro; Euro is sometimes accepted at major shops and restaurants in Copenhagen.</td>
</tr>
<tr>
<td>Registration</td>
<td>Registration is open from 16:00 and onwards.</td>
</tr>
<tr>
<td>Roaming service</td>
<td>The GSM network is used along with 3G and 4G.</td>
</tr>
<tr>
<td>Taxi</td>
<td>Taxis are all metered and a trip from Helsingør St. to the venue should cost around 10-12 Euro; a taxi from Copenhagen Airport could be as much as 150 Euro.</td>
</tr>
<tr>
<td>Venue</td>
<td>Konventum is located in Helsingør (Elsinore) approximately 45 km north of Copenhagen and 4 km from Helsingør St. Phone number (+45) 49280900.</td>
</tr>
</tbody>
</table>

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