

1 **Community recommendations on terminology and procedures used in flooding and**  
2 **low oxygen stress research**

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4 Rashmi Sasidharan<sup>1</sup>, Julia Bailey-Serres<sup>1,2</sup>, Motoyuki Ashikari<sup>3</sup>, Brian Atwell<sup>4</sup>, Timothy  
5 D Colmer<sup>5</sup>, Kurt Fagerstedt<sup>6</sup>, Takeshi Fukao<sup>7</sup>, Peter Geigenberger<sup>8</sup>, Kim Hebelstrup<sup>9</sup>,  
6 Robert D Hill<sup>10</sup>, Michael J Holdsworth<sup>11</sup>, Abdelbagi M Ismail<sup>12</sup>, Francesco Licausi<sup>13</sup>,  
7 Angelika Mustroph<sup>14</sup>, Mikio Nakazono<sup>15</sup>, Ole Pedersen<sup>16</sup>, Pierdomenico Perata<sup>13</sup>,  
8 Margret Sauter<sup>17</sup>, Ming-Che Shih<sup>18</sup>, Brian Sorrell<sup>19</sup>, Gustavo G Striker<sup>20</sup>, Joost T. van  
9 Dongen<sup>21</sup>, James Whelan<sup>22</sup>, Shi Xiao<sup>23</sup>, Eric JW Visser<sup>24</sup>, Laurentius ACJ Voesenek<sup>1</sup>

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11 <sup>1</sup>Institute of Environmental Biology, Utrecht University, Padualaan 8, 3584CH Utrecht,  
12 The Netherlands

13 <sup>2</sup>Center for Plant Cell Biology, Department of Botany and Plant Science, University of  
14 California, Riverside, California 92521-0124, USA

15 <sup>3</sup>Bioscience and Biotechnology Center, Nagoya University, Chikusa, Nagoya, Aichi 464-  
16 8601, Japan

17 <sup>4</sup> Department of Biological Sciences, Faculty of Science and Engineering, Macquarie  
18 University, Sydney, 2109 NSW, Australia.

19 <sup>5</sup>School of Plant Biology, The University of Western Australia, 35 Stirling Highway,  
20 Crawley, WA 6009, Australia

21 <sup>6</sup>Department of Biosciences, Viikki Plant Science Centre, P.O. Box 65, FI-00014 Helsinki  
22 University, Finland

23 <sup>7</sup>Department of Crop and Soil Environmental Sciences, Translational Plant Science  
24 Program, Fralin Life Science Institute, Virginia Tech, Blacksburg, Virginia 24061, USA

25 <sup>8</sup> Ludwig Maximilian University of Munich, Dept Biol 1, Grosshaderner Str 2-4, D-82152  
26 Planegg Martinsried, Germany.

27 <sup>9</sup> Department of Molecular Biology and Genetics, Aarhus University, Flakkebjerg, 4200  
28 Slagelse, Denmark

29 <sup>10</sup>Department of Plant Science, University of Manitoba, Winnipeg, Manitoba R3T 2N2,  
30 Canada

31 <sup>11</sup>Plant and Crop Sciences Division, School of Biosciences, University of Nottingham,  
32 LE12 5RD, UK.

33 <sup>12</sup> International Rice Research Institute, Los Banõs, Laguna, Philippines

34 <sup>13</sup> PlantLab, Institute of Life Sciences, Scuola Superiore Sant'Anna, Via Mariscoglio 34,  
35 56124, Italy

36 <sup>14</sup>Plant Physiology, University Bayreuth, Universitaetsstr. 30, 95440 Bayreuth,  
37 Germany

38 <sup>15</sup>Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya  
39 464-8601, Japan

40 <sup>16</sup>Freshwater Biological Laboratory, Department of Biology, University of Copenhagen,  
41 Universitetsparken 4, 3<sup>rd</sup> floor, 2100 Copenhagen, Denmark

42 <sup>17</sup>Plant Developmental Biology and Plant Physiology, Kiel University, 24118 Kiel,  
43 Germany

1 <sup>18</sup>Agricultural Biotechnology Research Center, Academia Sinica, Taiwan  
2 <sup>19</sup> Department of Bioscience, Aarhus University, Denmark  
3 <sup>20</sup> IFEVA, Universidad de Buenos Aires, CONICET, Facultad de Agronomía, Av. San  
4 Martin 4453, Buenos Aires, Argentina  
5 <sup>21</sup>Institute of Biology, RWTH Aachen University, 52074 Aachen, Germany  
6 <sup>22</sup>Department of Animal, Plant and Soil Science, School of Life Science, Australian  
7 Research Council Centre of Excellence in Plant Energy Biology, La Trobe University,  
8 Bundoora, Victoria 3086, Australia.  
9 <sup>23</sup> State Key Laboratory of Biocontrol and Guangdong Provincial Key Laboratory of  
10 Plant Resources, School of Life Sciences, Sun Yat-sen University, Guangzhou, 510275  
11 China  
12 <sup>24</sup>Department of Experimental Plant Ecology, Institute for Water and Wetland  
13 Research, Radboud University, Heyendaalseweg 135, 6525 AJ Nijmegen, The  
14 Netherlands  
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21 Word count (Main body of text): 2789

22 Number of figures: 0

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1 **SUMMARY**

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3 Flooding and low oxygen (O<sub>2</sub>) stress research is a rapidly advancing area that has seen  
4 tremendous progress in the last decade, including appreciation of sensing  
5 mechanisms and a role of O<sub>2</sub> as a positional signal in development. However,  
6 inconsistencies in nomenclature, terminology and experimental methods hamper  
7 interpretations, heightened by technical challenges in assessing O<sub>2</sub> concentrations at  
8 the cellular and subcellular level. Here we present an overview of the current state of  
9 O<sub>2</sub> monitoring technologies and provide a unified nomenclature in flooding and low  
10 O<sub>2</sub> stress research. We aim to create an awareness of how experimental conditions  
11 can influence *in planta* O<sub>2</sub> and advocate the universal usage of the stated guidelines  
12 to promote unambiguous experimental comparisons and the reproducibility needed  
13 for addressing the major challenges in this field.

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18 **INTRODUCTION**

19 Apart from playing a key role in important biochemical reactions, molecular oxygen  
20 (O<sub>2</sub>) and its by-products also have crucial signalling roles in shaping plant  
21 developmental programs and environmental responses. Even under normal  
22 conditions, sharp O<sub>2</sub> gradients can occur within the plant when cellular O<sub>2</sub> demand  
23 exceeds supply, especially in dense organs such as tubers, seeds and fruits. Spatial and  
24 temporal variations in O<sub>2</sub> concentrations are important cues for plants to modulate  
25 development (Considine *et al.*, 2016; van Dongen & Licausi, 2015). Environmental  
26 conditions can also expand the low O<sub>2</sub> regions within the plant. For example, excessive  
27 rainfall can lead to partial or complete plant submergence resulting in O<sub>2</sub> deficiency in  
28 the root or the entire plant (Voeselek & Bailey-Serres, 2015). Climate change-  
29 associated increases in precipitation events have made flooding a major abiotic stress  
30 threatening crop production and food sustainability. This increased flooding and  
31 associated crop losses highlight the urgency of understanding plant flooding  
32 responses and tolerance mechanisms.

1 Timely manifestation of physiological and morphological changes triggering  
2 developmental adjustments or flooding survival strategies requires accurate sensing  
3 of O<sub>2</sub> levels. Despite progress in understanding how plants sense and respond to  
4 changes in intracellular O<sub>2</sub> concentrations (van Dongen & Licausi, 2015), several  
5 questions remain unanswered due to a lack of high resolution tools to accurately and  
6 non invasively monitor (sub)cellular O<sub>2</sub> concentrations. In the absence of such tools, it  
7 is therefore extremely critical for researchers in the field to be aware of how  
8 experimental conditions can influence plant O<sub>2</sub> levels, and thus on the importance of  
9 accurately reporting specific experimental details. This also requires a consensus on  
10 the definition of frequently used terms.

11 At the 15th *New Phytologist* workshop on Flooding Stress (Voesenek *et al.*, 2016),  
12 community members discussed and agreed on unified nomenclature and standard  
13 norms for low O<sub>2</sub> and flooding stress research. This consensus on terminology and  
14 experimental guidelines is presented here. We expect that these norms will facilitate  
15 more effective interpretation, comparison and reproducibility of research in this field.  
16 We also highlight the current challenges in noninvasively monitoring and measuring  
17 O<sub>2</sub> concentrations in plant cells, outlining the technologies currently available, their  
18 strengths and drawbacks, and their suitability for use in flooding and low O<sub>2</sub> research.

19

## 20 **TERMINOLOGY**

21 The inconsistent and sometimes inaccurate usage of flooding and low O<sub>2</sub> stress-  
22 related terms together with incomplete details regarding experimental conditions  
23 have hindered the interpretation, reproducibility and comparison of independent  
24 studies in the field. Here, we define and clarify commonly used terms used in flooding  
25 and low-O<sub>2</sub> related experimental conditions.

26 **Flooding:** a general term referring to excessively wet conditions, i.e. where excess  
27 water replaces gas-spaces surrounding roots and/or shoots. Flooding encompasses  
28 the following terms that describe natural events or experiments.

- 29     • **Waterlogging or soil flooding:** only the root-zone is flooded (excessive water  
30         in the soil or other rooting media).

- 1 • **Partial waterlogging or soil flooding:** partial flooding of the root-zone. Details  
2 regarding depth and extent of soil flooding should be specified.
- 3 • **Submergence:** the entire plant (root and shoot) is under water.
- 4 • **Partial submergence:** the entire root system and part of above ground organs  
5 are under water. Details regarding the submergence depth in relation to plant  
6 height or distance from soil surface should be specified.

7

8 **Anaerobiosis:** literally means ‘life without molecular O<sub>2</sub>’. Plants can only survive a  
9 limited time without molecular O<sub>2</sub>. The term anaerobiosis frequently refers to the  
10 status of plants/organs exposed to a lack of O<sub>2</sub> for a short time, during which  
11 acclimation occurs through altered gene expression and metabolism.

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13 **Anaerobic metabolism:** describes cellular energy production from carbohydrates  
14 *without* the benefit of oxidative phosphorylation and engagement of cytochrome c  
15 oxidase as the final electron acceptor. Anaerobic metabolism occurs when O<sub>2</sub> is absent  
16 and is usually associated with (but not limited to) ethanolic and/or lactate  
17 fermentation. In plants, it is also associated with accumulation of alanine and gamma-  
18 aminobutyric acid due to altered metabolite fluxes involving the tricarboxylic acid  
19 cycle among others (Narsai, *et al.*, 2011; Van Dongen & Licausi, 2015; Voesenek &  
20 Bailey-Serres, 2015). It can occur in cells within an ‘*anoxic core*’ in tissues/organs (e.g.  
21 vascular tissues of roots (Berry & Norris, 1949; Thomson & Greenway, 1991) even  
22 under externally aerobic conditions and in densely packed tissues or organs with a low  
23 surface to volume ratio (e.g. developing fruits, tubers, seeds, meristems)  
24 (Geigenberger *et al.*, 2000; Gibbs & Greenway, 2003; Van Dongen & Licausi, 2015).

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26 **Defining –oxic conditions:** The terms hypoxia and anoxia are often used  
27 interchangeably, which limits experimental reproducibility and can lead to  
28 misunderstanding of associated physiological, biochemical and molecular processes.  
29 When accurate quantification of the O<sub>2</sub> status of biological samples or their  
30 environment is not possible, use of -oxic terms is valid, but care should be taken when

1 inferring conclusions about O<sub>2</sub> availability from these experiments. As a guideline, we  
2 describe common -oxic words, highlighting their limitations.

- 3 • **Anoxia:** describes complete absence of O<sub>2</sub> in a system. This is not the same as  
4 an O<sub>2</sub> concentration that is too small to measure because such a condition can  
5 be maintained when the diffusive flux of O<sub>2</sub> into the tissue is equal to the O<sub>2</sub>  
6 metabolism. True anoxic conditions are unlikely to occur in plant tissues where  
7 photosynthesis and respiration are key metabolic processes (Smith & Dukes,  
8 2013). Thus, this term should be limited to describing the *atmosphere* applied  
9 to biological samples or the environment under investigation. Most cases  
10 applying anoxic conditions involve replacing the natural atmosphere with an  
11 inert gas such as argon or nitrogen (e.g. Loreti *et al.*, 2005; Branco-Price *et al.*,  
12 2008). Some artificial flooding treatments have also been defined as anoxic,  
13 when water was degassed prior to submergence (Baud *et al.*, 2004). In this  
14 case, however, the degassed water will not remain O<sub>2</sub> free unless subsequently  
15 placed in an O<sub>2</sub> free environment. Finally, true anoxic conditions require  
16 darkness, since the photosynthetic light reaction generates molecular O<sub>2</sub>.
- 17 • **Normoxia:** The reference normoxic condition is often the O<sub>2</sub> availability in air  
18 at sea level on today's Earth, i.e. 20.95 %. However, O<sub>2</sub> concentrations within  
19 plant organs can be lower or higher under normoxic conditions (Van Dongen  
20 & Licausi, 2015; Pedersen *et al.*, 2016). Therefore, internal  
21 (organ/tissue/cellular) O<sub>2</sub> concentrations could deviate from the "normoxic  
22 environment". Under external normoxia, cells may be O<sub>2</sub>-limited due to high  
23 metabolic activity, as in meristems (Greve *et al.*, 2003), vascular tissues of roots  
24 (Armstrong & Beckett, 1987) or due to limited diffusion in bulky tissues  
25 (Pedersen *et al.*, 2006), or tubers (Geigenberger *et al.*, 2000). O<sub>2</sub> levels  
26 measured in these tissues over time in the experimental system is desirable.
- 27 • **Hypoxia:** describes O<sub>2</sub> concentrations below normoxic without necessarily  
28 implying any impact (i.e. hypoxic treatment refers to experiments in which a  
29 plant is exposed to lower O<sub>2</sub> conditions than air). Hypoxia is preferably used to  
30 selectively describe O<sub>2</sub> concentrations below which a specific process is  
31 affected (e.g. below the critical O<sub>2</sub> pressure (Armstrong *et al.*, 2009) for

1 respiration) or a response is activated. This may imply the need for additional  
2 terms to indicate ranges of O<sub>2</sub> concentrations (e.g. in the field of microbiology,  
3 micro-oxic often describes 0.5 to 5% O<sub>2</sub> (Pessi *et al.*, 2013)). When authors use  
4 hypoxia or alternative terms to describe reduced O<sub>2</sub> availability, provision of  
5 precise O<sub>2</sub> tensions or ranges is valuable. This can include flow rates or  
6 turbulence, medium composition and temperatures for the external medium  
7 and the bulkiness, respiration rate and density of experimental tissue(s).

8 • **Hyperoxia/superoxia:** describe O<sub>2</sub> concentrations above normoxia.  
9 Hyperoxia/superoxia can result from, for instance, underwater photosynthesis  
10 and reduced outwards diffusion rate of O<sub>2</sub> from photosynthetic organs to the  
11 environment ( Rich *et al.*, 2013; Pedersen *et al.*, 2016), or from water bodies  
12 to the atmosphere (Nikinmaa, 2014).

13 Although it is advisable that O<sub>2</sub> concentrations be described for each experimental  
14 system, authors may prefer to use -oxic conditions best suiting the study, as long as  
15 the description enables experimental replication. Detailed description of the O<sub>2</sub> levels  
16 assessed externally or internally (within the plant) or physical parameters that affect  
17 its availability will improve the reproducibility of observations and help design of  
18 models and meta-analyses.

19

## 20 **THE CHALLENGE OF MONITORING OXYGEN LEVELS IN PLANTS**

21 Flooding is a compound stress imposing changes in O<sub>2</sub> availability (and thus respiratory  
22 ATP production), CO<sub>2</sub>, light, ethylene, mineral nutrients and reactive oxygen species  
23 (Voeselek & Bailey-Serres, 2015; Voeselek & Sasidharan, 2013). The severity of the  
24 stress and the response elicited depends upon genotype, developmental age of the  
25 plant, organ, tissue, and other factors including flooding depth and duration, light  
26 availability, temperature, humidity and the amount of carbohydrate storage (such as  
27 sugars, starch, lipids, protein) in cells and tissues.

28 It is not easy to predict what physiological changes occur in a spatial and dynamic  
29 fashion during flooding at the cellular level, especially with respect to O<sub>2</sub>  
30 concentration. The way in which a flooding treatment is performed will strongly  
31 influence how fast plant tissues experience low O<sub>2</sub> stress. Different factors, including

1 light levels in the water, the temperature and the volume of the water used to  
2 submerge the plants, microbial activity in the submerged soil, and O<sub>2</sub> concentration of  
3 the water at the beginning of the experiment will all influence how the O<sub>2</sub> availability  
4 to the plant changes during the treatment. Therefore, careful monitoring and  
5 reporting of the O<sub>2</sub> concentration around submerged plant tissue is required.  
6 Polarographic electrodes (such as the Clark-type electrode) are still most widely used  
7 for this. However, fiber-optic based sensor methods have become more popular  
8 during recent years (Rolletschek *et al.*, 2009; Ast *et al.*, 2012), since these are fast and  
9 selective. Moreover, optical sensors have the advantage that the same sensor can be  
10 used to measure molecular O<sub>2</sub> concentrations in solution as well as in air, and the  
11 baseline of the measurement is more stable as compared to polarographic methods  
12 which makes optical sensing more suited for long-term (days to weeks)  
13 measurements.

14 To avoid technical difficulties in controlling the O<sub>2</sub> concentration around a plant by  
15 submergence, many studies use a chamber filled with O<sub>2</sub>-free or O<sub>2</sub>-poor air. This has  
16 the advantage that O<sub>2</sub> concentrations can be changed much faster as compared to a  
17 submergence treatment, and that the actual external concentration can be controlled  
18 precisely. One should be aware, however, that a treatment with air containing little  
19 O<sub>2</sub> does not mimic submergence, but only changes one out of many parameters that  
20 are affected by submergence.

21 Apart from the importance of controlling the environmental O<sub>2</sub> concentration during  
22 experimental treatments, there is a strong need to obtain precise information about  
23 the plant internal O<sub>2</sub> concentration as well. To date, measurements of plant internal  
24 O<sub>2</sub> have been only accomplished via invasive means (Ast *et al.*, 2012; Ast & Draaijer,  
25 2014; van Dongen & Licausi, 2015). Most commonly, a small sensor needle is inserted  
26 into a plant organ and O<sub>2</sub> concentrations are measured at the tip of the needle. The  
27 smallest needle type sensors that currently exist are based on the Clark-type sensor  
28 system (Revsbech, 1989) and commercially available sensors have a diameter of  
29 around 4 µm. These sensors are extremely fragile. More robust glass fibre-based  
30 optical sensors typically have a diameter of about 50 µm. A disadvantage of needle-  
31 type sensors is that the tissue will be damaged upon insertion, which can lead to local  
32 changes in the rate of respiratory O<sub>2</sub> consumption. Moreover, external O<sub>2</sub> is likely to



1 diffuse through the insertion wound into the interior of the tissue, which could lead  
2 to an overestimation of the actual *in planta* O<sub>2</sub> concentration.

3 An alternative invasive method to determine local differences or changes in O<sub>2</sub>  
4 concentration is by using O<sub>2</sub> sensitive reporter foil (Tschiersch *et al.*, 2012). Here, a  
5 special camera is used to determine O<sub>2</sub> concentration-dependent light emission from  
6 a special coated sensor foil that is placed on the surface of plant tissue (Jensen *et al.*,  
7 2005). This method has been used to describe local differences in O<sub>2</sub> concentration of  
8 plant organs such as stems and seeds that were cut in order to access the interior  
9 tissues with the sensor foil. By doing so, these measurements allowed detection of  
10 differential O<sub>2</sub> consumption patterns within the plant organ (Tschiersch *et al.*, 2012).  
11 More recently, nano particles coated with a fluorescent dye have been successfully  
12 used in rhizosphere studies (Koren *et al.*, 2015). These nano particles are possible  
13 future candidates for O<sub>2</sub> studies at the cell level when working with large, transparent  
14 model cells such as cells of *Chara*.

15 To date, no method exists that enables non-invasive analysis of plant internal O<sub>2</sub>  
16 concentrations. The best alternative that is currently being applied makes use of  
17 reporter proteins (such as GUS, GFP or Luciferase) that are expressed under the  
18 control of low- O<sub>2</sub> induced promotor sequences (Gasch *et al.*, 2016). Interpretation of  
19 the expression pattern of the reporter protein allows conclusions about relative  
20 variation in the O<sub>2</sub> concentration between regions or through time. It will not provide,  
21 however, an exact value for the actual local concentration of O<sub>2</sub>. Moreover, the  
22 reaction time of such reporter systems is relatively long, making it difficult to  
23 investigate rapid changes. Several other suggestions are being discussed to design  
24 alternative non-invasive molecular O<sub>2</sub> reporter systems. FRET (fluorescent resonance  
25 energy transfer)-based methods in which the FRET efficiency is affected by O<sub>2</sub>-  
26 dependent protein maturation has already been applied successfully in bacterial cell  
27 cultures (Pötzkei *et al.*, 2012), but there are no reports yet of the successful application  
28 of such O<sub>2</sub> sensors in plants.

29 In medical research, various non-invasive O<sub>2</sub> monitoring techniques are being used,  
30 including Positron Emission Tomography (PET) and nuclear magnetic resonance  
31 (NMR) technology (Roussakis *et al.*, 2015). In plants, such methods have not been  
32 reported yet to determine O<sub>2</sub> gradients, because of the poor resolution and because

1 homogenous application of the required radioisotopes or contrast agents (such as  
2 Fluorine-19 ( $^{19}\text{F}$ )-based probes) appears difficult in plants. Further research to  
3 develop methods to determine plant internal  $\text{O}_2$  concentrations will remain of utmost  
4 importance for the research field to develop further.

5

## 6 **EXPERIMENTAL SYSTEMS**

7 It is extremely important that researchers carefully detail the experimental imposition  
8 of flooding or low  $\text{O}_2$  stress. We suggest that, in addition to details essential to any  
9 methods description, the following details specific to low  $\text{O}_2$  and flooding studies are  
10 necessary:

### 11 **Stress conditions:**

- 12 ○ Type of flooding (waterlogging, partial or complete submergence)  
13 should include depth relative to shoot height. Investigators are  
14 encouraged to define terms used in their system, e.g. stagnant  
15 flooding. If hydroponics are used, information on aeration,  $\text{O}_2$  status,  
16 light and medium composition are needed.
- 17 ○ Flooding in a natural or artificial environment should include  
18 information on light, flow, turbidity, pH, inorganic carbon  
19 concentration and temperature of the water. It is beneficial to record  
20 the rate of decline of  $\text{O}_2$  in the soil, air and water. Soil flooding can also  
21 be documented from soil redox potential.
- 22 ○ Hypoxia experiments should provide details regarding the system used  
23 to achieve low  $\text{O}_2$  conditions (and state the  $\text{O}_2$  concentrations),  
24 including time taken to achieve the condition. Further information can  
25 include: chamber size, flow rate through the system, and details of  
26 application. The gas used to lower  $\text{O}_2$  levels must be stated.
- 27 ○ In experimental setups determining  $\text{O}_2$  flux into roots from  $\text{O}_2$   
28 containing bathing media, experimenters should be aware that when  
29 roots are attached to shoots, fluxes to the root can come not just from  
30 the media but also internally from the shoot (Armstrong & Armstrong,  
31 2014).

- 1           ○ Recovery conditions (post-submergence or post-hypoxia) should be
- 2           described, including light levels, temperature, humidity, and watering
- 3           regime post-drainage. Rate of soil drainage (changes in soil water
- 4           content) and changes in soil redox potential are also valuable.
- 5           ○ Zeitgeber time (hours after dawn) and illumination when experiments
- 6           start and terminate should be mentioned.
- 7           ○ Plant density and orientation of growth on medium should be included.

8

9   **Scoring survival:** A recovery period following the removal of flooding/hypoxia/anoxia  
10 stress is essential for scoring survival (Striker, 2012). Plants should be photographed  
11 immediately before and after the treatment and at the end of the recovery period.  
12 When scoring damage, quantitative rather than qualitative data are more  
13 reproducible and can be analysed statistically (e.g., chlorophyll levels, biomass, green  
14 leaf area).

15

## 16 **CONCLUSIONS**

17 Careful descriptions of growth and treatment conditions, especially factors that can  
18 influence both plant external and internal O<sub>2</sub> concentrations are essential for clarity,  
19 reproducibility and progress in the research on plant responses to flooding and low  
20 O<sub>2</sub>. Reporting on O<sub>2</sub> concentrations, whenever possible, using the most suitable,  
21 currently available methods is recommended. Ultimately, the challenge is to also  
22 achieve an understanding of the spatial and temporal dynamics of the major flooding  
23 signals O<sub>2</sub>, ethylene, nitric oxide, reactive oxygen species and low-energy, their  
24 interactions, and how signalling modulates response from the subcellular to the whole  
25 plant level. Furthermore, the focus of many studies has been on short-term molecular  
26 signatures often under severe conditions, whereas responses associated with long-  
27 term, less severe and more chronic O<sub>2</sub> limitations that influence developmental  
28 plasticity deserve greater attention.

## 29 **References**

- 1 **Armstrong W, Armstrong J. 2014.** Plant internal oxygen transport (Diffusion and Convection) and  
2 measuring and modelling oxygen gradients. *Low Oxygen Stress in Plants: Oxygen sensing and adaptive*  
3 *responses to hypoxia. Plant Cell Monographs.* 267-298.
- 4 **Armstrong W, Beckett PM. 1987.** Internal aeration and the development of stelar anoxia in submerged  
5 roots. A multishelled mathematical model combining axial diffusion of oxygen in the cortex with radial  
6 losses to the stele, the wall layers and the rhizosphere. *New Phytologist.* **105:** 221-245.
- 7 **Armstrong W, Webb T, Darwent M, Beckett PM. 2009.** Measuring and interpreting respiratory critical  
8 oxygen pressures in roots. *Annals of Botany* **103:** 281-293.
- 9 **Ast C, Draaijer A. 2014.** Methods and techniques to measure molecular oxygen in plants. *Low Oxygen*  
10 *Stress in Plants: Oxygen sensing and adaptive responses to hypoxia. Plant Cell Monographs.* 397-417.
- 11 **Ast C, Schmäzlin E, Löhmannsröben HG, van Dongen JT. 2012.** Optical oxygen micro- and nanosensors  
12 for plant applications. *Sensors* **12:** 7015-7032.
- 13 **Baud S, Vaultier MN, Rochat C. 2004.** Structure and expression profile of the sucrose synthase  
14 multigene family in Arabidopsis. *Journal of Experimental Botany* **55:** 397-409
- 15 **Berry LJ, Norris WE. 1949.** Studies of onion root respiration. II. The effect of temperature on the  
16 apparent diffusion coefficient in different segments of the root tip. *Biochemistry Biophysics Acta* **3:** 607-  
17 614.
- 18 **Branco-Price C, Kaiser KA, Jang CJH, Larive CK, Bailey-Serres J. 2008.** Selective mRNA translation  
19 coordinates energetic and metabolic adjustments to cellular oxygen deprivation and reoxygenation in  
20 *Arabidopsis thaliana.* *The Plant Journal* **56:** 743-755
- 21 **Considine MJ, Diaz-Vivancos P, Kerchev P, Signorelli S, Agudelo-Romero P, Gibbs DJ, Foyer CH. 2016.**  
22 Learning to breathe: Developmental phase transitions in oxygen status. *Trends in Plant Science.*  
23 <http://dx.doi.org/10.1016/j.tplants.2016.11.013>
- 24 **Gasch P, Fundinger M, Muller JT, Lee T, Bailey-Serres J, Mustroph A. 2015.** Redundant ERF-VII  
25 transcription factors bind an evolutionarily-conserved cis-motif to regulate hypoxia-responsive gene  
26 expression in Arabidopsis. *The Plant Cell.* **28:** 160-180.
- 27 **Geigenberger P, Fernie AR, Gibon Y, Christ M, Stitt M. 2000.** Metabolic activity decreases as an  
28 adaptive response to low internal oxygen in growing potato tubers. *Biological Chemistry* **381:** 723-740
- 29 **Gibbs J, Greenway H. 2003.** Mechanisms of anoxia tolerance in plants. I. Growth, survival and anaerobic  
30 catabolism. *Functional Plant Biology* **30:** 1-47
- 31 **Greve, T.M., Borum, J., Pedersen, O. 2003.** Meristematic oxygen variability in eelgrass (*Zostera*  
32 *marina*). *Limnology and Oceanography* **48:** 210-216.  
33
- 34 **Jensen SI, Kühl M, Glud RN, Jørgensen LB, Priemé A. 2005.** Oxic microzones and radial oxygen loss  
35 from roots of *Zostera marina.* *Marine Ecology Progress Series* **293:** 49-58.  
36
- 37 **Koren K, Brodersen KE, Jakobsen SL, Kühl M. 2015.** Optical sensor nanoparticles in artificial  
38 sediments - a new tool to visualize O<sub>2</sub> dynamics around the rhizome and roots of seagrasses.  
39 *Environmental Science & Technology* **49:** 2286-2292.
- 40 **Lorbiecke R, Sauter M. 1999.** Adventitious root growth and cell-cycle induction in deepwater rice. *Plant*  
41 *Physiology* **119:** 21-30

- 1 **Loreti E, Poggi A, Novi G, Alpi A, Perata P. 2005.** A genome-wide analysis of the effects of sucrose on  
2 gene expression in Arabidopsis seedlings under anoxia. *Plant Physiology* **137**: 1130-1138
- 3 **Narsai R, Rocha M, Geigenberger P, Whelan J, van Dongen JT. 2011.** Comparative analysis between  
4 plant species of transcriptional and metabolic responses to hypoxia. *New Phytologist* **190**: 472-487
- 5 **Pedersen O, Vos H, Colmer T. 2006.** Oxygen dynamics during submergence in the halophytic stem  
6 succulent *Halosarcia pergranulata*. *Plant Cell and Environment* **29**: 1388-1399
- 7 **Pedersen O, Colmer TD, Borum J, Zavala-Perez A, Kendrick GA. 2016.** Heat stress of two tropical  
8 seagrass species during low tides – impact on underwater net photosynthesis, dark respiration and diel  
9 *in situ* internal aeration. *New Phytologist*. **210**:1207-1218
- 10 **Pessi G, Braunwalder R, Grunau A, Omasits U, Ahrens CH, Eberl L. 2013.** Response of *Burkholderia*  
11 *cenocepacia* H111 to Micro-Oxia. *PLoS One* **8**: e72939
- 12 **Potzkei J, Kunze M, Drepper T, Gensch T, Jaeger K, Buchs J. 2012.** Real-time determination of  
13 intracellular oxygen in bacteria using a genetically encoded FRET-based biosensor. *BMC Biology* DOI:  
14 10.1186/1741-7007-10-28
- 15 **Revsbech NP. 1989.** An oxygen microelectrode with a guard cathode. *Limnology and Oceanography* **34**:  
16 474-478.
- 17 **Rich SM, Pedersen O, Ludwig M, Colmer TD. 2013.** Shoot atmospheric contact is of little importance  
18 to aeration of deeper portions of the wetland plant *Meionectes brownii*: submerged organs mainly  
19 acquire O<sub>2</sub> from the water column or produce it endogenously in underwater photosynthesis. *Plant,*  
20 *Cell and Environment* **36**: 213-223.
- 21 **Rolletschek H, Stangelmayer A, Borisjuk L. 2009.** Methodology and significance of microsensor-based  
22 oxygen mapping in plant seeds – an overview. *Sensors* **9**: 3218-3227.
- 23 **Roussakis E, Li Z, Nichols AJ, Evans CL. 2015.** Oxygen-sensing methods in biomedicine from the  
24 macroscale to the microscale. *Angewandte Chemie International Edition* **54**: 8340-8362.
- 25 **Smith NG, Dukes JS. 2013.** Plant respiration and photosynthesis in global-scale models: incorporating  
26 acclimation to temperature and CO<sub>2</sub>. *Global Change Biology* **19**: 45-63
- 27 **Striker GG. 2012.** Time is on our side: the importance of considering a recovery period when assessing  
28 flooding tolerance in plants. *Ecological Research* **27**: 983-987
- 29 **Tschiersch H, Liebsch G, Borisjuk L, Stangelmayer A, Rolletschek H. 2012.** An imaging method for  
30 oxygen distribution, respiration and photosynthesis at a microscopic level of resolution. *New*  
31 *Phytologist*. **196**: 926-936.
- 32 **Thomson CJ, Greenway H. 1991.** Metabolic evidence for stelar anoxia in maize roots exposed to low  
33 oxygen concentrations. *Plant Physiology*. 96:1294-1301.
- 34 **Van Dongen JT, Licausi F. 2015.** Oxygen sensing and signaling. *Annual Review of Plant Biology* **66**: 345-  
35 367
- 36 **Voesenek LACJ, Sasidharan R. 2013.** Ethylene - and oxygen signalling - drive plant survival during  
37 flooding. *Plant Biology* **15**: 426-435

1 **Voesenek LACJ, Bailey-Serres J. 2015.** Flood adaptive traits and processes: an overview. *New*  
2 *Phytologist* **206**: 57-73

3 **Voesenek LACJ, Bailey-Serres J. 2015.** Air conditional. *Nature Plants*. DOI: 10.1038/NPLANTS.2015.95

4 **Voesenek LACJ, Sasidharan R, Visser EJW, Bailey-Serres J. 2016.** Flooding stress signaling through  
5 perturbations in oxygen, ethylene, nitric oxide and light. *New Phytologist* **209**: 39-43

6

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