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RESEARCH ARTICLE

Root acclimations to soil flooding prime rice (*Oryza sativa* L.) for subsequent conditions of water deficit

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Abstract

Background and aims The root barrier to radial O_2 loss is a trait induced during soil flooding restricting oxygen loss from the roots to the anoxic soil. It can also restrict radial water loss, potentially providing tolerance towards drought during conditions of water deficit. Several root traits (aerenchyma and xylem vessels area) respond in a similar way to soil flooding and low soil water potentials. Therefore, we hypothesised that root acclimations to soil flooding prime plants to withstand conditions of water deficit.

Methods We raised plants in hydroponics mimicking contrasting soil water conditions (aerated controls for well-watered soils; stagnant, deoxygenated solutions for flooded soils, and aerated solutions with different PEG6000 concentrations to mimic conditions of water deficit). We used O_2 microsensors and gravimetric measurements to characterize the formation of a barrier to radial O_2 loss during conditions of water deficit, and measured key anatomical root traits using light microscopy.

Results Several root traits were induced in stagnant conditions as well as in conditions of water deficit, including the barrier to radial O_2 loss. The tightness of the barrier to water loss was similar in both stagnant and PEG6000 treatments. Moreover, plants growing in stagnant conditions tolerated a following severe condition of water deficit, whereas those growing in mimicked well-watered conditions did not.

Conclusions We demonstrated that plants growing in stagnant conditions can withstand following severe conditions of water deficit. We propose that key root traits, such as the barrier to radial O_2 loss, which are induced in stagnant conditions as well as

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mild conditions of water deficit, prime the plants for a following severe condition of water deficit.

Keywords Aerenchyma · Cortex to stele ratio · Drought · Permeance to water · Radial water loss · ROL barrier · Root traits · Soil flooding · Waterlogging

Abbreviations

DI	Deionized
P_a	Apparent permeance
PAM	Pulse amplitude modulation
PEG6000	Polyethylene glycol 6000
RH	Relative humidity
ROL	Radial oxygen loss
RWL	Radial water loss
SA:V	Surface area to volume ratio

Introduction

Crop production across the world is threatened by the ongoing climate change (IPCC 2019). The changes in frequency and intensity of precipitation, which can lead to low (drought) or excess (flooded) soil water content are already causing a reduction in yields of staple crops such as rice (Ray et al. 2019). Therefore, identifying traits that can help crop production under a combination of stresses is of high priority (Rivero et al. 2022; Yamauchi et al. 2021). In the present study, we demonstrated that plants acclimated to soil flooding are better at tolerating subsequent situations of water deficit, and we propose that this is due to the barrier to radial O_2 loss, which also restricts radial water loss (Peralta Ogorek et al. 2021; Song et al. 2023).

Lowland irrigated rice (paddy rice) can be exposed to fluctuating soil water potentials. The water for agricultural use is becoming less available (Rijsberman 2006) and this, combined with the high water demand for paddy rice production, has led to the development of an alternate wetting and drying irrigation method (Bouman et al. 2007). Here, the water-saturated soil of the paddy field is allowed to dry to a certain soil depth whereafter water is replenished; this approach greatly reduces total water usage compared to continuous soil flooding without significant yield losses (Bouman et al. 2007). Further pushing the alternate wetting and drying threshold can increase water savings but these thresholds rely on the current and shifting climatological conditions (Dela Cruz et al. 2022; Lampayan et al. 2014). Therefore, a predicted safe threshold can change exposing the plants to lower soil water content than expected. In addition, a well-timed drainage of paddy fields remove soil phytotoxins such as H_2S (Amin et al. 2011), but if this coincides with insufficient rainfalls or onset of drought, the rice will suffer from water deficit.

The adverse effects caused by low soil water potential leads to physiological responses typically associated to several trade-offs. Rice is particularly sensitive to drought (Bouman et al. 2007) and low water availability causes reduction in leaf area and early senescence (Wopereis et al. 1996). Reduced stomatal conductance restricts loss of water during low water availability and thereby maintain leaf turgor. However, it also limits gas exchange (O_2, CO_2) and therefore the photosynthetic rate, ultimately reducing biomass production (Wang et al. 2018). The reduction in photosynthesis further causes oxidative stress in the leaf tissues due to energy and electron transfer imbalance in the photosystem II reaction centre (Goltsev et al. 2012). Consequently, survival comes at a cost, and root plasticity appears as a key component to help in the survival process.

Several key root traits enhance drought resistance, but may also come with trade-offs. Gas-filled spaces (aerenchyma) are formed as a response to low soil water content, saving water per root volume so that a longer root can grow with the same investment in carbon and water (Zhu et al. 2010). As a consequence, longer roots are able to tap into deeper soil layers with higher water availability (Kato et al. 2007), but aerenchyma formation can also accelerate water loss from roots and lead to root shrinking as a consequence of cortex collapse (Duddek et al. 2022; Song et al. 2023). Root shrinking in dry soils may lead to a loss of root-soil contact, compromising water uptake (Carminati et al. 2009). However, the accelerated water loss as a consequence of aerenchyma formation can be counteracted by increasing root thickness, forming a barrier to radial water loss (RWL), and/or thicker roots (Song et al. 2023). Thicker roots in combination with multiseriate cortical sclerenchyma are mechanically stronger and thereby better suited at penetrating dry soils (Schneider et al. 2021; Yu et al. 1995) that become dense and difficult to penetrate (Bengough et al. 2006). Finally, low soil water availability can

induce suberization of the endodermis and exodermis (Enstone et al. 2002; Lo Gullo et al. 1998) reducing RWL but also radial apoplastic water flow (Franke and Schreiber 2007; Taleisnik et al. 1999). Interestingly, some of the above key root traits are also relevant in soils with excess water (Yamauchi et al. 2021).

Flooded soils present a contrasting set of challenges to the roots. O_2 diffusion is 10.000-fold slower in water than in air, which limits the O_2 supply to the roots in flooded soils (Armstrong et al. 1991; Ponnamperuma 1972). Rice constitutively forms aerenchyma, creating a low resistance diffusive pathway for gases between shoot and roots (Colmer 2003a), and aerenchyma formation is further enhanced in flooded soils, enhancing the transport capacity of O_2 to the growing root tips (Colmer and Voesenek 2009). Radial O_2 loss from the roots to the anoxic soils can be reduced by thicker roots through the reduction in SA:V (Pedersen et al. 2020). However, the most effective trait restricting oxygen loss is the root barrier to radial O_2 loss, where suberization-lignification of the exodermal cell walls results in high resistance to O_2 diffusion (Colmer 2003a; Ranathunge et al. 2011). Soil flooding (Colmer 2003a) and soil phytotoxins (Peralta Ogorek et al. 2023a) induce the formation of the barrier to radial O_2 loss. Interestingly, it was demonstrated that low water potentials in the root medium, simulated using polyethylene glycol 6000, triggered the formation of a root barrier to radial O_2 loss in rice (Song et al. 2023). The combination of these traits enhances longitudinal O_2 diffusion to the root tip and enables root survival and elongation in the anoxic soil (Shiono et al. 2014).

Due to the overlap in beneficial root traits during conditions of low as well as excess soil water, we hypothesised that rice acclimated to soil flooding possesses an advantage under subsequent conditions of water deficit, which can happen in many rice growing systems (Amin et al. 2011; Bouman et al. 2007). To test the hypothesis, we measured stomatal conductance, F_v/F_m, and conducted low water potential shock experiments to evaluate desiccation of the plants at the shoot level. We also characterized and compared the formation of the barrier to radial O_2 loss and other key root traits, such as aerenchyma formation and cortex to stele ratio, under the various treatments. We used hydroponically grown rice applying three treatments, *i*) aerated nutrient solution to mimic well-watered soils, *ii*) stagnant, deoxygenated nutrient solution to mimic soil flooding (Wiengweera et al. 1997), and *iii*) aerated nutrient solution with polyethylene glycol 6000 (PEG6000) to mimic low soil water potential (Michel 1983). We used O_2 microsensors to diagnose the formation of the barrier to root radial O_2 loss barrier, gravimetric measurements to establish the apparent permeance (P_a) to water, and key root traits were measured on root cross-sections using light microscopy.

Materials and methods

Plant material and nutrient solutions

Seeds of IR42, a moderately drought tolerant variety of rice (*Oryza sativa* L.) (Ponnamperuma 1979), were germinated for 3 d in darkness at 28 °C. The seedlings were transferred to a 25% strength aerated nutrient solution for 7 d and then moved to 3.6 L black pots with 100% strength aerated nutrient solution with 4 plants in each pot; a pot was considered one replicate. Aeration was achieved using air pumps and foam stoppers were used to hold the seedlings with the roots in the solution and the shoot in the air. After 21 d, half of the plants were placed in a stagnant, deoxygenated nutrient solution. Aerated nutrient solutions mimic a well-watered soil, and stagnant, deoxygenated nutrient solutions mimic soil flooding (Wiengweera et al. 1997). After at least 7 d (total 28 d), plants from aerated and stagnant conditions were transferred to an aerated nutrient solution with polyethylene glycol 6000 (PEG6000) that mimics low soil water potentials; the percentage of PEG6000 added and the resulting water potential is indicated in each experiment. The water potential of the solutions with different PEG6000 percentages was estimated using Eq. 2 described in Michel (1983) and confirmed by measuring the osmotic potential using a dew point hygrometer (WP4, Decagon Devices Inc., Pullman, USA). The composition of the nutrient solution, as well as photon flux and dark light cycle, is described in Peralta Ogorek et al. (2021) with the following two exceptions: (1) MES was increased to 7.5 mM and (2) final pH was between 6.3 and 6.5. Aerated or stagnant nutrient solutions were renewed weekly, and aerated PEG6000 solutions were renewed twice a week.

Leaf desiccation as a response to water deficit

To test whether plants acclimated to stagnant, deoxygenated conditions would be primed to withstand a

following condition of water deficit, plants growing in aerated or stagnant, deoxygenated conditions were transferred to a 25% PEG6000 (-0.8 MPa) aerated solution to visualize the wilting of the leaves during 24 h under a controlled exposure to a severely low water potential. A higher percentage of PEG6000 was used in this experiment to facilitate the visualization of leaf wilting. Time-lapse photos of the shoots were taken using a GoPro Hero 8 camera (GoPro Inc., California, USA).

Key anatomical root traits in contrasting growth conditions

We measured anatomical acclimations of root traits to the various growth conditions, i.e., aerated or stagnant, deoxygenated conditions, or after transferring them for 7 d to 20% PEG6000 (-0.5 MPa) aerated nutrient solution. Target roots (10–14 cm long) were cut into 10 mm segments corresponding to positions 7 to 8 cm behind the root tip, infiltrated with 70% ethanol using a vacuum pump and fixed in 5% agar. Segments were sectioned into 100 µm thickness using a vibrating microtome (Leica VT1200, Wetzlar, Germany). Photos of the cross-sections were taken using a microscope-mounted camera, and analysed using ImageJ (v1.52a). The data obtained were: aerenchymal area, xylem vessel area, whole root area, and cortical and stelar area.

Diagnosing of the barrier to radial O_2 loss

To detect the formation of the barrier to radial O_2 loss, the apparent permeance (P_a) to O_2 was measured in roots without a pre-formed barrier to radial O_2 loss from plants growing in an aerated nutrient solution without or with 20% PEG6000 (-0.5 MPa). This concentration of PEG6000 created a tolerable stress for the plants, i.e., the leaves wilted but did not completely lose turgor. In parallel, mannitol was also used to create a water potential of -0.5 MPa in the root medium following Eq. 1 from Michel (1983). We also tested mannitol since PEG6000 solutions are relatively viscose and therefore could create a *quasi* stagnant condition, triggering the formation of the barrier to radial O_2 loss, whereas the mannitol solution is of much lower viscosity and would not create a *quasi* stagnant conditions. In this way, the potential formation of the barrier to radial O_2 loss can be attributed to the water potential in the solution and not to the viscosity. After 3 d in 20% PEG6000 or mannitol, 25 mm segments (representing the position 35 to 60 mm behind the root tip) from 10 to 14 cm-long roots were severed and the cut ends sealed with lanoline, mounted on a metal mesh using rubber bands, and placed inside a 2 L aquarium (200*100*100 mm). An O_2 microsensor (OX25, Unisense A/S, Aarhus, Denmark) was inserted 125 to 175 µm into the root cortex, aided with a boom-stand dissection microscope (Leica WILD M3B, WILD LEITZ, Heerbrugg, Switzerland) and a motorized micromanipulator controlled by Logger software (part of SensorSuite v3.2, Unisense A/S). The aquarium was filled with a DI saturated with 100% O_2 to measure O_2 intrusion into the segments for 15 to 30 min (Peralta Ogorek et al. 2021). The bulk water O_2 concentration was monitored using an O_2 minioptode (Opto-MR, Unisense A/S) and temperature was monitored using a temperature sensor (ZNTC, Unisense A/S). Both O_2 sensors were calibrated using the manufacturer´s recommended two-point calibration procedure. The intrusion rate was determined from the linear slope of 10 data points representing 10 s when the O_2 concentration inside the segment was at air-equilibrium (270.8 µM at 23 °C) and corrected to 1 mm of root diameter, as intrusion rates are influenced by surface area to volume ratio (see Peralta Ogorek et al. (2021)). The intrusion rates were also corrected for root surface area and gradient difference inside and outside the root segments to obtain P_a to O_2 following the equation by Lendzian (2006):

$$Pa = \frac{F}{A \times \Delta C} \quad (1)$$

where P_a (m s^{-1}) is obtained by dividing the intrusion rate (F, mol s^{-1}) by the surface area of the segment (A, m^2) and the gradient driving force (ΔC, mol m^{-3}).

The P_a to H_2O vapour was used to further characterize the formation of a barrier to radial O_2 loss and its tightness (Peralta Ogorek et al. 2021). Plants without or with a barrier to radial O_2 loss from aerated or stagnant, deoxygenated nutrient solutions, or after transferring them to a 20% PEG6000 aerated solution for 7 d had target roots (10 to 14 cm-long) severed to 10.0 to 11.5 cm with the tip intact. The roots were gently blotted using paper towels and placed on mesh inside a 4-digit analytical balance (Mettler Toledo Analytical Balance ME54) connected

to the software LabX direct balance (v2.4), keeping the balance chamber at room temperature and RH at 18–30% (HOBO UX100-011 Temperature and RH data logger, Onset). The RH was maintained hanging bags with silica gel inside the balance chamber. Changes in root mass were recorded every 30 s for 1 h, at which point the segments were oven dried for 24 h at 70 °C to remove all the remaining moisture to determine the total water content and to calculate the cumulated water loss (% of water loss relative to total water content). Changes in root diameter caused by root shrivelling were recorded using an USB microscope camera (AM7025X, Dino-Lite Europe, Almere, The Netherlands) and its corresponding software (Dino-capture 2.0, v. 1.5.44C) to create empirical models predicting root diameter changes (for details, see Song et al. (2023)) with time during desiccation in the balance chamber (Fig. S1). Rates of radial water loss (RWL) were calculated on the basis of the amount of water lost and the dynamic root surface area as predicted by the root diameter models. Finally, the P_a to H_2O vapour was calculated using the RWL rates when 35% of the cumulated water was lost following Eq. 1. In a follow-up experiment, we assessed P_a to H_2O in roots from plants growing in aerated or stagnant solutions, or after moving the aerated control plants to a 20% PEG6000 solution but removing the root tip prior to the measurements. The procedure was as described above with the following modifications: (i) we used 5 cm segments without the root tip (representing position 3 to 8 cm behind the root tip) instead of 10.0–11.5 cm-long roots, (ii) the rates of RWL used to calculate P_a to H_2O were extracted when 15% of cumulated water loss instead of 35% to facilitate comparison with the literature, (iii) lateral roots were removed, and (iv) we used a fixed instead of the dynamic root surface area. Like for P_a to O_2, the rates of RWL at 15% or 35% cumulated water loss were corrected to 1 mm root diameter, as thinner roots would have higher RWL rates but not due to a higher P_a to H_2O.

Histochemical staining of lignin and suberin in roots

The formation of the barrier to radial O_2 loss parallels with suberin and lignin depositions in the outer parts of the root (Kotula et al. 2009). Therefore, we conducted suberin and lignin staining to confirm the presence of these biopolymers in the different growth conditions, using the same root segments as for key anatomical root traits (see above). Lignin depositions were detected using the HCl-Phloroglucinol method (Jensen 1962; Vallet et al. 1996). Briefly, root cross sections were incubated for 3.5 min in a 2% solution (w/v) of phloroglucinol dissolved in 96% ethanol and mounted in a drop of 18% HCl. Cinnamaldehyde groups of lignin were detected under white light microscopy (Olympus BX60, Olympus Optical CO., LTD Tokyo, Japan) as they produced a red-pink colour. For suberin staining, root cross sections were incubated for 1 h in a 0.01% (w/v) Fluorol Yellow 088 solution dissolved in polyethylene glycol 400. Stained suberin depositions produced yellow fluorescence (Brundrett et al. 1991) under UV light (Nikon ECLIPSE C*i*, Excitation filter Ex 365/10, Dichroic mirror DM-400, Barrier filter BA-400, camera Nikon DS-F*i*3).

Root hydraulic conductivity

We assessed whether the barrier to radial O_2 loss and other root acclimations to stagnant, deoxygenated conditions would impact the capacity for longitudinal water flow of the root system by measuring the root hydraulic conductivity. We used a Scholander pressure bomb (3000F01 Plant Water Status Console, Soilmoisture Equipment Corp., California, USA) following the procedure of Miyamoto et al. (2001) with minor modifications. Plants between 25 and 30 d-old from aerated or stagnant, deoxygenated conditions had all their leaves cut and tillers shortened. The main shoot protruded out of the pressure chamber, while the remaining tillers were kept inside the pressure chamber with their cut ends sealed with paraffin wax (melting point 52–54°C, VWR Chemicals) and clamps. The root system inside the chamber was submerged in DI water, and a pressure of 0.4 MPa was applied for 30 to 60 min to obtain a stable water flow. Then, water protruding from the cut surface of the main shoot was collected using 3–5 pre-weighed Eppendorf tubes containing cotton (sampling time 45–120 s). The pressure was reduced to 0.3, 0.2, and 0.1 MPa, repeating the sampling procedure for each pressure. The water collected was plotted for each pressure point to calculate the slope and obtain the root hydraulic conductance ($m^3\ s^{-1}\ MPa^{-1}$). The root system was then cut and scanned to calculate the total root length and total root surface area using

RhizoVision Explorer software (v. 2.0.3). Finally, root hydraulic conductance was divided by root surface area and multiplied by total root length to obtain the area-specific root hydraulic conductivity ($m^3\ m^{-1}\ s^{-1}\ MPa^{-1}$).

Stomatal conductance and pulse amplitude modulation fluorometry measurements

To investigate whether plants pre-treated in aerated or stagnant conditions responded differently to low water potentials in the root medium, stomatal conductance and pulse amplitude modulation (PAM) measurements were conducted. Plants from aerated or stagnant, deoxygenated conditions were transferred to a 20% PEG6000 (-0.5 MPa) aerated nutrient solution for 7 d. Between 6 and 8 h into the light cycle, the stomatal conductance of the adaxial and abaxial sides of either the 2nd, 3rd or 4th leaf were measured and summed to obtain the total stomatal conductance for each leaf. The device was calibrated every day before measuring as indicated in the manufacturer's manual. Next, the lights were turned off and, after allowing the leaves to acclimate to darkness for 30 min, PAM measurements were conducted on the same leaves as stomatal conductance to determine the F_v/F_m of the photosystem II using a JUNIOR-PAM device (Heinz Walz GmbH, Effeltrich, Germany). Both measurements started 24 h into the PEG6000 treatment and readings were taken every day for 5 days.

Leaf water potential

To assess the typical leaf water potential, measurements were conducted on either the 2nd, 3rd or 4th leaf from plants growing in aerated or stagnant conditions. Between 6 to 11 h into the light cycle, leaves were cut, immediately wrapped in cling film, and mounted in a Scholander pressure bomb (3000F01 Plant Water Status Console, Soilmoisture Equipment Corp., California, USA) with the cut section protruding out and the rest of the leaf inside the pressure chamber. Pressure was applied using compressed N_2 until water emerged from the xylem bundles.

In addition, we assessed whether the leaf wilting observed in plants transferred to a 25% PEG6000 aerated solution was due to water loss from the leaves or to water being pulled from the leaves into the PEG6000 solution via the roots. For this, we induced stomatal closure by covering plants growing in aerated or stagnant, deoxygenated conditions with a double black plastic bag and added moist paper towels inside to maintain a high RH (> 75%) for a minimum of 2 h. In this way, water loss from the leaves was strongly reduced. Afterwards, the covered plants from aerated or stagnant, deoxygenated conditions were moved to a 25% PEG6000 aerated solution and their leaf water potential was measured as described above, while still maintaining a high RH inside the bags by frequently replacing the moist paper towels. Mature leaves were sampled every 30–60 min for 300 min, plus 2 more measurements the following day.

Turgor loss point

Leaf water potential at turgor loss point was estimated following the procedure by Petruzzellis et al. (2019). Plants growing in aerated or stagnant, deoxygenated conditions were covered with double black plastic bags for 24 h to completely hydrate the leaves, and expanded mature leaves were sampled and wrapped in cling film. Their fresh mass was quickly recorded and the leaves were then placed in an oven at 70 °C for 24 h to obtain the dry mass. Finally, the leaf dry matter content was obtained dividing the dry mass by the fresh mass. In parallel, another group of leaves were sampled, wrapped in cling film, immersed for 2 min in liquid nitrogen and grounded into fine pieces. They were stored at -20 °C in sealed plastic vials until conducting the measurements. The water potential of the grounded material, corresponding to its incorrect osmotic potential at full turgor, was obtained using a dew point hygrometer (WP4, Decagon Devices Inc., Pullman, USA) waiting 10 min for the material to thaw before measuring. The correct osmotic potential at full turgor was obtained from the leaf dry matter content and finally used to obtain the turgor loss point following the equations:

$$\pi_0 = \left(0.5305 \times \pi_{0,incorr}\right) - (0.0019 \times LDMC) \quad (2)$$

$$\Psi_{tlp} = 1.31 \times \pi_0 - 0.03 \quad (3)$$

where π_0 and $\pi_{0,incorr}$ are the correct and incorrect osmotic potential at full turgor (MPa), respectively, LDMC is the leaf dry matter content (mg g^{-1}), and Ψ_{tlp} is the water potential at turgor loss point (MPa).

Statistical analyses

GraphPad Prism software (v.8.4.3) was used for statistical analyses. Each test applied is indicated in the corresponding figure caption, along with the number or replicates and if any data transformation was required to fulfil a statistical test assumptions (data normality, homogeneity of variance). Data shown in all figures is non-transformed.

Results

The barrier to radial O_2 loss does not reduce root hydraulic conductivity but delays wilting at the shoot level

We measured root hydraulic conductivity to confirm that the barrier to radial O_2 loss and other root acclimations to stagnant conditions would not affect the water transport capacity to the shoot. Indeed, no significant differences were found between the longitudinal hydraulic conductivity of roots from plants growing in aerated or stagnant conditions, being $2.11 \times 10^{-6} \pm 7.35 \times 10^{-7}$ and $4.87 \times 10^{-6} \pm 1.11 \times 10^{-6}$, respectively ($m^3\ m^{-1}\ s^{-1}\ MPa^{-1}$, mean $\pm$ *SE*, two-tailed *t*-test, $n = 4$–5). In addition, neither root surface area (0.060 $m^2 \pm 0.007$ for aerated and 0.060 $m^2 \pm 0.005$ for stagnant; mean $\pm$ *SE*) nor total root length (48.9 m $\pm$ 5.1 for aerated and 44.7 m $\pm$ 5.3 for stagnant; mean $\pm$ *SE*) differed significantly (two-tailed *t*-test, $n = 4$–5) between plants grown in aerated or stagnant, deoxygenated nutrient solution.

Furthermore, we tested whether root acclimations to stagnant conditions, being largely similar to those induced in PEG6000 conditions, would allow plants to tolerate a severe and sudden condition of water deficit. For this, we transferred plants from aerated or stagnant, deoxygenated conditions into a 25% PEG6000 (-0.8 MPa) aerated solution (Fig. 1). Within 10 min in 25% PEG6000, plants from both growth conditions showed a slight leaf rolling. Thereafter, plants from aerated conditions further rolled and some leaves started to bend after 4 h. After 24 h, the leaves of plants from aerated conditions were completely wilted. In stark contrast, plants from stagnant, deoxygenated conditions did not show any other symptoms besides the initial leaf rolling and the leaves still maintained turgor after 24 h at -0.8 MPa.

Root anatomical traits responded to growth conditions regardless of their initial growth conditions

Key anatomical root traits responded to the different growth conditions, and in the following only significant responses are further analysed. Aerenchymal area was 66% smaller in control roots compared with roots in stagnant, deoxygenated conditions, and control roots also had 48% lower aerenchymal area than roots moved from control conditions to 20% PEG6000 (-0.5 MPa) aerated solution (Fig. 2A). Roots were thinner in aerated controls compared to root grown in stagnant conditions. Moreover, roots grown in aerated controls and moved to PEG6000 did not change their thickness. However, root thickness decreased when plants were moved from stagnant conditions to PEG6000 (Fig. 2B); the latter response concurs with previous observations in six different rice genotypes that all decreased their root diameter under conditions of soil water deficit (Henry et al. 2012). The aerenchyma to whole root ratio integrates responses in aerenchyma and cortical area, and the ratio of control roots were 38% lower than in stagnant, deoxygenated conditions, and 44% lower than in roots moved from control conditions to 20% PEG6000 aerated solution (Fig. 2C). The cortex to stele ratio is another root trait responding to contrasting water availability; the ratio of control roots was only half of that in stagnant, deoxygenated conditions. Interestingly, the ratio of roots formerly in stagnant, deoxygenated conditions but moved to PEG6000 was reduced also to half of that in control roots (Fig. 2D). Besides, the water transporting tissues also responded to the treatments so that xylem vessel area was 1.4-fold higher in control roots compared with all other treatments (Fig. 2E). Finally, the ratio of xylem vessel area to stele area declined as a response to stagnant, deoxygenated conditions and to treatments with PEG (Fig. 2F).

In summary, these results indicate that the key anatomical traits were all highly sensitive to the treatments and significant acclimations took place at the root anatomical level. However, we observed substantial overlap between key anatomical traits developed under soil flooding and drought, and therefore the roots formed under waterlogging prime the plant to withstand a subsequent period of water deficit.

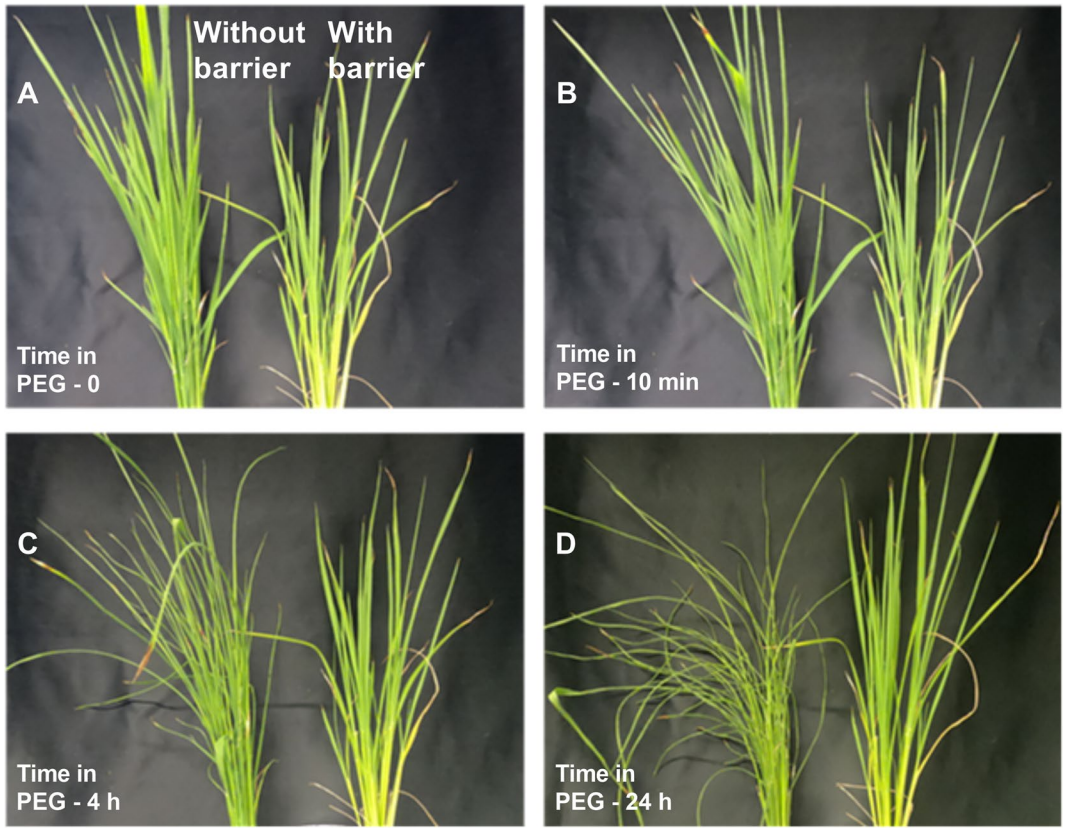

Fig. 1 Desiccation of rice (*Oryza sativa* L.) leaves after transferring plants growing in aerated or stagnant, deoxygenated nutrient solutions to a 25% PEG6000 aerated nutrient solution. Example of plants from aerated (left: without a barrier to radial oxygen loss) or stagnant (right: with a barrier to radial oxygen loss) conditions before moving to a 25% PEG6000 (-0.8 MPa) solution **A**, after 10 min **B**, after 4 h **C**, and after 24 h **D**. For data on shoot and root dry mass, see Fig. S6 and for the complete set of replicates, see Fig. S7. Plants were 28 d-old, and plants with a barrier grew at least 7 d in stagnant conditions before the measurements

Moderate conditions of water deficit induce the formation of the barrier to radial oxygen loss

Roots responded to low water potential in the nutrient solution by forming a barrier to radial O_2 loss with similar tightness to that of the barrier formed in a stagnant, deoxygenated nutrient solution. The P_a to O_2 indicates the induction of the barrier to radial o2 loss; P_a to O_2 was 3.7-fold higher in control roots compared to roots growing for 3 d in a 20% PEG6000 aerated solution (Fig. 3A; see Fig. S2A for an example of O_2 intrusion measurement). After 7 days of exposure to PEG6000, we calculated the P_a to H_2O vapour based on radial water loss rates (RWL) when 35% cumulated water was lost and found that control roots had a P_a to H_2O vapour 3.6-fold higher than the roots in a 20% PEG6000 aerated solution (Figs. 3B, S2B,C,D). Moreover, we benchmarked the tightness of the PEG6000-induced barrier to that of a barrier to radial O_2 loss induced in stagnant, deoxygenated conditions and found no significant difference between their P_a to H_2O vapour. Since the PEG6000 solution is relatively viscous, the higher viscosity could potentially mimic the stagnant, deoxygenated condition (even with strongly forced aeration) and thereby trigger the barrier formation. We therefore simulated a -0.5 MPa water potential solution using mannitol (Pandey et al. 2004), which is not viscous, and still found a significant decline in the average P_a to O_2 from 3.13×10^{-7} to 9.60×10^{-8} within 18 h of exposure (one-tailed *t*-test, $P < 0.0189$, $n = 3$), indicative of a barrier formation.

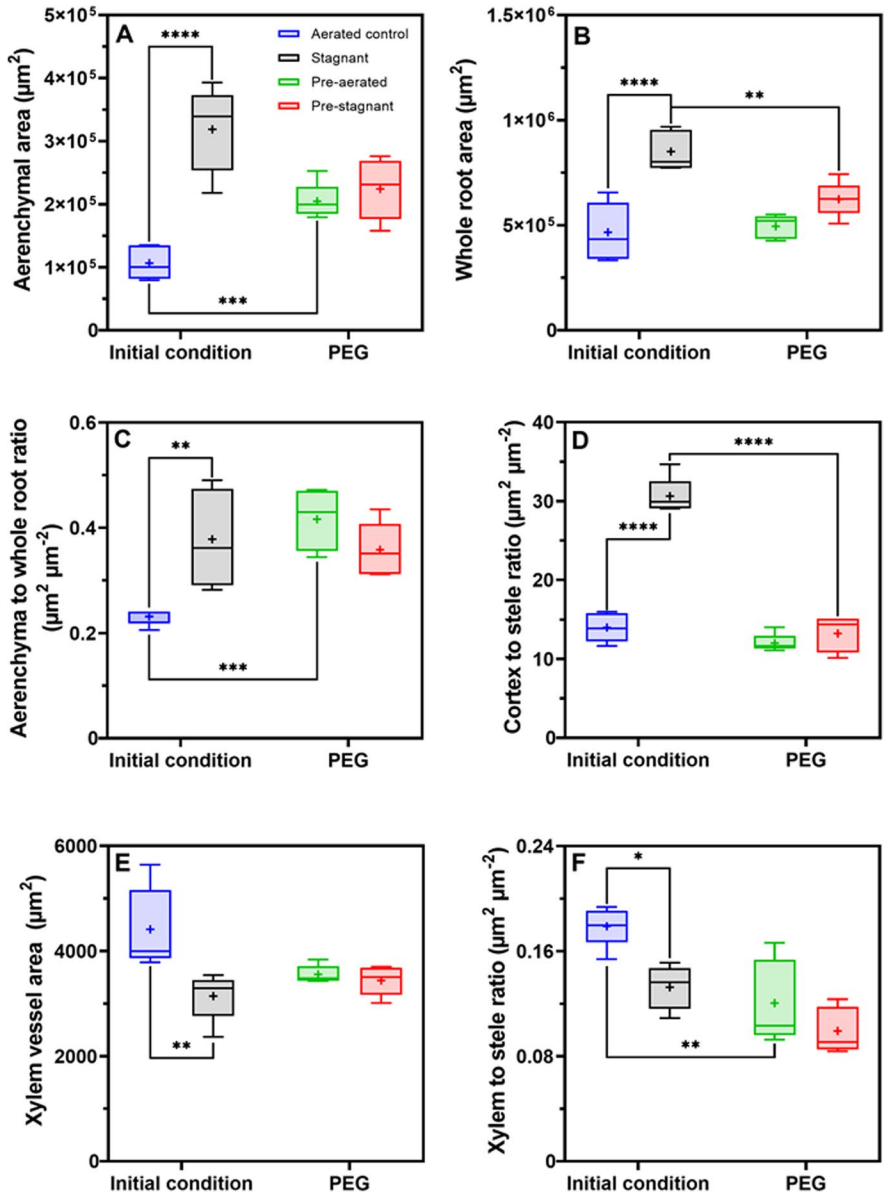

Fig. 2 - Key anatomical traits of rice (*Oryza sativa* L.) roots 70 to 80 mm behind the root tip growing in aerated or stagnant, deoxygenated nutrient solutions, or after transferring them to a 20% PEG6000 aerated nutrient solution. Key anatomical traits were measured on roots of plants growing in aerated or stagnant solution, or after transferring them to a 20% PEG6000 (-0.5 MPa) aerated solution. After 7 d in these conditions, new target roots (10–14 cm long) had been formed and were severed, infiltrated with 70% ethanol, fixed in 5% agar, and 100 µm thick cross sections were cut at position 70–80 mm behind the root tip. Panel **A** shows aerenchymal area, **B** whole root area, **C** aerenchyma to whole root ratio, **D** cortex to stele ratio, **E** xylem vessel area, and **F** xylem to stele ratio. Statistical comparisons were conducted using a two-way ANOVA on raw (**D, E, F**) or transformed data (**A, C** log-transformed; **B**, Y-squared) followed by a Tukey test. Asterisks indicate significant differences (*, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; ****, $P \leq 0.0001$); $n = 5$ for all; mean, +; median, horizontal line; 2nd and 3rd quartiles, box; minimum and maximum values, whisker. Non-transformed data are shown

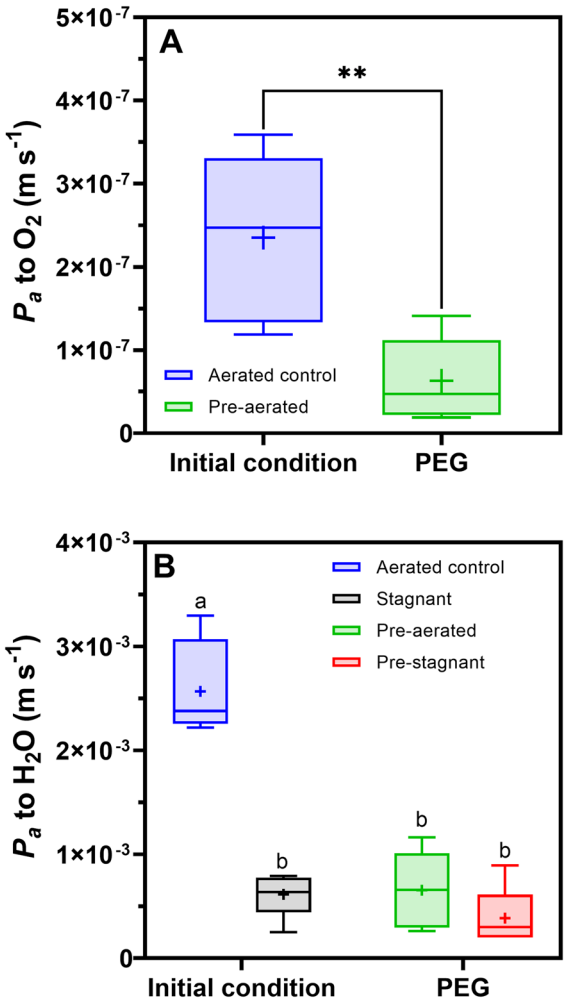

Fig. 3 Apparent permeance (P_a, m s^{-1}) to O_2 and H_2O vapour ▶ in rice (*Oryza sativa* L.) roots growing in aerated or stagnant, deoxygenated nutrient solutions, or after transferring them to a 20% PEG6000 aerated nutrient solution. Panel **A** shows P_a to O_2 of roots growing in aerated conditions without or with 20% PEG6000 (-0.5 MPa). Plants without a barrier to radial O_2 loss grown in aerated conditions were moved to a 20% PEG6000 aerated nutrient solution for 3 d. Target roots (10–14 cm long) were severed, shortened to 25 to 30 mm segments (corresponding to position 35 to 60 mm behind the root tip), their cut ends were sealed with lanolin, and fixed onto a metal mesh inside an aquarium. An O_2 microsensor was inserted 125–175 µm into the cortex and the aquarium was filled with DI water purged with pure O_2 to obtain radial O_2 intrusion rates and calculate P_a to O_2. The statistical comparison was conducted with a two-tailed t-test. Significant differences are shown using **, $P \leq 0.01$; $n=5$; mean, +; median, horizontal line; 2nd and 3rd quartiles, box; minimum and maximum values, whiskers. Panel **B** shows P_a to H_2O. Target roots from plants pre-treated in aerated or stagnant, deoxygenated nutrient solution and moved to a 20% PEG6000 aerated nutrient solution for 7 d were severed and shortened to 10–11.5 cm length, keeping the root tip intact. The roots were gently blotted with paper towels and placed onto a metal mesh inside a balance chamber to register decline in mass as H_2O evaporated. Radial water loss (RWL) rates were obtained when 35% of the total pool of water was lost, adjusted by the dynamic root surface area (based on the changes on root diameter, see Fig. S1), and corrected to 1 mm root diameter. Rates of RWL were finally used to calculate the P_a to H_2O. Statistical comparison was conducted using a two-way ANOVA followed by a Tukey test. Significant differences are shown using different letters, indicating $P \leq 0.0001$; $n=4$–5; mean, +; median, horizontal line; 2nd and 3rd quartiles, box; minimum and maximum values, whiskers

Plants growing in aerated or stagnant, deoxygenated conditions have a similar turgor loss point and leaf water potential

Potential differences in the turgor loss point from plants in aerated or stagnant, deoxygenated conditions could explain the differences in the observed desiccation response to 25% PEG6000 in the nutrient solution. However, plants grown under either aerated or stagnant conditions had a similar turgor loss point. In addition, there were no differences in the typical leaf water potential (LWP) of transpiring plants growing in aerated or stagnant conditions (Fig. S3A).

To test if the observed leaf desiccation in 25% PEG6000 conditions was prevented by root acclimations to stagnant, deoxygenated conditions, plants from aerated or stagnant conditions were covered with plastic bags for 2 h and kept at a high RH (> 75%) in order to induce stomatal closure and prevent desiccation through water loss via the leaves. Still covered, the plants were then transferred to a 25% PEG6000 aerated solution. Surprisingly, the LWP increased over time after exposure to low water potential in the root medium regardless of the previous growth condition (Fig. S3B). However, plants pre-treated in stagnant conditions maintained a lower LWP for the first 180 min, after which LWP was similar to those pre-treated in aerated conditions (between -0.94 and -0.58 MPa on average). Moreover, plants pre-treated in aerated conditions showed clear wilting signs in their leaves after 24 h in 25% PEG6000, despite having a similar LWP to those pre-treated

in stagnant conditions, which did not show any noticeable wilting symptoms. In other words, we found that leaves from aerated conditions wilted whereas those from stagnant, deoxygenated conditions did not, regardless of the LWP.

Roots respond to conditions of water deficit by enhancing lignin deposition in the outer parts of the root

The depositions of lignin and suberin of roots of rice were detected using phloroglucinol – HCl and Fluorol Yellow 088 (Fig. 4). The results show that the lignification of exodermal cells responded to the different growth conditions, while the outer parts of the roots were fully suberized in whatever growth conditions. Lignin depositions in the sclerenchyma of plants growing in aerated, stagnant, pre-aerated plants moved to PEG or pre-stagnant plants moved to PEG conditions were observed (Fig. 4A to D), whereas lignin staining was weak in aerated controls (Fig. 4A, white arrowheads, see Fig. S8 for higher magnification). Moreover, lignin depositions were detected surrounding exodermal cells of roots of rice growing in stagnant, pre-aerated plants moved to PEG or pre-stagnant plants moved to PEG conditions (Fig. 4B to D, black arrows), but no lignified exodermal cells were observed in cross-sections of roots growing in aerated nutrient solutions (Fig. 4A). On the other hand, the exodermal cells were fully suberized in roots of rice growing in aerated, stagnant, pre-aerated plants moved to PEG or pre-stagnant plants moved to PEG conditions (Fig. 4E to H, yellow arrowheads).

Stomatal conductance and F_v/F_m measurements

Both stomatal conductance and F_v/F_m responded to low water potential in the nutrient solution. Stomatal conductance of plants growing in aerated or stagnant, deoxygenated conditions (without PEG6000) were similar across the different days of measurements (Fig. S4A). After 24 h in 20% PEG6000, plants formerly growing in aerated or stagnant conditions reduced stomatal conductance compared to their initial growth condition by 40% and 35%, respectively. The reduction in stomatal conductance for plants previously growing in aerated conditions and moved to PEG6000 was maintained until 48 h, after which the stomatal conductance with its respective aerated control was similar. Moreover, the stomatal conductance of plants previously growing in aerated conditions and moved to PEG6000 was higher compared to plants formerly in stagnant conditions and moved to PEG6000 at 72 h. The latter had still a lower stomatal conductance than its initial condition (stagnant) at 96 h. Nevertheless, after 120 h, the stomatal conductance of all plants in control and PEG6000 conditions were similar.

Plants growing in aerated control or stagnant conditions showed similar F_v/F_m values. F_v/F_m measurements from these conditions were generally similar, except at 48 h where aerated control conditions readings were slightly lower than plants in stagnant, deoxygenated conditions (Fig. S4B). Once moved to 20% PEG6000, F_v/F_m readings of plants formerly growing in aerated conditions were similar to their controls. For unknown reasons, the F_v/F_m measurements on plants growing in stagnant conditions and moved to PEG6000 were higher than (i) plants in stagnant conditions, and (ii) to plants formerly growing in aerated conditions and moved to PEG6000. These readings were the same after 48 h of starting the PEG6000 treatment. At 72 h, no differences were found between controls and/or treatments. However, at 96 and 120 h, the F_v/F_m values of PEG6000 treated plants were higher than their respective controls. Regardless of these statistical differences, The the F_v/F_m values are all within the normal spectrum of unstressed rice in general and IR42 (the genotype used) in particular (Mohan and Gupta 2015), so we do not emphasize further on these statistical differences in the following discussion.

In summary, the stomatal conductance of plants transferred to PEG6000 pre-treated in aerated or stagnant, deoxygenated conditions showed a similar pattern. Stomatal conductance was significantly lower than their respective controls for the first 24 to 48 h followed by an acclimation. In PEG6000, plants pre-treated in aerated conditions had lower F_v/F_m for the first 72 h than those pre-treated in stagnant conditions, again followed by acclimation.

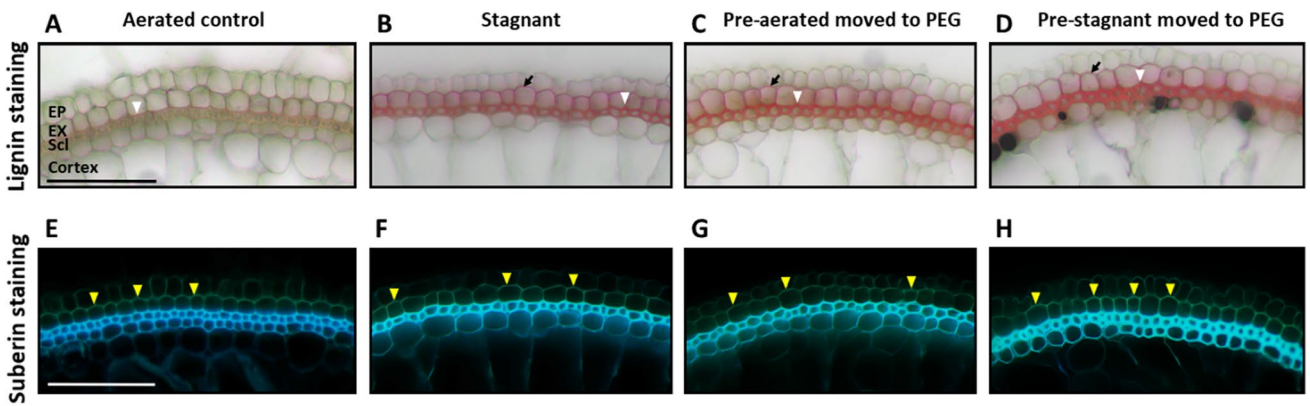

Fig. 4 Histochemical staining of suberin and lignin in rice (*Oryza sativa* L.) roots growing in aerated or stagnant, deoxygenated nutrient solutions, or after transferring them to a 20% PEG6000 aerated nutrient solution. Histochemical staining of cinnamaldehyde groups of lignin were conducted on roots formed in the above conditions using the phloroglucinol-HCl approach in root cross-sections (position 70 – 80 mm behind the root tip) from 35-day-old plants. Lignin depositions are shown in panel **A** for roots growing in aerated conditions, **B** stagnant, deoxygenated conditions, **C** after transferring plants grown in aerated nutrient solutions to 20% PEG6000 aerated nutrient solutions, and **D** after transferring plants grown in stagnant, deoxygenated nutrient conditions to 20% PEG6000 aerated nutrient solutions. In aerated conditions, lignified sclerenchyma was detected (white arrowhead, see Fig. S8 for higher magnification). However, a stronger lignification of the sclerenchyma was detected (white arrowhead) in stagnant, pre-aerated moved to PEG, or pre-stagnant moved to PEG growth conditions, compared to aerated controls. Moreover, lignification of the exodermis (black arrows) was detected in stagnant, pre-aerated moved to PEG, or pre-stagnant moved to PEG growth conditions, but not in aerated controls. Suberin depositions were detected using Fluorol Yellow 088 and are shown in panel (**E**) aerated conditions, (**F**) stagnant, deoxygenated conditions, (**G**) after transferring plants grown in aerated nutrient solutions to 20% PEG6000 aerated nutrient solutions, and (**H**) after transferring plants grown in stagnant, deoxygenated nutrient conditions to 20% PEG6000 aerated nutrient solutions (plant age and position similar to lignin staining). Suberin depositions were detected in all growth conditions showing a complete suberization of the exodermis (yellow arrowheads). Example of cells with suberin deposition are indicated with yellow arrows. Ep, epidermis cells; Ex, exodermis cells; Scl, sclerenchyma cells. Bar, 100 µm

Discussion

The goal of this study was to test if plants acclimated to soil flooding possessed advantages to following conditions of water deficit through alterations in key root traits. Accordingly, we found strong evidence that plants acclimated to stagnant conditions (mimicking soil flooding) were able to withstand a sudden change in the water potential of the root medium, whereas non-acclimated plants severely wilted. Different irrigation methods intend to reduce the usage of water in paddy rice (i.e., alternate wetting and drying), but could lead to periodic low soil water content since it requires temporal drying of the soil (Amin et al. 2011; Bouman et al. 2007; Lampayan et al. 2014). Moreover, rainfed lowland rice can also experience water shortage due to changes in rain patterns (Ponnamperuma 1979). This highlights the relevance of the present study as roots formed in flooded soils are primed to withstand following conditions of water deficit. Below, we discuss key root and shoot traits possibly involved in the contrasting responses to low water potential in the root medium.

Low water potentials induce the formation of the barrier to radial oxygen loss

We found that low water potential in the nutrient solution can act as a signal for the formation of the root barrier to radial O_2 loss. In addition, the tightness to water vapour of the barrier induced by the low water potential was similar to the barrier induced by stagnant conditions (Fig. 3B). Interestingly, comparing radial water loss rates of pre-aerated plants moved to PEG6000 when 15% of the cumulated water content was lost (instead of 35% as in Fig. 3B) to those previously reported show a significant difference (1610 µmol H_2O m^{-2} s^{-1}, this study, and 323 µmol H_2O m^{-2} s^{-1} in Song et al. (2023), *t*-test $p < 0.0001$). This difference is likely explained by this study using root segments with the tip region intact. Here, the barrier is not formed and thus the tip region can act as a window for radial water loss (Peralta Ogorek et al. 2021). Consequently, we conducted radial water loss measurements and apparent permeance (P_a) to water vapour calculations using root segments without the tip from plants in aerated, stagnant, and pre-aerated moved to 20% PEG6000 conditions (Fig. S5). However, even after removing the tip, the P_a to H_2O in 20% PEG6000 was still similar to that in roots with the tip, and therefore had higher radial water loss rates than those from roots growing in 10% PEG6000 (Song et al. 2023). This suggests that while the plants in the present study were able to survive in a 20% PEG6000 solution, this sudden and stressful condition restricted the capacity to form a strong barrier as water and nutrients were allocated to other vital functions such as root elongation, or root hair formation (Bodner et al. 2015). Moreover, we also found that the P_a to H_2O of root segments without their tips was significantly higher in roots growing in 20% PEG6000 compared to stagnant-grown roots. These root segments without the tip did not include more mature parts of the roots closer to the root-shoot junction compared with those used in the P_a to H_2O measurements with the tip. Therefore, this finding indicates that a 20% PEG6000 solution induced a tight barrier in the more mature parts of the roots, which compensated a weaker barrier formed close to the root tip, whereas the barrier induced in stagnant conditions is tighter along the entire length of the root except for the tip region. Consequently, we propose to establish a dose–response curve of root acclimations to different PEG6000 concentrations, mimicking different degrees in soil dryness, or different, controlled degrees of soil water content to study responses in the apoplastic barrier of rice roots.

To characterize the chemical components of the barrier to radial O_2 loss in stagnant, deoxygenated conditions and water deficit conditions, we conducted the histochemical staining of suberin and lignin of roots of rice (Fig. 3). Lignification of the sclerenchyma and exodermal cells were detected in all growth conditions except the aerated controls, whereas suberization was found similarly strong in the four growth conditions, as previously found in mature regions of root in stagnant conditions (Kotula et al. 2009). These results are further evidence that perhaps the gas-tight feature of the barrier to radial O_2 loss or radial water loss barrier in roots of rice is mainly given by lignin instead of suberin deposition (Peralta Ogorek et al. 2023a).

Several key anatomical roots traits induced by stagnant conditions are also induced by conditions of water deficit

Plants growing in stagnant, and pre-aerated or pre-stagnant moved to 20% PEG6000 enhanced

aerenchyma formation, and aerenchyma to whole root ratio also increased compared to control plants in aerated conditions (Fig. 1A, C). In the present study, we chose to express inducible aerenchyma as aerenchyma to whole root ratio because it highlights the amount of aerenchyma induced in relationship to all the potential changes of the root tissues (e.g., changes in stele or cortex size). However, inducible aerenchyma can also be expressed as aerenchyma to cortex ratio and surprisingly, aerenchyma to cortex ratio decreased under low soil water potentials in several lowland rice genotypes (Henry et al. 2012). We also calculated the aerenchyma to cortex ratio and still found that this ratio increased as response to a low water potential (data not shown). In accordance with our findings, water deficit in maize induced aerenchyma formation and this was correlated to drought tolerance as it reduced root respiration and therefore enabled root growth (Zhu et al. 2010). Roughly, twofold higher aerenchyma to whole root ratio would lead to almost twofold longer roots for the same investment in resources (water, carbon, nutrients), demonstrating the benefit of aerenchyma formation as a response to resource limitation including water. Roots acclimated to soil flooding already have high amounts of aerenchyma and therefore have lower maintenance requirements (e.g. respiration per unit of root length), ultimately enabling deeper root penetration (Armstrong 1979). However, roots with higher amounts of aerenchyma are also prone to higher radial water loss, and thus other root acclimations are needed to compensate this trade-off such as the formation of an apoplastic barrier in the root exodermis (Song et al. 2023).

The cortex to stele ratio is also known to respond to differences in soil water availability. We found that the cortex to stele ratio in aerated controls (mimicking well-watered soils) were similar to those of species of wild *Poaceae* at high soil water contents (Yamauchi et al. 2021). Typically, values of cortex to stele ratios were between 2 and 8 for lower soil water contents (Yamauchi et al. 2021), but roots growing in aerated controls did not respond once moved to 20% PEG6000 (present study), having cortex to stele ratio values of 11 to 14. In contrast, cortex to stele ratio increased significantly under stagnant conditions (29 to 35) allowing for more aerenchyma space and thereby a more effective O_2 diffusion (Pedersen et al. 2020). However, the transition from stagnant to PEG6000 conditions resulted in a decline of the cortex to stele ratio, and the functional importance of this decline is a reduced consumption of water and energy (Kong et al. 2021).

Interestingly, traits directly related to root hydraulic conductivity also responded significantly to growth conditions. Aerated controls had the highest xylem vessel to stele ratio, but this ratio was reduced once the aerated controls were moved to 20% PEG6000. Similarly, conditions of water deficit also reduced the size and number of xylem vessels in 6 genotypes of rice and this response was proposed to reduce the risk of cavitation (Henry et al. 2012) to which rice is particularly susceptible (Mostajeran and Rahimi-Eichi 2008). Xylem vessel to stele ratio were already lower under stagnant conditions, highlighting the potential benefit of root acclimations to soil flooding to prepare the plants for a following period of low soil water potential.

Changes in root thickness can be influenced by soil water availability. Indeed, root thickness showed a contrasting response to contrasting soil water availability mimicked in hydroponics, with thin roots in aerated controls and thick roots in stagnant conditions, as previously shown for rice (Pedersen et al. 2020). Surprisingly, plants moved from aerated controls to PEG6000 did not form thinner roots, as would otherwise be expected based on observations in rice growing in dry soil (Henry et al. 2012). The lack of response perhaps indicates that 20% PEG6000 solutions only imposed a mild drought stress to this rice genotype (IR42), which is characterized at moderately drought tolerant (Ponnamperuma 1979). Regardless of this lack of response in this particular genotype, the general pattern is that rice forms thick roots in flooded soils (Colmer 2003b), but thin in dry soils (Gowda et al. 2011), indicating that some traits of roots acclimated to soil flooding are disadvantageous for following conditions of water deficit. Thinner roots require less resources as longer roots can be formed with the same carbon investment (Kong et al. 2021), although a higher SA:V accelerates water loss (Song et al. 2023). However, the water loss trade-off of thin roots can be counteracted by the formation of a tight barrier to radial water loss (Peralta Ogorek et al. 2021; Song et al. 2023; Taleisnik et al. 1999).

Root acclimations to soil flooding prevents the wilting of the shoot under severe conditions of water deficit

Plants acclimated to stagnant conditions were clearly able to survive sudden water potential changes of the root medium. Stagnant-grown plants showed higher resistance to wilting than aerated-grown plants in a low water potential medium (Fig. 1). In principle, four factors could explain the observed responses: (i) stomatal conductance, (ii) turgor loss point, (iii) leaf water potential, and/or (iv) root hydraulic conductivity. Reducing stomatal conductance restricts the loss of water from the leaves (Wang et al. 2018) but we found no differences in stomatal conductance between aerated controls and stagnant plants. Moreover, once moved to PEG6000, the stomatal closure was similar within the first 48 h regardless of the initial growth condition (Fig. S4A). Similarly, a lower turgor loss point would increase the tolerance to low soil water potentials (Bartlett et al. 2014) but again, we found no differences in the turgor loss point. Moreover, we did not observe differences in the typical leaf water potential between the aerated- and stagnant-grown plants (Fig. S3A) and were also similar to the leaf water potential of rice growing in a greenhouse (Henry et al. 2012). Finally, root hydraulic conductivity was similar in both aerated and stagnant conditions, likely due to the presence of root tips from where water could bypass the barrier to radial O_2 loss. However, the root to leaf blade ratio was 1.9-fold higher in aerated compared to stagnant controls (Fig. S6A). A greater leaf mass would lose water faster and could potentially explain the observed differences in leaf desiccation under severe conditions of water deficit. Consequently, a follow-up experiment was conducted where the shoot of plants from aerated and stagnant conditions were trimmed so that differences in root to leaf blade ratio were eliminated (Fig. S6B) before being exposed to a 25% PEG6000 solution for 24 h (Fig. S7). Nevertheless, the desiccation pattern observed for untrimmed and trimmed plants remained unchanged: plants from aerated conditions wilted significantly faster than those from stagnant conditions. Since we found no significant differences in any of the above key factors potentially influencing shoot desiccation, we propose that the restricted radial water loss from roots with a barrier to radial O_2 loss is the main factor delaying wilting responses in the shoot, as the barrier prevents water being pulled from the plant to the low water potential root medium (Song et al. 2023).

It is worth noting that accumulation of PEG6000 was observed in the shoot of aerated and stagnant controls, even though PEG6000 is argued not to enter the root apoplast due to its large molecular weight (Hohl and Schopfer 1991; Osmolovskaya et al. 2018; Verslues et al. 1998). PEG6000 in the leaves could drag water from the entire leaf apoplast and thereby increase the amount of water in the xylem bundles, confounding LWP measurements (Fig. S3B). Nevertheless, leaf wilting was observed only minutes after exposing the roots to low water potential (Fig. 2B,C) and little amounts of PEG6000 would have been translocated to the shoot at the onset of wilting responses, considering the stomatal closure and restricted water uptake due to the low water potential in the root medium. Consequently, we rule out that apoplastic transport of PEG6000 had caused the observed leaf rolling responses.

Conclusions and outlook

We have demonstrated that rice acclimated to soil flooding gains some advantages when conditions of water deficit follow. We propose that key root traits such as the presence of the barrier to radial O_2 loss, higher aerenchymal area, and a lower xylem vessel area to stele ratio all prime the plants for conditions of water deficit. This was evidenced by contrasting responses at the shoot level in plants previously growing in aerated or stagnant conditions and then moved to PEG6000. We hypothesize that the presence of the barrier to radial O_2 loss is the most relevant root trait to prevent shoot desiccation under severe and short-term conditions of water deficit, as it prevents water from being pulled from the plant to the root medium. Such a situation would occur in a drying rice soil, where water uptake takes place in the deep soil and where the proximal parts of the roots are exposed to bone dry conditions. Consequently, and due to the multifaceted functions of the barrier to radial O_2 loss, we have recently proposed to refer to this root trait as the "outer apoplastic barriers" in order to emphasize on the barrier function(s) and location rather than on a single solute, i.e., molecular O_2 (Peralta Ogorek et al. 2023b).

Interestingly, aerenchyma formation in roots of dryland crops, such as in wheat, barley and upland rice, is a common phenomenon. It has frequently been reported in the literature, yet its potential role in reducing water loss from roots during conditions of soil water deficit has not been explored so far. However, a recent modelling approach suggested that cortical aerenchyma actually increases radial water loss (Song et al. 2023), so priming of dryland crops by a period of waterlogging to withstand a subsequent drought would likely only be effective if these could also form an outer apoplastic barrier. However, when considering the potentially enhanced drought tolerance of the crops by pre-waterlogging treatment/or priming, one needs to be aware that, under field conditions the responses of plants to soil water deficits will not be the same as those when exposing to PEG6000 treatment in hydroponically grown plants. Radial water loss from roots is a not common feature of soil-grown plants during moderate soil water deficits, and it probably primarily occurs during very severe soil water deficits at night, or when the topsoil is bone-dry. Thus, the question whether a waterlogging pre-treatment could enhance subsequent drought tolerance of dryland crops remains speculative.

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Author contribution LLPO, AN, and OP planned and designed the study. LLPO performed all experiments except root anatomy and histochemical staining conducted by ZS, and turgor loss point measurements conducted by MT. LLPO and OP wrote the manuscript with input from all co-authors.

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Data availability The data supporting the findings of this study are available from the corresponding authors, LLPO or OP, upon request.

Declarations

Conflict of interest No conflict of interest declared.

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