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Research

Root aeration in rice (*Oryza sativa*): evaluation of oxygen, carbon dioxide, and ethylene as possible regulators of root acclimatizations

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Summary

- Adventitious roots of rice (*Oryza sativa*) acclimatize to root-zone O_2 deficiency by increasing porosity, and induction of a barrier to radial O_2 loss (ROL) in basal zones, to enhance longitudinal O_2 diffusion towards the root tip.
- Changes in root-zone gas composition that might induce these acclimatizations, namely low O_2, elevated ethylene, ethylene—low O_2 interactions, and high CO_2, were evaluated in hydroponic experiments.
- Neither low O_2 (0 or 0.028 mol m^{-3} O_2), ethylene (0.2 or 2.0 μl l^{-1}), or combinations of these treatments, induced the barrier to ROL. This lack of induction of the barrier to ROL was despite a positive response of aerenchyma formation to low O_2 and elevated ethylene. Carbon dioxide at 10 kPa had no effect on root porosity, the barrier to ROL, or on growth.
- Our findings that ethylene does not induce the barrier to ROL in roots of rice, even though it can enhance aerenchyma formation, shows that these two acclimatizations for improved root aeration are differentially regulated.

Key words: adventitious roots, aerenchyma, flooding, internal aeration, oxygen transport, radial oxygen loss, root porosity, waterlogging.

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Introduction

Internal aeration is crucial for plant growth in waterlogged soils (Armstrong, 1979; Jackson & Armstrong, 1999). Roots of rice contain large volumes of aerenchyma (Barber *et al.*, 1962; Clark & Harris, 1981), providing a low-resistance pathway for diffusion of O_2 within the roots. In addition, rice roots also contain a barrier against radial O_2 loss (ROL) from the basal zones (Armstrong, 1971; Colmer, 2003a), so that ROL occurs predominately from short lateral roots and the apical few centimetres of the main axes of adventitious roots (Colmer, 2003b). The aerenchyma and barrier to ROL enhance longitudinal diffusion of O_2 towards the root tip, and thus root elongation into anoxic substrates (Armstrong, 1979). These two traits are regarded as key features contributing to waterlogging tolerance in many plants (Armstrong, 1979; Jackson & Drew, 1984; Justin & Armstrong, 1987; Colmer, 2003b).

Roots of rice constitutively form aerenchyma, although the amount can be further enhanced by soil waterlogging (Armstrong, 1971; Pradhan *et al.*, 1973; Das & Jat, 1977). Recent studies of root aeration in rice have shown enhanced formation of aerenchyma when O_2 deficiency was imposed in hydroponics, and that a barrier to ROL was also induced in the basal root zones of plants grown in deoxygenated stagnant nutrient solution (Colmer *et al.*, 1998; Colmer, 2003a). The present study evaluated (1) whether induction of a barrier to ROL, as previously documented for rice grown in stagnant compared with aerated nutrient solution (Colmer, 2003a), also occurs in rice grown in waterlogged vs drained soil, and (2) possible regulators involved in enhanced aerenchyma formation and induction of the tight barrier to ROL in adventitious roots of rice. Low O_2, elevated ethylene, ethylene–low O_2 interactions and high CO_2 were evaluated as changes in

root-zone gas composition that might promote the observed acclimatizations in root aeration, in experiments using well-defined treatments in hydroponics. Waterlogged soils are usually anoxic (i.e. lack O_2), contain high levels of CO_2 and HCO_3^- (ratio depends on pH), and elevated levels of the gaseous phytohormone ethylene (Ponnamperuma, 1984), therefore these changes in gas composition were the focus of our research. Moreover, ethylene signalling had previously been implicated in enhanced aerenchyma formation in rice (Justin & Armstrong, 1991) and other species (e.g. *Zea mays*, Drew *et al.*, 1979).

Materials and Methods

A paddy cultivar of rice (*Oryza sativa* L.), Calrose, was used in all experiments.

Experiment in soil

The inducible nature of the barrier to ROL in adventitious roots of rice, as dependent upon root-zone conditions, was described in experiments with aerated vs stagnant deoxygenated nutrient solutions (Colmer *et al.*, 1998; Colmer, 2003a). That roots of rice exhibit a barrier to ROL when grown in waterlogged soil was shown previously (Armstrong, 1971); however, experiments to directly assess the inducible barrier had not been conducted for soil-grown plants (i.e. ROL measurements for roots grown in drained vs. waterlogged soil were not available).

Seeds were de-hulled, washed in 0.4% (w : v) sodium hypochlorite for 30 s, rinsed thoroughly with deionized water, and then imbibed for 3 h in aerated 0.5 mol m^{-3} $CaSO_4$ at 30°C. Seeds were then placed on filter paper soaked with 0.5 mol m^{-3} $CaSO_4$ in a Petri dish, all within a humid container inside a controlled-environment chamber (12 h light/12 h dark cycle; 30°C/25°C; relative humidity 70%/80%; 500 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR) during light period). After 24 h, three seeds that had germinated were sown at a depth of 7.5 mm into each of eight pots (400 mm high, 150 mm diameter) containing a 3 : 1 mixture of washed river sand : potting mix, and watered daily with the same nutrient solution (except without 2-(*N*-morpholino) ethanesulfonic acid (MES)) as used in the solution culture experiments (described below). After 7 d, plants were thinned to two per pot. Treatments were imposed 15 d after imbibition; two pots were sampled for an 'initial harvest', three pots were continued as drained controls and three pots were waterlogged with nutrient solution to 10 mm above the soil surface. The pots were positioned randomly inside the chamber.

Twenty-five days after imbibition (i.e. after 10 d of treatments), the soil was gently washed away from the roots of plants in each pot, and ROL was measured along two roots ('shorter' and 'longer' roots) on one plant from each pot (described later). The second plant in each pot was used to measure numbers of tillers, numbers of adventitious roots, length of the longest adventitious root, and shoot and root dry masses were determined after harvested tissues were oven-dried at 60°C.

Experiments in nutrient solution

Four experiments were conducted using nutrient solution culture; in either a controlled-environment room or chamber (12 h light/12 h dark cycle; 30°C/25°C; relative humidity 70%/80%; PAR at 500 μmol m^{-2} s^{-1} during the light period).

In each experiment, seeds were de-hulled, washed in 0.4% (w : v) sodium hypochlorite for 30 s, rinsed thoroughly with deionized water, and then imbibed for 3 h in aerated 0.5 mol m^{-3} $CaSO_4$ at 30°C. Seeds were then placed on plastic mesh floating on aerated quarter-strength nutrient solution (composition given in next paragraph) in a container covered with aluminium (Al) foil. Seedlings were exposed to light 3 d after imbibition. Five days after imbibition, seedlings were transferred to 4.5-l pots containing full-strength nutrient solution. Each pot (internal diameter of 190 mm) held four seedlings, with one seedling from each pot sampled for measurements of shoot and root dry weights at the time treatments were imposed. Treatments, timings of various operations, and numbers of replicates are given below for each experiment.

The composition of the nutrient solution at full concentration (mol m^{-3}) was: K^+, 3.95; Ca^{2+}, 1.50; Mg^{2+}, 0.40; NH_4^+, 0.625; NO_3^-, 4.375; SO_4^{2-}, 1.90; $H_2PO_4^-$, 0.20; Na^+, 0.20; H_4SiO_4, 0.10; and the micronutrients (mmol m^{-3}) were Cl, 50; B, 25; Mn, 2; Zn, 2; Ni, 1; Cu, 0.5; Mo, 0.5; Fe-EDTA, 50. The solution also contained 2.5 mol m^{-3} MES and the pH was adjusted to 6.5 using KOH, increasing the final K^+ concentration to 5.6 mol m^{-3}. A few days after transplanting, $FeSO_4$ was added to each pot at 7.2 mmol m^{-3} as a final concentration. All chemicals used were analytical grade. In all experiments, pots were arranged in a completely randomized design, all the solutions were renewed every 7 d, and there were three or four replicate pots of each treatment.

Nutrient solution experiment 1 evaluated the effects of aerated, N_2-flushed, or stagnant deoxygenated nutrient solution (i.e. 3 root-zone O_2 treatments × 4 replicate pots) on growth, aerenchyma formation, root porosity, and patterns of ROL along adventitious roots. Five-day-old seedlings were transplanted into 4.5-l pots containing nutrient solution that was either (1) aerated, (2) flushed with high-purity N_2, or (3) contained 0.1% (w : v) agar and had been deoxygenated overnight before being siphoned into each pot. The dilute agar prevents convective movements in the solution ('stagnant' treatment) so that it may mimic the changes in gas composition found in waterlogged soils (e.g. decreased O_2 and increased ethylene) better than other methods used to impose root-zone O_2 deficiency in solution culture (Wiengweera *et al.*, 1997). Measurements were taken 32–33 d after imbibition.

Nutrient solution experiment 2 evaluated the effects of ethylene at 0, 0.2 or 2.0 μl l^{-1} in aerated nutrient solution (i.e.

3 ethylene levels × 4 replicate pots) on growth, aerenchyma formation, root porosity and patterns of ROL along adventitious roots. These concentrations of ethylene were used since 1–2 μl l^{-1} ethylene can enhance aerenchyma formation in roots of rice (Justin & Armstrong, 1991), and the 0.2 μl l^{-1} treatment was also included as this concentration of ethylene stimulated root extension in several genotypes of rice, whereas > 1 μl l^{-1} ethylene inhibited root extension in some genotypes (Smith & Robertson, 1971).

Five-day-old seedlings were transplanted into 4.5-l pots containing aerated nutrient solution, and then the plants were grown until 15-d-old. The pots were similar to those used in Experiment 1, except the bottom of each pot had a 500 mm inlet pipe (internal diameter 20 mm) attached to ensure a long path-length for the gases to be dissolved into solution and well-mixed before reaching the roots. Treatment concentrations of ethylene were achieved by mixing ethylene in air (BOC gases, Perth, Australia) with compressed air, using flow meters to deliver the desired volumes of the two gases to a mixing chamber, before distribution to the individual pots. To avoid ethylene build-up in the controlled-environment room, an outlet tube was attached to the sealed lid of each pot, and the exiting gases were vented to outside the building. Measurements were taken 24–27 d after imbibition.

Nutrient solution experiment 3 investigated whether ethylene interacts with O_2 status in signalling root acclimatizations. The effects of ethylene (0 or 2.0 μl l^{-1}) and O_2 in the medium ('aerated'; 'hypoxic', 0.028 mol m^{-3} O_2; 'anoxic', high-purity N_2) were assessed in a full factorial design (i.e. 2 ethylene levels × 3 O_2 levels × 3 replicate pots). Growth, aerenchyma formation, root porosity, and patterns of ROL along adventitious roots, were measured. Five-day-old seedlings were transplanted into 4.5-l pots (each with the inlet pipe as in experiment 2) containing aerated nutrient solution, and then the plants were grown until 15-d-old. Gases were mixed as described for experiment 2, but the ethylene stock was in high-purity N_2 rather than in air, and ethylene concentrations were checked using a gas chromatograph (Synspec GC 955-100 equipped with a photo-ionization detector and a 2 m stainless steel column of Haysep R mesh 80/100 (Synspec bv., Groningen, the Netherlands), at 105°C and using N_2 as carrier gas) and O_2 using an O_2-electrode. Out-let tubes from the lid of each pot vented exiting gases outside the building. Measurements were taken 23–25 d after imbibition.

Nutrient solution experiment 4 investigated whether high (*c.* 10 kPa) CO_2 affects growth, aerenchyma formation, porosity, or patterns of ROL along adventitious roots of rice. The 10 kPa CO_2 treatment was applied in pots flushed with high-purity N_2, to mimic both the increased CO_2 and low O_2 in waterlogged soils. Pots flushed with high-purity N_2 only were also included, to enable direct assessments of the effects of 10 kPa CO_2. Pots with deoxygenated stagnant agar solution were included as a positive control and aerated pots were also included (i.e. 4 root-zone treatments × 3 replicate pots). Five-day-old seedlings were transplanted into 4.5-l pots containing aerated nutrient solution and the plants were grown until 14-d-old. The pots were the same as used in experiment 2. Gases were mixed using flow meters, and CO_2 concentration was checked using a CO_2 analyser (Model S151; Qubit Systems, Kingston, Ontario, Canada). Out-let tubes vented gases exiting the pots to outside the building. No change in CO_2 concentration in the controlled environment room was detected (measured on three occasions). Measurements were taken 28–29 d after imbibition.

Measurement of root extension rates

Towards the end of each treatment period (see Tables) two adventitious roots on one plant in each pot were marked near the base of each, using xylene-free ink. The lengths of these roots were measured at the time of marking, and again 24 h later, so that rates of root extension were determined. Roots selected for these measurements were of similar lengths to those used in the ROL measurements.

Measurements of root porosity and aerenchyma

Porosity (% gas spaces per unit tissue volume) was measured on samples of adventitious roots by determining root buoyancy before and after vacuum infiltration of the gas spaces in the roots with water (Raskin, 1983), using the equations as modified by Thomson *et al.* (1990). Adventitious roots (100–200 mm in length) were excised from two plants in each treatment pot, cut into 50 mm segments, and used in the measurements. The ages of the plants, and times of exposure to the various treatments, are given for each data set in the Tables.

In two of the experiments, cross-sections were taken at 20 mm and 40 mm behind the tip, and viewed using a microscope (Jenamed 2; Carl Zeiss, Jena, Germany) fitted with a video camera linked to a computer. The proportion of each cross-section occupied by aerenchyma was determined using VIDEO PRO 32 software (Leading Edge Pty Ltd, Adelaide, Australia). In experiment 2, root samples were fixed (5% glutaraldehyde in 50 mol m^{-3} phosphate buffer at pH 7), dehydrated in a alcohol series, and embedded in glycol methacrylate (O'Brien & McCully, 1981). Cross-sections (2 μm) were cut using a glass knife and Sorvall microtome, and stained with 0.05% toluidine blue (pH 4.4) before viewing. In experiment 3, cross-sections were cut from fresh root samples using a hand-held razor blade. Lengths of the adventitious roots used in these measurements are given in the Tables.

Radial O_2 loss (ROL) measurements

When in an O_2-free root medium, ROL at any position along a root depends upon (1) the concentration of O_2 within the root, (2) the permeability of the outer tissues of the root to O_2

diffusion and (3) the respiratory demand in the outer layers (Armstrong, 1979). For roots dependent upon internal diffusion of O_2 from the shoots as the only source, the O_2 concentration within the cortical gas-spaces at any given distance from the root–shoot junction is determined by the cumulative physical resistance along the diffusion pathway and O_2 consumption along the path. Oxygen concentrations within the cortical gas-spaces decline in a curvilinear fashion from the root base to the apex (Armstrong, 1979). For roots without a barrier to ROL, ROL therefore decreases with distance from the root–shoot junction as the concentration of O_2 inside the root declines towards the apex (Armstrong, 1979). For roots with a barrier to ROL, ROL is typically very low in the basal regions of the main axis (i.e. towards the root–shoot junction) and relatively high near the apex (e.g. as shown for rice; Armstrong, 1971), despite the internal O_2 concentration at the apex being lower than further back along the root. This pattern of very low ROL from basal zones, but with high rates from near the tip, persists even when respiration is inhibited (e.g. by cooling roots of rice to 3°C; Armstrong, 1971), indicating that the barrier to ROL largely results from a physical resistance to radial diffusion of O_2 across the external cell layers of the root. The marked differences in ROL profiles between roots of rice grown in aerated or in stagnant conditions enable assessment of whether a barrier to ROL is present or absent in the roots (Colmer *et al.*, 1998; Colmer, 2003a).

Rates of ROL at selected distances behind the apex of intact adventitious roots when in an O_2-free root medium were measured using cylindrical root-sleeving O_2 electrodes (Armstrong & Wright, 1975; Armstrong, 1994). Root systems of intact plants were immersed in transparent chambers (50 × 50 × 250 mm, breadth × width × height) containing deoxygenated solution of composition: 0.1% (w : v) agar and K^+ 5.0 mol m^{-3}, Cl^- 5.0 mol m^{-3}, Ca^{2+} 0.5 mol m^{-3}, SO_4^{2-} 0.5 mol m^{-3}. The shoot base was held with wet cotton wool in a rubber lid sealed onto the top of each chamber, so that the shoots were in air. The chambers and plants were located in a 30°C constant temperature room with PAR of 150 μmol $m^{-2}s^{-2}$ at shoot height.

For each plant transferred into the experimental system, an adventitious root was inserted through a cylindrical O_2 electrode (internal diameter 2.25 mm, height 5.0 mm) fitted with guides to keep the root near the centre of the electrode. In all experiments (except nutrient solution experiment 4) measurements were taken for one 'shorter' (*c.* 60 mm long) and one 'longer' (*c.* 120 mm long) adventitious root on each plant (lengths of roots measured are given in the figure captions). Defined length classes were measured in ROL experiments since root length influences ROL at any given position (see earlier), and therefore a standardized approach removes this variability so that clear patterns for replicated measurements can be elucidated. Two length classes of roots were measured so as to broaden the scope of our study. Three or four individual plants from each treatment, each from a different replicate pot, were measured. Plants were left for at least 2 h before the first ROL recordings. Measurements were then taken along each root with the centre of the electrode positioned at 5, 10, 20, 30, 50 or 80 mm behind the root apex (80 mm only for 'longer' roots). Root diameters at the positions measured for ROL were determined using a vernier microscope, or a microscope with a calibrated eye-piece reticule.

Statistical analyses

Data on growth, root porosity, and aerenchyma were evaluated using analysis of variance (ANOVA). Means were compared using least significant differences (LSD) at the 5% probability level. For ROL data, means ± standard errors were calculated and graphed as a function of distance behind the root tip.

Results and Discussion

Patterns of ROL along adventitious roots, and growth of rice, in drained or waterlogged soil

Roots of rice grown in well-drained soil did not form a 'tight' barrier to ROL; when transferred to a deoxygenated medium for the measurements, O_2 fluxes from the more basal regions were high (e.g. 5.1-fold higher at 80 mm for 'longer' roots) relative to rates at 5 mm behind the apex (Fig. 1). Roots of plants grown in waterlogged soil showed 2.5-fold ('shorter' roots) and 17-fold ('longer' roots) higher rates of ROL from near the root tips, compared with roots of similar lengths from the drained treatment (Fig. 1). The higher amounts of O_2 reaching the tips of roots in waterlogged soil is consistent with enhanced formation of aerenchyma in rice roots when in waterlogged, compared with drained, soil (Armstrong, 1971; Pradhan *et al.*, 1973; Das & Jat, 1977; Justin & Armstrong, 1991). For 'shorter' roots of plants exposed to soil waterlogging, O_2 fluxes from the basal regions remained high (Fig. 1a). By contrast, for the longer roots, O_2 fluxes from the basal regions were substantially lower (i.e. only 22%) than for the more apical positions; a pattern of ROL consistent with induction of a barrier to ROL in these roots (Armstrong, 1979; Colmer, 2003b). However, ROL from the basal zones of roots grown in waterlogged soil in the present experiment was somewhat higher than in an earlier study of rice from waterlogged soil (Armstrong, 1971) or from stagnant deoxygenated agar solution (Colmer, 2003a).

Growth responses of rice to soil waterlogging are shown in Table 1. Shoot dry mass responded positively to waterlogging, it increased by 41% ($P < 0.05$), as did numbers of adventitious roots (91% increase, $P < 0.001$). By contrast, the maximum lengths achieved by the roots in waterlogged soil were restricted when compared with those in drained soil (Table 1, $P < 0.05$), presumably owing to O_2-deficiency as tips grow further from the O_2 source (cf. Armstrong, 1979). Stimulation of adventitious root initiation in response to soil waterlogging has also been described for other cultivars of rice (Colmer, 2003a).

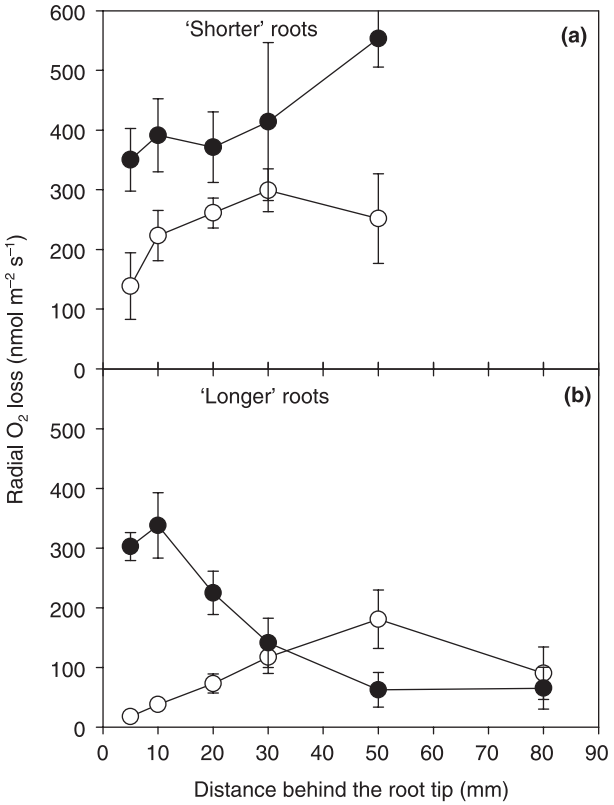

Fig. 1 Rates of radial O_2 loss (ROL) along 'shorter' (a) and 'longer' (b) intact adventitious roots of rice (*Oryza sativa*) in an O_2-free root medium with shoots in air, at 30°C ($n = 3 \pm$ SE). Plants were grown for 15 d in drained soil, after which treatments of drained (open circles) or waterlogged (closed circles) were imposed for the final 10 d before measurements. Lengths of the roots used in the measurements were: (a) 'shorter' roots, 57–75 mm; (b) 'longer' roots, 111–154 mm. Defined length classes were measured since root length influences ROL at any given position (see the Materials and Methods section), and therefore a standardized approach removes this variability so that clear patterns for replicated measurements can be elucidated. Two length classes of roots were measured so as to broaden the scope of our study.

Table 1 Parameters of growth and development for 25-d-old rice (*Oryza sativa*) in drained or waterlogged soil

Parameter	Treatment Drained	Waterlogged
Shoot dry mass (g)	0.391 ± 0.035	0.554* ± 0.032
Root dry mass (g)	0.099 ± 0.012	0.126 ± 0.013
Number of tillers	2.0 ± 0.0	2.7 ± 0.3
Number of adventitious roots	30 ± 1.0	58*** ± 1.2
Longest adventitious root (mm)	285 ± 23	215* ± 3

Data are mean ± SE; $n = 3$. Plants were grown for 15 d in drained soil, after which treatments were imposed for the final 10 d. At the time treatments were imposed, plants had 3.2–3.7 main-stem leaves, and (mean ± SE) shoot and root dry mass (g) of 0.0338 ± 0.006 and 0.0054 ± 0.0005, respectively.
Significant differences between treatment means are indicated by: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Patterns of ROL along adventitious roots, porosity, and growth of rice, as influenced by aerated, N_2-flushed or stagnant deoxygenated nutrient solution

Growth in stagnant deoxygenated nutrient solution (containing 0.1% agar to prevent convective movements) induced a barrier to ROL in 10 cultivars of rice from diverse origins (Colmer, 2003a). Stagnant agar solution was used as this method best mimics in hydroponics, the changes in gas composition (i.e. decreased O_2, increased ethylene) that occur in waterlogged soils (Wiengweera *et al.*, 1997). We tested whether root-zone O_2 deficiency *per se* induces aerenchyma formation and the barrier to ROL in roots of rice, by comparing responses of plants in N_2-flushed, stagnant deoxygenated 0.1% agar solution, and in aerated nutrient solution.

For roots of plants raised in aerated solution before being transferred into an O_2-free medium for the ROL measurements, ROL was least near the root tip and increased towards the root–shoot junction (Fig. 2 a and b). Patterns were similar for the 'shorter' and 'longer' roots, except that ROL had declined somewhat at the most basal position measured for the 'longer'

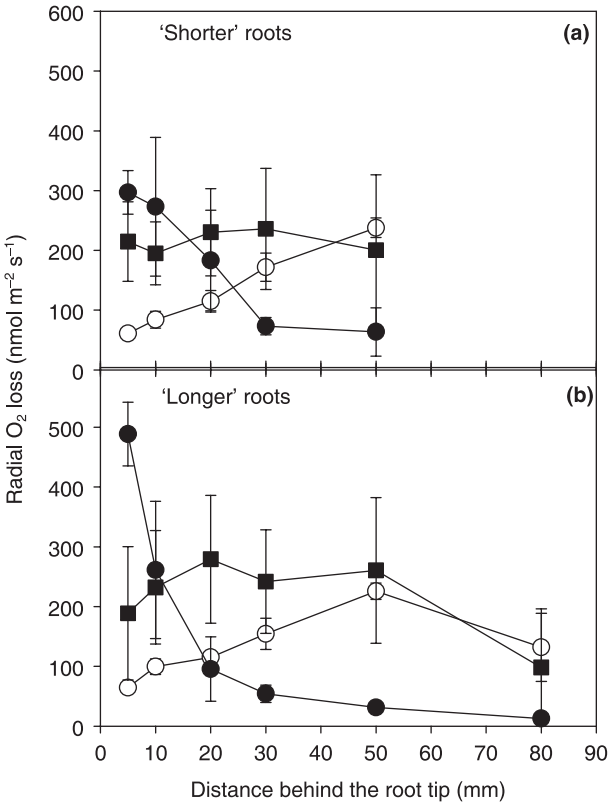

Fig. 2 Rates of radial O_2 loss (ROL) along 'shorter' (a) and 'longer' (b) intact adventitious roots of rice (*Oryza sativa*) in an O_2-free root medium with shoots in air, at 29°C ($n = 3 \pm$ SE). Plants were grown in aerated (open circles), N_2-flushed (closed squares) or stagnant 0.1% agar deoxygenated (closed circles) nutrient solution. Plants were 29- to 34-d-old, with treatments imposed on day 5. Lengths of the roots used in the measurements were: (a) 'shorter' roots, 66–78 mm; (b) 'longer' roots, 116–131 mm: (Nutrient solution experiment 1.)

Table 2 Parameters of growth and development for 32- to 33-d-old rice (*Oryza sativa*) in aerated, N_2-flushed or stagnant (0.1% agar) deoxygenated nutrient solution

Parameter	Treatment		
	Aerated	N_2-flushed	Stagnant agar
Shoot dry mass (g)	$1.59^a \pm 0.142$	$1.90^a \pm 0.366$	$0.86^b \pm 0.156$
Root dry mass (g)	0.35 ± 0.032	0.50 ± 0.164	0.39 ± 0.070
Number of tillers	5.0 ± 1.0	7.0 ± 1.0	3.0 ± 0
Number of adventitious roots	64 ± 2	94 ± 18	74 ± 9
Longest adventitious root (mm)	$345^a \pm 18$	$210^b \pm 7$	$254^c \pm 12$
Extension[1] (mm d^{-1})			
Shorter roots	$32^a \pm 1.3$	$20^b \pm 1.8$	$24^b \pm 3.0$
Longer roots	$32^a \pm 2.1$	$20^b \pm 0.3$	$26^b \pm 3.3$
Root porosity[2] (% gas volume)	$24.8^a \pm 0.7$	$34.7^b \pm 2.4$	$40.6^c \pm 1.6$

Data are mean ± SE; $n = 4$. Treatments were imposed on 5-d-old seedlings. Initial dry mass (g): shoots, 0.0028 ± 0.002; roots, 0.0020 ± 0.001.
Significant differences ($P < 0.05$) between means across a row are indicated by different letters (letters absent across a row when there were no differences). (Nutrient Solution Experiment 1.)
[1]Rates of extension were measured for the 24-h period when plants were 30- to 31-d-old.'Shorter' roots were 63–81 mm and 'longer' roots were 114–137 mm, at the commencement of the measurements.
[2]Measured for adventitious roots of 100–200 mm.

roots. In marked contrast, roots of plants grown in stagnant deoxygenated agar solution had highest ROL near the tips and lowest at the more basal positions (e.g. for 'longer' roots, ROL was 27-fold higher at 5 mm behind the tip than at 80 mm). The 4.9- to 7.6-fold higher ROL rates near the tip of roots from the stagnant treatment indicate higher O_2 concentrations within these roots compared with those of the plants from the aerated treatment, and are consistent with the 1.6-fold higher porosity of roots from the stagnant treatment (Table 2; $P < 0.001$). However, at 50 mm behind the tip of roots from the stagnant treatment, rates of ROL were only 27% (shorter roots) and 14% (longer roots) of those from roots raised in aerated solution, despite the distinct possibility of higher O_2 within the roots from the stagnant treatment (see earlier), indicating a substantial increase in the resistance to O_2 movement from the aerenchyma to the root exterior in the basal zones of these roots grown in stagnant deoxygenated agar. These data support our earlier observations that a 'tight' barrier against ROL is induced in roots of rice when grown in stagnant conditions (Colmer *et al.*, 1998; Colmer, 2003a).

The effect of an O_2-deficient rooting medium *per se*, was tested by growing plants in pots of nutrient solution flushed with N_2. Patterns of ROL along roots of plants from this treatment were somewhat intermediate to those along roots of plants from the aerated and stagnant treatments. ROL near the root tip had increased (Fig. 2), but ROL from the more basal zones also remained high. So, although the N_2 treatment resulted in a 1.4-fold increase in root porosity (Table 2, $P < 0.001$), enhancing O_2 diffusion to the root tips and thus ROL at these apical positions, the spatial patterns of ROL along these roots were rather 'flat', indicating at best only a partial induction of a barrier to ROL. Clearly, resistance to radial movement of O_2 in the basal zones of roots from the N_2-flushed treatment was less than in roots from the stagnant agar treatment.

Shoot dry mass of plants in aerated and N_2-flushed solutions were not statistically different, whereas plants in the stagnant agar treatment had lower shoot dry mass (Table 2; $P < 0.05$). Unlike the shoots, root dry mass did not differ for plants in the three treatments ($P = 0.551$). Thus, root : shoot ratio of rice plants increased from 0.22 in aerated solution to 0.45 in stagnant agar solution.

Reduced tillering by plants in the stagnant agar (Table 2) would have contributed to the reduction in shoot dry mass. An additional experiment (not shown), with increasing levels of mineral nitrogen (supplied as NH_4NO_3) in the stagnant agar, demonstrated that the reduced tillering and lower shoot dry mass were caused by N-deficiency. Addition of 2.5 mol m^{-3} NH_4NO_3, above the 5 mol m^{-3} N (1 : 7, NH_4^+ : NO_3^-) already present in the stagnant agar solution, resulted in growth being equal to that in aerated solution (see also Rubinigg *et al.*, 2002). Thus, although Wiengweera *et al.* (1997) had determined that 5 mol m^{-3} mineral N was sufficient to prevent diffusion limitations on N supply for wheat (waterlogging sensitive) when in stagnant 0.1% agar, albeit only over 8 d, this level of N was not sufficient for rice (waterlogging tolerant) with a higher growth rate in stagnant agar solution (whole-plant relative growth rate (RGR) of rice was 0.194 d^{-1} (Rubinigg *et al.*, 2002) and in wheat it was 0.103 d^{-1} (Wiengweera *et al.*, 1997)); in our experiment rice was grown in stagnant agar for 28 d (with solutions renewed every 7 d).

Effects of ethylene on patterns of ROL along adventitious roots, porosity and growth of rice in aerated nutrient solution

We tested the hypothesis that ethylene might induce a barrier to ROL in roots of rice. Our rationale being that: (1) ethylene had previously been shown to invoke several acclimatizations to

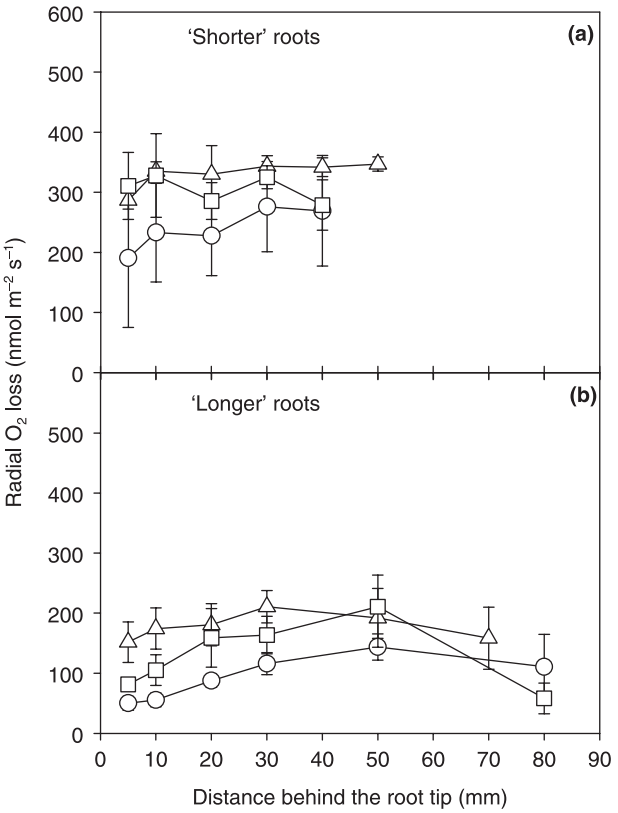

Fig. 3 Rates of radial O_2 loss (ROL) along 'shorter' (a) and 'longer' (b) intact adventitious roots of rice (*Oryza sativa*) in an O_2-free root medium with shoots in air, at 29°C ($n = 4 \pm$ SE). Plants were grown in aerated nutrient solution without ethylene (open circles), or with 0.2 (open triangles) or 2.0 (open squares) μl l^{-1} ethylene. Plants were 24- to 27-d-old, with treatments imposed on day 15. Lengths (mm) of the roots used in the measurements were: (a) without ethylene, 66 ± 6; 0.2 μl l^{-1} ethylene, 70 ± 5; 2.0 μl l^{-1} ethylene, 72 ± 4; (b) without ethylene, 118 ± 6; 0.2 μl l^{-1} ethylene, 118 ± 6; 2.0 μl l^{-1} ethylene, 116 ± 9. (Nutrient solution experiment 2.)

flooding in roots of other species (e.g. aerenchyma formation, Drew *et al.*, 1979; adventitious rooting, Visser *et al.*, 1996), including enhanced aerenchyma formation in roots of rice (Justin & Armstrong, 1991); and (2) one major difference between soil and stagnant agar solution (barrier to ROL induced) and N_2-flushed treatments (barrier not induced) is that ethylene can be purged away in N_2-flushed systems, whereas it accumulates in stagnant agar (Wiengweera *et al.*, 1997) and in waterlogged soil (Smith & Russell, 1969) with consequences for ethylene-induced acclimatizations such as root porosity (wheat, Wiengweera *et al.*, 1997; rice, Table 2 present study).

Patterns of ROL did not differ for adventitious roots of rice grown in aerated nutrient solution, with 0, 0.2 or 2.0 μl l^{-1} ethylene (Fig. 3). The only difference was that ROL was higher at the apical positions of the 'longer' roots treated with ethylene, which was probably due to higher volumes of aerenchyma in the roots treated with 2.0 μl l^{-1} ethylene (Table 3, $P < 0.05$). So, although the patterns of ROL along the roots were rather 'flat', again indicating a somewhat higher resistance to radial movement of O_2 from the aerenchyma to the root exterior in basal positions, compared with more apical positions, applied ethylene did not induce development of a 'tight' barrier to ROL.

Table 3 Effects of ethylene on rates of extension and aerenchyma formation in adventitious roots of 24-d-old rice (*Oryza sativa*) in aerated nutrient solution, with treatments imposed on day 15

Parameter	Ethylene treatment (μl l^{-1}) Control (0)	0.2	2.0
Extension[1] (mm d^{-1})			
Shorter roots	20 ± 1.5	22 ± 1.5	18 ± 1.2
Longer roots	19^a ± 1.5	17^a ± 0.8	13^b ± 2.3
Aerenchyma (% cross-sectional area)			
20 mm behind tip	2.4^a ± 2.4	2.6^a ± 1.3	15.8^b ± 4.8
40 mm behind tip	19.2^a ± 4.5	26.3^a ± 4.0	31.8^b ± 0.6

Data are mean ± SE; $n = 4$, root extension data; $n = 3$, aerenchyma data. Rates of root extension were measured over the final 24 h. Aerenchyma was measured at two distances behind the tip of 'longer' adventitious roots (100–120 mm in length), with three cross-sections per position for each replicate root. (Nutrient solution experiment 2.) Significant differences ($P < 0.05$) between means across a row are indicated by different letters (letters absent across a row when there were no differences).

[1]'Shorter' roots were 60–79 mm and 'longer' roots were 97–125 mm at the commencement of the measurements.

The lack of response of the barrier to ROL to ethylene is rather convincing, especially since the plants showed other physiological responses to ethylene treatment. For plants treated with 2.0 μl l^{-1} ethylene, aerenchyma in roots was 1.7-fold (40 mm behind tip) and 6.6-fold (20 mm behind tip) higher (Table 3, $P < 0.05$), and extension rates of 'longer' roots declined to 68% of that in controls (Table 3, $P < 0.05$). Exogenous ethylene had no effect on the other parameters of growth and development that we measured (i.e. numbers of adventitious roots, numbers of tillers and shoot and root dry mass) other than stem height was increased by 15% (data not shown).

For two other cultivars of rice, ethylene at 1–2 μl l^{-1} in aerated solution also enhanced aerenchyma formation in adventitious roots; however, in one genotype aerenchyma development was only simulated in younger tissues towards the root tip, whereas in the other genotype ethylene also increased aerenchyma in mature zones (Justin & Armstrong, 1991). For one of the two genotypes studied by Justin & Armstrong (1991), ethylene also increased, by at most 26%, the number of adventitious roots per plant. Ethylene at 2 μl l^{-1} also caused substantial decreases in the mean lengths of roots of both genotypes (Justin & Armstrong, 1991). Data on mean root lengths can be skewed if the amounts of recently emerged roots differ between treatments or genotypes, therefore we measured rate of root extension in our study. Ethylene at 2 μl l^{-1} did not affect rates of extension of 'shorter' roots, whereas 'longer'

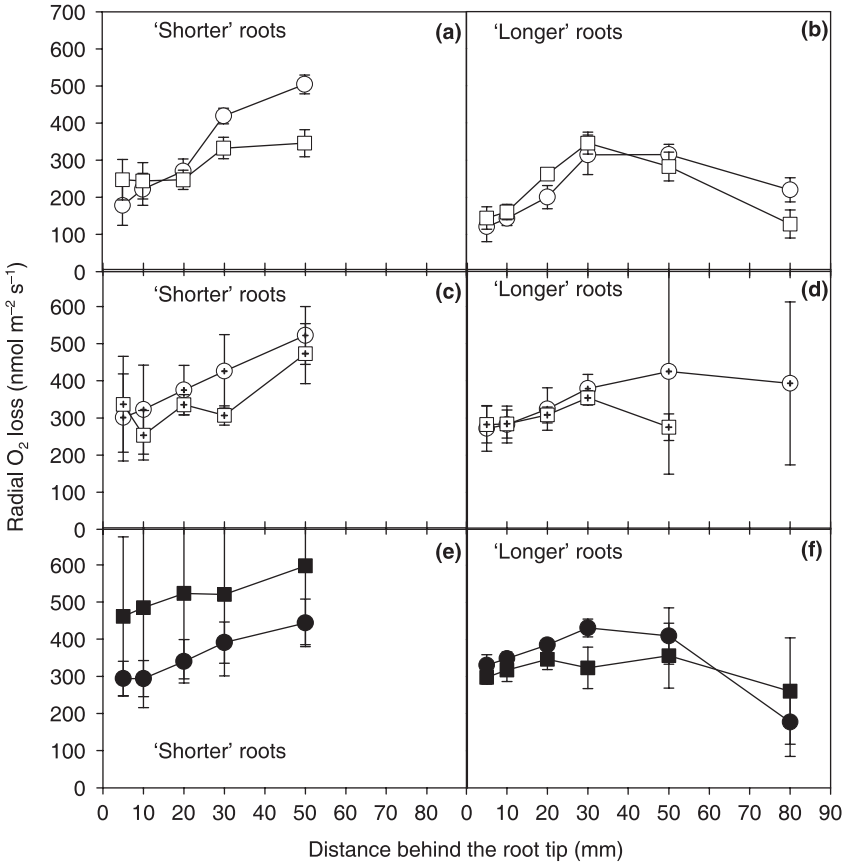

Fig. 4 Rates of radial O_2 loss (ROL) along 'shorter' (a,c,e) and 'longer' (b,d,f) intact adventitious roots of rice (*Oryza sativa*) in an O_2-free root medium with shoots in air, at 28.5–30°C ($n = 3 \pm$ SE). Plants were grown in: (a,b) aerated nutrient solution without (open circles) or with 2.0 μl l^{-1} (open squares) ethylene; (c,d) hypoxic (2.2% O_2) nutrient solution without (open circles containing +) or with 2.0 μl l^{-1} (open squares containing +) ethylene; (e,f) N_2-flushed nutrient solution without (closed circles) or with 2.0 μl l^{-1} (closed squares) ethylene. Plants were 23- to 25-d-old, with treatments imposed on day 15. Lengths (mm) of the roots used in the measurements were: (a) aerated without ethylene 67 ± 2, aerated with 2.0 μl l^{-1} ethylene 77 ± 8; (b) aerated without ethylene 113 ± 1, aerated with 2.0 μl l^{-1} ethylene 113 ± 10; (c) hypoxic without ethylene 61 ± 3, hypoxic with 2.0 μl l^{-1} ethylene 71 ± 5; (d) hypoxic without ethylene 102 ± 8, hypoxic with 2.0 μl l^{-1} ethylene 85 ± 4; (e) N_2-flushed without ethylene 68 ± 4, N_2-flushed with 2.0 μl l^{-1} ethylene 77 ± 8; (f) N_2-flushed without ethylene 106 ± 9, N_2-flushed with 2.0 μl l^{-1} ethylene 91 ± 15. (Nutrient solution experiment 3.)

roots were reduced to only 68% of the rate in controls (Table 3). Rice genotypes differ markedly in sensitivity of root extension to ethylene, 1 μl l^{-1} ethylene in air did not affect growth of one genotype, whereas it reduced the most sensitive of the seven cultivars tested to 55% of the control value (Smith & Robertson, 1971). Two genotypes showed enhanced elongation by up to 20% when exposed to low (e.g. c. 0.2 μl l^{-1}) concentrations of ethylene (Smith & Robertson, 1971), as did a different genotype of rice in another study (Konings & Jackson, 1979), whereas such responses were not apparent for five other rice genotypes (Smith & Robertson, 1971) or for 'Calrose' in the present study (Table 3). Even more sensitive than rice, were responses of root extension to ethylene in another wetland species *Rumex palustris* (Visser *et al.*, 1997) and of several dryland crop species (Smith & Robertson, 1971; Konings & Jackson, 1979).

Effects of ethylene–O_2 interactions on patterns of ROL along adventitious roots, porosity, and growth of rice in nutrient solution

Before ruling out a role of ethylene in induction of the barrier to ROL, we conducted a second experiment in order to confirm our previous findings, and to test for possible ethylene–O_2 interactions. Sensitivity of some tissues/organs to ethylene can be increased by low O_2, as demonstrated for petioles of *R. palustris* (Voesenek *et al.*, 1997). Alternatively, effects of ethylene and low (but not zero) O_2 can be additive; for example, effects of these gases on elongation of the coleoptile of rice seedlings (Satler & Kende, 1985). In our experiment, roots of rice plants were treated with 0 or 2 μl l^{-1} ethylene, in aerated, hypoxic, or anoxic nutrient solution.

Ethylene did not induce a barrier to ROL in roots exposed to any of the three O_2 regimes, for either 'shorter' or 'longer' roots (Fig. 4). Thus, the lack of a significant response of the barrier to ethylene in aerated conditions was confirmed (Fig. 4a,b), and extended to rule out a role of ethylene, even when roots were in hypoxic (Fig. 4c,d) or in N_2-flushed solutions (Fig. 4e,f). As a positive control, the capacity for these same roots to develop a barrier to ROL was demonstrated by transferring three plants (one from each O_2 treatment with 2 μl l^{-1} ethylene) to stagnant agar solution following the ROL measurements, and then measuring the same roots again 2 d later. Roots of all three plants that were tested then formed a barrier to ROL (data not shown).

Despite the lack of any significant effect of ethylene on the barrier to ROL (discussed in preceding paragraph), 2 μl l^{-1} ethylene enhanced aerenchyma formation at 20 mm behind the tip, particularly when combined with hypoxia, whereas there was no effect of ethylene on aerenchyma when applied

Table 4 Effects of ethylene and root-zone O_2 treatments on aerenchyma and length of the longest adventitious root of 24-d-old rice (*Oryza sativa*) plants, with treatments imposed on day 15

	0 μl l^{-1} Ethylene			2 μl l^{-1} Ethylene		
Parameter	20.6% O_2	2.2% O_2	0% O_2	20.6% O_2	2.2% O_2	0% O_2
Longest adventitious root (mm)	$228^{a} \pm 15$	$163^{b} \pm 8$	$147^{b,e} \pm 7$	$197^{c} \pm 8$	$119^{d,f} \pm 10$	$138^{e,f} \pm 5$
Aerenchyma (%) 20 mm behind tip	7.9 ± 2.3	9.5 ± 1.6	10.2 ± 0.8	11.8 ± 5.7	$21.9^{*} \pm 1.5$	12.7 ± 2.0
Aerenchyma (%) 40 mm behind tip	21.2 ± 2.7	21.6 ± 1.3	27.3 ± 2.2	26.5 ± 2.6	28.3 ± 1.9	23.2 ± 2.0

Data are mean ± SE; $n = 3$. Aerenchyma was measured for adventitious roots of *c.* 100 mm in length. Two-way ANOVA showed a significant ($P < 0.001$) ethylene–O_2 interaction for root length, so differences ($P < 0.05$) between means across this row of data are indicated by different letters. For aerenchyma, the ethylene–O_2 interaction was not significant ($P = 0.238$ and 0.011, at 20 mm and 40 mm, respectively); however, ethylene alone had a significant effect ($P = 0.02$) on aerenchyma formed at 20 mm behind the tip, being higher in the hypoxic treatment when exposed to 2.0 μl l^{-1} ethylene (indicated by *). (Nutrient solution experiment 3.)

to roots in N_2-flushed solution (Table 4). Furthermore, ethylene reduced the lengths of adventitious roots in aerated solution (86% of control), and when in combination with hypoxia, ethylene had an even more adverse effect on maximum root lengths (reduced to 52% of control) (Table 4, $P < 0.01$ for ethylene–O_2 interaction in two-way ANOVA). In addition, ethylene increased stem height by 10% for plants in aerated solution, but had no effect on plants in the low O_2 treatments. However, numbers of adventitious roots, numbers of tillers, shoot dry mass and root dry mass, were all not significantly different between plants in the various treatments (data not shown).

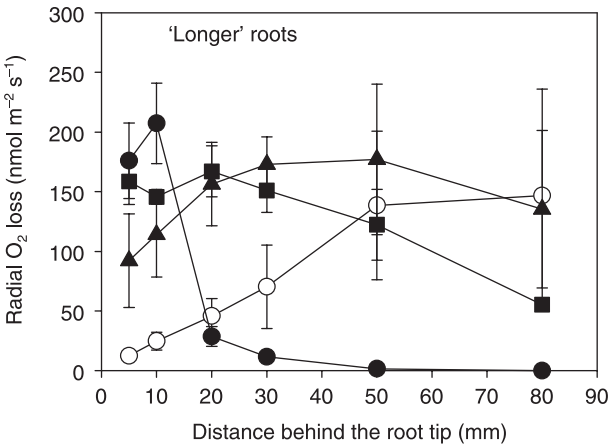

Fig. 5 Rates of radial O_2 loss (ROL) along 'longer' intact adventitious roots of rice (*Oryza sativa*) in an O_2-free root medium with shoots in air, at 30°C ($n = 3 \pm$ SE). Plants were grown in aerated (open circles), N_2-flushed (closed squares), N_2-flushed plus 10% CO_2 (closed triangles) or stagnant deoxygenated 0.1% agar (closed circles) nutrient solution. Plants were 28- to 29-d-old, with treatments imposed on day 14. Lengths (mm) of the roots used in the measurements were: aerated 121 ± 2; N_2-flushed 115 ± 6; N_2-flushed plus 10% CO_2 129 ± 4; stagnant deoxygenated 0.1% agar 129 ± 1. (Nutrient solution experiment 4.)

Effects of CO_2 on patterns of ROL along adventitious roots, porosity, and growth of rice in N_2-flushed nutrient solution

Carbon dioxide accumulates to high levels (e.g. tens of kPa) in flooded rice-growing soils (Ponnamperuma, 1984), and as high CO_2 had been implicated in acclimatization to submergence by the ethylene-insensitive stems of *Potamogeton pectinatus* (Summers & Jackson, 1996), we considered it worthwhile to test effects of 10 kPa CO_2 on root aeration in rice, when grown in N_2-flushed nutrient solution. The ROL measurements in this experiment were taken only for 'longer' roots.

As expected from our earlier experiments, aerated controls showed no barrier to ROL, whereas the barrier was induced in roots of plants grown in stagnant agar solution (Fig. 5). Plants grown in N_2-flushed solution showed enhanced ROL from the apical few cm, and then a somewhat flat profile with distance behind the tip, similar to that shown earlier (Figs 2 and 4e,f). A concentration of 10 kPa CO_2 did not change the general pattern of ROL along roots grown in N_2-flushed solution (Fig. 5), indicating that high CO_2 does not induce a barrier to ROL in roots of rice.

Few studies have evaluated responses of roots to the high concentrations of CO_2 (i.e. several kPa) that occur in many waterlogged soils (reviewed by Greenway *et al.*, In press); therefore, we present here data on growth of rice when exposed to 10 kPa CO_2 added to N_2 in the root-zone (Table 5). A concentration of 10 kPa CO_2 in N_2 had no effect in addition to N_2 alone, on root porosity, rate of root extension, length of the longest adventitious root, root dry mass or shoot dry mass (Table 5). The lack of an effect of 10 kPa CO_2 in N_2 on root porosity contrasts with an earlier observation that 11 kPa CO_2 in air caused modest reductions in aerenchyma formed in roots of rice (Jackson *et al.*, 1985). The lack of an effect of high CO_2 on shoot and root dry mass is consistent with results of Boru *et al.* (2003), who found that rice plants in nutrient solution bubbled with up to 50 kPa CO_2 in N_2 for 14 d had shoot and root dry mass equal to that of plants in N_2-flushed solution; although maximum root length was reduced by 25% in this earlier study. Even when bubbled with

Table 5 Parameters of growth and development for 32- to 33-d-old rice (*Oryza sativa*) in aerated, N_2-flushed, N_2-flushed plus 10% CO_2 or stagnant (0.1% agar) deoxygenated nutrient solution

	Treatments			
Parameter	Aerated	N_2-flushed	N_2-flushed + 10% CO_2	Stagnant agar
Shoot dry mass (g)	$1.37^{a} \pm 0.08$	$1.13^{b} \pm 0.09$	$1.05^{b} \pm 0.03$	$0.79^{c} \pm 0.03$
Root dry mass (g)	0.35 ± 0.04	0.28 ± 0.04	0.26 ± 0.03	0.41 ± 0.03
Longest adventitious root (mm)	$320^{a} \pm 4$	$193^{b} \pm 6$	$208^{b} \pm 7$	$280^{c} \pm 7$
Rate of root extension[1] (mm d^{-1})	$34^{a} \pm 2.6$	$23^{b} \pm 2.2$	$26^{b} \pm 0.9$	$28^{a,b} \pm 2.6$
Porosity[2] (% gas volume) of adventitious roots	$21.9^{a} \pm 1.4$	$33.3^{b,c} \pm 1.7$	$30.9^{b} \pm 1.3$	$36.5^{c} \pm 1.0$

Data are mean ± SE; $n = 3$. Plants were 28- to 29-d-old with treatments imposed on day 14 (nutrient solution experiment 4).
Significant differences ($P < 0.05$) between means across a row are indicated by different letters (letters absent across a row when there were no differences).
[1]Rates of extension were measured for the 24-h period when plants were 27- to 28-d-old. Roots were 101–112 mm, at the commencement of the measurements.
[2]Measured for adventitious roots of 100–200 mm.

100% CO_2 for 1 month in nutrient solution at pH 6, the mass of rice plants was only reduced to 82% of that when flushed with 100% N_2 (Tanaka & Navasero, 1967). Neither 11 kPa CO_2 in air (Jackson *et al.*, 1985), nor 10 kPa CO_2 in N_2 (Table 5), affected root extension rates. By contrast, 10 kPa CO_2 reduced extension of roots of *R. palustris* to 80% of the control (Visser *et al.*, 1997). In summary, high levels of CO_2 (i.e. 10 kPa) added to a N_2-flushed root-zone did not have adverse effects on rice.

Conclusions

The present study shows that the inducible nature of the barrier to ROL in adventitious roots of rice, as reported previously for plants in stagnant deoxygenated agar solution vs aerated solution (Colmer *et al.*, 1998; Colmer, 2003a), is of relevance to soil-grown plants (Fig. 1b). Several wetland species have now been documented to possess an inducible barrier to ROL, whereas many others contain a constitutive barrier (Visser *et al.*, 2000; McDonald *et al.*, 2002; Garthwaite *et al.*, 2003). The possible role of changes in root-zone gas composition in regulating the induction of this acclimatization of roots to flooded soils, however, had not been evaluated. We tested whether low O_2, elevated ethylene, low O_2–elevated ethylene or elevated CO_2 are cues for inducing the barrier to ROL, as these are major components of the altered environment in flooded soils (Ponnamperuma, 1984); and in the case of ethylene, had been shown to enhance other acclimatizations to soil flooding (aerenchyma in roots of rice, Justin & Armstrong, 1991). However, none of the levels and combinations of O_2, ethylene and CO_2 that we tested induced a barrier to ROL in roots of rice.

In addition to changes in levels of O_2, ethylene and CO_2, other aspects of soil chemistry are altered by flooding; for example, carboxylic acids, Fe^{2+}, Mn^{2+}, H_2S, and/or S^{2-} can be produced by microorganisms in anaerobic soils (Ponnamperuma, 1984). When applied to roots of rice, carboxylic acids (Armstrong & Armstrong, 2001) and sulphide (Armstrong & Armstrong, 2005), caused ROL to decline from normally permeable regions (i.e. from apical positions but also from laterals that normally do not form a barrier to ROL). These experiments by Armstrong & Armstrong (2001, 2005) were designed to evaluate toxicity effects, therefore, relatively high concentrations were used so that (1) root extension ceased, or at best continued at only 16% of the prestress rate, and (2) tissues appeared to suffer injury. Thus, the reductions in ROL from roots of rice exposed to toxic levels of carboxylic acids or sulphide were associated with injury (Armstrong & Armstrong, 2001, 2005), so further work is still required to elucidate the signal responsible for induction of the barrier to ROL in healthy roots.

In roots of many wetland species, the subapical portions become very impermeable to ROL (Armstrong, 1979; Colmer, 2003b), and this was previously noted to 'seemingly coincide with aerenchyma formation' (Jackson & Armstrong, 1999). Our present finding that ethylene does not induce the barrier to ROL in roots of rice, even though it enhances aerenchyma formation (Tables 3 and 4; present study; Justin & Armstrong, 1991) implies that these two root aeration traits, considered to act synergistically to enhance O_2 diffusion to the root apex (Armstrong, 1971; Colmer, 2003b), are differentially regulated.

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References

Armstrong W. 1971. Radial oxygen losses from intact rice roots as affected by distance from the apex, respiration, and waterlogging. *Physiologia Plantarum* **25**: 192–197.

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

Armstrong W. 1994. Polarographic oxygen electrodes and their use in plant aeration studies. *Proceedings of the Royal Society of Edinburgh* **102B**: 511–527.

Armstrong J, Armstrong W. 2001. Rice and *Phragmites*: effects of organic acids on growth, root permeability, and radial oxygen loss to the rhizosphere. *American Journal of Botany* **88**: 1359–1370.

Armstrong J, Armstrong W. 2005. Rice: sulphide-induced barriers to root radial oxygen loss, Fe^{2+} and water uptake, and lateral root emergence. *Annals of Botany* **96**: 625–638.

Armstrong W, Wright EJ. 1975. Radial oxygen loss from roots: the theoretical basis for the manipulation of flux data obtained by the cylindrical platinum electrode technique. *Physiologia Plantarum* **35**: 21–26.

Barber DA, Ebert M, Evans NTS. 1962. The movement of ^{15}O through barley and rice plants. *Journal of Experimental Botany* **13**: 397–403.

Boru G, van Toai T, Alves J, Hua D, Knee M. 2003. Responses of soybean to oxygen deficiency and elevated root-zone carbon dioxide concentration. *Annals of Botany* **91**: 447–453.

Clark LH, Harris WH. 1981. Observations on the root anatomy of rice (*Oryza sativa* L.). *American Journal of Botany* **68**: 154–161.

Colmer TD. 2003a. Aerenchyma and an inducible barrier to radial oxygen loss facilitate root aeration in upland, paddy and deep-water rice (*Oryza sativa* L.). *Annals of Botany* **91**: 301–309.

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

Colmer TD, Gibbered MR, Wiengweera A, Tinh TK. 1998. The barrier to radial oxygen loss from roots of rice (*Oryza sativa* L.) is induced by growth in stagnant solution. *Journal of Experimental Botany* **49**: 1431–1436.

Das DK, Jat RL. 1977. Influence of three soil-water regimes on root porosity and growth of four rice varieties. *Agronomy Journal* **69**: 197–200.

Drew MC, Jackson MB, Giffard S. 1979. Ethylene-promoted adventitious rooting and development of cortical air spaces (aerenchyma) in roots may be adaptive responses to flooding in *Zea mays* L. *Planta* **147**: 83–88.

Garthwaite AJ, von Bothmer R, Colmer TD. 2003. Diversity in root aeration traits associated with waterlogging tolerance in the genus *Hordeum*. *Functional Plant Biology* **30**: 875–889.

Greenway H, Armstrong W, Colmer TD. 2006. Conditions leading to high CO_2 (> 5 kPa) in waterlogged-flooded soils and possible effects on root growth and metabolism. *Annals of Botany*. (In press.)

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

Jackson MB, Drew MC. 1984. Effects of flooding on growth and metabolism of herbaceous plants. In: Kozlowski TT, ed. *Flooding and plant growth*. New York, NY, USA: Academic Press, 47–128.

Jackson MB, Fenning TM, Jenkins W. 1985. Aerenchyma (gas-space) formation in adventitious roots of rice (*Oryza sativa* L.) is not controlled by ethylene or small partial pressures of oxygen. *Journal of Experimental Botany* **36**: 1566–1572.

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

Justin SHFW, Armstrong W. 1991. Evidence for the involvement of ethene in aerenchyma formation in adventitious roots of rice (*Oryza sativa* L.). *New Phytologist* **118**: 49–62.

Konings H, Jackson MB. 1979. A relationship between rates of ethylene production by roots and the promoting or inhibiting effects of exogenous ethylene and water on root elongation. *Zeitschrift für Pflanzenphysiologie* **92**: 385–397.

McDonald MP, Galwey NW, Colmer TD. 2002. Similarity and diversity in adventitious root anatomy as related to root aeration among a range of wet- and dry-land grass species. *Plant, Cell & Environment* **25**: 441–451.

O'Brien TP, McCully ME. 1981. *The study of plant structure: principles and selected methods*. Melbourne, Australia: Thermacarpi.

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

Pradhan SK, Varade SB, Kar S. 1973. Influence of soil water conditions on growth and root porosity of rice. *Plant and Soil* **38**: 501–507.

Raskin I. 1983. A method for measuring leaf volume, density, thickness, and internal gas Volume. *Hortscience* **18**: 698–699.

Rubinigg M, Stulen I, Elzenga JTM, Colmer TD. 2002. Spatial patterns of radial oxygen loss and nitrate net flux along adventitious roots of rice raised in aerated or stagnant solution. *Functional Plant Biology* **29**: 1475–1481.

Satler SO, Kende H. 1985. Ethylene and the growth of rice seedlings. *Plant Physiology* **79**: 194–198.

Smith KA, Robertson PD. 1971. Effect of ethylene on root extension of cereals. *Nature* **234**: 148–149.

Smith KA, Russell RS. 1969. Occurrence of ethylene, and its significance, in anaerobic soil. *Nature* **222**: 769–771.

Summers JE, Jackson MB. 1996. Anaerobic promotion of stem extension in *Potamogeton pectinatus*. Roles for carbon dioxide, acidification and hormones. *Physiologia Plantarum* **96**: 615–622.

Tanaka A, Navasero SA. 1967. Carbon dioxide and organic acids in relation to the growth of rice. *Soil Science and Plant Nutrition* **13**: 25–30.

Thomson CJ, Armstrong W, Waters I, Greenway H. 1990. Aerenchyma formation and associated oxygen movement in seminal and nodal roots of wheat. *Plant, Cell & Environment* **13**: 395–403.

Visser EJW, Bogemann GM, Blom CWPM, Voesenek LACJ. 1996. Ethylene accumulation in waterlogged *Rumex* plants promotes formation of adventitious roots. *Journal of Experimental Botany* **47**: 403–410.

Visser EJW, Nabben RHM, Blom CWPM, Voesenek LACJ. 1997. Elongation by primary lateral roots and adventitious roots during conditions of hypoxia and high ethylene concentrations. *Plant, Cell & Environment* **20**: 647–653.

Visser EJW, Colmer TD, Blom CWPM, Voesenek LACJ. 2000. Changes in growth, porosity, and radial oxygen loss from adventitious roots of selected mono- and dicotyledonous wetland species with contrasting types of aerenchyma. *Plant, Cell & Environment* **23**: 1237–1245.

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

Wiengweera A, Greenway H, Thomson CJ. 1997. The use of agar nutrient solution to simulate lack of convection in waterlogged soils. *Annals of Botany* **80**: 115–123.

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