Chapter 4

Morphological and physiological responses of rice to limited phosphorus supply in aerated and stagnant solution culture

4.1 Introduction

In the rainfed lowlands, rice experiences intermittent anaerobic and aerobic conditions in the root zone (Zeigler and Puckridge, 1995). Deep root activity in aerobic soil is supported by oxygen uptake directly from the air-filled soil pores, whereas in anaerobic flooded soils oxygen supply is via internal diffusion in aerenchyma (Armstrong, 1979). Root acclimatization to the changing water regimes may in turn affect plant morphological features, physiological function and nutrient uptake efficiency.

In anaerobic condition, roots acclimate by increasing the internal supply of oxygen to the root tip from the atmosphere, via formation of aerenchyma (Justin and Armstrong, 1987; Drew *et al.*, 1994; Drew, 1997). This modification of the root cortex by aerenchyma formation enhances longitudinal oxygen diffusion, but might also decrease symplastic nutrient transport across the roots to the stele (Drew and Saker, 1986; Kronzucker *et al.*, 1998). Furthermore, in order to minimize radial oxygen loss (ROL) from the main axes of adventitious roots, rice forms a barrier to gas diffusion near the root exterior (Colmer *et al.*, 1998) believed to be related to increased suberization of the exodermis (Ranathunge *et al.*, 2004). Although such a barrier might cause an inhibition of nutrient absorption by roots (Koncalova, 1990), direct studies of this are few, and the data available indicate that nutrient uptake might

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not be affected (Rubinigg *et al.*, 2002). Furthermore, it has been proposed that the axial root with aerenchyma is inefficient in nutrient uptake, and that new fine lateral roots are induced for nutrient absorption (Kirk and Du, 1997; Kirk, 2003). The fine lateral roots comprise the bulk of the external surface, and the laterals are connected directly into the main water and solute transport vessels in the stele of the primary root (Matsuo and Hoshikawa, 1993). Moreover, roots of rice acclimate to growth in anaerobic condition by increasing the number of adventitious roots per plant, which presumably also contributes to waterlogging tolerance (Colmer, 2003b). Rice plants grown in aerobic soils have a different structure of roots; maximum root lengths are greater in aerobic than in anaerobic media, and plants in aerobic media have fewer adventitious roots and these have lower porosity and a much less pronounced barrier against ROL (Colmer, 2003b).

The intermittently waterlogged conditions that occur in soils of the rainfed lowland rice ecosystem are likely to have adverse effects on P nutrition of rice on low P soils, due to changes in P availability with varied water regimes (Huguenin-Elie *et al.*, 2003; Seng *et al.*, 2004) and possibly due to adverse effects of soil oxidation/reduction cycles on root function. The adaptations of plants for increased P acquisition include mycorrhizal symbioses, rhizosphere modification by secretion of organic acids (Gardner *et al.*, 1983; Lipton *et al.*, 1987; Lu *et al.*, 1999), and proton release (Kirk, 2003). Moreover, roots also increase surface area by root hair elongation and proliferation (Bates and Lynch, 1996; Ma *et al.*, 2001). For rice in anaerobic root conditions, low P supply enhanced adventitious root elongation and lateral root development and elongation (Kirk and Du, 1997). Fan *et al*. (2003) reported that in maize roots, aerenchyma formation can be induced by low P status,

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and this in turn reduced respiration and P requirement in the roots. A similar decline in respiratory costs of root growth in rice with low P may enhance its adaptation to P-deficient flooded soils. However, the adaptation of rice roots in low P soils to the transitions from aerobic to anaerobic conditions and vice versa has not been studied. This study evaluated both of prolonged and early responses of morphology and physiology in rice to changes in oxygen and P supplies, and how these in turn affect P uptake and plant growth.

4.2 Materials and Methods

The experiments were conducted in a solution culture in a glasshouse at Murdoch University and root porosity, radial oxygen loss from roots and root respiration were measured in plant physiology laboratory at the University of Western Australia, Western Australia.

4.2.1 Experiment 1: Australian rice in alteration of oxygen and P supplies in solution culture

During the experiment, minimum/maximum temperatures were 25/30°C and 13 hrs of day lengths. The experiment examined responses of an Australian rice cultivar (Amaroo) in aerated nutrient solution culture at 200 μM P supply (high P) for two weeks, then transition to P levels were 1.6 (low P) and 200 (high P) μM in stagnant and aerated nutrient solution cultures. There were four replicates. Six (two plants of each replicate) 14 day-old seedlings were transplanted in each 10 L pot. The rice plants were harvested at one, two weeks (in high P aerated nutrient solution culture), three and four weeks (after transition to treatments). Maximum root length,

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maximum shoot length, number of leaves, root and tiller, porosity of adventitious root (percentage of gas space per volume), root and shoot dry weights, root/shoot ratio, aerenchyma development, and the barrier cell in root tissue were assessed. A sample of three root tips (50 mm in length) was collected from each pot (one root tip/plant) for examination of the barrier forming in root tissue and evaluated by cross sectioning and scanning under a fluorescent microscope. Autofluorescence of cells in root cross-sections were measured from fresh "thick" adventitious roots (100-120 mm in length). Roots were sectioned by free-hand at 10, 20 and 70 mm behind the apex, using a razor blade. Roots were sampled at 0 and 8 days. Root cross-sections were viewed under blue excitation U-MNB2 (excitation BP 460-490 nm, barrier filter IF 520, Dichromatic mirror 500) using an Olympus BX51 Photomicroscope and photographed with an Olympus DP70 Camera.

Autofluorescence can be from walls impregnated with phenolic compound or lipid complexes; so can indicate lignin, suberin and/or other phenolic compounds in cell walls (O' Brien and McCully, 1981; Yeung, 1998). The relative intensity of autofluorescence of cell walls in the layer of sclerenchymatous fibres on the outer side of the root cortex was scored on a 3-point scale: 0, when intensity of autofluorescence of the walls in the sclerenchymatous layers was equal to that of the cell layers on either side of it; 1, when intensity of autofluorescence was greater than that in cell walls of cell layers on either side of it and the thickness of the walls were equal to the walls of cell layers on either side of it; 2, when the intensity of autofluorescence was greater than those in cell walls of the cell layers on either side of it and had thicker walls than those of cell layers on either side of it. Scores for autofluorescence

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intensity were made for cross-sections at 10, 20 and 70 mm behind the root tip, for three replicates (each replicate was a cross-section from an individual root).

Photosynthesis efficiency or photosynthesis rate (μmol m^2 s^{-1}) of plant was measured by CIRAS-2 Portable Photosynthesis System. The used method of measuring the gas exchanges of leaves is to enclose them in a cuvette, pass a known flow rate of air over the leaf, and measure the change in concentration of CO_2 and H_2O in the air. CIRAS-s has internal air sampling pumps with mass flow controllers that pump the air through the cells at about 100mls/min. It also contained the cuvette air supply system, which full control of both the CO_2 and H_2O concentrations.

The measurements in maximum root length, maximum shoot length, number of leaves, root and tiller, root and shoot dry weights, root/shoot ratio and aerenchyma formation were the same as describe in the experiments in Chapter 2 and 3.

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Table 4.2.1 Treatments of oxygen (aerated; A and stagnant; S) and phosphorus (High P; 200 μM and Low P; 1.6 μM) levels imposed on Australian rice (cv. Amaroo) in different periods.

Treatments	Time in treatments (week)	
	1-2	3-4
AA200	Aerated at high P	Aerated at high P
AA1.6	Aerated at high P	Aerated at low P
AS200	Aerated at high P	Stagnant at high P
AS1.6	Aerated at high P	Stagnant at low P
SS200	Stagnant at high P	Stagnant at high P
SS1.6	Stagnant at high P	Stagnant at low P
SA200	Stagnant at high P	Aerated at high P
SA1.6	Stagnant at high P	Aerated at low P

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4.2.2 Experiment 2: Short term responses of Australian rice to alteration of O_2 and P supplies

During the experiment, minimum/maximum temperatures were 35/42°C and 12 hrs of day lengths. The early physiological and morphological responses of Australian rice root (Amaroo) were examined when transition to stagnant and aerated solution at low P availability. There were three replicates. Six (one plant for each harvest) 14 day-old seedlings were transplanted to each 10 L pot of full strength nutrient solution culture for 14 days before transition. The rice plants were harvested at 14 days in aerated high P supply nutrient solution culture (before transition to treatments), 1, 2 and 8 days after transition to stagnant and aerated at 1.6 (low P) and 200 (high P) μM. Maximum root length, number of leave, root and tiller, porosity of adventitious root (percentage of gas space per volume), root and shoot dry weights, root/shoot ratio, radial oxygen loss (ROL) from the adventitious root and nutrient accumulation were assessed.

Root porosity was measured from whole root system of one plant which was cut into 50 mm segments. Porosity (percentage of gas spaces per unit tissue volume) of roots from each plant was evaluated by measuring root buoyancy before and after vacuum infiltration of the gas spaces in the roots with water. ROL was measured for two replicate plants (an adventitious root on an individual plant was one replicate). Intact 101-127 mm adventitious roots were selected for ROL measurements in an O_2-free root medium using cylindrical root-sleeving O_2 electrodes in a temperature controlled room (30°C) with photosynthetically active radiation at shoot height of 150 μmol m^{-2} s^{-1}. Root systems of intact plants were immersed in a transparent chamber (50 x 50 x 250 mm; breadth x width x height) of 0.1 % (w/v) agar solution with 5.0

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mol m^{-3} KCl and 0.5 mol m^{-3} $CaSO_4$. The shoots were in air and the stem base was held with wet cotton wool in a rubber lid sealed onto the top of the chamber. An intact adventitious root was selected and gently guided through a root-sleeving O_2 electrode (height 5 mm; internal diameter 2.25 mm) with guides to keep the root located towards the centre of the cylindrical electrode. ROL measurements were taken at 10, 20, 30, 40, 50 and 70 mm behind the root tip. Root diameters at these positions along the roots were measured using a microscope with a calibrated eye-piece reticule. Plant growth measurement was the same as describe in the experiments in Chapter 2 and 3.

4.2.3 Experiment 3: Confirmed the responses of Australian rice to alteration of O_2 and P supplies

During the experiment, minimum/maximum temperatures were 35/42°C and 12 hrs of day lengths. ROL and root respiration rates of Australian rice (Amaroo) were measured when transition to stagnant and aerated solution at low P supply. There were three replicates. Six (one plant for each harvest) 14 day-old seedlings were transplanted to each 10 L pot of full strength nutrient solution culture for 14 days before transition. The rice plants were harvested at 14 days in aerated with high P supply nutrient solution culture (before transition to treatments), 1, 2 and 8 days after transition to stagnant and aerated at 1.6 (low P) and 200 (high P) μM. Porosity of adventitious root (% of gas space per volume), root and shoot dry weights, root/shoot ratio, radial oxygen loss (ROL) from the adventitious root, root respiration rate and the autofluoresced barrier cell in root tissue were measured.

Root respiration rate were measured as root oxygen consumption. Roots in aerated solution culture at both high and low P supply were measured for oxygen

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consumption rates. Adventitious roots were separated into the 0-20 mm tip and 20-40 mm zone from the tip. The 20-40 mm zone contains expanded cells, whereas the 20 mm tip contains expanding and dividing cells, as well as some expanded cells. Locations further behind the tip were not used as lateral roots are present and/or the outer cell layers can have low permeability to oxygen, both situations would complicate interpretations of the data from these measurements. Each zone of root was cut into 5 mm segments for the measurements which lasted at most 30 min; small root segments minimize the influence of boundary layers. The 5 mm root segments were placed in a sealed cuvette of known volume which contained air-saturated treatment solutions (composition as used in the growth medium) with rapid mixing via a magnetic stir-bar. The rate of oxygen consumption was measured at 30°C using the polarographic technique and equipment as described in Lambers *et al.* (1993). Plant growth, root porosity, ROL and autofluoresced barrier cell in root tissue measurement was the same as describe in the previous experiment.

4.2.4 Statistical analyses

Data on root and shoot growth, root porosity, ROL, root oxygen consumption rate and tissue P were analyzed using linear models as general analyses of variance (ANOVA) to determine the main effects and interactions among treatments. Effects of treatments at each harvest time were analyzed as separate experiments. Means were compared using Least Significant Differences (L.S.D.) at P = 0.05.

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4.3 Results

4.3.1 Experiment 1: Australian rice in alteration of O_2 and P supplies in solution culture

At first week, plants in both of aerated and stagnant nutrient solution at 200 μM P supply were not different on growth. They started to differ in root numbers, plant in stagnant culture had more roots resulted in the higher root/shoot ratio by two weeks, similar in photosynthesis efficiency (Table 4.3.1.1). Root porosity (%) was increased in stagnant solution culture, consistent with aerenchyma formation (Table 4.3.1.2). Aerenchyma formation in root of plant grown in stagnant culture was increased at 50 mm from the tip and dramatically increased at 20 mm from the root base. One week after transition, the maximum root length of aerated plants was increased when transition to aerated solution at low P supply (AA1.6), but it was 30 % decreased when aerated plants were transferred to stagnant at low P (AS1.6). Whereas, the transition from plant in stagnant culture to aerated culture increased the maximum root length, especially when transferred to aerated at low P supply (SA1.6), the increase was 30 % (Table 4.3.1.3). Root/shoot ratio was the lowest for plants kept in aerated culture in low and adequate P (AA1.6 and AA200), while the highest of root/shoot ratio was in plants in stagnant that were transfer to low P supply (SS1.6). Root/shoot ratio was increased in the period after transfer on most plants in stagnant culture regardless of whether they were transferred from aerated or stagnant culture. Photosynthesis efficiency was depressed by transition to low P supply, while stagnant culture promoted the P effect on photosynthesis efficiency. Most plants in aerated culture were enhanced on the photosynthesis efficiency, the same as plant in stagnant at high P supply (SS200) (Table 4.3.1.3). The aerenchyma formation was increased

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when grown in stagnant in both high and low P supply (SS200 and SS1.6) for the aerenchyma formation at 20 and also in stagnant in both high and low P supply (SS200 and AS1.6) for aerenchyma formation at 50 mm form the root tip (Table 4.3.1.4). The changes, however were not reflected in corresponding changes in root porosity. After transition for two weeks, the aerated culture in both high and low P supply clearly increased the maximum root length to 73.2 cm and 74.8 cm compared with 56.2 cm and 64.5 cm in plants that were kept in stagnant culture (Table 4.3.1.5). Phosphorus deficiency in aerated solution culture promoted maximum shoot length by 17 – 25 % higher in SA1.6 and 10 - 20 % higher in AA1.6 conditions. In contrast, number of leaves per plant was increased when grown in aerated at high P supply (AA200). These resulted in increase in shoot dry weight of plant in aerated at both high or low P supply (AA200, AA1.6, SA200 and SA1.6), while shoot dry weight of plant in stagnant at low P supply solution culture (SS1.6) was dramatically depressed by 57 % compared with plants in aerated at high P supply culture (AA200) (Table 4.3.1.6). Although, root dry weight was not affected by condition of solution culture but root/shoot ratio was increased when plant grown in stagnant at low P supply (SS1.6) and was decreased when plant grown in aerated at high P supply solution culture, whereas root/shoot ratio of plants in other conditions was intermediate between aerated at high P supply and stagnant at low P supply conditions (Table 4.3.1.6). Photosynthesis efficiency of plants in aerated at both low and high P supply was enhanced by 11 – 22 % higher than photosynthesis efficiency of plant in all stagnant solution cultures (Table 4.3.1.7). Root porosity (%) of plant in all nutrient solution conditions was not different, while the aerenchyma formation (%) adventitious root was only appeared at 50 mm from the root tip (Table 4.3.1.7).

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Plants in all stagnant nutrient solution cultures developed 23 – 54 % more aerenchyma at 50 mm from the tip than plant in aerated solution culture at both high and low P supply.

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Table 4.3.1.1 The number of adventitious roots per plant and root/shoot ratio of Amaroo when grown in aerated and stagnant nutrient solution culture at high P supply for two weeks.

Conditions	Root numbers plant^{-1}	Root/Shoot Ratio	Photosynthesis rate (μmol m^2 s^{-1})
Aerated at high P	24.0 B	0.24 B	4.20 B
Stagnant at high P	35.3 A	0.34 A	11.85 A
F-test	Con *	Con *	Con *
$LSD_{(P<0.05)}$	3.8	0.09	3.80

* significant at P<0.05. Con indicates F-test for culture condition effect. The difference between culture conditions in the same column is indicated by upper case letters.

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Table 4.3.1.2 Root porosity (%) of a whole root system and aerenchyma formation (%) at 50 mm from the root tip and 20 mm from the root base of Amaroo when grown in aerated and stagnant nutrient solution culture at high P supply for two weeks.

Conditions	Root porosity (%)	Aerenchyma formation (%)	
		50 mm from tip	20 mm from root base
Aerated at high P	18.57 B	26.67 B	32.67 B
Stagnant at high P	24.90 A	55.00 A	85.00 A
F-test	Con *	Con *	Con *
$LSD_{(P<0.05)}$	6.06	18.97	29.00

* significant at $P<0.05$. Con indicates F-test for culture condition effect. The difference between culture conditions in the same column is indicated by upper case letters.

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Table 4.3.1.3 The maximum root length (cm), root/shoot ratio and photosynthesis rate (μmol m^2 s^{-1}) of Amaroo after transferred from aerated or stagnant nutrient solution culture at high P supply to aerated and stagnant nutrient solution culture at high or low P supply for one week.

Conditions	Maximum root length (cm)	Root/Shoot Ratio	Photosynthesis rate (μmol m^2 s^{-1})
AA 200	26.8 C	0.27 E	26.85 A
AA 1.6	31.2 AB	0.29 DE	25.46 AB
AS 200	23.5 CD	0.30 CDE	22.59 BC
AS 1.6	19.7 D	0.33 BC	19.52 C
SS 200	22.2 D	0.34 B	25.59 AB
SS 1.6	22.7 CD	0.41 A	22.46 BC
SA 200	29.0 BC	0.31 BCD	26.40 AB
SA 1.6	33.2 A	0.33 BC	23.34 ABC
F-test	Con *	Con *	Con *
$LSD_{(P<0.05)}$	4.1	0.04	4.02

* significant at P<0.05. Con indicates F-test for culture condition effect. The difference between culture conditions in the same column is indicated by upper case letters.

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Table 4.3.1.4 Root porosity (%), aerenchyma formation (%) at 20 and 50 mm from root tip of Amaroo after transferred from aerated or stagnant nutrient solution culture at high P supply to aerated and stagnant nutrient solution culture at high or low P supply for one week.

Conditions	Root porosity (%)	Aerenchyma formation (%)	
		20 mm from tip	50 mm from tip
AA 200	22.65	2.67 B	55.00 B
AA 1.6	15.56	0.00 B	45.00 B
AS 200	27.62	0.00 B	58.33 B
AS 1.6	31.02	4.33 B	85.00 A
SS 200	43.23	16.67 AB	80.00 A
SS 1.6	38.98	33.33 A	58.33 B
SA 200	41.20	1.67 B	58.33 B
SA 1.6	46.68	33.33 A	40.00 B
F-test	Con ^{ns}	Con *	Con *
$LSD_{(P<0.05)}$	11.64	19.27	23.84

* significant at $P<0.05$. Con indicates F-test for culture condition effect. The difference between culture conditions in the same column is indicated by upper case letters.

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Table 4.3.1.5 The maximum root and shoot length (cm) and number of leaves per plant of Amaroo after transferred from aerated or stagnant nutrient solution culture at high P supply to aerated and stagnant nutrient solution culture at high or low P supply for two weeks.

Conditions	Maximum root length (cm)	Maximum shoot length (cm)	Number of leaves
AA 200	74.8 A	34.2 D	17.7 A
AA 1.6	73.2 A	42.3 B	14.2 B
AS 200	54.2 C	22.5 F	11.5 CDE
AS 1.6	55.0 C	26.7 E	9.5 E
SS 200	64.5 B	24.2 EF	11.3 CDE
SS 1.6	56.2 C	24.5 EF	10.3 DE
SA 200	73.5 A	38.3 C	14.3 B
SA 1.6	74.8 A	46.2 A	13.0 BC
F-test	Con *	Con *	Con *
$LSD_{(P<0.05)}$	6.0	3.8	2.7

* significant at $P<0.05$. Con indicates F-test for culture condition effect. The difference between culture conditions in the same column is indicated by upper case letters.

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Table 4.3.1.6 Root and shoot dry weight (g plant^{-1}) and root/shoot ratio of Amaroo after transferred from aerated or stagnant nutrient solution culture at high P supply to aerated and stagnant nutrient solution culture at high or low P supply for two weeks.

Conditions	Root dry weight (g plant^{-1})	Shoot dry weight (g plant^{-1})	Root/Shoot Ratio
AA 200	0.315	1.514 A	0.21 B
AA 1.6	0.326	1.246 ABC	0.27 AB
AS 200	0.225	0.762 DE	0.30 AB
AS 1.6	0.256	0.752 DE	0.33 AB
SS 200	0.303	0.922 CD	0.33 AB
SS 1.6	0.253	0.650 E	0.39 A
SA 200	0.295	1.275 AB	0.23 AB
SA 1.6	0.312	1.163 BC	0.27 AB
F-test	Con ^{ns}	Con *	Con *
$LSD_{(P<0.05)}$	-	0.341	0.17

* significant at $P<0.05$. Con indicates F-test for culture condition effect. The difference between culture conditions in the same column is indicated by upper case letters.

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Table 4.3.1.7 Photosynthesis rate (μmol m^2 s^{-1}), root porosity (%) of a whole root system and aerenchyma formation (%) at 50 mm from the root tip of Amaroo after transferred from aerated or stagnant nutrient solution culture at high P supply to aerated and stagnant nutrient solution culture at high or low P supply for two weeks.

Conditions	Photosynthesis rate (μmol m^2 s^{-1})	Root porosity (%)	Aerenchyma formation (%) at 50 mm from tip
AA 200	23.39 AB	30.54	46.67 C
AA 1.6	24.16 A	34.56	50.00 C
AS 200	20.28 BC	35.03	81.67 A
AS 1.6	19.43 C	15.94	76.67 AB
SS 200	20.89 BC	28.90	86.67 A
SS 1.6	20.79 BC	17.37	76.00 A
SA 200	24.81 A	26.76	58.33 BC
SA 1.6	24.77 A	34.89	40.00 C
F-test	Con *	Con ns	Con *
$LSD_{(P<0.05)}$	3.28	-	20.10

* significant at P<0.05. Con indicates F-test for culture condition effect. The difference between culture conditions in the same column is indicated by upper case letters.

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4.3.2 Experiment 2: Short term responses of Australian rice to alteration of O_2 and P supplies

The results of two experiments (experiment 4.2.2 and 4.2.3) were combined, due to the experiment 4.2.3 was confirmed the previously and was provided a total of four replicates for ROL measurements. Overall, plants in stagnant solution had shorter roots than those in aerated solution (Table 4.3.2.1). After four days of treatments, the longest adventitious roots in aerated solution were 7 % longer at low P than at high P. By contrast, in stagnant solution the longest root at low P supply was 8 % shorter than at high P. The differences in adventitious root lengths were more clearly distinguished after 8 days of treatments (Table 4.3.2.1).

Stagnant culture for eight days increased the number of adventitious roots per plant by 12-19 % (Table 4.3.2.2). However, P treatments did not influence the number of adventitious roots per plant in either stagnant or in aerated solutions (Table 4.3.2.2). Tiller numbers were 20-33 % greater in plants in aerated solution compared with those in stagnant solution. Moreover, the tiller numbers were decreased by low P supply under both root oxygen treatments. Likewise, the low oxygen treatment decreased shoot dry weight and low P also resulted in lower shoot dry weights (Table 4.3.2.3). The differential effect of stagnant conditions on root and shoot growth resulted in higher root/shoot ratio in stagnant than in aerated solution (Table 4.3.2.3).

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Porosity of the whole root system increased within eight days after transfer from aerated to stagnant solution, from 23.6 to 28.8 % at low P supply and to 30.6 % at high P supply (Fig. 4.3.2.1). The porosity of roots in aerated solution at low P supply was maintained at 23.6 % and slightly decreased to 21.1 % at high P supply in aerated solution. Transverse sections taken to examine the percentage of root cross-

sectional area occupied by aerenchyma showed that at 20 mm from the tip, aerenchyma formation in the roots of plants grown in stagnant solution was increased by 18 %. Rice roots grown in stagnant solution had twice as much aerenchyma formation at 70 mm than in aerated roots (data not shown). The observed increase in aerenchyma supported the trends of increased root porosity of rice in stagnant culture (Fig. 4.3.2.1).

The relative intensity of autofluorescence indicates the secondary thickened walls or walls impregnated with phenolic compounds or lipid-complexes in the layer of fibre cells, as well as in the hypodermal layer and the layer immediately inside the fibre cells, in response to stagnant solution treatment for eight days (Table 4.3.2.4). In aerated solution, the intensity of autofluorescence of cell walls in these outer cell layers remained unchanged during the eight days of the experiment. The greatest increase in autofluorescence of cell walls in these outer cell layers for roots in stagnant compared with aerated solution was evident in root sections at 70 mm, rather than 20 mm, from the tip (Fig. 4.3.2.2).

Only one day after transfer of aerated roots to stagnant solution, rates of ROL were unchanged at 10 mm behind the root tip, but had decreased markedly to low rates in the more basal positions measured at 20-70 mm behind the tip (Fig. 4.3.2.3). The ROL rates from the roots in stagnant solution were not affected by P treatment. For plants from aerated solution, those at high P supply had higher ROL rates (when transferred to the oxygen-free root medium for the ROL measurements) than the adventitious roots of plants grown in aerated solution at low P supply.

Two days after transfer, the ROL rates at 10 mm behind the tip of roots of plants grown in stagnant culture were higher than for roots of plants grown in aerated

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solution. During the first two days, low P supply decreased ROL rates for roots of plants in aerated culture compared with those with high P, but this difference was no longer present at later times. Furthermore, the ROL rates along the adventitious root axis for all plants gradually decreased with time, to be lowest after eight days (Fig. 4.3.2.3). While ROL rates from the roots on day 8 in aerated culture were decreased five-fold from the first day in treatments, these were still much higher than the ROL from roots of plants in stagnant solution, except at 10 mm behind the tip. The stagnant culture after eight days decreased the ROL rates to almost zero along the basal positions measured at 30 – 70 mm behind the tip, whereas ROL near the tips was still relatively high compared to the earlier times (Fig. 4.3.2.3 d).

Root oxygen consumption rates for root tissues of plants grown in aerated solution at low or high P supply for four days were measured for two zones of the roots (0-20 mm and 20-40 mm zones behind the tip). The 0-20 mm tips had 44-52 % higher oxygen consumption rates on a fresh weight basis than the 20-40 mm segments. Root oxygen consumption rates on a fresh mass basis in both tissue zones were not affected by P supply (Table 4.3.2.5).

Two days after transition to stagnant culture, relative P uptake was decreased by 90 % at low P supply (Fig. 4.3.2.4). At four days, relative P uptake in stagnant plant declined also at high P compared to plants in aerated nutrient solution. Relative P uptake also declined in aerated solution at low P during the last four days in treatments.

Phosphorus content (mgP per plant) of plants after one day did not differ among treatments (Table 4.3.2.6). Thereafter, P content for plants grown in stagnant solution at low P supply declined relative to aerated plants, so that after eight days

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values were 40 % lower than in plants in aerated solution. The low oxygen also affected the P content for plants at high P supply, by halving P content % in stagnant culture. At eight days, the low P treatment decreased by 37 % the P accumulation in plants grown in both aerated or in stagnant solution culture (Table 4.3.2.6).

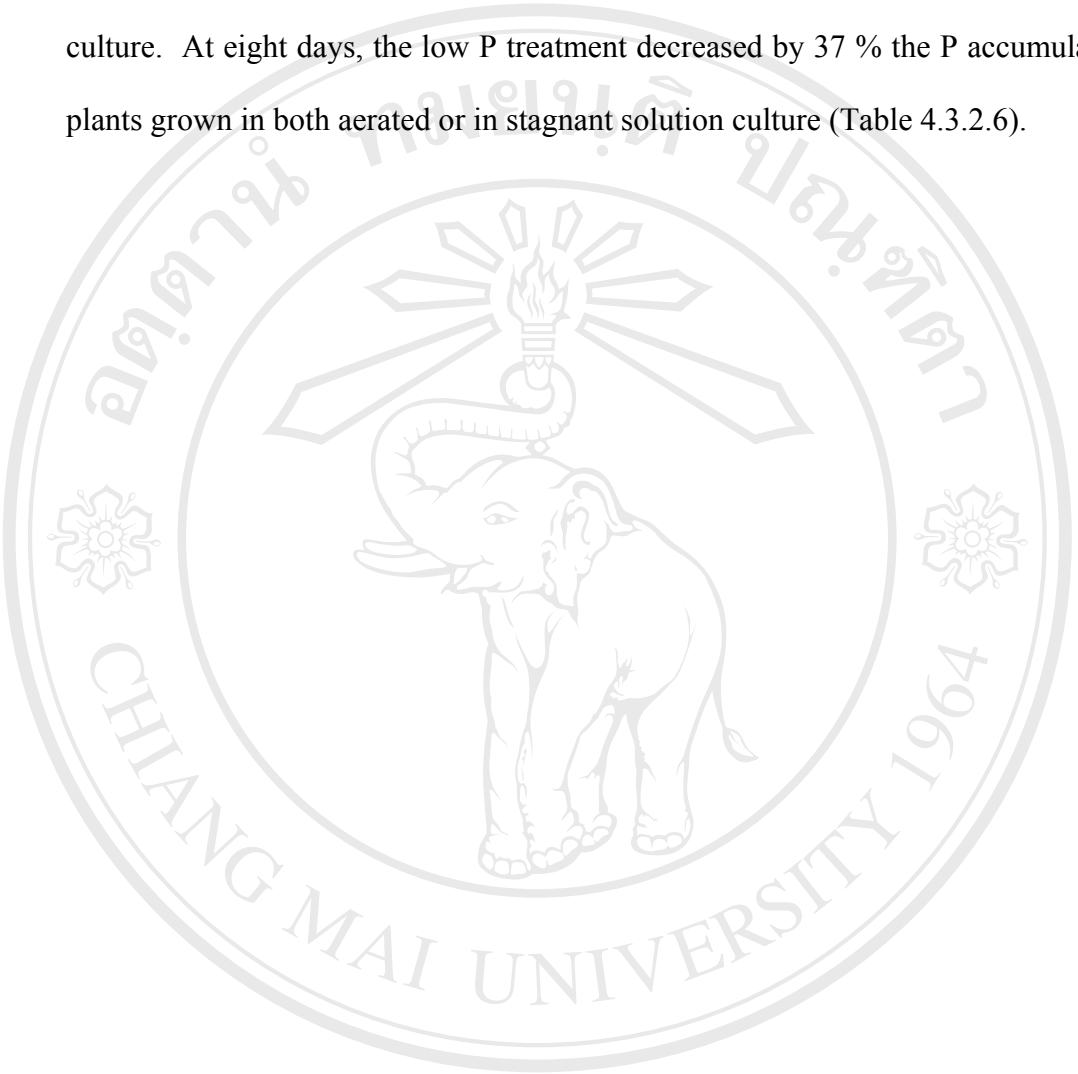

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Table 4.3.2.1 Length of longest roots of rice (cv. Amaroo) grown in aerated solution with high P (200 μM) and then transferred to aerated or stagnant nutrient solution at low (1.6 μM) or high (200 μM) P supply for four and eight days. Values are means of three replicates ± standard errors.

P levels	Culture	Lengths of longest roots (cm)		
		Initial	4 days	8 days
Low P	Aerated	34.8 ± 0.59	40.3 ± 2.17	45.2 ± 1.42
	Stagnant	34.8 ± 0.59	32.7 ± 0.67	32.5 ± 3.87
High P	Aerated	34.8 ± 0.59	37.4 ± 2.42	40.7 ± 4.87
	Stagnant	34.8 ± 0.59	35.6 ± 1.50	37.1 ± 0.95
F-test			O x P*	O x P*
$LSD_{(P<0.05)}$			2.4	2.5

* significant at P< 0.05, O x P indicates F test for solution aeration and P interaction effects.

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Table 4.3.2.2 Adventitious root and tiller numbers of rice (cv. Amaroo) grown in aerated solution with high P (200 μM) level (initial condition) and then transferred to aerated or stagnant nutrient solution at low (1.6 μM) or high (200 μM) P supply for eight days. Values are means of three replicates ± standard errors.

Conditions		Adventitious root numbers (roots/plant)	Tiller numbers (tillers/plant)
Initial		55 ± 2.1	3.9 ± 0.3
Low P	Aerated	142 ± 18.4	10.0 ± 0.9
	Stagnant	161 ± 9.8	6.7 ± 0.6
High P	Aerated	143 ± 24.0	12.0 ± 1.0
	Stagnant	176 ± 7.5	8.0 ± 0.0
F-test		O*	O* and P*
$LSD_{(P<0.05)}$		22.5	0.7 and 0.7

* Significant at P< 0.05, O and P indicates F test for solution aeration and P effects.

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Table 4.3.2.3 Root and shoot growth of rice (cv. Amaroo) in aerated solution with high P (200 μM) supply (initial) and then transferred to aerated or stagnant nutrient solution at low (1.6 μM) or high (200 μM) P supply for eight days. Values are means of three replicates ± standard errors.

Conditions		Root dry weight (g plant^{-1})	Shoot dry weight (g plant^{-1})	Relative Growth Rate (g plant^{-1} day^{-1})	Root/Shoot ratio
Initial		0.17 ± 0.01	0.65 ± 0.03	-	0.26 ± 0.010
Low P	Aerated	0.83 ± 0.03	3.23 ± 0.12	0.159 ± 0.002	0.26 ± 0.001
	Stagnant	0.77 ± 0.09	2.19 ± 0.27	0.137 ± 0.013	0.35 ± 0.002
High P	Aerated	0.80 ± 0.09	3.24 ± 0.30	0.171 ± 0.011	0.25 ± 0.004
	Stagnant	0.91 ± 0.04	2.66 ± 0.03	0.173 ± 0.004	0.34 ± 0.012
F-test		ns	O*	O*	O*
$LSD_{(P<0.05)}$		-	0.35	0.018	0.01

* Significant at P< 0.05, O indicates F test for solution aeration effects.

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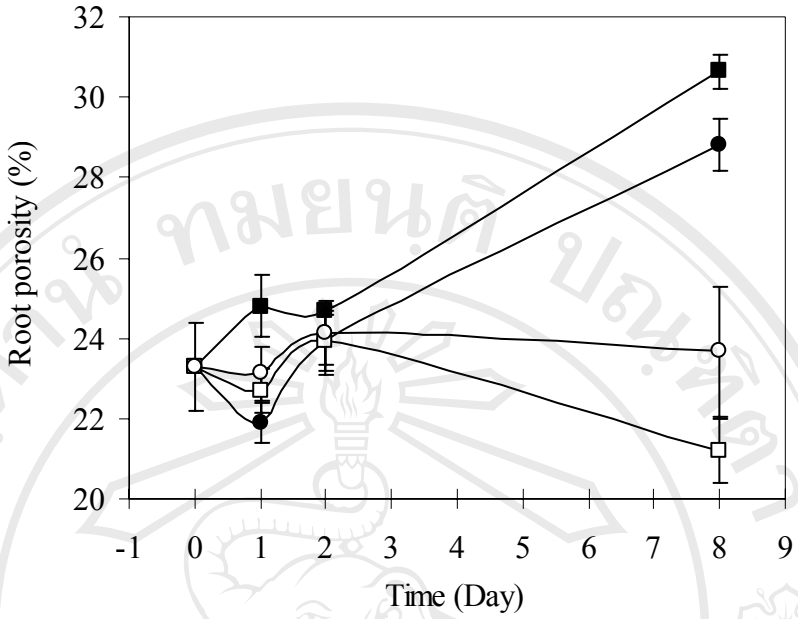

Figure. 4.3.2.1 Root porosity (% gas volume per unit root volume) of rice (cv. Amaroo) grown in aerated solution with high (200 μM) P (HP) then transferred to aerated (open symbols) and stagnant (closed symbols) solutions at 1.6 μM P (○),and 200 μM P (□). Root porosity (%) was measured for the whole root system of each plant. Bars represent standard errors of three replicates.

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Table 4.3.2.4 Score for fluorescence of lignin and/or suberin in the layer of sclerenchymatous fibre cells in rice roots when grown in aerated solution with high P (200 μM) and then transferred to aerated or stagnant nutrient solution at low (1.6 μM) or high (200 μM) P supply for eight days. Values are means of three replicates ± standard errors.

Distance of sections from the root apex (mm)	Score of fluorescence of lignin and/or suberin in outer cell layers in rice roots*			
	Low P		High P	
	Aerated	Stagnant	Aerated	Stagnant
10	1.00 ± 0.00	2.00 ± 0.00	1.00 ± 0.00	1.67 ± 0.33
20	1.33 ± 0.33	1.50 ± 0.41	1.33 ± 0.33	1.67 ± 0.33
70	2.00 ± 0.00	2.00 ± 0.00	1.33 ± 0.33	2.00 ± 0.00

* The thickness of cell walls in the layer of sclerenchymatous fiber was scored on a 3 point scale: 0, when no fluorescence was detected in the cell wall; 1, when the thickness of fluorescence was equal to wall thickness of the cell layers on either side of it and; 2, when the thickness of fluorescence was greater than cell wall thickness of the cell layers on either side of it.

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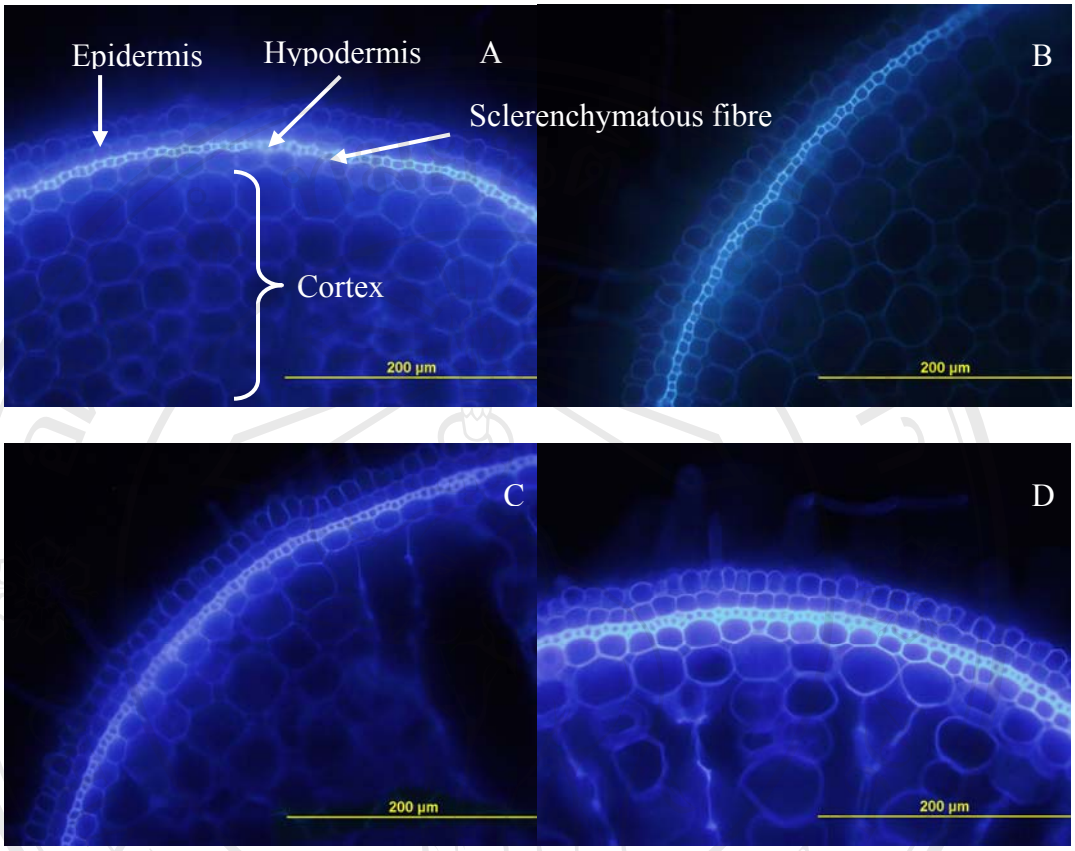

Figure. 4.3.2.2 Typical transverse sections of rice roots showing autofluorescence of the walls in the outer cell layers. Plants were grown for eight days after transfer to high P level (200 µM) in aerated nutrient solution at 20 mm (A) and 70 mm (B) from the apex or in stagnant nutrient solution at 20 mm (C) and 70 mm (D). Increased autofluorescence in response to the stagnant treatment was evident for the walls of the sclerenchymatous fibres, as well as those in the hypodermal cell layer and the cells to the immediate interior of the fibre cells. Average scoring of sections in triplicate is shown in Table 4.3.2.4.

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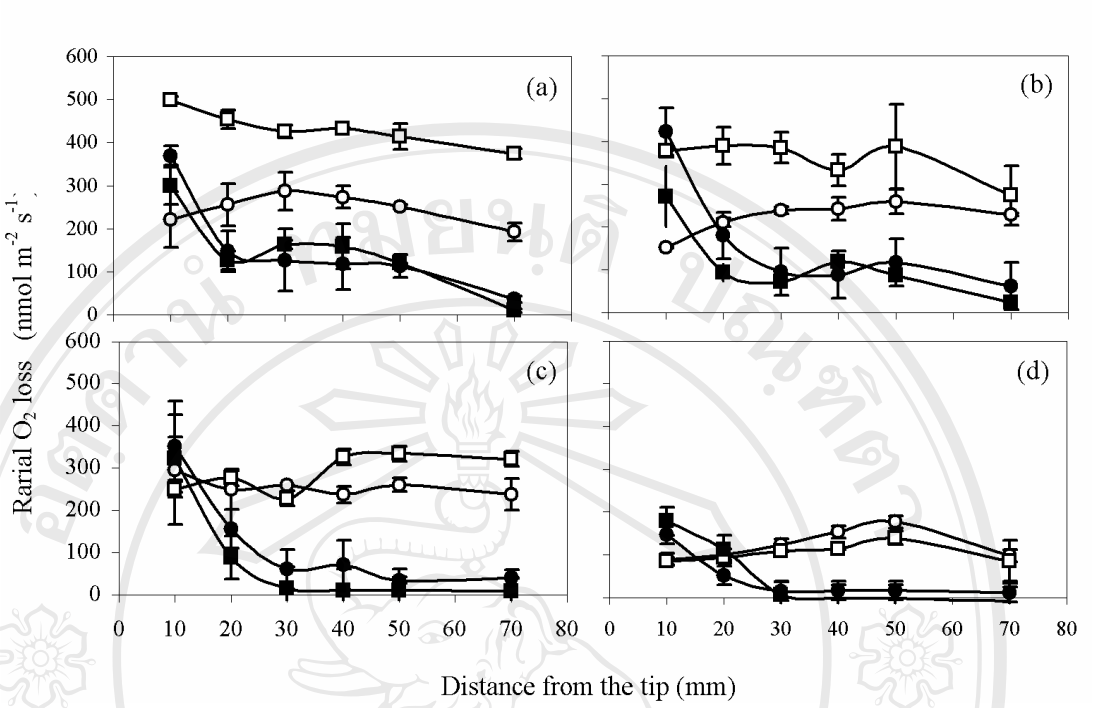

Figure. 4.3.2.3 Rate of radial O_2 loss (ROL) along adventitious roots of rice (cv. Amaroo) after transition to treatment solutions; aerated at 1.6 µM P (○), and 200 µM P (□) and stagnant at 1.6 µM P (●) and 200 µM P (■). Rates of ROL were measured from one 101-127 mm adventitious root of each of four 28 d-old plants in each treatment, with the treatment imposed for 1 (a), 2 (b), 4 (c) or 8 (d) days. Bars represent standard errors of four replicates.

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Table 4.3.2.5 O_2 consumption rate (n moles O_2 g^{-1} Fresh Weight s^{-1}) of rice (cv. Amaroo) roots grown in aerated solution with high P (200 μM) and then transferred to aerated or stagnant nutrient solution at low (1.6 μM) or high (200 μM) P supply for four days. Roots were cut into 5 mm segments for each zone and measured in the same solution as the growth medium. Values are means of four replicates ± standard errors.

Part of root	O2 consumption rate (n moles O2 g-1 FW s-1)	
	1.6 μM P	200μM P
0-20 mm tip	4.67 ± 0.75	5.22 ± 0.47
20-40 mm mature zone	2.61 ± 0.02	2.50 ± 0.30

Significant at P< 0.05, for a part of root, 0.97. FW; Fresh weight.

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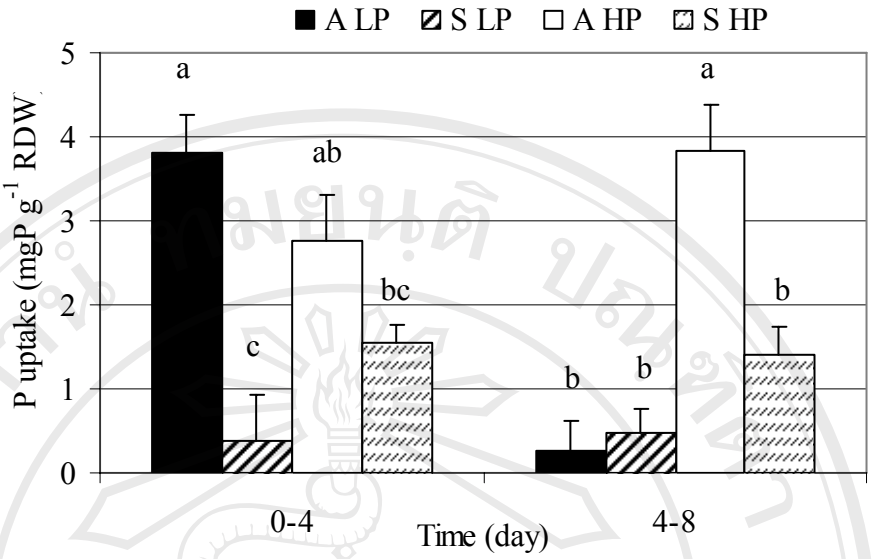

Figure. 4.3.2.4 Phosphorus uptake of rice (mg P g^{-1} root dry weight) after transfer to treatment at 0-4 days and 4-8 days; aerated at 1.6 μM P (A LP), and 200 μM P (A HP) and stagnant at 1.6 μM P (S LP) and 200 μM P (S HP) nutrient solution culture. Plants were 28 d-old (initial) and the uptake was calculated as the final P content minus the initial P content. Bars represent means + standard errors of three replicates. Different letters indicate significant differences at 5% level.

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Table 4.3.2.6 Phosphorus content in whole rice plants (mg plant^{-1}) grown in aerated solution with high P (200 μM) and then transferred to aerated or stagnant nutrient solution at low (1.6 μM) or high P supply for 1, 2, 4 and 8 days. Values are means of three replicates ± standard errors.

Time (day after transition)	Phosphorus content (mg per plant)				F-test
	Low P		High P		
	Aerated	Stagnant	Aerated	Stagnant	
Initial	0.62 ± 0.03	0.62 ± 0.03	0.62 ± 0.03	0.62 ± 0.03	ns
1	0.66 ± 0.07	0.66 ± 0.06	0.66 ± 0.09	0.62 ± 0.06	ns
2	0.86 ± 0.06	0.65 ± 0.05	0.81 ± 0.07	0.93 ± 0.01	O x P*
4	1.28 ± 0.08	0.69 ± 0.10	1.10 ± 0.12	0.89 ± 0.04	O x P*
8	1.36 ± 0.04	0.82 ± 0.11	2.16 ± 0.12	1.31 ± 0.06	O* / P*

* Significant at P< 0.05, O, P and O x P indicate F test for solution aeration, P and solution aeration and P interaction effects. ns indicates no significant difference. $LSD_{(P<0.05)}$ for solution aeration and P interaction effects on phosphorus content in whole plant were as follows: two days after transition, 0.13; four days after transition, 0.28; for solution aeration effects on phosphorus content in whole plant at eight days after transition, 0.19.

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4.4 Discussion

The responses of plants in the prolonged of aerated and stagnant nutrient solution were studied in the winter (15 - 25 °C) resulted in no different in the growth of plant in first week. However, at two weeks, Australian rice; Amaroo cultivar similarly respond to stagnant culture the same as Thai rice cultivar in previous studies in Thailand and other reported as increasing root production, root porosity and aerenchyma formation(Colmer *et al.*, 1998; Colmer, 2003a). The transition plant to adverse conditions both of oxygen and phosphorus levels for one week dramatically affected the elongation of root, especially plant in low P supply. The increase in root growth was the nutrient acquisitive mechanism of plant by increase root surface areas and penetrate to nutrient source at deep soil (Kirk and Du, 1997; Lu *et al.*, 1999; Kirk, 2003), resulted in increase in root/shoot ratio, especially plant in stagnant at low P supply which faced with both of oxygen and phosphorus stress (Kirk and Du, 1997; Lu *et al.*, 1999). The influence of phosphorus stress also reduced the photosynthesis pathway, it may resulted from phosphorus is the important element as component of high energy substrate (ATP) and sugar phosphate substrates (Marschner, 1995). The effect of phosphorus stress was enhanced in stagnant nutrient solution, due to phosphate was fixed with agar and reducing in convection of nutrient solution (Wiengweera *et al.*, 1997). Therefore, plant growth in stagnant at low P supply was more retardation.

In experiment 4.2.2 and 4.2.3 was determined the early responses of rice within only eight days in treatments. Whereas, the roots of plants grown in aerated solution had a porosity within the range found previously in 12 rice cultivars (20 – 26 %) grown in aerated solution culture for 35 days (Colmer, 2003b), and had high rates

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of ROL from basal zones when measured in an oxygen-free medium. Before transfer into stagnant solution, plants had been grown in aerated solution until early tillering (average 3.9 tillers per plant). After roots were exposed to stagnant culture for only one day, ROL declined markedly in basal zones. However, near the tips of the roots, ROL did not decline after transfer to stagnant solution, indicating that oxygen supply was not limiting to the distal portions of these roots. The decreasing ROL rates from basal zones of roots of rice, and many other plant species, have been interpreted in terms of a physical barrier to restrain radial oxygen diffusion from the root (Armstrong, 1979; Colmer *et al.*, 1998; McDonald *et al.*, 2002; Colmer, 2003a, b). The barrier can enhance longitudinal oxygen transport via the aerenchyma to the root tip, by reducing losses to the rhizosphere. The rapid decline in ROL from basal zones preceded the increase in aerenchyma development for plants in stagnant culture. Root porosity was only slightly changed after two days in stagnant culture, but after 4-8 days more aerenchyma formation in roots was evident as an increase in the porosity (Fig. 4.3.2.1). This study is the first to demonstrate that the barrier to ROL can be induced in existing roots, an important addition to earlier work that showed induction of the barrier in roots that had formed in a stagnant medium (Colmer *et al.*, 1998; Colmer, 2003b).

Roots of rice contain a layer of sclerenchymatous fibre cells (with thickened secondary walls) on the outer side of the cortex, that becomes the exodermis (i.e. outer-most layer) when the two outer cell layers (epidermis and hypodermis) are lost, at least in soil-grown roots (Clark and Harris, 1981). Cross-sections of roots taken immediately before treatments were imposed, showed autofluorescence of cell walls in this layer was evident at 70 mm from the apex, whereas at 10 and 20 mm the walls

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of the cells destined to become the layer of sclerenchymatous fibres exhibited very little autofluorescence (Table 4.3.2.4). Autofluorescence in the cell walls in this layer increased after eight days in roots that had been in stagnant solution, showing impregnated with phenolic compounds or lipid-complexes (e.g. lignin and/or suberin or other phenolic compounds) in these walls. The walls of cells in the hypodermal layer (layer immediately exterior to the fibres) and those cells in the layer to the immediate interior of the fibres, also showed increased autofluorescence, again indicating impregnation of materials also into these walls. Whether the barrier to the ROL relates directly to the intensity of autofluorescence in the layer of sclerenchymatous fibre cells, or to that for the adjacent cell layers, requires further work using more specific histochemical stains, to assess anatomical changes in these layers within the same 2 days time frame after transfer to stagnant solution when ROL had already decreased.

Root acclimation to oxygen deficiency was reflected in changes in root morphology, but not until four days after transfer to stagnant solution. The morphological responses, which included decreases in root elongation and new adventitious root production and increased root porosity, occurred too late to explain changes in ROL at day 1-2; nevertheless, these changes might be significant for functioning of the root system in the longer term. The increased root porosity is essential for oxygen transport to tips of roots in anoxic media, especially as roots extend deeper into an oxygen-free medium (Armstrong, 1979). New adventitious roots help to offset the decreased root depth (restricted by the distance that internal oxygen can diffuse along roots; Armstrong, 1979) by adding to total root length and surface area for water and nutrient absorption. These new roots are therefore

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presumed to be of importance for longer term growth of rice in waterlogged soil. Even when these new roots form a barrier to ROL in basal parts of the main axis, water (Ranathunge *et al.*, 2004) and nutrient (Rubinigg *et al.*, 2002) uptake should still proceed near the root tips, and presumably even more so if lateral roots are formed (Kirk, 2003).

After only one day at low P in aerated solution, ROL rates at all positions measured along roots were decreased (plants transferred into an oxygen-free root medium for measurements), but only temporarily for the first two days and then the P effect disappeared (Fig. 4.3.2.3). The disappearance of the P effect on ROL rate may result from changes in sinks for oxygen in the root due to P deficiency. It is not expected to be due to the formation of a barrier to ROL, since the roots were aerated. Similarly, increased porosity in low P roots of aerated solution should have decreased the oxygen consumption in the cortex for respiration (Fan *et al.*, 2003), as well as enhanced internal oxygen transport due to the decreased physical resistance to gas diffusion (Armstrong, 1979). Both the decreased resistance and lower respiratory consumption of oxygen would be expected to increase oxygen supply to individual roots. However, on a whole-plant basis, low P also increased root/shoot ratio in aerated solution which may have increased the overall requirement for oxygen in root respiration from day 4 onwards.

Oxygen deficiency depressed P uptake in first four days and keep depressed for the last four days at both low and high P supply (Fig. 4.3.2.4). By contrast, in aerated solution P uptake remain increased until day 8 but there was a sharp decline at low P. One possible cause of the drop in the P uptake at low P in stagnant solution is the formation of a P depletion zone around the roots. Depletion of P in the

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rhizosphere is well known in soil (Kirk and Saleque, 1995) due to P uptake by the root and slow diffusion of P to the root surface. Stagnant solution culture is designed to minimize convective movement of solution. Wiengweera *et al*. (1997) also reported that stagnant solution can limit nutrient uptake by wheat due to the formation of depletion zones. Such depletion zones would be expected to develop more quickly at low P concentrations and this is consistent with the earlier decline in the P uptake at low P than at high P for plants in stagnant solution in the present study. A second possibility for declining P uptake is the formation of the barrier to ROL: the barrier to ROL has been suggested by several authors to decrease nutrient uptake (reviewed in Colmer, 2003a, although conversely other authors have suggested such effects might not occur, see Rubinigg *et al*., 2002). Moreover, Kirk (2003) argued that nutrient uptake by rice in an anoxic medium is mostly by fine lateral roots, and these normally remain permeable to oxygen, except under unusual rhizosphere conditions such as sulphide toxicity (Armstrong and Armstrong, 2005). Nevertheless, we consider that depletion zones adjacent to roots in stagnant agar probably were the major cause of decreased P uptake.

Radial oxygen loss (ROL) in stagnant solution does not fully simulate processes affecting P uptake that occur in soil. In soil, ROL causes oxidation of ferrous iron which acidifies the rhizosphere and increases the pool of plant available P (Kirk and Saleque, 1995). Hence, in anoxic soils, ROL might increase solubilization of soil P in the rhizosphere. Stagnant solution contains no pool of P that can be solubilised by rhizosphere acidification.

Root morphological changes can be important acclimations to improve P uptake under low P supply in anoxic condition (Kirk and Du, 1997). In the present

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study, the morphological changes in roots under low P included reduced root elongation and increased number of adventitious roots, but these changes were observed at four to eight days and hence followed the decrease in P uptake in stagnant solution. The morphological changes in roots will presumably be significant for the longer term adaption of rice to P acquisition in low P anoxic soil. Fine lateral roots on adventitious roots are considered the dominant surface for nutrient uptake (Kirk, 2003). Lorenzen *et al*. (2001) found the plants in low oxygen solution had reduced root length at low oxygen which they suggested may have a negative effect on nutrient acquisition. However, increased numbers of adventitious roots under low P may offset the decrease in maximum root length under anoxic conditions, hence increased numbers of adventitious roots should help to maintain or increase root surface area which is important for P uptake under low P condition (Wissuwa, 2003).

Based on the results of Fan *et al.* (2003) with maize and bean, we expected that low P in stagnant solution would increase root porosity. However, in the present study, P had no consistent effect on root porosity. Rice differs from dry land crops such as maize and bean in that it has high root porosity even under aerated conditions. Hence, the benefits of lower respiratory costs in roots has already been captured by aerated rice and may explain why low P failed to further increase root porosity in rice roots under stagnant solution. Moreover, Armstrong (1979) clearly showed that most benefit from increased root porosity is derived from the reduced resistance to internal oxygen diffusion, with the decreased demand for oxygen a secondary benefit. The present study also examined root porosity over a short period of 8 days compared to 12 to 42 days as reported by Fan *et al*.(2003). Indeed, Lu *et al*. (1999) found no effect of low P on root porosity in three rice cultivars at 14 days and an increase in root

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porosity for two out of three cultivars at 28 days. Hence it would appear that increased aerenchyma in rice roots under low P supply is a longer term acclimation of plants, or possibly a secondary response, to P deficiency.

In summary, only one day after transfer to stagnant conditions, rice roots had apparently formed a barrier to radial oxygen loss (ROL) which decreased ROL by 90 % in sub-apical root zones, but not at the root tip. After four days in stagnant conditions, plants also showed increases in numbers of adventitious roots, root dry mass and root/shoot ratio. By contrast, maximum root length and shoot growth were decreased in stagnant conditions. Only four days after transition to stagnant condition, P uptake declined, especially at low P supply. In aerated solutions, roots increased P uptake until eight days, before uptake at low P supply decreased to the same levels as in stagnant solution at day 8. The effect of P deficiency was more severe on rice growth and P uptake of plants in stagnant than in aerated nutrient solution culture. In conclusion, roots responded rapidly to oxygen deficiency with decreased ROL in sub-apical root zones, at least two days before any change in root porosity or morphology was evident. Phosphorus uptake also decreased under oxygen deficiency, showing that a sudden decline in root-zone oxygen adversely affects P nutrition of rice.

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