Chapter 6

General Discussion

Waterlogged soils depress root aeration. Rice acclimates to waterlogging by changes in both of root morphology and physiology. It has been suggested that these changes in roots depress the nutrient uptake efficiency and there is chear evidence of reduced root length. However, in chapter 2 rice in waterlogged soil generally grew better than in drained soil. Rice adapts to oxygen deficient condition by developing aerenchyma to facilitate internal transport of oxygen from the shoot atmosphere to the root tips (Justin and Armstrong, 1987; Drew *et al.*, 1994; Drew, 1997; Aschi-Smiti *et al.*, 2003; Colmer, 2003a). On the other hand, waterlogged soil helps to reduce weed competition, provides a favourable pH and microbiological environment for rice root, and increases nutrient availability. This study found that nutrient content (N, P, K) in rice plant was generally increased when grown in waterlogged soil. These may reflect the fact that in waterlogged soil, nitrogen as ammonium form is used by rice, besides soil reduction also increases the availability of phosphate (Ponnamperuma, 1972) to enhance rice growth. In addition, roots of rice acclimate to growth in waterlogged condition by increasing the number of adventitious roots per plant, which presumably also contributes to waterlogging tolerance (Colmer, 2003b), and it increased root surface area for nutrient uptake (Kirk and Du, 1997).

Aerated or drained soil had greater oxygen supply for rice roots but unfavorable chemical properties for rice such as low availability of nitrogen, phosphorus and iron (Ponnamperuma, 1975). Nutrient flow to rice roots in drained soil is impaired because of low moisture supply. In this study low moisture in drained soil depressed root elongation of two rainfed cultivars and one upland cultivar. By contrast, the previous reports indicated that rice plants in drained soil had greater root length which enhanced water and nutrient uptake at depth in soils (Morita and Abe, 1996). Deep root activity in aerobic soil is supported by oxygen uptake directly from the air-filled soil pores. Moreover, some rice in aerated soil increased P nutrient uptake by symbiotic associations with mycorrhizal fulgi.

The above studies related to experiments where roots were either exposed to aerated or waterlogged soils. However, in the rainfed lowland ecosystems rice roots grow in changing water regimes in soil. In soil culture the study of responses of intact roots to aerobic and anaerobic conditions is rather difficult and complicated. Moreover, in soil culture one cannot control the required condition such as oxygen levels, nutrient available, soil pH, microbe activity and toxic substrances. Therefore, nutrient solution culture was developed and for rice growth to determine the responses of rice shoots and root to the changing of oxygen and nutrient supply to simulate the changes in soil in the rainfed lowlands.

This study examined rice responses to aerobic and anaerobic root conditions when grown in aerated and stagnant nutrient solution cultures Aerated culture with air bubbling throughout in solution simulated the aerobic or well drained soil condition. Stagnant culture was prepared by mixing with 0.1 % agar in nutrient solution to simulated the anaerobic condition, which restricted atmospheric oxygen diffusion and convection in the solution (Wiengweera *et al.*, 1997). Nutrient solution culture facilitated environmental control and determination rice root responses. The nutrient composition for rice in both aerated and stagnant culture in this experiment was based on Yoshida (1979) as modified by Mcdonald *et al*. (2001b). However, rice in aerated culture in preliminary experiments developed iron deficiency symptoms on leaves at the seedling stage. Moreover, iron plaque formed on the root surface and iron precipates at the bottom. Therefore, the iron supply as Fe EDTA form was increased from 50 µM (McDonald *et al.*, 2001a) up to 100 µM. Rice plants were healthy at 100 µM of Fe EDTA, although the iron plaque and precipitation were still present. In previous research it was found that iron plaque ($Fe(OH)_3$) outside roots increased phosphorus absorption from the growth medium, but higher phosphorus concentration in shoot corresponded with amounts of iron plaque (Zhang *et al.*, 1999b). Rice growth with other iron forms ($FeSO_4$ and $FeCl_2$) and higher supply (200 µM) were tested and compared with Fe EDTA at 100 µM. Fe EDTA was deservedly used due to as it led to less precipitation of Fe oxides and slow release of the iron available form for rice uptake. Iron supply at 100 and 200 µM were little or no different on rice growth enhancement. Therefore, nutrient solution culture conditions for rice growth were optimized by using aerated solution with continuous bubbling for aerobic conditions, pr 0.1 % agar mixed (Wiengweera *et al.*, 1997) for anaerobic condition, both with Fe EDTA at 100 µM. Moreover, the varied P availability when the water regime changing (Snyder, 2002) was also considered and determined the responses of rice to limited P supply.

Responses of rice to well drained and waterlogged soils were different from rice in aerated and stagnant nutrient solution cultures. Rice grown in aerated solution was greater in dry matter than in stagnant culture. Rice growth in stagnant culture was depressed by oxygen deficient supply for root and suffered more under limited P supply. Roots changed in both physiology and morphology during acclimation to low concentrations of oxygen and P supply. Prolonged stagnant culture presented the clear increase in root porosity, which it was corresponded with aerenchyma formation in roots. Dramatic changes of rice roots developed within 2-4 days when they were transferred to anaerobic conditions. Root may suffer under stress conditions, especially under limited P supply and stagnant culture which it was oxygen deficient condition. Rice roots excessively increased root elongation under limited P supply for P acquisition at depth, which it was consistent with the previous reports (Kirk and Du, 1997; Wissuwa, 2003). Moreover, root elongation under limited P supply was enhanced when plant grown in aerated condition resulted from adequate oxygen maintained aerobic respiration and energy production of root, hence roots had more energy to provide the elongation. Plants in stagnant transferred to aerated at low P supply similarly increased root elongation as plants in preceding aerated culture. In contrast, transferred plant to stagnant culture was depressed by oxygen deficiency, hence aerenchyma in root were developed for oxygen transport to root tip. Moreover, Kirk and Du (1997) and Fan *et al*.(2003) suggested that the aerenchyma development by the degradation of the root cortex reduced the respiratory oxygen demand and reduced root phosphorus requirement. Numerous aerenchymatous roots production by plant in stagnant culture provided the adequate oxygen supply instead of increasing root elongation. However, increasing root surface area by new adventitious roots production also increased nutrient uptake, although these root was low efficiency. Due to aerenchymatous roots were reduced symplastic transport pathway in cortex, besides the barrier of radial oxygen loss were formed for oxygen preserve in root (Colmer *et al.*, 1998; Colmer, 2003b) and it may impeded nutrient (Rubinigg *et al.*, 2002; Colmer, 2003a, b; Fan *et al.*, 2003) and water (Ranathunge *et al.*, 2003; Ranathunge *et al.*, 2004) uptake. However, Fan *et al.* (2003) suggested that aerenchyma formation might reduced opportunity for mycorrhizal colonization, decreased resistance to the longitudinal spread or pathogens and decreased capacity for metabolic storage. Changing in morphology of root was clearly acclimated when grown in prolonged condition for two weeks. Furthermore, the shorterm responses of rice after transferred to limited P condition was evident within eight days. Apparently, physiological responses of rice to limited P supply in solution culture were presented in only 1-2 days after transition by reducing in radial oxygen loss (ROL) along adventitious roots. In addition, pattern of ROL along rice root in aerated culture was contrast with pattern of ROL in stagnant culture. The different pattern of ROL corresponded with an inducible barrier to ROL, which the basal zones remain permeable to ROL when grown in aerated culture but growth in stagnant culture induced a 'tight' barrier to ROL (Colmer *et al.*, 1998; Colmer, 2003b, a). This study confirmed the inducible barrier to ROL by scanning the root cross section under fluorescent microscope and found the fluoresced cell at sclerenchymatous fibres. Although the barrier to ROL was induced within 2 days but the nutrient uptake was not impeded until after four days, which morphology of root was responded. The root morphological changing such root elongation, aerenchymatous root production influentially contributed nutrient uptake and shoot growth. However, the overall responses of rice to limited P supply in aerated and stagnant cultures were apparently observed when grown in prolonged conditions.

In addition, the adapted ability of rice plant to aerobic or anaerobic conditions was differed by growth stage and cultivar. The water fluctuation in chapter 2 confirmed that lack of water supply in any growth stage, especially at the seedling stage depressed root growth and nutrient uptake, which the effect distinctly presented in tillering and panicle initiation stages. This period is the most important for rice productivity because the number of tillers and root development during this stage determined the success of reproductive stage and yield (De Datta, 1981). Moreover, the cultivar differences in growth under waterlogged and well-drained conditions suggested that wetland rice (CNT1 and KDML105) was generally affected by water fluctuate soil, while upland rice (KN) was maintainable. Apparently, KN formed more aerenchyma than others but it increased root elongation for nutrient acquirement to maintain their growth. However, some adapted character to waterlogging overlapped between upland and lowland.

Responses of different species or genotypes to the same stress were frequently presented in both of different and similar including overlapping. The present study in chapter 5 determined the responses of ten Thai and three Australian rice cultivars to complicated conditions of P stress under aerobic and anaerobic conditions. Apparently, most cultivars correspondingly responded to treatments, however they varied among cultivars in each treatment. For example, some cultivars such as CNT1, RD7 and PCB1 were higher in root number production than others when grown in stagnant at high P but they were lower than NSG19 when grown in aerated at high P. The preference in different conditions of each cultivar may presume that these cultivars had special mechanism for adapt to that condition. Such as, RD7 produced more root numbers than others when grown under limited P condition. Hence, RD7 may had high P uptake efficiency, high P accumulation in plant and retranslocated to root when they faced with low P supply in growth media. By contrast, all cultivars in stagnant at low P supply was severely depressed to the same growth. These conditions consisted both of oxygen and P deficiency until there was no characteristic of any cultivars can be recovered. Moreover, the short term responses of two cultivars (CNT1 and RD7) to limited P supply in aerated and stagnant cultures were confirmed the similar trend as previous but they were different in some characteristics and degree of tolerant. Rice growth in four P levels were strongly confirmed the different responses of rice cultivars and it was more complicated. Amaroo produced more tiller than other cultivars in all phosphorus levels, however tiller numbers of Amaroo at 1.6 µM P were less than other levels. Moreover, Amaroo increased tiller number in aerated solution, whereas Kyeema increased in stagnant culture. Overall responses of rice genotypes in different conditions can be concluded that among rice genotypes may be different, similar or overlapping in responsive characteristics depending on closeness of relation or familiar habitat. However, several responses of some cultivars can not explained due to they may had the individual responsive mechanisms for special condition. Likewise, previos study has demonstrated that graminaceous species had the phytosiderophores which can mobilize Fe^{+3} in soil and transfer to root, hence graminaceous species were higher in iron accumulation in root than dicotyledonous when they were grown in calcareous soil (Zhang *et al.*, 1999a). In addition, Jan and Pettersson (1993) reported that three upland rice cultivars were different in degree of aluminium tolerant, moreover they had the different mechanism to reduce the aluminium concentration in plant. These may assumed that the genotypic variation in responses to environment associated with diverse mechanism.

Conclusion

The main findings of this thesis was to advance understanding of both morphological and physiological responses of rice when transferred from aerobic to anaerobic conditions, which is relevant to the adaption of rice to the rainfed rice ecosystem. These understanding might help agronomist to adjust the fertilization regimes of rice grown in prolonged waterlogged soil or when soil suffer intermittent changes in soil water regimes. Water regimes in rice field should not constrain at depth to expose the oxygen diffusion from atmosphere reach to root. Flowing water also enhanced the diffusion and dissolution of atmospheric oxygen to soil solution. Besides, the different responsive characteristics and degree of waterlogging tolerant of rice genotypes in responses to limited P supply in aerobic and anaerobic conditions may be the key for plant breeders to select the traits that may help to improved and increased the waterlogging tolerant cultivars. However, the responsive characteristics need to research on the mechanism of alternate from aerobic to anaerobic and *vice versa* conditions. That mechanism may be selected and improved for high adaptive efficiency of rainfed rice.

Improvements suggested in nutrient solution composition may help other researchers for their experiments on rice and also other plant species. However, the stagnant nutrient solution conditions need further research. The stagnant solution can limit nutrient uptake 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. Therefore, the depletion zones around rhizosphere in stagnant solution should be eliminated to understand the actual responses as in waterlogged soil.