Chapter 3

Solution Culture: Method development for using stagnant nutrient solution culture

3.1 Introduction

Rice is grown under a range of soil water conditions from well drained to waterlogged soil. Well drained soil has air in soil pores which supplies oxygen for plant respiration by roots and microorganisms in soil. Waterlogged soil has decreased oxygen in soil pores due to displacement by water, and rapid depletion. In irrigated clay soil for example, at 15 cm depth from the soil surface, oxygen in soil declined to close to zero 48 hrs after irrigation (Thongbai *et al.*, 2001). In waterlogged soil, redox potential was lower and soil pH increased to about 6-7, and availability of phosphorus, iron, and manganese were increased (Ponnamperuma, 1972). In contrast, availability of nitrogen as nitrate form and the mineralization of organic matter were decreased. Therefore, rice plants in well drained and waterlogged soils experience profoundly different oxygen supply and nutrient availability in the root zone.

The study of physiological and morphological response of roots to waterlogged and well drained soils is hampered by the difficulty of recovering roots without damaging them, and by the imprecise understanding of soil nutrient regimes. Therefore, nutrient solution culture is commonly used in the study of the impact of nutrient and oxygen availability on roots growth and function. Moreover, solution cultures facilitate the study of transitions between aerobic to anaerobic conditions on

root function. Rubinigg *et al*. (2002) and Malik *et al.* (2003) used solution culture to study root adaptation and uptake efficiency which grown in aerated and then transferred to stagnant condition or vice versa. Moreover, nutrient availability for rice uptake in nutrient solution was examined by Kirk and Du (1997) studied phosphorus uptake of rice in deoxygenated nutrient solution culture. Solution culture is helpful also for detecting the change of nutrient levels or release of protons and organic acids from roots (Kirk and Du, 1997).

Nutrient solution culture or hydroponics is popular in vegetable production. There are many variations of nutrient solution composition such as Yoshida, Kimura B, Hoagland etc (Hoagland and Snyder, 1933; Kimura, 1955; Yoshida, 1976 cited by Prom-u-thai, 2006). For rice the Yoshida formula is most commonly nutrient solution culture with adjustments as necessary to fit the study aims. Generally, the nutrient solution conditions aim to simulate key aspects of soil conditions. Oxygen supply is a most important factor in root growth. In vegetable productions flowering of nutrient solution relies on oxygen dissolved in nutrient solution and this adequately supplies small root system of vegetable plants. However, the supply of dissolved oxygen solution is not sufficient for plants with large or deep root systems. Aerated nutrient solution cultures simulate high oxygen supply of well drained soils by continuously bubbling air through solution. By contrast, stagnant nutrient solution cultures are created by adding 0.1%w/v agar to conventional nutrient solutions to simulate lack of convection in waterlogged soils (Wiengweera *et al.*, 1997). Aerated and stagnant nutrient solution cultures are often used to simulate the effects of aerobic and anaerobic soils on rice root morphology and physiology (Wiengweera *et al.*, 1997; Lu *et al.*, 1999; Rubinigg *et al.*, 2002; Colmer, 2003a; Colmer *et al.*, 2006). There is

evidence for different root structure and anatomy in aerated and stagnant conditions that mimic differences between well drained and waterlogged soils. When nutrient and water supply are not limiting, rice growth in aerated solution culture is better than in stagnant solution culture (Colmer, 2003a).

Notwithstanding the common use of the Yoshida nutrient solution, there are few studies that have reported on the effects of oxygen or nutrients supply on rice in solution culture. Therefore, a series of experiments was carried out to compare rice growth in soil and solution culture (experiment 3.2.1), to determine the response of a diverse range of rice cultivars to aerated and stagnant nutrient solution culture (experiment 3.2.2), to determine the effects of iron form and concentration for rice growth under varied of oxygen supply (experiment 3.2.3), to determine the suitable of oxygen levels for rice growth in aerated and stagnant solution culture (experiment 3.2.4), and to establish the sufficiency levels of oxygen for rice growth in aerated and stagnant solution culture (experiment 3.2.5).

3.2 Materials and Methods

3.2.1 Experiment 1: Comparing of rice growth in soil and nutrient solution culture

Growth of KDML105 was compared in soil cultures (W+; waterlogged soil and W0; well drained soil) and nutrient solution (S; stagnant nutrient solution and A; aerated nutrient solution). In waterlogged soils, the soil surface was submerged under 10 cm water. In well drained soils was daily watering of the plant but no water standing in the pot. In nutrient solution cultures, the composition was (mol m^{-3}) 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}): Cl 50, B 25, Mn 2, Zn 2, Ni 1, Cu 0·5, Mo 0·5 (McDonald *et al.*, 2001b). Stagnant nutrient solution, 0.1% agar was used to prevent convective circulation of solution (Wiengweera *et al.*, 1997), thus simulating the slow gas movement which occurs in waterlogged soil. In aerated nutrient solution, air was bubbled in solution continuously. There were three replicates per treatment. Five 14 day-old rice plants were transplanted in each pot. Each plastic pot was lined with a plastic bag and contained 10 kg of soil (San Sai series) for soil cultures. Basal fertilizer was applied at the rate of 0.37 g N/pot as urea, 0.26 g P/pot and 0.26 g K/pot two weeks after transplanting. For nutrient solution, each plastic pot contained 10 litres of nutrient solution cultures which was renewed every week. There were separate plants from the same pot for each harvest at 2, 3, 4, 5 and 7 weeks. At each harvest maximum root length, maximum shoot length, number of leaves and root, root and shoot dry weight, and aerenchyma appearance (% of root cross sectional area) at 10, 20, 30 and 50 mm from root tip were assessed. The number of leaves and roots were counted on a separate plant. The measurement on maximum root and shoot length, root and shoot dry weights, aerenchyma appearance and nutrient analysis was the same as the previous describe (chapter 2).

3.2.2 Experiment 2: Comparing of rice growth in stagnant and aerated nutrient solution culture

Root growth of 15 rice cultivars was compare in stagnant and aerated nutrient solution culture. Fifteen rice cultivars of diverse origin were chosen; three cultivars used previously (Kae Noi, KN; Chainat 1, CNT1; and KDML105), three wetland

cultivars (Supanburi 1, SPB1; Phitsanuloke 60, PSL60; and Kong Laung 1, KL1), three upland cultivars (R258; Sew Mae Jan, SMJ; and Nam Roo, NR), three rainfed cultivars (Muey Nong 68M, MN68M; RD6; and Nam Sa Gui1 9, NSG19) and three deepwater rice cultivars (Tong Bai Aeng Nag, TBAN; Tong Bai Aeng Bao, TBAB; and Prachinburi 1, PCB1). The composition of nutrient solution was the same as experiment 1. There were four replicates. Thirty (for each cultivar there were two plants per pot) seven days-old rice plants were transplanted in each pot. Each plastic pot contained 60 litres of nutrient solution which was renewed every week. The rice plants were harvested after four weeks growth. Maximum root length, maximum shoot length, number of leaves and root, root and shoot dry weight, and root/shoot ratio were assessed. The measurement on plant growth was the same as the previously described for experiment 1. Root/shoot ratio was calculated as root dry weight divided by shoot dry weight.

3.2.3 Experiment 3: Iron compound and concentration for rice growth in solution culture

This experiment was conducted in 2 parts. In the first part, KDML 105 was grown with three iron compounds in stagnant and aerated nutrient solution cultures and five schedules of oxygen bubbling in aerated nutrient solution. Three iron compounds were $FeSO_4.7H_2O$, $FeCl_3.6H_2O$ and $C_{10}H_{12}FeN_2NaO_8$ (Fe EDTA), at 100 μM Fe for all compounds. Five schedules of oxygen bubbling in aerated nutrient solution culture were 0, 3, 6, 12 and 24 hours per day. In nutrient solution cultures, the composition was the same as previously except for the iron treatments. There were three replicates. Five 2 cm root length (three days after germination) seedlings were transplanted in each pot. Each plastic pot contained 10 litres of nutrient solution

cultures that was renewed every week. Plants supplied with $FeSO_4.7H_2O$ and $FeCl_3.6H_2O$ were harvested after two weeks growth. Thereafter iron levels were increased for the second part to Fe EDTA at 100, 150 and 200 μM, and times of oxygen bubbling to 0 (stagnant) and 3 hrs per day (aerated). The rice plants were harvested after a further one week under the new conditions. The measurements for the first part were root and shoot dry weight. For the second part, maximum root length, maximum shoot length, number of leaves, root and tiller and root and shoot dry weight were assessed. The measurement on plant growth was the same as the previously described for experiment 1.

3.2.4 Experiment 4: Comparing three levels of oxygen supply for rice growth in solution culture

Response of three rice cultivars (KDML105, CNT1 and KN) was determined in nutrient solution cultures at 3 levels of oxygen concentration: 0-3 (1 mgO_2: stagnant), 8-9 (3 mgO_2 without air or oxygen bubbling: still) and 18-21 (8 mgO_2 with air or oxygen bubbling 24 hrs per day: aerated) % O_2. The composition of nutrient solution culture was the same as experiment 3, and used Fe EDTA at 100 μM for iron supply. There were three replicates. Four seedlings with 2 cm root length (three days after imbibition) were transplanted in each pot. Each black plastic pot contained 10 litres of nutrient solution, renewed every week and adjusted to pH 6.5 every day. The rice plants were harvested four weeks after transplanting. Maximum root length, maximum shoot length, number of leaves, root and tiller, leaf greenness (SPAD value) and root and shoot dry weight were assessed. The measurement on plant growth was the same as the previously described for experiment 1.

3.2.5 Experiment 5: Comparing four levels of oxygen supply for rice growth in solution culture

CNT1 was grown in nutrient solution cultures at four levels of oxygen concentrations. The levels of oxygen concentration were 0.0-0.5 % O_2 (stagnant with 0.1 % agar and flushed before agar addition with N_2: N_2 flush), 1.0-3.0 % O_2 (mixed 0.1 % agar: stagnant), 9-12 % O_2 (solution without 0.1 % agar or bubbling: still) and 18-20 % O_2 (solution with air bubbling 24 hrs per day: aerated). There were three replicates. Four seedlings with 2 cm root length (three days after germination) were transplanted in each pot. Each black plastic pot contained 10 litres of nutrient solution cultures, renewed every week and adjusted to pH 6.5 every day. The rice plants were harvested at four and six weeks after transplanting. Maximum root length, maximum shoot length, number of leaves, root and tiller, root and shoot dry weights and nutrient accumulation in plants were assessed. The measurement on plant growth was the same as the previously described for experiment 1.

3.2.6 Statistic analysis

Analysis of variance was conducted using a factorial treatment combination arranged in a Randomized Complete Block Design (RCBD). Depending on the experiment, data were analyzed using one- or two-way analysis on variance (ANOVA) to determine the main effects and interactions among cultivar, water treatment. Means were compared using Least Significant Difference (LSD) at $P<0.05$.

3.3 Results

3.3.1 Experiment 1: Comparing of rice growth in soil and nutrient solution culture

After two weeks in treatments, rice plants in stagnant nutrient solution culture had higher on leaf numbers than other treatments. Similarly, root dry weight and total plant dry weight in stagnant culture was higher in waterlogged soil (Table 3.3.1.1). Nutrient solution culture increased by 19 – 25 % maximum shoot length and nearly 60 % of number of leaves (Table 3.3.1.2), while number of roots, root and shoot dry weight was increased when grown in stagnant nutrient solution culture (Table 3.3.1.3). After four weeks, maximum root length was started to decrease in stagnant solution culture, while maximum root length of plants in other conditions was the same length and 32 – 39 % higher than plant in stagnant culture. However, plants in stagnant culture produced more numbers of roots and root dry weight than others (Table 3.3.1.4). Shoot growth of plant in stagnant culture at four weeks was also dramatically increased and resulted from more tillers and greater shoot length (Table 3.3.1.5). Plant growths were increased with time (5-7 weeks) and the difference between soil and solution culture was accentuated whereas the difference in shoot dry weight between stagnant and aerated solution diminished (Table 3.3.1.6). Root growth in aerated solution culture was intermediate between plant growth in stagnant culture and in soils, however at seven weeks shoot growth in aerated solution recovered to the same level as plants in stagnant culture.

Table 3.3.1.1 Root, shoot and total plant dry weight (g plant^{-1}) of KDML105 when grown in soil (waterlogged; W+, well drained; W0) and nutrient solution (Aerated; A and Stagnant; S) for two weeks.

Culture conditions	Dry weight (g plant^{-1})		
	Root	Shoot	Total
W+	0.012 A	0.028 BC	0.186 A
W0	0.007 B	0.021 C	0.138 B
S	0.012 A	0.057 A	0.344 A
A	0.007 B	0.037 B	0.222 B
F-test	Con*	Con*	Con*
$LSD_{(P<0.05)}$	0.004	0.014	0.004

* 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.

Table 3.3.1.2 A maximum shoot length (cm) and number of leaves of KDML105 when grown in soil (waterlogged; W+, well drained; W0) and nutrient solution (Aerated; A and Stagnant; S) cultures for three weeks.

Culture conditions	Shoot growth	
	Maximum shoot length (cm)	Number of leaves
W+	29.9 B	4.73 B
W0	28.2 BC	3.87 B
S	39.9 A	10.8 A
A	34.8 AB	9.65 A
F-test	Con*	Con*
$LSD_{(P<0.05)}$	5.66	2.20

* 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.

Table 3.3.1.3 The number of roots and shoot and root dry weights (g plant^{-1}) of KDML105 when grown in soil (waterlogged; W+, well drained; W0) and nutrient solution (Aerated; A and Stagnant; S) for three weeks.

Culture Conditions	Number of roots	Root dry weight (g plant^{-1})	Shoot dry weight (g plant^{-1})
W+	19.88BC	0.020B	0.053C
W0	16.60C	0.020B	0.053C
S	29.53A	0.066A	0.273A
A	21.50B	0.026B	0.139B
F-test	Con*	Con*	Con*
$LSD_{(P<0.05)}$	4.70	0.009	0.056

* 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.

Table 3.3.1.4 Root growth of KDML105 when grown in soil (waterlogged; W+, well drained; W0) and nutrient solution (Aerated; A and Stagnant; S) for four weeks.

Culture conditions	Root growth		
	Maximum root length (cm)	Number of roots	Root dry weight (g plant^{-1})
W+	27.92 A	29.53 BC	0.053 C
W0	30.07 A	25.40 C	0.049 C
S	18.82 B	41.35 B	0.240 A
A	31.00 A	74.05 A	0.151 B
F-test	Con*	Con*	Con*
$LSD_{(P<0.05)}$	5.58	12.30	0.044

* 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.

Table 3.3.1.5 Shoot growth of KDML105 when grown in soil (waterlogged; W+, well drained; W0) and nutrient solution (Aerated; A and Stagnant; S) cultures for four weeks.

Culture conditions	Shoot growth	
	Maximum shoot length (cm)	Shoot dry weight (g plant^{-1})
W+	38.19 B	0.145 C
W0	33.98 C	0.128 C
S	50.73 A	1.022 A
A	46.95 A	0.546 B
F-test	Con*	Con*
$LSD_{(P<0.05)}$	3.77	0.141

* 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.

Table 3.3.1.6 Root and shoot growth of KDML105 when grown in soil (waterlogged; W+, well drained; W0) and nutrient solution (Aerated; A and Stagnant; S) cultures for five and seven weeks.

Culture conditions	5 weeks		7 weeks	
	Root dry weight (g plant^{-1})	Shoot dry weight (g plant^{-1})	Root dry weight (g plant^{-1})	Shoot dry weight (g plant^{-1})
W+	0.109 C	0.423 C	0.838 C	1.796 B
W0	0.100 C	0.317 C	0.771 C	2.439 B
S	0.503 A	1.717 A	2.761 A	7.907 A
A	0.358 B	1.311 B	1.814 B	7.406 A
F-test	Con*	Con*	Con*	Con*
$LSD_{(P<0.05)}$	0.117	0.325	0.59	1.85

* 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.

3.3.2 Experiment 2: Comparing of rice growth in stagnant and aerated nutrient solution culture

Plant growth of all cultivars was increased when grown in stagnant solution for four weeks, apart from the maximum root length. Maximum root length of most cultivars was promoted when grown in stagnant solution culture but KN, PSL60, MN, RD6 and PCB1 had the same maximum root length regardless of solution cultures (Table 3.3.2.1). Whereas, shoot length of all cultivars was increased when grown in stagnant solution culture, shoot length of deepwater rice types was higher than other types (Table 3.3.2.1). Root and leaf numbers (Table 3.3.2.3), and root and shoot dry weight (Table 3.3.2.3) were also increased when grown in stagnant solution culture.

Table 3.3.2.1 Root and shoot lengths (cm) of 15 rice cultivars when grown in aerated (A) and stagnant (S) nutrient solution for four weeks.

Cultivars	Root length (cm)		Shoot length (cm)	
	A	S	A	S
KDML105	17.0	15.2	19.4	45.4
CNT1	14.3	18.4	20.8	40.2
KN	18.3	20.8	17.6	42.1
SP1	15.0	17.1	18.8	36.1
PSL60	16.3	16.2	17.2	38.8
KL1	15.9	18.3	15.9	38.7
R258	17.4	22.8	18.6	46.6
SMJ	15.8	17.8	21.0	46.1
NR	16.3	20.9	16.3	41.0
MN	18.5	18.3	22.8	41.8
RD6	13.9	14.2	18.0	42.1
NSG19	13.6	15.3	31.7	42.8
TBAN	13.3	16.5	19.8	49.3
TBAB	13.6	16.3	25.4	53.8
PJ1	16.0	16.6	20.3	47.9
F-test	Con x Cul*		Con x Cul*	
$LSD_{(P<0.05)}$	1.5		3.6	

* significant at P< 0.05. Con x Cul indicates F-test for culture condition and cultivar interaction effects.

Table 3.3.2.2 Root and leaf numbers of 15 rice cultivars when grown in aerated (A) and stagnant (S) nutrient solution for four weeks.

Cultivars	Root numbers		Leaves numbers	
	Aerated	Stagnant	Aerated	Stagnant
KDML105	15.0	36.3	4.6	9.9
CNT1	27.1	37.4	3.8	11.1
KN	9.8	22.1	3.0	7.0
SP1	19.1	41.0	4.4	12.3
PSL60	13.1	33.6	3.9	11.5
KL1	12.9	34.4	4.3	10.6
R258	11.0	28.6	3.5	9.6
SMJ	12.6	33.3	3.5	8.4
NR	10.1	26.5	3.0	9.6
MN	17.9	41.8	4.1	9.0
RD6	13.4	32.8	3.6	8.8
NSG19	23.3	42.5	5.4	9.9
TBAN	14.0	30.9	4.4	10.3
TBAB	15.4	30.0	4.4	9.8
PJ1	16.6	37.4	4.8	11.9
F-test	Con x Cul*		Con x Cul*	
$LSD_{(P<0.05)}$	4.4		1.1	

* significant at P< 0.05. Con x Cul indicates F-test for culture condition and cultivar interaction effects.

Table 3.3.2.3 Root and shoot dry weight (g plant^{-1}) of 15 rice cultivars when grown in aerated (A) and stagnant (S) nutrient solution for four weeks.

Cultivars	Root dry weight (g plant^{-1})		Shoot dry weight (g plant^{-1})	
	Aerated	Stagnant	Aerated	Stagnant
KDML105	0.015	0.079	0.051	0.293
CNT1	0.012	0.119	0.043	0.366
KN	0.014	0.112	0.047	0.279
SP1	0.015	0.124	0.051	0.371
PSL60	0.011	0.083	0.029	0.344
KL1	0.010	0.121	0.030	0.345
R258	0.012	0.143	0.034	0.367
SMJ	0.017	0.114	0.043	0.290
NR	0.012	0.147	0.025	0.367
MN	0.013	0.109	0.039	0.325
RD6	0.007	0.065	0.026	0.223
NSG19	0.036	0.110	0.114	0.332
TBAN	0.008	0.099	0.031	0.368
TBAB	0.023	0.112	0.062	0.341
PJ1	0.013	0.140	0.039	0.440
F-test	Con x Cul*		Con x Cul*	
$LSD_{(P<0.05)}$	0.018		0.055	

* significant at P< 0.05. Con x Cul indicates F-test for culture condition and cultivar interaction effects.

3.3.3 Experiment 3: Iron compound and concentration for rice growth in solution culture

The results of this experiment were separated to 2 stages. First stage was in three different iron compounds. After only three days in Fe treatments, rice seedling exhibited iron deficiency symptom on partially emerged leaves. Severe chlorosis was presented on leaves when grown in with $FeSO_4$ and $FeCl_3$ nutrient solution culture. By contrast, in Fe EDTA, rice leaves showed less severe iron deficiency symptoms and growth was better than with the other iron compounds. After two weeks in treatments, rice plants in both $FeSO_4$ and $FeCl_3$ nutrient solution showed very severe iron deficiency symptoms and began dying. Therefore, the rice plants in both $FeSO_4$ and $FeCl_3$ nutrient solution culture were harvested.

Plants supplied with Fe EDTA transferred to solution at 100, 150 and 200 μM Fe EDTA for one more week to examine their recovery from iron deficiency symptoms. The rice plants when grown at 100 μM Fe EDTA recovered faster and grew better than at 150 and 200 μM Fe EDTA, especially when the solution was bubbled with air for 3 hrs per day. Growth was the poorest at 200 μM Fe EDTA with air bubbling (Table 3.3.3.1 and 3.3.3.2).

Table 3.3.3.1 Growth of rice plants when grown in nutrient solution at 3 levels of Fe EDTA; 100, 150 and 200 μM in no oxygen bubbling (0) or 3 hrs per day of air bubbling (3).

Fe levels / Time of oxygen bubbling	Shoot length (cm)	Root length (cm)	Root numbers	Leaf numbers	Tiller numbers
0/100	58.8 A	19.8 A	33.3 AB	13.5 A	2.7 A
0/150	58.3 AB	20.2 A	33.8 A	12.1 AB	2.6 A
3/100	57.7 AB	19.4 A	32.9 AB	12.6 A	2.71 A
3/150	52.7 BC	16.8 B	26.9 BC	10.2 BC	1.9 B
3/200	50.3 C	16.3 B	23.9 B	9.7 C	1.8 B
F-test	Con*	Con*	Con*	Con*	Con*
$LSD_{(P<0.05)}$	5.79	2.31	6.55	2.28	0.60

* 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.

Table 3.3.3.2 Dry weight (g plant^{-1}) of rice plants when grown in nutrient solution culture at 3 levels of Fe EDTA; 100, 150 and 200 μM in no oxygen bubbling (0) or 3 hrs per day of oxygen bubbling (3).

Fe levels / Time of oxygen bubbling	Shoot dry weight (g plant^{-1})	Root dry weight (g plant^{-1})
0/100	0.40 A	0.06 A
0/150	0.40 A	0.06 A
3/100	0.38 A	0.06 A
3/150	0.24 B	0.03 B
3/200	0.19 B	0.03 B
F-test	Con*	Con*
$LSD_{(P<0.05)}$	0.06	0.03

* 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.

3.3.4 Experiment 4: Comparing three levels of oxygen supply for rice growth in solution culture

Apart from maximum root length which was decreased, plant growth in all cultivars was promoted when grown in still solution. Maximum root length in all cultivars was the longest when rice plant grown in aerated solution, while the maximum root length in all cultivars was dramatically reduced when grown in stagnant solution (Table 3.3.4.1). Although aerated solution promoted the maximum root length in all cultivar, KDML105 roots reached only 63 - 66 % of the maximum length of KN and CNT1 respectively. Root numbers of all cultivars was increased by still solution, whereas CNT1 also produced more root numbers in aerated solution (Table 3.3.4.2). KN was the lowest in root numbers; it was only half of CNT1 and KDML105 in still solution, and only 28 % and 60 % in root numbers of CNT1 and KDML105 when grown in aerated solution. Root numbers of all cultivars were reduced when grown in stagnant culture, especially in CNT1 and KDML105 were 40 % and 44 % reduction when comparing in aerated solution. By contrast, root numbers of KN in stagnant culture was equal to those in aerated solution. All cultivars had higher root dry weight in still solution than others, and CNT1 was the highest in root dry weight (Table 3.3.4.3). Shoot growth in all cultivars (maximum shoot length, number of leaves, number of tillers and shoot dry weight) was significantly increased when grown in still solution. Shoot dry weight was strongly inhibited when grown stagnant culture with 73-85 % decrease in shoot dry weight compared to still culture (Table 3.3.4.4). Unlike other cultivars which had much higher tiller numbers that responded to solution aeration, tiller numbers of KN in stagnant culture were similarly

low under all aeration conditions (3.3.4.5). Greenness of rice leaves in all cultivars was generally highest in still and in stagnant conditions (Table 3.3.4.6).

Table 3.3.4.1 Maximum root length (cm) of three rice cultivars when grown in stagnant, still and aerated nutrient solution for four weeks.

Cultivars / Conditions	Stagnant	Still	Aerated
KN	19.9 Ac	Ab 27.7	Aa 45.8
CNT1	21.7 Ac	Ab 29.0	Aa 43.6
KDML105	14.7 Bc	Bb 23.1	Ba 28.8
F-test		Con x Cul*	
LSD $_{(P<0.05)}$		4.3	

* significant at P< 0.05. Con x Cul indicates F-test for culture condition and cultivar interaction effects. The difference between culture conditions in the same row is indicated by lower case letters. The difference between cultivars in the same column is indicated by upper case letters.

Table 3.3.4.2 Root numbers of three rice cultivars when grown in stagnant, still and aerated nutrient solution for four weeks.

Cultivars / Conditions	Stagnant	Still	Aerated
KN	25.3 Bb	Ba 50.0	Cb 27.9
CNT1	59.9 Ab	Aa 119.4	Aa 100.0
KDML105	39.1 Bc	Aa 116.8	Bb 70.2
F-test		Con x Cul*	
LSD $_{(P<0.05)}$		16.3	

* significant at P< 0.05. Con x Cul indicates F-test for culture condition and cultivar interaction effects. The difference between culture conditions in the same row is indicated by lower case letters. The difference between cultivars in the same column is indicated by upper case letters.

Table 3.3.4.3 Root dry weight (g/plant) of three rice cultivars when grown in stagnant, still and aerated nutrient solution for four weeks.

Cultivars / Conditions	Stagnant	Still	Aerated
KN	Ab 0.183	Ca 0.455	Cb 0.274
CNT1	Ac 0.333	Aa 1.010	Ab 0.752
KDML105	Ac 0.153	Ba 0.748	Bb 0.447
F-test		Con x Cul*	
LSD $_{(P<0.05)}$		0.199	

* significant at P< 0.05. Con x Cul indicates F-test for culture condition and cultivar interaction effects. The difference between culture conditions in the same row is indicated by lower case letters. The difference between cultivars in the same column is indicated by upper case letters.

Table 3.3.4.4 Shoot dry weight (g/plant) of three rice cultivars when grown in stagnant, still and aerated nutrient solution for four weeks.

Cultivars / Conditions	Stagnant	Still	Aerated
KN	Ab 0.632	Ba 2.335	Bb 1.177
CNT1	Ac 0.950	Aa 3.926	Ab 2.796
KDML105	Ac 0.545	Aa 3.639	Ab 2.106
F-test		Con x Cul*	
LSD $_{(P<0.05)}$		0.696	

* significant at P< 0.05. Con x Cul indicates F-test for culture condition and cultivar interaction effects. The difference between culture conditions in the same row is indicated by lower case letters. The difference between cultivars in the same column is indicated by upper case letters.

Table 3.3.4.5 Tiller numbers of three rice cultivar when grown in stagnant, still and aerated nutrient solution for four weeks.

Cultivars / Conditions	Stagnant	Still	Aerated
KN	2.0 Ba	Ca 3.9	Ca 3.0
CNT1	5.0 Ac	Aa 14.9	Ab 12.2
KDML105	2.8 ABc	Ba 9.1	Bb 6.0
F-test		Con x Cul*	
LSD $_{(P<0.05)}$		2.35	

* significant at P< 0.05. Con x Cul indicates F-test for culture condition and cultivar interaction effects. The difference between culture conditions in the same row is indicated by lower case letters. The difference between cultivars in the same column is indicated by upper case letters.

Table 3.3.4.6 SPAD values of three rice cultivars when grown in stagnant, still and aerated nutrient solution for four weeks.

Cultivars / Conditions	Stagnant	Still	Aerated
KN	Aa 37.5	Aa 35.8	Ab 23.4
CNT1	Bb 32.2	Aa 37.2	Ac 26.9
KDML105	ABa 34.4	Aa 36.8	Ab 27.5
F-test		Con x Cul*	
LSD $_{(P<0.05)}$		3.55	

* significant at P< 0.05. Con x Cul indicates F-test for culture condition and cultivar interaction effects. The difference between culture conditions in the same row is indicated by lower case letters. The difference between cultivars in the same column is indicated by upper case letters.

3.3.5 Experiment 5: Comparing four levels of oxygen supply for rice growth in solution culture

After four weeks, only maximum root length of CNT1 was affected by oxygen concentration in nutrient solution culture (Table 3.3.5.1). Aeration with air bubbling increased maximum root length whereas ceasing air treatment decreased root length. Stagnant solution with or without N_2 flushing decreased root length to a similar extent.

After six weeks, all plant growth characteristics responded to oxygen concentration in nutrient solution cultures (Table 3.3.5.2). Increased root length was obtained in plants in still solution. In contrast, number of roots was the highest in plant grown in stagnant solutions and those in still solution. Shoot dry weight of plant by contrast was the greatest when grown in N_2 flushing solution.

Phosphorus and Potassium contents in plants after four weeks increased in plant grown in still culture and decreased in plants grown in stagnant solution (Table 3.3.5.3). Nitrogen contents were higher in grown in aerated solution than those grown in stagnant culture.

Table 3.3.5.1 Plant growth characteristics of Chainat 1 when grown in different culture conditions of oxygen concentration in nutrient solution for four weeks.

Culture conditions	Maximum root length (cm)	Maximum shoot length (cm)	Number of tillers	Number of leaves	Number of roots	Root dry weight (g plant^{-1})	Shoot dry weight (g plant^{-1})
N_2 flushing	21.83 C	48.61	7.64	27.17	66.61	0.430	1.068
Stagnant	22.37 C	50.08	8.08	27.42	61.75	0.414	1.034
Still	31.93 B	50.70	9.58	32.33	71.08	0.394	1.276
Aerated	43.99 A	49.04	8.14	27.39	56.67	0.365	1.022
F-test	Con*	Con^{ns}	Con^{ns}	Con^{ns}	Con^{ns}	Con^{ns}	Con^{ns}
LSD $_{(P<0.05)}$	4.04	-	-	-	-	-	-

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

Table 3.3.5.2 Plant growth characteristics of Chainat1 when grown in different culture conditions of oxygen concentration in nutrient solution for six weeks.

Culture conditions	Maximum root length (cm)	Maximum shoot length (cm)	Number of tillers	Number of leaves	Number of roots	Root dry weight (g plant^{-1})	Shoot dry weight (g plant^{-1})
N_2 flushing	39.6 B	58.7 B	13.4 B	50.9 B	178.9 B	2.390 A	7.470 A
Stagnant	41.4 B	58.8 B	14.9 B	58.0 B	193.2 AB	1.728 C	5.737 B
Still	35.6 B	67.2 A	30.2 A	100.6 A	208.7 A	2.481 A	4.818 B
Aerated	64.3 A	68.1 A	25.1 A	83.7 A	168.8 B	2.052 B	4.444 B
F-test	Con*	Con*	Con*	Con*	Con*	Con*	Con*
LSD $_{(P<0.05)}$	10.8	2.2	6.1	18.55	28.8	0.291	1.611

* 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.

Table 3.3.5.3 Nutrient content (mg plant^{-1}) of Chainat1 when grown in different culture conditions of oxygen concentration in nutrient solution for six weeks.

Culture conditions	Nutrient content (mg plant^{-1})		
	N	P	K
N_2 flushing	19.16 BC	3.15 A	55.91 A
Stagnant	14.06 C	2.42 B	42.51 B
Still	25.95 A	2.31 B	47.47 AB
Aerated	24.88 AB	1.94 B	32.99 C
F-test	Con*	Con*	Con*
LSD $_{(P<0.05)}$	6.65	0.67	8.74

* 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.

3.4 Discussion

Nutrient solution culture dramatically increased plant growth especially in stagnant nutrient solution culture when compared with soil culture. These indicated that solution culture had higher nutrient available for plant uptake due to mass flow and diffusion of nutrient from medium to root were enhanced by water sufficiency in solution. Nutrient ions in solution culture were changed less chemical processes of oxidation or reduction when compare with soil pH was easily adjusted to maintain the nutrient availability. Moreover, there is no nutrient fixed by soil particle or competition with micro-organisms in soils. Growth in stagnant solution culture was better than in aerated and well drained soil culture, so could represent growth in waterlogged soil. Although, the length of root of rice in stagnant culture was differently reduced from the length of rice root in waterlogged soil which the same as in well drained soil and aerated solution culture. These resulted from oxygen in the atmosphere can not diffuse through the agar film to support the deep root and oxygen transportation to deep root may be limited. While, root of rice grown in waterlogged soil was supported by both oxygen transport from shoot and the diffused oxygen through the soil water, although diffused oxygen was a few but it was useful for rice in waterlogged soil to support both of root and micro-organism activity. Plant in prolonged stagnant solution was enhanced both of shoot and root growth, while aerated culture had lower root growth and had the same shoot growth as grown in stagnant culture. These may resulted from rice in aerated culture had high partition on shoot mass production, differed from plant in stagnant culture. Rice in stagnant culture produced more root numbers to increase in root surface area for nutrient uptake and increase in aerenchymatous root for oxygen transport. However, this

study was no consistent with the previous study and facts which plant in sufficient oxygen supply as aerated nutrient solution culture was better growth than plant in lack of oxygen supply in medium. In this study, rice in aerated culture faced with iron deficiency in the seedling stage, younger leaves presented the yellow colour. These may resulted in iron suffered plant in aerated culture was lower in early growth than plant in stagnant culture, then in prolonged treatment, aerated plants was recovered from iron stress and rose up the growth as plant in stagnant culture. These supported that plant in stagnant culture was not better in growth than aerated solution, they need more study to investigation.

The comparing the plant growth in soil and solution culture was indicated that they were acceptable and used in the experiment 3.2.2 to determined and confirmed the responses of fifteen rice cultivars to aerated and stagnant solution. After four weeks in solution culture, plant growth of all cultivars was higher in stagnant solution culture. However, the iron deficient stress was also found in plant in aerated culture. Moreover, algae were plenty grown in nutrient solution in both stagnant and aerated culture, resulted from the light emitted through the transparent solution pot. Thus, the growth data of this experiment can not determined the response of rice when grown in nutrient solution culture. The experiment 3.2.3 was conducted to improve the nutrient solution condition in both of iron and oxygen supply. Rice plant in nutrient solution with Fe-EDTA was healthier than other iron form. Both $FeSO_4$ and $FeCl_3$ were easily precipitated in solution especially in air bubbling. Oxygen from air bubbling may enhanced the precipitation and became plaque at root surface. This plaques was not only unavailable iron form but also impeded other nutrient uptake by reducing root surface areas. After rice plants were transition to the different concentration of Fe

EDTA for a week, solution culture with Fe EDTA at 100 μM promoted plant growth in both no air bubbling and 3 hrs of air bubbling, while higher concentration of iron especially at 200 μM and 3 hrs air bubbling was the lowest in plant growth contribution. In Kimura B or Huagland or Yoshida nutrient solution formula recommend the adequate iron concentration at only 50 μM but rice need higher concentration, therefore at 100 mM was optimum level for rice culture. High iron concentration may affect on balance of other nutrient, especially air bubbling may enhanced the precipitation by oxidation or reduction in solution culture. Moreover, 3 hrs per day of air bubbling in solution culture may affect the fluctuation of plant adaptation, the changing from 3 hrs in aerobic activity to 21 hrs anaerobic activity. Plants in 3 hrs air bubbling may suffer and stress, resulted in growth was reduced. The results of the experiment 3 was convinced that Fe EDTA at 100 μM was adequate level of iron supply but the oxygen supply for aerated culture was not consistent with the well drained soil condition. Therefore, the experiment 3.2.4 was conducted to determine the suitable oxygen supply for aerated solution culture as aerated without air bubbling and aerated with thoroughly air bubbling and for stagnant solution culture as stagnant (0.1 % agar) conditions. This experiment, growth of three rice cultivars as previous experiment in waterlogged and well drained soil culture was also determined when grown in different oxygen supply in nutrient solution. Plant growth of all cultivars was different from the previous nutrient solution experiment, it was higher when grown in aerated without air bubbling for four weeks, excepted root length was enhance by aerated bubbling treatment. It was indicated that in aerated without air bubbling had sufficient on both of nutrient availability and oxygen supply for rice growth. However, the longer root length of plant in aerated with air bubbling

was implied that oxygen can not pass through to the deep zone which it was important for deep root penetration. Although, plant in aerated without air bubbling was shorter root length, the higher on root numbers can compensated the root surface area for nutrient uptake, which it was confirmed by the root dry weight and shoot production in all cultivars. Stagnant nutrient solution culture adversely affected on growth of previous experiments, but it was consistent with the other reports that rice growth was reduced in stagnant culture (Wiengweera *et al.*, 1997; Colmer *et al.*, 1998; Colmer, 2003a; Colmer *et al.*, 2006) which it resulted from nutrient uptake impediment of root structure when grown in stagnant culture (Colmer and Bloom, 1998; Rubinigg *et al.*, 2002; Huang *et al.*, 2003b; Wiengweera and Greenway, 2004).

Oxygen supply in aerated and stagnant culture was suspected. Therefore, experiment 5 was conducted to confirm the suitable oxygen supply in both aerated and stagnant culture. The length of root was indicator for adequate oxygen supply in aerated culture, which at four weeks of plant in both aerated without and with air bubbling was the same length but at six weeks clearly presented on significant longer root length of plant in aerated with oxygen bubbling. It was clearly on prolong growth in aerated solution culture need more oxygen supply from the air bubbling due to the increase in demand of oxygen by increased root numbers and increase in nutrient requirement for increase growth with time. In lack of oxygen condition, root both stagnant and stagnant with N_2 flush conditions similarly limited the oxygen supply for root elongation but root dry mass was dramatically increased in plant grown in stagnant with N_2 flush. These result was consistent with higher phosphorus uptake of plant in stagnant with N_2 flush, which phosphorus in plant directly promoted the root growth (Marschner, 1995). However, the root/shoot ratio of plant

in both stagnant and stagnant with N_2 flush solution culture was the same. Likewise, the plant in aerated with and without air bubbling had the same on plant distribution. Therefore, the aerated nutrient solution for rice culture was ensured for adequate oxygen supply by air bubbling. While, the stagnant nutrient solution for rice culture was added 0.1 % agar (w/v) as report of Wiengweera *et al*. (1997) to prevent the oxygen diffusion and convection. Although the N_2 flush enhanced the growth but the plant distribution was the same as in stagnant culture. Moreover, N_2 gas was more expensive. Therefore, the study of plant responses in waterlogged and well drained soils was acceptably mimicked by stagnant and aerated nutrient solution culture due to the gap of oxygen supply was significantly different.