Chapter 3

Manganese acquisition in Mn efficient and inefficient rice genotypes

3.1 Introduction

Manganese efficiency in plants is described by many mechanisms, beginning with how the Mn is acquired from the soil. Plant acquisition of nutrients from soil is depending on a range of biological and chemical processes at the soil-root interface. Primary among these is rhizosphere activities which can influence the availability of mineral nutrients by inducing pH-changes, root exudation of organic acids, phenolics and activities of microbial populations that depend on the plant for energy source and nutrition (Marschner, 1995).

Manganese is absorbed by plants mainly as the free Mn^{2+} ion, but it exists in the soil as different oxidized forms including Mn(III) and Mn(IV) oxide. Reduction of Mn(III, IV) to the available Mn^{2+} occurs acidification and reduction (Marschner, 1988). The availability of Mn to plants is governed by redox processes, plant-derived organic compounds in the rhizosphere may increase availability of Mn by providing a source of electrons for Mn reduction. It is now widely accepted that plants are able to increase the availability of nutrients by exuding various organic substances into the rhizosphere. Exuded compounds can alter micronutrient availability directly, by influencing the solubility and equilibrium of nutrient chemical forms (Uren, 1981; Uren and Reisenauer, 1988). Chelation of Mn^{2+} by carboxylate anions (the conjugated bases of carboxylic acids) suppresses its reoxidation and increases the

mobility of the reduced Mn in the rhizosphere (Marschner, 1995). Characterization of exudates components may help to define the Mn solubilising ability of plants and processes involved in Mn acquisition from soil. Increased release of root exudates in response to nutrient deficiency of Mn has been observed in many plant species such as sunflower (*Helianthus annuus* L.) roots could reduce insoluble Mn(IV) oxides (Uren, 1981). Lucerne (*Medicago sativa*) plants have been shown to exude a variety of carboxylates under Mn deficiency (Gherardi and Rengel, 2003, 2004). The mechanism of direct dissolution of Mn oxides by plant roots is yet to be fully elucidated.

This chapter reports of studies the mechanisms of Mn acquisition of rice by root exudates in sand and solution culture. Rice genotypes differing in tolerance to Mn deficiency were grown under various Mn treatments ranging from deficiency to sufficiency. Possible effects of root exudation from Mn efficient genotypes were assessed on its Mn inefficient neighbor. Growth patterns were observed and root exudates analyzed to determine how the genotypes response to low Mn supply and whether observed responses may explain differential Mn-deficiency tolerance in rice.

3.2 Materials and Methods

3.2.1 Experiment 3.2.1 Manganese acquisition of rice in sand culture

Sub experiment 1 **The influence of a Mn efficient genotype Mn nutrition of an Mn inefficient plants growing nearby**

Two rice genotypes, PSL1 (Mn inefficient) and KDML105 (Mn efficient) identified in the last chapter, were grown together in the same pot and separately in sand culture (Figure 3.1) with two levels of applied Mn (0 and 0.5 mg Mn/L) in Yoshida solution that was applied to the sand twice daily, one liter per pot each time. The seeds were placed on a moistened paper in petri dish until germinated. Germinated seeds were transplanted to pot (0.30 m diameter and 0.30 m deep) filled with washed river quartz sand at 6 seedlings per pot. Planting was done in 3 ways: PSL1 and KDML105 (GP1 and GP2, respectively) in separate pots and PSL1 + KDML105 together in the same pot (designated GP3 for PSL1 and GP4 for KDML105), with 3 plants each. The nutrient solution modified from Yoshida solution (Table 2.1). All pots were flushed with filtered-water every week to avoid accumulation of salts in the sand. There are three replicates per treatment.

Measurement and plant analysis

Data were recorded every week until 8 week after transplanting including: chlorophyll content in YEB-1 (next youngest leaf blade below YEB) (a chlorophyll meter SPAD 502), number of leaves plant^{-1} and tillers plant^{-1}. Then, harvest measurements including: shoot dry weight (g plant^{-1}) and root dry weight (g plant^{-1}). The samples will be determined for Mn concentration in all plant part by dry-ashing and atomic absorption spectrometry (Delhaize *et al*., 1984).

Figure 3.1 Model of PSL1 and KDML105 were grown together in pairs in the same pot and separately.

Sub experiment 2 **Ability to use different forms of Mn in Mn efficient and Mn inefficient genotypes**

Two rice genotypes, PSL1 (Mn inefficiency) and KDML105 (Mn efficiency) were grown in sand culture with 4 treatments of applied Mn as follows: 1) 0 mg Mn/L 2) 0.5 mg Mn/L from Mn-EDTA 3) 0.25 mg Mn/L from Mn-EDTA and 0.25 mg Mn/L. from $KMnO_4$ 4) 0.5 mg Mn/L from $KMnO_4$. The seeds were placed on a moistened paper in petri dish until germinated. Germinated seeds were transplanted to pot filled with washed river quartz sand at 5 seedlings per pot. Pots were supplied twice daily with complete nutrient solution. The nutrient solution was modified by Insalud (2006) (Table 2.3). All pots were flushed with filtered-water every week to avoid accumulation of salts in the sand. There are three replicates per treatment.

Measurement and plant analysis

Data were recorded at 30 days after transplanting including: chlorophyll content in YEB-1, number of leaves plant^{-1} and tillers plant^{-1}. Then, harvest measurements including: shoot dry weight (g plant^{-1}) and root dry weight (g plant^{-1}). The samples will be determined for Mn concentration in all plant part by dry-ashing and atomic absorption spectrometry (Delhaize *et al*., 1984).

3.2.2 Experiment 3.2.2 Manganese acquisition of rice in solution culture

Sub experiment 1 **Comparing the responses of rice to manganese deficiency with and without aeration of the nutrient solution**

Two rice genotypes, PSL 1 and KDML 105 were grown in solution culture with two status of O_2 and non O_2 in Mn deficiency. There were two conditions; without and with oxygen supply by air bubbling in nutrient solution. Ten days-old rice plants were transplanted in each pot at 5 seedlings per pot. Each plastic pot contained 10 L of nutrient solution cultures without added Mn. The solution was modified by Insalud (2006) (Table 2.3). The solution was renewed every week and pH values were adjusted daily to 5.5±0.05 with 1N HCl or 1N NaOH. There are three replicates per treatment. The responses to Mn deficiency with and without air bubbling were evaluated by measuring chlorophyll content in YEB-1, number of leaves plant^{-1} and tillers plant^{-1}. .

Sub experiment 2 **Acidification and reduction power of root exudate from Mn efficient and inefficient rice genotypes**

Two rice genotypes, PSL1 (Mn inefficiency) and KDML105 (Mn efficiency) were grown in solution culture with two levels of applied Manganese (0 and 0.5 mg Mn/L). Ten days after germination, five plants of each variety were transplanted to plastic pots containing 10 L of nutrient solution (Table 2.3) (Insalud, 2006). The solution was renewed every week and pH values were adjusted daily to 5.5±0.05 with 1N HCl or 1N NaOH. There are three replicates per treatment.

Reduction of Mn(IV)

Filter paper were soaked in 10 m*M* $KMnO_4$ for 7 hours. When in contact with the filter paper, permanganate is reduce to Mn oxide (brown) as it oxidizes cellulose in the filter paper (Uren, 1981). After soaking, the filter papers were rinsed thoroughly with distilled water and placed on square plate for drying. The dry papers can be stored for months without loss of colour intensity. The demonstrate MnIV reduction in the rhizosphere, the plastic box contain the impregnated filter paper and removed plant root surface is placed on the filter paper and covered by filter paper.

pH

Plants from each repicate pot were carefully washed with filtered-water. Plants were transferred to a collection vessel (500 mL volume) containing de-ionised water (DI) with pH value adjusted to 7.0±0.05 with 1N HCl or 1N NaOH.

Measurement

Data were recorded included chlorophyll content in YEB-1, number of leaves $plant^{-1}$ and tillers $plant^{-1}$ at 2 weeks until 4 weeks after transplanting. The samples

were determined for reduction of MnIV-oxide applied on fillter paper. Reduction of MnIV was indicated by whith zones on the MnIV impregnated filter paper. The reduction of pH was detected by pH meter.

3.2.3 Statistic analysis

Analysis of variance was conducted based on a factorial model with treatment arranged in a Completely Randomized Design (CRD). Data were analyzed using two-way analysis of variance (ANOVA) to determine the main effects and interactions among genotypes, Mn treatment. The comparison of mean was used with Least Significant Difference (LSD) at $P<0.05$.

3.3 Results

Experiment 3.2.1 Manganese acquisition of rice in sand culture

Sub experiment 1 **The influence of a Mn efficient genotype Mn nutrition of a Mn inefficient plants growing nearby**

This experiment has shown that root exudates may have a role to play in Mn acquisition of KDML105 in Mn deficiency. This was indicated by the improved of Mn nutrition in the Mn inefficient PSL 1 when grown in the same pot as KDML 105. The relative YEB-1 chlorophyll content, number of leaves and tillers (measurement in $Mn_0/Mn_{0.5}$) of PSL1 were all significantly higher when grown with KDML105 than when it was alone. Significant increasing in YEB-1 chlorophyll content began to be observed from 3rd week after transplanting. That the relative YEB-1 chlorophyll around 100 in KDML105 planted separately or together with PSL1 and PSL1 planted with KDML105 means that in these treatments there was no difference between plants in Mn_0 and in $Mn_{0.5}$. In contrast the significantly lower relative YEB-1 chlorophyll content of PSL1 indicated an effect of Mn deficiency in this Mn inefficient genotype (Figure 3.2). The same effect of KDML105 influenced PSL1 growing together in the same Mn_0 pot was also observed on relative number of tillers (Figure 3.3) and leaves (Figure 3.4) and from 5th week after transplanting.

Dry weight

Shoot dry weight

The effect of Mn deficiency in Mn_0 on shoot dry weight was clearly observed at 4 weeks after transplanting, but there was no difference between the genotypes. Differences between genotypes and planting combination became significant at 8 weeks after transplanting (Table 3.1). At 8 weeks after transplanting, Mn inefficient

PSL1 and efficient KDML105 showed expected response to Mn when grown separately. When grown separately, shoot dry weight of PSL1 in Mn_0 was only 55% of that in $Mn_{0.5}$ and also significantly lower than KDML105 in Mn_0. In contrast, KDML105 showed only a slight depression in shoot dry weight in Mn_0 compared with $Mn_{0.5}$. When grown together in the same Mn_0 pot, shoot dry weight of both KDML105 and PSL1 were significantly higher than that of PSL1 in Mn_0, and showing no significant difference from when they were in $Mn_{0.5}$. Response to Mn of the genotypes with different Mn efficiency growing separately or together is clearly distinguished be the relative shoot dry weight (dry weight in $Mn_0/Mn_{0.5}$). It was lower in PSL1 at 55% than KDML105 at 80%, and higher when the genotypes were grown together, 104% for PLS1 and 115% for KDML105.

Root dry weight

At 4 weeks after transplanting, there was a significant interaction between Mn levels and genotypes with their different planting system on root dry weight (GP x Mn $P < 0.001$). In Mn_0, root dry weight was lowest in PSL1 planted separately, while PSL1 planted with KDML105 and KDML105 planted separately or together with PSL1 had about the same root dry weight, which were twice or more that of PSL1 planted separately in Mn_0 (Table 3.2).

The same difference in root dry weight response to Mn of Mn efficient and inefficient genotypes with their different planting system was observed at 8 weeks after transplanting. By this time, the relative root dry weight (dry weight in $Mn_0/Mn_{0.5}$) was lowest in PSL1 at 42% when planted alone compared with 82% in KDML105 planted alone and 86% in PSL1 and 122% in KDML015, when the two genotypes were planted together.

Relative total dry weight

Relative total dry weight did not differ significantly between genotypes and planting at 4 weeks after transplanting (Figure 3.5 (A)). At 8 weeks after transplanting, Mn inefficient PSL1 growing alone had significantly lower relative total dry weight (total DW in Mn_0/$Mn_{0.5}$) than Mn efficient KDML105. But PSL1 planted together with KDML105 had about the same relative total dry weight approaching 100, i.e. total dry weight in Mn_0 was almost the same as that in $Mn_{0.5}$, which was the case with KDML105, planted alone or together with PSL1 (Figure 3.5 (B)).

Manganese concentration

YEB

At 4 weeks after transplanting, there was significant difference between genotypes and planting system and Mn levels in term of Mn concentration in YEB (Table 3.3). Manganese deficiency condition increased YEB Mn concentration of all genotypes when compared to Mn sufficiency condition. In Mn_0, Mn concentration in YEB was similar in all genotypes. In $Mn_{0.5}$, PSL1 planted separately or together with KDML105 had the highest Mn concentration in YEB, whereas KDML105 planted separately or together with PSL1 were the lowest.

At 8 weeks after transplanting, YEB Mn concentration did not differ significantly in all genotypes and planting system (Table 3.3).

Shoot

In response to Mn deficiency in term of Mn concentration in shoot, there was significant difference between genotypes and planting system and Mn levels at both 4 and 8 weeks (Table 3.4). At 4 weeks after transplanting, it was found that KDML105

planted separately or together with PSL1 and PSL1 planted with KDML105 had higher Mn concentration in shoot than PSL1 growing alone in Mn_0. Shoot Mn concentrations of PSL1 growing alone increased when grown in $Mn_{0.5}$, whereas PSL1 planted with KDML105 were similar when compared with $Mn_{0.5}$.

At 8 weeks after transplanting, shoot Mn concentration in Mn_0 of PSL1 was the lowest when growing alone but increased to the same level as Mn efficient KDML105 when grown together. Increasing Mn level in the nutrient solution from Mn_0 to $Mn_{0.5}$, it increased Mn concentration in the shoot of PSL1 when grown separately but did not affect it when grown together with KDML105 (Table 3.4).

Root

For Mn concentration in root, there was significant difference between genotypes and planting system and Mn levels in both 4 and 8 weeks after transplanting (Table 3.5). At 4 weeks after transplanting, root Mn concentration in Mn_0 of PSL1 was the lowest when growing alone but was increased to the same level as Mn efficient KDML105 when grown together. Mn concentration in root of PSL1 grown separately or together with KDML105 and KDML105 planted with PSL1 increased when increasing Mn level in the nutrient solution from Mn_0 to $Mn_{0.5}$ (Table 3.5).

At 8 weeks after transplanting, Mn concentration in root of KDML105 grown separately, KDML105 and PSL1 growing in the same pot were similar whereas it was decreased in PSL1 grown separately compared to Mn sufficiency condition.

Manganese content

Shoot

At 4 weeks after transplanting, shoot Mn content was not affected between genotypes and planting system and Mn levels (Table 3.6).

At 8 weeks after transplanting, shoot Mn content in Mn_0 of PSL1 was the lowest when growing alone but was increased to the same level as Mn efficient KDML105 when grown together. KDML105, however, had significantly higher relative Mn content when grown together with PSL1 than grown alone. Increasing Mn level in the nutrient solution from Mn_0 to $Mn_{0.5}$ tripled Mn content in the shoot of PSL1 when grown separately but did not affect it when grown together with KDML105.

Root

At 4 weeks after transplanting, in Mn_0 root Mn content of PSL1 growing alone did not differ significantly in PSL1 and KDML105 grown together but were lower than that of KDML105 growing alone (Table 3.7). Increasing Mn in the nutrient solution to $Mn_{0.5}$ increased root Mn contents in all genotypes and planting system, but most strongly in PSL1 growing alone.

At 8 weeks after transplanting, root Mn content in Mn_0 of PSL1 growing alone was the lowest. The root Mn of PSL1 strongly responded on increasing from Mn_0 to $Mn_{0.5}$ whereas the root Mn of KDML105 and PSL1 that was growing with KDML105 were not affected by Mn levels in the nutrient solution (Table 3.7).

Whole plant

At 4 weeks after transplanting, whole plant Mn content of PSL1 growing alone in Mn_0 was lower than that of KDML105 (Table 3.8). Increasing Mn in the

nutrient solution to $Mn_{0.5}$ increased root Mn contents in all genotypes and planting system.

At 8 weeks after transplanting, whole plant Mn content in Mn_0 was the lowest in PSL1 growing alone and it was increased to the same level as KDML105 when grown together with Mn efficient genotype. Increasing Mn to $Mn_{0.5}$ increased whole shoot Mn in PSL1 growing by itself but not KDML105 and PSL1 growing together with KDML105.

Manganese uptake efficiency

At 4 weeks after transplanting, relative Mn uptake efficiency (plant Mn per g root DW in $Mn_0/Mn_{0.5}$) did not differ significantly between genotypes or planting system (Figure 3.6 (A)). By 8 weeks after transplanting, however, the relative Mn uptake efficiency of PSL1 was significantly lower when it was growing alone than when grown together with KDML105, which was about the same as that for KDML105 (Figure 3.6 (B)).

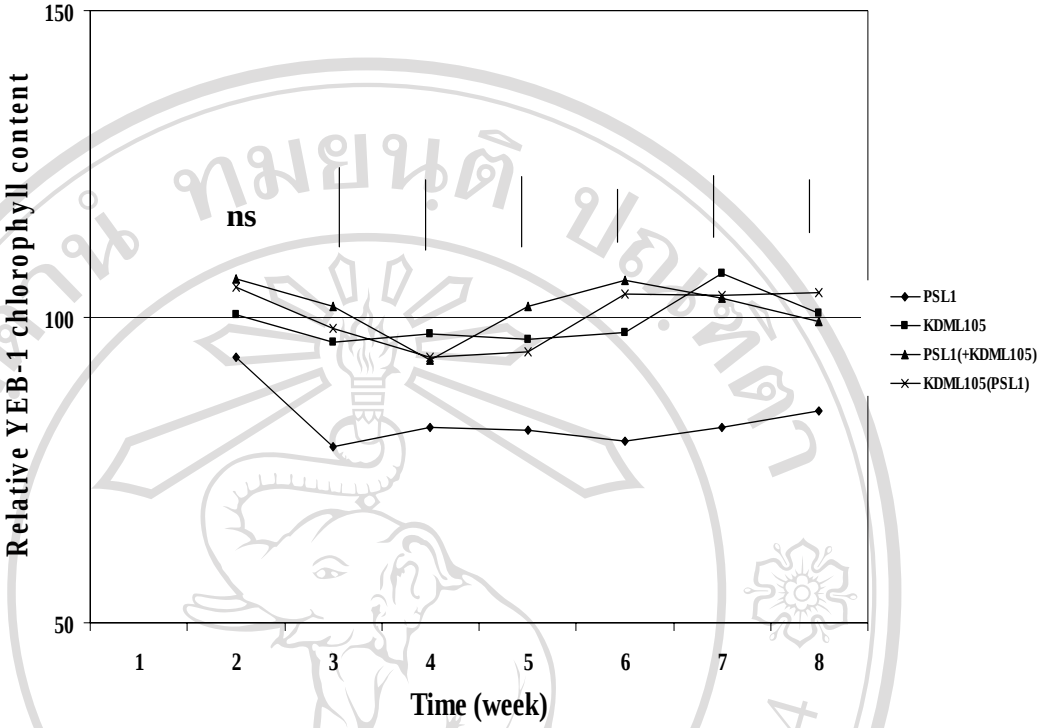

Figure 3.2 Relative Mn_0 / $Mn_{0.5}$ of chlorophyll content in YEB-1 at 1 - 8 weeks after transplanting. (Mn_0 = 0 ppm and $Mn_{0.5}$ = 0.5 ppm)

The difference between varieties is indicated by LSD at $P < 0.05$ presented by each bar.

ns = nonsignificant at $P < 0.05$.

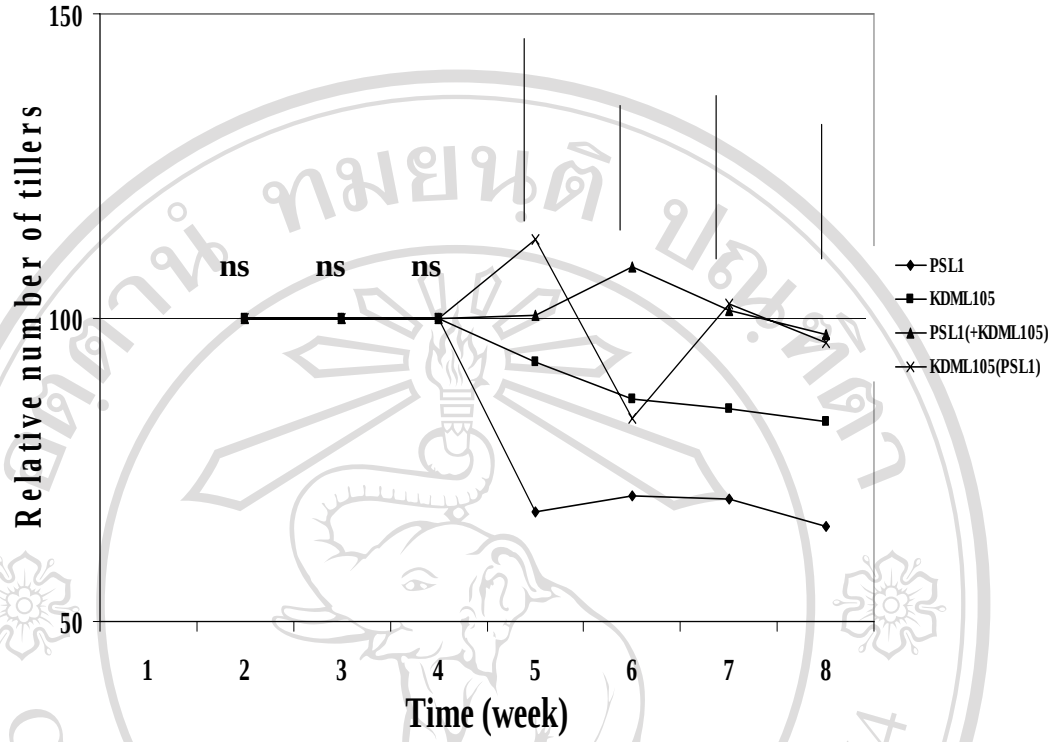

Figure 3.3 Relative Mn_0 / $Mn_{0.5}$ of number of tillers at 1 - 8 weeks after transplanting**.** The difference between varieties is indicated by LSD at $P < 0.05$ presented by each bar.

ns = nonsignificant at $P < 0.05$.

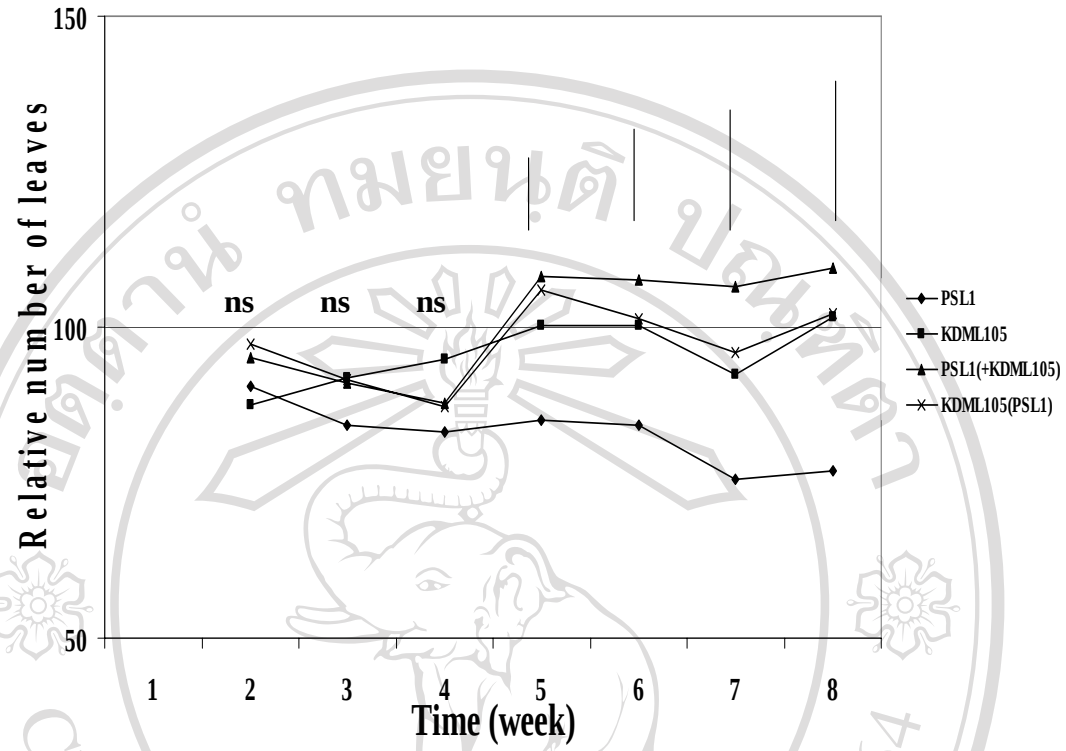

Figure 3.4 Relative Mn_0 / $Mn_{0.5}$ of number of leaves at 1 - 8 weeks after transplanting.

The difference between varieties is indicated by LSD at $P < 0.05$ presented by each bar.

ns = nonsignificant at $P < 0.05$.

Table 3.1 Shoot dry weight (g plant^{-1}) of two rice genotypes grown together in pairs in the same pot and separately in sand culture at 4 and 8 weeks after transplanting**.**

Genotype and planting	Mn level (ppm)			Relative Shoot
	0	0.5	Mean	Dry weight
4 weeks				
PSL1 (P1)	0.159	0.266	0.213	60.55
KDML105 (P2)	0.175	0.234	0.205	78.36
PSL1(+KDML105)(P3)	0.153	0.329	0.241	46.30
KDML105(PSL1)(P4)	0.170	0.282	0.226	62.96
Mean	0.16b	0.28a	0.22	62.04
F-test	GP^{ns}	Mn***	GPxMn^{ns}	GP^{ns}
$LSD_{(0.05)}$		0.0346		
8 weeks				
PSL1 (V1)	1.74bC	3.19aA	2.47	55.44C
KDML105 (V2)	2.39bAB	2.97aB	2.68	80.45BC
PSL1(+KDML105)(V3)	2.04aBC	2.01aC	2.02	103.58AB
KDML105(PSL1)(V4)	2.75aA	2.39aC	2.57	115.20A
Mean	2.23	2.64	2.44	88.67
F-test	GP**	Mn**	GPxMn***	GP**
$LSD_{(0.05)}$			0.4414	28.585

^{ns}, ** and *** non significant, significant at $P < 0.01$ and $P < 0.001$, respectively. GP, Mn and GPxMn indicated F-test for genotype and planting, Mn level and genotype and planting and Mn level interaction effects, respectively. The difference between genotype and planting is the same column is indicated by upper case letters. The difference between Mn level in the same row is indicated by lower case letters.

Table 3.2 Root dry weight (g plant^{-1}) of two rice genotypes grown together in pairs in the same pot and separately in sand culture at 4 and 8 weeks after transplanting**.**

Variety	Mn level (ppm)			Relative Root
	0	0.5	Mean	Dry weight
4 weeks				
PSL1 (V1)	0.018bA	0.104aB	0.061	17.51B
KDML105 (V2)	0.040aA	0.062aC	0.051	67.84A
PSL1(+KDML105)(V3)	0.038bA	0.143aA	0.091	27.18B
KDML105(PSL1)(V4)	0.038bA	0.109aB	0.073	35.44B
Mean	0.034	0.105	0.069	36.99
F-test	GP**	Mn***	GP*Mn**	GP*
$LSD_{(0.05)}$			0.0283	29.959
8 weeks				
PSL1(V1)	0.25bA	0.60aA	0.42	41.82C
KDML105(V2)	0.29aA	0.36aB	0.33	82.25B
PSL1(+KDML105)(V3)	0.31aA	0.36aB	0.34	86.42B
KDML105(PSL1)(V4)	0.32aA	0.26aC	0.29	121.69A
Mean	0.29	0.39	0.34	83.05
F-test	GP**	Mn***	GP*Mn***	GP***
$LSD_{(0.05)}$			0.0956	26.169

** and *** Significant at $P < 0.01$ and $P < 0.001$, respectively. GP, Mn and GPxMn indicated F-test for genotype and planting, Mn level and genotype and planting and Mn level interaction effects, respectively. The difference between genotype and planting is the same column is indicated by upper case letters. The difference between Mn level in the same row is indicated by lower case letters.

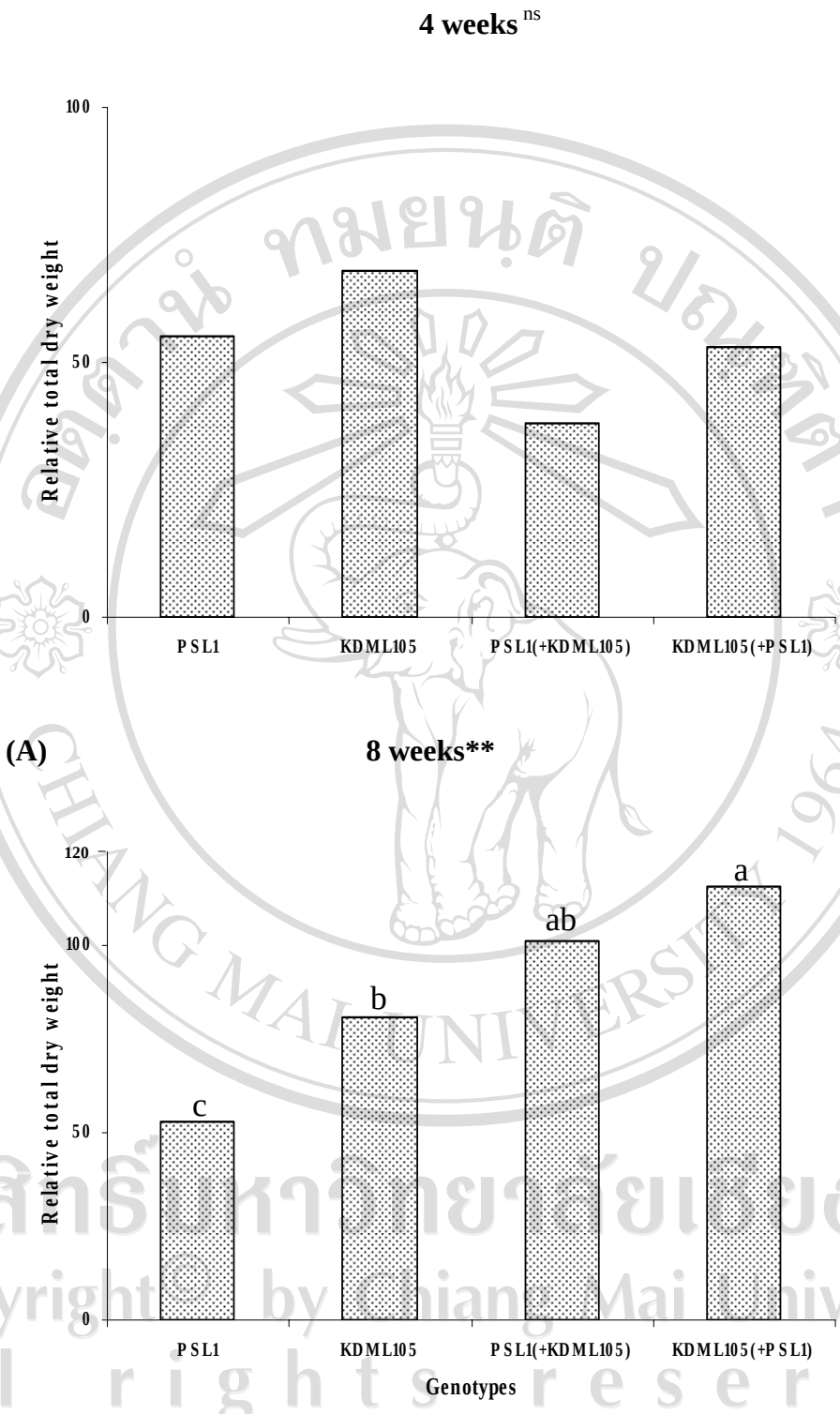

Figure 3.5 Relative total dry weight of two rice genotypes at Mn_0 compared with $Mn_{0.5}$ in sand culture at (A) 4 weeks and (B) 8 weeks after transplanting**.**

ns and ** non significant and significant at $P < 0.01$, respectively.

Table 3.3 Mn concentration in YEB (mg Mn kg^{-1}) of two rice genotypes grown together in pairs in the same pot and separately in sand culture at 4 and 8 weeks after transplanting**.**

Genotype and planting	Mn level (ppm)		
	0	0.5	Mean
4 weeks			
PSL1(P1)	9.46bA	76.06aA	42.76
KDML105(P2)	9.57bA	60.69aB	35.13
PSL1(+KDML105)(P3)	8.70bA	72.14aA	40.42
KDML105(PSL1)(P4)	7.52bA	62.00aB	34.76
Mean	8.81	67.72	38.27
F-test	GP**	Mn***	GPxMn*
LSD(0.05)			6.842
8 weeks			
PSL1(P1)	12.52bA	141.60aA	77.06
KDML105(P2)	12.01bA	120.39aB	66.20
PSL1(+KDML105)(P3)	13.67bA	138.21aA	75.94
KDML105(PSL1)(P4)	12.19bA	121.14aB	66.67
Mean	12.60	130.34	71.47
F-test	GP**	Mn***	GPxMn**
LSD(0.05)			8.649

*, ** and *** significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively. GP, Mn and GPxMn indicated F-test for genotype and planting, Mn level and genotype and planting and Mn level interaction effects, respectively. The difference between genotype and planting is the same column is indicated by upper case letters. The difference between Mn level in the same row is indicated by lower case letters.

Table 3.4 Mn concentration in shoot (mg Mn kg^{-1}) of two rice genotypes grown together in pairs in the same pot and separately in sand culture at 4 and 8 weeks after transplanting**.**

Genotype and planting	Mn level (ppm)		
	0	0.5	Mean
4 weeks			
PSL1(P1)	107.8bB	182.2aA	145.0
KDML105(P2)	142.6aA	161.7aA	152.1
PSL1(+KDML105)(P3)	149.4aA	169.9aA	159.7
KDML105(PSL1)(P4)	136.4bA	161.4aA	148.9
Mean	134.0	168.8	151.4
F-test	GP ^{ns}	Mn***	GPxMn**
LSD(0.05)			23.412
8 weeks			
PSL1(P1)	257.3bB	400.6aA	329.0
KDML105(P2)	323.2aA	268.0aC	295.6
PSL1(+KDML105)(P3)	339.6aA	327.9aB	333.7
KDML105(PSL1)(P4)	333.5bA	268.9aC	301.2
Mean	313.4	316.4	314.9
F-test	GP ^{ns}	Mn^{ns}	GPxMn***
LSD(0.05)			57.775

^{ns}, ** and *** non significant, significant at $P < 0.01$ and $P < 0.001$, respectively. GP, Mn and GPxMn indicated F-test for genotype and planting, Mn level and genotype and planting and Mn level interaction effects, respectively. The difference between genotype and planting is the same column is indicated by upper case letters. The difference between Mn level in the same row is indicated by lower case letters.

Table 3.5 Mn concentration in root (mg Mn kg^{-1}) of two rice genotypes grown together in pairs in the same pot and separately in sand culture at 4 and 8 weeks after transplanting**.**

Genotype and planting	Mn level (ppm)		
	0	0.5	Mean
4 weeks			
PSL1(P1)	122.18bB	326.40aAB	224.29
KDML105(P2)	245.62aA	319.04aAB	282.33
PSL1(+KDML105)(P3)	196.09bAB	360.04aAB	278.06
KDML105(PSL1)(P4)	187.78bAB	429.86aA	308.82
Mean	187.92	358.83	273.38
F-test	GP *	Mn***	GPxMn*
LSD(0.05)			76.797
8 weeks			
PSL1(P1)	437.7bB	728.0aA	582.8
KDML105(P2)	532.1aAB	449.3aB	490.7
PSL1(+KDML105)(P3)	682.9aA	484.8aB	583.8
KDML105(PSL1)(P4)	621.3aAB	454.3aB	537.8
Mean	568.5	529.1	548.8
F-test	GP^{ns}	Mn^{ns}	GPxMn*
LSD(0.05)			218.69

^{ns}, * and *** non significant, significant at $P < 0.05$ and $P < 0.001$, respectively. GP, Mn and GPxMn indicated F-test for genotype and planting, Mn level and genotype and planting and Mn level interaction effects, respectively. The difference between genotype and planting is the same column is indicated by upper case letters. The difference between Mn level in the same row is indicated by lower case letters.

Table 3.6 Mn content in shoot (mg Mn plant^{-1}) of two rice genotypes grown together in pairs in the same pot and separately in sand culture at 4 and 8 weeks after transplanting**.**

Genotype and planting	Mn level (ppm)		
	0	0.5	Mean
4 weeks			
PSL1(P1)	0.017	0.049	0.033
KDML105(P2)	0.025	0.038	0.032
PSL1(+KDML105)(P3)	0.023	0.056	0.039
KDML105(PSL1)(P4)	0.023	0.046	0.035
Mean	0.022b	0.047a	0.035
F-test	GP^{ns}	Mn*	GPxMn^{ns}
LSD(0.05)			0.011
8 weeks			
PSL1(P1)	0.447bC	1.264aA	0.855
KDML105(P2)	0.770aB	0.797aB	0.783
PSL1(+KDML105)(P3)	0.685aB	0.661aBC	0.673
KDML105(PSL1)(P4)	0.917aA	0.643bC	0.780
Mean	0.705	0.841	0.773
F-test	GP **	Mn*	GPxMn***
LSD(0.05)			0.894

^{ns}, *, ** and *** non significant, significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively. GP, Mn and GPxMn indicated F-test for genotype and planting, Mn level and genotype and planting and Mn level interaction effects, respectively. The difference between genotype and planting is the same column is indicated by upper case letters. The difference between Mn level in the same row is indicated by lower case letters.

Table 3.7 Mn content in root (mg Mn plant^{-1}) of two rice genotypes grown together in pairs in the same pot and separately in sand culture at 4 and 8 weeks after transplanting**.**

Genotype and planting	Mn level (ppm)		
	0	0.5	Mean
4 weeks			
PSL1(P1)	0.003bC	0.0336aB	0.018
KDML105(P2)	0.010bA	0.0195aC	0.015
PSL1(+KDML105)(P3)	0.008bB	0.0509aA	0.029
KDML105(PSL1)(P4)	0.007bB	0.0458aA	0.026
Mean	0.007	0.374	0.022
F-test	GP***	Mn***	GPxMn***
LSD(0.05)			0.0075
8 weeks			
PSL1(P1)	0.11bB	0.43aA	0.27
KDML105(P2)	0.16aAB	0.16aB	0.16
PSL1(+KDML105)(P3)	0.21aA	0.17aB	0.19
KDML105(PSL1)(P4)	0.20aA	0.12aB	0.16
Mean	0.17	0.22	0.20
F-test	GP**	Mn*	GPxMn***
LSD(0.05)			0.0894

*, ** and *** significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively. GP, Mn and GPxMn indicated F-test for genotype and planting, Mn level and genotype and planting and Mn level interaction effects, respectively. The difference between genotype and planting is the same column is indicated by upper case letters. The difference between Mn level in the same row is indicated by lower case letters.

Table 3.8 Mn content in whole plant (mg Mn plant^{-1}) of two rice genotypes grown together in pairs in the same pot and separately in sand culture at 4 and 8 weeks after transplanting**.**

Genotype and planting	Mn level (ppm)		
	0	0.5	Mean
4 weeks			
PSL1(P1)	0.041bB	0.188aB	0.115
KDML105(P2)	0.083bA	0.142aC	0.113
PSL1(+KDML105)(P3)	0.067bAB	0.249aA	0.158
KDML105(PSL1)(P4)	0.068bAB	0.228aA	0.148
Mean	0.065	0.202	0.133
F-test	GP**	Mn***	GPxMn**
LSD(0.05)			0.0395
8 weeks			
PSL1(P1)	0.86bB	2.77aA	1.81
KDML105(P2)	1.44aA	1.50aB	1.47
PSL1(+KDML105)(P3)	1.62aA	1.14aB	1.38
KDML105(PSL1)(P4)	1.92aA	1.21aB	1.57
Mean	1.46	1.65	1.56
F-test	GP^{ns}	Mn^{ns}	GPxMn***
LSD(0.05)			2.016

^{ns}, ** and *** non significant, significant at $P < 0.01$ and $P < 0.001$, respectively. GP, Mn and GPxMn indicated F-test for genotype and planting, Mn level and genotype and planting and Mn level interaction effects, respectively. The difference between genotype and planting is the same column is indicated by upper case letters. The difference between Mn level in the same row is indicated by lower case letters.

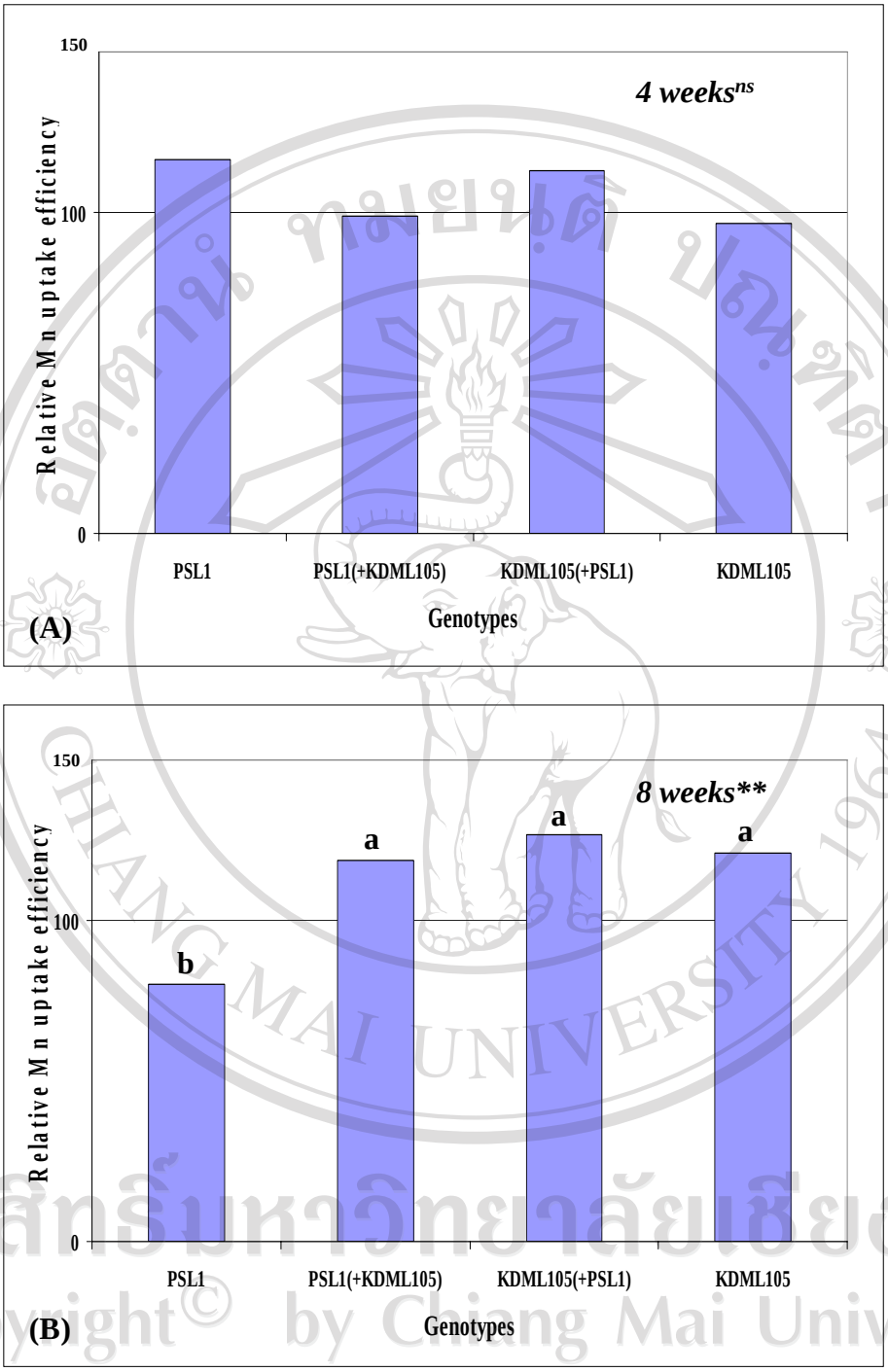

Figure 3.6 Relative Mn uptake efficiency at Mn_0 compared with $Mn_{0.5}$ of rice genotypes grown in sand culture at (A) 4 weeks and (B) 8 weeks after transplanting. (Mn uptake efficiency= (Mn content of shoot + Mn content of root) / root dry weight)

Sub experiment 2 **Ability to use different forms of Mn in Mn efficient and Mn inefficient genotypes**

Rice genotypes grown in different Mn levels and forms differed in their YEB-1 chlorophyll content, number of leaves and tillers. YEB-1 chlorophyll content of KDML105 did not differ in all Mn levels and forms. YEB-1 chlorophyll content in 0.5Mn (II) of PSL1 was the highest followed by in 0.25Mn(II)+0.25Mn(IV) and 0.5Mn (IV), whereas in 0Mn was the lowest. In 0Mn and 0.5Mn (IV), YEB-1 chlorophyll content of KDML105 were higher than PSL1 but KDML105 and PSL1 in 0.5Mn (II) and 0.25Mn(II)+0.25Mn(IV) were similar (Table 3.9).

Number of leaves of KDML105 did not differ significantly in all Mn levels and forms. In 0.5Mn (II) and 0.5Mn (IV) of PSL1 had number of leaves higher than in 0Mn and 0.25Mn(II)+0.25Mn(IV). Number of leaves in 0Mn and 0.25Mn(II)+0.25Mn(IV) of KDML105 were higher than PSL1 but KDML105 and PSL1 were similar in 0.5Mn (II) and 0.5Mn(IV) (Table 3.10).

Mn levels and forms did not affect on number of tillers of KDML105 but affected on PSL1. Number of tillers of PSL1 in 0.5Mn (II), 0.5Mn (IV) and 0.25Mn(II) +0.25Mn(IV) were higher than in 0Mn. In 0Mn, number of tillers of KDML105 were higher than PSL1 but did not differ in other Mn levels and forms (Table 3.11).

Dry weight

Mn levels and forms did not affect on shoot dry weight of all genotypes but affected on root dry weight (Table 3.12). Root dry weight of PSL1 did not differ significantly in all Mn levels and forms. In 0.5Mn (II) and 0.5Mn (IV), root dry weight of KDML105 was higher than in 0Mn and 0.25Mn(II)+0.25Mn(IV). In 0Mn,

root dry weight of KDML105 was lower than PSL1 whereas KDML105 and PSL1 in other levels and forms of Mn were similar (Table 3.13).

Manganesen concentration

The concentration of Mn in YEB was significantly different between genotypes and Mn levels and forms (Table 3.14). In 0.5Mn (II), Mn concentration in YEB of KDML105 and PSL1 was the highest following by 0.25Mn(II)+0.25Mn(IV), whereas it was the lowest in 0.5Mn (IV) and 0Mn. Shoot Mn concentration of all genotypes did not differ in all Mn levels and forms (Table 3.15). In 0.25Mn(II)+0.25Mn(IV), Mn concentration in root of KDML105 and PSL1 was higher than other Mn levels and forms. Root Mn concentration of KDML105 in 0.25Mn(II)+0.25Mn(IV), 0.5Mn (IV) and 0Mn was higher than PSL1 whereas it was similar in 0.5Mn (II) (Table 3.16).

Manganesen content

The effect of Mn levels and forms on shoot Mn content was clear but there was no difference between the genotypes (Table 3.17). Differences between genotypes and Mn levels and forms combination became significant in root Mn content. Mn content in root of PSL1 was not significant in all Mn levels and forms. In 0.5Mn (IV) and 0.25Mn(II)+0.25Mn(IV), Mn content in root of KDML105 was higher than PSL1 (Table 3.18).

Mn levels and forms affected on Mn uptake efficiency in all genotypes. Mn uptake efficiency of KDML105 did not differ significantly in all Mn levels and forms. Mn uptake efficiency in 0.5Mn (II) of PSL1 was the highest whereas it was the lowest in other Mn levels and forms. In 0Mn, 0.5Mn (IV) and 0.25Mn(II) +0.25Mn(IV), Mn uptake efficiency of KDML105 was higher than PSL1 (Table 3.19).

Table 3.9 Response to Mn (II) and Mn (IV) of YEB-1 chlorophyll content (SPAD unit) in two rice genotypes grown at 30 days after transplanting.

Variety	Mn level (ppm)				Mean
	0Mn	0.5Mn (II)	0.5Mn (IV)	0.25Mn (II) +0.25Mn (IV)	
PSL1	21.65cB	30.17aA	24.21bcB	26.97abA	25.75
KDML105	29.29aA	31.11aA	31.20aA	29.51aA	30.28
Mean	25.47	30.64	27.70	28.24	28.01
F-test	V***	Mn***	VxMn***		
LSD			4.0516		

*** Significant $P < 0.001$. V, Mn and VxMn indicated F-test variety, Mn level and variety and Mn level interaction effects, respectively. The difference between varieties in the same column is indicated by upper case letters. The difference between Mn levels in the same row is indicated by lower case letters.

Table 3.10 Response to Mn (II) and Mn (IV) of number of leaves (plant^{-1}) in two rice genotypes grown at 30 days after transplanting.

Variety	Mn level (ppm)				Mean
	0Mn	0.5Mn (II)	0.5Mn (IV)	0.25Mn (II) +0.25Mn (IV)	
PSL1	7.75bB	9.53aA	8.73abA	6.80bB	8.20
KDML105	9.13aA	9.47aA	8.60aA	8.37aA	8.89
Mean	8.44	9.50	8.67	7.58	8.55
F-test	V***	Mn***	VxMn **		
LSD			1.211		

*** Significant $P < 0.001$. V, Mn and VxMn indicated F-test for variety, Mn level and variety and Mn level interaction effects, respectively. The difference between varieties in the same column is indicated by upper case letters. The difference between Mn levels in the same row is indicated by lower case letters.

Table 3.11 Response to Mn (II) and Mn (IV) of number of tillers (plant^{-1}) in two rice genotypes grown at 30 days after transplanting.

Variety	Mn level (ppm)				Mean
	0Mn	0.5Mn (II)	0.5Mn (IV)	0.25Mn (II) +0.25Mn (IV)	
PSL1	1.07bB	1.85aA	1.27abA	1.67abA	1.46
KDML105	1.87aA	1.93aA	1.72aA	1.73aA	1.81
Mean	1.47	1.89	1.49	1.70	1.64
F-test	V**	Mn*	VxMn *		
LSD			0.7066		

*, ** Significant $P < 0.05$ and $P < 0.01$, respectively. V, Mn and VxMn indicated F-test for variety, Mn level and variety and Mn level interaction effects, respectively. The difference between varieties in the same column is indicated by upper case letters. The difference between Mn levels in the same row is indicated by lower case letters.

Table 3.12 Response to Mn (II) and Mn (IV) of shoot dry weight (g plant^{-1}) in two rice genotypes grown at 30 days after transplanting.

Variety	Mn level (ppm)				Mean
	0Mn	0.5Mn (II)	0.5Mn (IV)	0.25Mn (II) +0.25Mn (IV)	
PSL1	4.74	5.62	4.41	3.65	4.60
KDML105	4.68	5.76	5.12	3.65	4.80
Mean	4.71b	5.69a	4.77b	3.65b	4.70
F-test	V^{ns}	Mn**	VxMn ^{ns}		
LSD		1.6789	2.9517		

^{ns} and ** non significant and significant at $P < 0.01$, respectively. V, Mn and VxMn indicated F-test for variety, Mn level and variety and Mn level interaction effects, respectively. The difference between varieties in the same column is indicated by upper case letters. The difference between Mn levels in the same row is indicated by lower case letters.

Table 3.13 Response to Mn (II) and Mn (IV) of root dry weight (g plant^{-1}) in two rice genotypes grown at 30 day after transplanting.

Variety	Mn level (ppm)				Mean
	0Mn	0.5Mn (II)	0.5Mn (IV)	0.25Mn (II) +0.25Mn (IV)	
PSL1	0.85aA	0.64aA	0.78aA	0.63aA	0.73
KDML105	0.47bB	0.64aA	0.81aA	0.55bA	0.62
Mean	0.66	0.64	0.79	0.59	0.67
F-test	V**	Mn**	VxMn ***		
LSD			0.2198		

*, ** and *** Significant $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively. V, Mn and VxMn indicated F-test for variety, Mn level and variety and Mn level interaction effects, respectively. The difference between varieties in the same column is indicated by upper case letters. The difference between Mn levels in the same row is indicated by lower case letters.

Table 3.14 Response to Mn (II) and Mn (IV) of Mn concentration in YEB (mg Mn kg^{-1}) in two rice genotypes grown at 30 days after transplanting.

Variety	Mn level (ppm)				Mean
	0Mn	0.5Mn (II)	0.5Mn (IV)	0.25Mn (II) +0.25Mn (IV)	
PSL1	7.54cA	112.86aA	7.02cA	32.86bA	40.07
KDML105	8.34cA	107.60aA	6.34cA	30.37bA	38.16
Mean	7.94	110.23	6.68	31.61	39.12
F-test	V^{ns}	Mn***	VxMn*		
$LSD_{0.05}$			9.622		

* and *** significant at $P < 0.05$ and $P < 0.001$, respectively. V, Mn and VxMn indicated F-test for variety, Mn level and variety and Mn level interaction effects, respectively. The difference between varieties in the same column is indicated by upper case letters. The difference between Mn levels in the same row is indicated by lower case letters.

Table 3.15 Response to Mn (II) and Mn (IV) of shoot Mn concentration (mg Mn kg^{-1}) in two rice genotypes grown at 30 days after transplanting.

Variety	Mn level (ppm)				Mean
	0Mn	0.5Mn (II)	0.5Mn (IV)	0.25Mn (II) +0.25Mn (IV)	
PSL1	430.76	882.99	468.30	617.64	599.92B
KDML105	562.69	932.98	569.91	892.49	739.52A
Mean	496.73c	907.99a	519.11c	755.07b	669.72
F-test	V***	Mn***	VxMn ^{ns}		
$LSD_{0.05}$			142.77		

^{ns} and *** non significant and significant at $P < 0.001$, respectively. V, Mn and VxMn indicated F-test for variety, Mn level and variety and Mn level interaction effects, respectively. The difference between varieties in the same column is indicated by upper case letters. The difference between Mn levels in the same row is indicated by lower case letters.

Table 3.16 Response to Mn (II) and Mn (IV) of root Mn concentration (mg Mn kg^{-1}) in two rice genotypes grown at 30 days after transplanting.

Variety	Mn level (ppm)				Mean
	0Mn	0.5Mn (II)	0.5Mn (IV)	0.25Mn (II) +0.25Mn (IV)	
PSL1	356.24bB	391.92abA	355.29bB	452.40aB	388.96
KDML105	463.76bA	479.11bA	478.07bA	747.28aA	542.06
Mean	410.00	435.52	416.68	599.84	465.51
F-test	V***	Mn***	VxMn *		
$LSD_{0.05}$			94.923		

* and *** Significant at $P < 0.05$ and $P < 0.001$, respectively. V, Mn and VxMn indicated F-test for variety, Mn level and variety and Mn level interaction effects, respectively. The difference between varieties in the same column is indicated by upper case letters. The difference between Mn levels in the same row is indicated by lower case letters.

Table 3.17 Response to Mn (II) and Mn (IV) of shoot Mn content (mg Mn plant^{-1}) in two rice genotypes grown at 30 days after transplanting.

Variety	Mn level (ppm)				Mean
	0Mn	0.5Mn (II)	0.5Mn (IV)	0.25Mn (II) +0.25Mn (IV)	
PSL1	2.04	4.96	2.05	2.24	2.82
KDML105	2.70	5.54	2.93	3.29	3.62
Mean	2.37b	5.25a	2.49b	2.76b	3.22
F-test	V^{ns}	Mn**	VxMn ^{ns}		
$LSD_{0.05}$			1.995		

^{ns} and ** non significant and significant at $P < 0.01$, respectively. V, Mn and VxMn indicated F-test for variety, Mn level and variety and Mn level interaction effects, respectively. The difference between varieties in the same column is indicated by upper case letters. The difference between Mn levels in the same row is indicated by lower case letters.

Table 3.18 Response to Mn (II) and Mn (IV) of root Mn content (mg Mn plant^{-1}) in two rice genotypes grown at 30 days after transplanting.

Variety	Mn level (ppm)				Mean
	0Mn	0.5Mn (II)	0.5Mn (IV)	0.25Mn (II) +0.25Mn (IV)	
PSL1	0.30aA	0.25aA	0.28aB	0.29aB	0.28
KDML105	0.22cA	0.31bcA	0.39abA	0.41aA	0.33
Mean	0.26	0.28	0.33	0.35	0.30
F-test	V*	Mn*	VxMn *		
$LSD_{0.05}$			0.097		

* Significant at $P < 0.05$. V, Mn and VxMn indicated F-test for variety, Mn level and variety and Mn level interaction effects, respectively. The difference between varieties in the same column is indicated by upper case letters. The difference between Mn levels in the same row is indicated by lower case letters.

Table 3.19 Response to Mn (II) and Mn (IV) of Mn uptake efficiency (mg Mn g^{-1} root DW) in two rice genotypes grown at 30 days after transplanting.

Variety	Mn level (ppm)				Mean
	0Mn	0.5Mn (II)	0.5Mn (IV)	0.25Mn (II) +0.25Mn (IV)	
PSL1	2.75bB	8.19aA	2.99bB	3.97bB	4.48
KDML105	7.21aA	7.87aA	6.78aA	6.61aA	7.12
Mean	4.98	8.03	4.88	5.29	5.80
F-test	V***	Mn***	VxMn ***		
$LSD_{0.05}$			1.348		

*** Significant at $P < 0.001$. V, Mn and VxMn indicated F-test for variety, Mn level and variety and Mn level interaction effects, respectively. The difference between varieties in the same column is indicated by upper case letters. The difference between Mn levels in the same row is indicated by lower case letters.

Experiment 3.3.2 Manganese acquisition of rice in solution culture

Sub experiment 1 **Comparing the responses of rice to manganese deficiency with and without aeration of the nutrient solution**

YEB-1 chlorophyll content of rice genotypes grown with and without oxygen supply in nutrient solution did not differ in all genotypes at 15 days after transplanting (DAT) (Figure 3.7). At 30 DAT, YEB-1 chlorophyll content of KDML105 was not affected by oxygen supply, whereas PSL1 increased when grown in nutrient solution with oxygen supply by air bubbling. YEB-1 chlorophyll content of KDML105 in all conditions was higher than PSL1 as well as at 15 DAT (Figure 3.8). Beside, Root length of all genotypes was not effected when grown with and without oxygen supply in absence of Mn (Table 3.20) like in shoot lenght. Shoot length of all genotypes did not differ between oxygen supply (Table 3.21).

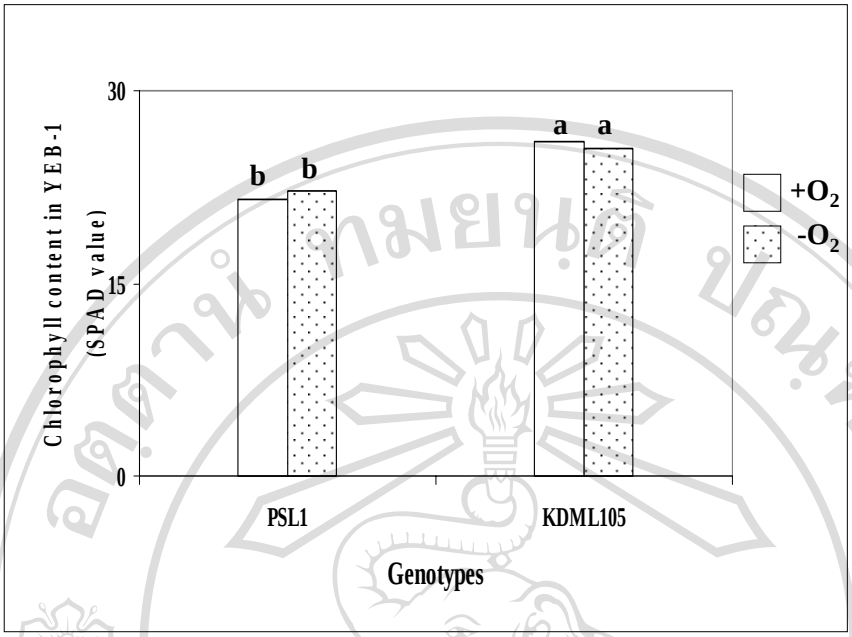

Figure 3.7 Response to Mn deficiency of chlorophyll content in YEB-1 (SPAD unit) of KDML105 and PSL1 grown in nutrient solution with and without oxygen supply ($+O_2$ and $-O_2$) at 15 days after transplanting.

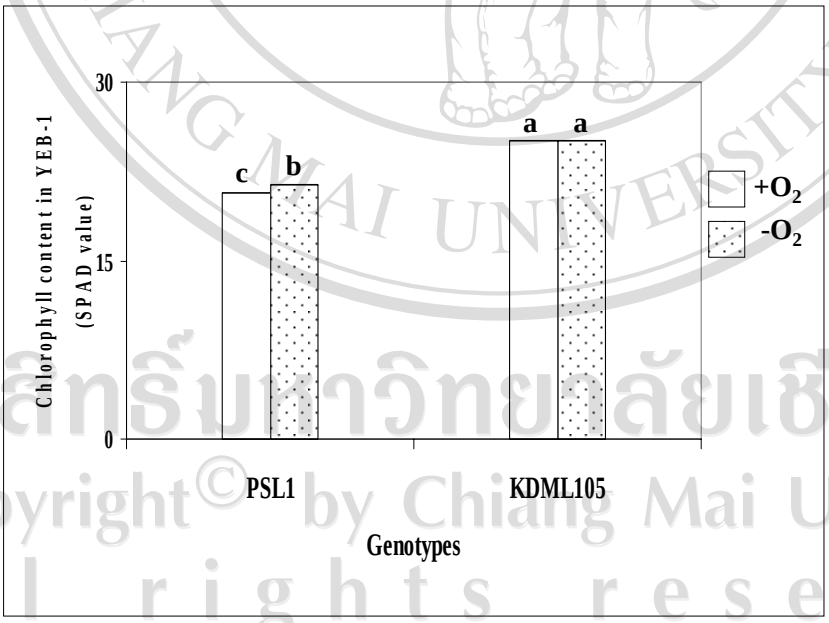

Figure 3.8 Response to Mn deficiency of chlorophyll content in YEB-1 (SPAD unit) of KDML105 and PSL1 grown in nutrient solution with and without oxygen supply ($+O_2$ and $-O_2$) at 30 days after transplanting.

Table 3.20 Response to Mn deficiency of root length (cm) of KDML105 and PSL1 grown in nutrient solution with and without oxygen supply ($+O_2$ and $-O_2$) at 15 and 30 days after transplanting (DAT).

Variety	Mn level (ppm)		Mean
	0	0.5	
15 DAT			
PSL 1	17.93	17.33	17.63
KDML105	14.72	16.28	15.50
Mean	16.33	16.81	16.57
F-test	V*	O^{ns}	VxO^{ns}
$LSD_{(0.05)}$	1.7797		2.5168
30 DAT			
PSL 1	28.90	27.99	28.45
KDML105	28.17	26.64	27.40
Mean	28.54	27.31	27.92
F-test	V^{ns}	O^{ns}	VxO^{ns}
$LSD_{(0.05)}$			7.9533

^{ns} and * Non significant, significant at $P < 0.05$, respectively. V, O and VxO indicated F-test for variety, Mn level and variety and Mn level interaction effects, respectively. The difference between varieties in the same column is indicated by upper case letters. The difference between oxygen supply in the same row is indicated by lower case letters.

Table 3.21 Response to Mn deficiency of shoot length (cm) of KDML105 and PSL1 grown in nutrient solution with and without oxygen supply ($+O_2$ and $-O_2$) at 15 and 30 days after transplanting (DAT).

Variety	Oxygen supply		Mean
	$+O_2$	$-O_2$	
15 DATs			
PSL 1	42.04	43.34	42.69
KDML105	40.14	40.70	40.42
Mean	41.09	42.02	41.56
F-test	V^{ns}	O^{ns}	VxO^{ns}
$LSD_{(0.05)}$			3.2479
30 DAT			
PSL 1	89.67	73.61	81.64
KDML105	86.67	81.40	84.04
Mean	88.17	77.51	82.84
F-test	V^{ns}	O*	VxO^{ns}
$LSD_{(0.05)}$		8.9044	12.593

^{ns} and * Non significant, significant at $P < 0.05$, respectively. V, O and VxO indicated F-test for variety, Mn level and variety and Mn level interaction effects, respectively. The difference between varieties in the same column is indicated by upper case letters. The difference between oxygen supply in the same row is indicated by lower case letters.

Sub experiment 2 **Acidification and reduction power of root exudate from Mn efficient and inefficient rice genotypes**

Chlorophyll content

Mn deficiency did not affect on YEB-1 chlorophyll content of two rice genotypes at 2 weeks after transplanting but affected on YEB-1 chlorophyll content at 3 and 4 weeks after transplanting (Table 3.22). At 3 weeks after transplanting, YEB-1 chlorophyll content of KDML105 was higher than PSL1 in Mn deficiency condition but did not differ in Mn sufficiency condition. YEB-1 chlorophyll content of PSL1 decreased when grown in Mn deficiency condition. At 4 weeks after transplanting, YEB-1 chlorophyll content of KDML105 and PSL1 increased when grown in Mn sufficiency condition. However, YEB-1 chlorophyll content of KDML105 was higher than PSL1 in Mn deficiency condition.

Number of leaves

Number of leaves of KDML105 was higher than PSL1 in Mn deficiency but did not differ in Mn sufficiency at 2 and 3 weeks after transplanting. Number of leaves of PSL1 increased when grown in Mn sufficiency condition. At 4 weeks after transplanting, number of leaves of KDML105 was higher than PSL1 in all Mn conditions. Number of leaves of KDML105 and PSL1 increased when grown in Mn sufficiency condition (Table 3.23).

Number of tillers

Mn deficiency did not affect on number of tillers of two rice genotypes at 2 weeks after transplanting. At 3 and 4 weeks after transplanting, number of tillers of KDML105 was higher than PSL1 in Mn deficiency condition. In Mn sufficiency, KDML105 and PSL1 were similar at 3 weeks after transplanting. KDML105 was

higher than PSL1 at 4 weeks after transplanting. Number of tillers of PSL1 increased when grown in Mn sufficiency (Table 3.24).

Root exudates

The possibility that some root exudates may be the basis for Mn efficiency in KDML 105 was demonstrated by MnIV reduction indicated by the decolourization of filter paper impregnate with Mn oxide. KDML 105 grown without added Mn could reduce MnIV oxide by reducing root exudates (mainly organic acid) (Figure 3.9). When KDML105 was grown with added Mn, it could not reduced MnIV oxide on filter peper. On the other hand, PSL1 could not reduced MnIV oxide on filter peper when grown in both with and without added Mn (Figure 3.10).

Moreover, KDML105 could realeasea root exudate at lower pH in rhizospere solution under Mn deficiency condition. Whereas, PSL1 in all conditions and KDML105 in Mn sufficiency condition could not reduced pH in rhizosphere solution (Table 3.25).

Table 3.22 Response to Mn of YEB-1 chlorophyll content (SPAD unit) of two rice genotypes grown at 2 – 4 weeks after transplanting.

Variety	Mn level (ppm)		Mean
	0	0.5	
2 weeks			
PSL 1	20.39	23.25	21.82
KDML105	22.94	23.18	23.06
Mean	21.67	23.21	22.44
F-test	V^{ns}	Mn^{ns}	$VxMn^{ns}$
$LSD_{(0.05)}$			2.6260
3 weeks			
PSL 1	20.85bB	28.68aA	24.77
KDML105	24.49aA	26.82aA	25.66
Mean	22.67	27.75	25.21
F-test	V^{ns}	Mn***	VxMn**
$LSD_{(0.05)}$			2.4168
4 weeks			
PSL 1	22.47bB	28.10aB	25.28
KDML105	28.50bA	30.88aA	29.69
Mean	25.48	29.49	27.49
F-test	V**	Mn**	VxMn*
$LSD_{(0.05)}$			1.9985

^{ns}, *, ** and *** Non significant, significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively. V, Mn and VxMn indicated F-test for variety, Mn level and variety and Mn level interaction effects, respectively. The difference between varieties in the same column is indicated by upper case letters. The difference between Mn levels in the same row is indicated by lower case letters.

Table 3.23 Response to Mn of number of leaves (plant^{-1}) of two rice genotypes grown at 2 -4 weeks after transplanting.

Variety	Mn level (ppm)		Mean
	0	0.5	
2 weeks			
PSL 1	10.58bB	12.72aA	11.65
KDML105	13.07aA	13.43aA	13.25
Mean	11.82	13.08	12.45
F-test	V**	Mn**	VxMn*
$LSD_{(0.05)}$			1.1687
3 weeks			
PSL 1	14.89bB	16.87aA	15.88
KDML105	17.07aA	17.11aA	17.09
Mean	15.98	16.99	16.48
F-test	V**	Mn*	VxMn*
$LSD_{(0.05)}$			1.1405
4 weeks			
PSL 1	16.94bB	20.75aB	18.85
KDML105	21.11bA	23.17aA	22.14
Mean	19.03	21.96	20.49
F-test	V***	Mn***	VxMn*
$LSD_{(0.05)}$			0.894

*, ** and *** Significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively. V, Mn and VxMn indicated F-test for variety, Mn level and variety and Mn level interaction effects, respectively. The difference between varieties in the same column is indicated by upper case letters. The difference between Mn levels in the same row is indicated by lower case letters.

Table 3.24 Response to Mn of number of tillers (plant^{-1}) of two rice genotypes grown at 2 -4 weeks after transplanting.

Variety	Mn level (ppm)		Mean
	0	0.5	
2 weeks			
PSL 1	2.56	2.33	2.45
KDML105	2.56	2.39	2.47
Mean	2.56	2.36	2.46
F-test	V^{ns}	Mn^{ns}	VxMn^{ns}
$LSD_{(0.05)}$			0.7201
3 weeks			
PSL 1	3.08bB	3.56aA	3.32
KDML105	4.06aA	4.11aA	4.09
Mean	3.57	3.84	3.70
F-test	V***	Mn**	VxMn*
$LSD_{(0.05)}$			0.264
4 weeks			
PSL 1	3.08bB	3.50aB	3.29
KDML105	3.75aA	4.75aA	4.25
Mean	3.42	4.13	3.77
F-test	V***	Mn***	VxMn**
$LSD_{(0.05)}$			0.2718

^{ns}, *, ** and *** Non significant, significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively. V, Mn and VxMn indicated F-test for variety, Mn level and variety and Mn level interaction effects, respectively. The difference between varieties in the same column is indicated by upper case letters. The difference between Mn levels in the same row is indicated by lower case letters.

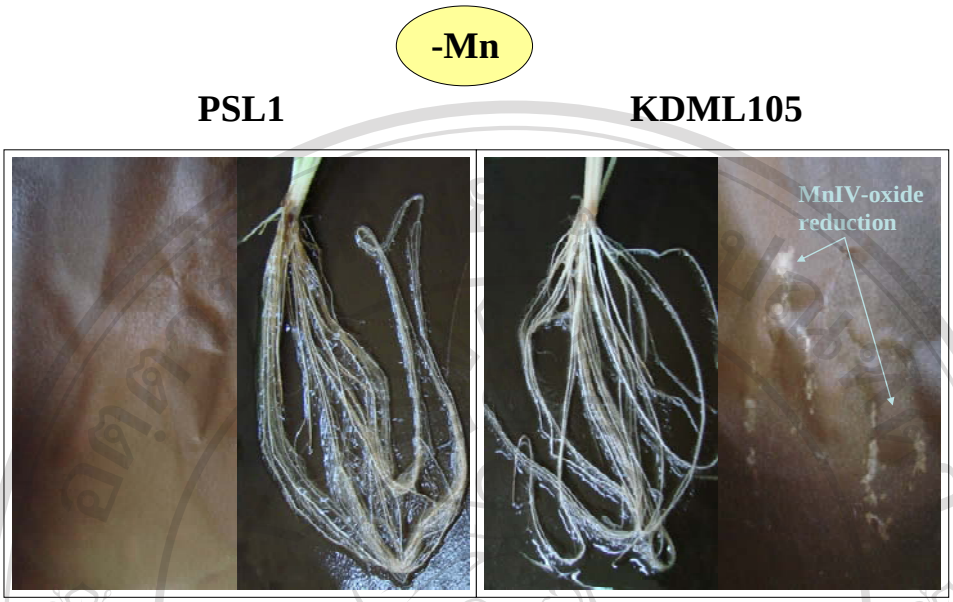

Figure 3.9 Response to Mn deficient of root exudate by reduction of MnIV-oxide applied on filter paper of two rice genotypes grown at 4 weeks after transplanting.

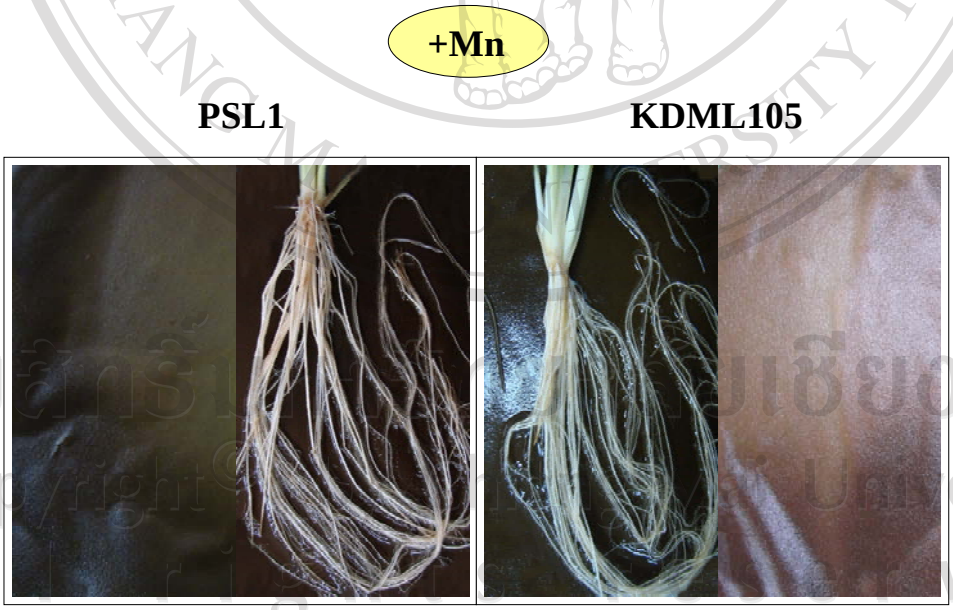

Figure 3.10 Response to Mn sufficient of root exudate by reduction of MnIV-oxide applied on filter paper of two rice genotypes grown at 4 weeks after transplanting.

Table 3.25 Response to Mn of number of root exudate by reduce pH in rhizosphere solution of two rice genotypes grown at 4 weeks after transplanting. pH_1= pH in de-ionised water (DI) that were adjusted to 7.0±0.05. pH_2= pH in DI water after plants transfer.

Variety	Mn level (ppm)				Mean
	0		0.5		
	pH_1	pH_2	pH_1	pH_2	
PSL 1	7.03aA	7.03aA	7.04aA	7.03aA	7.03
KDML105	7.02bA	6.46cB	7.05aA	7.04aA	6.89
Mean	7.02	6.74	7.04	7.03	6.96
F-test	V***	Mn***	VxMn***		
$LSD_{(0.05)}$			0.0125		

*** Significant at $P < 0.001$. V, Mn and VxMn indicated F-test for variety, Mn level and variety and Mn level interaction effects, respectively. The difference between varieties in the same column is indicated by upper case letters. The difference between Mn levels in the same row is indicated by lower case letters.

3.4 Discussion

As discussed by Rengel (2000), putative mechanisms of tolerance to Mn deficiency may rely on greater excretion of various substances into the rhizosphere by plant roots. In addition, the facilitative root interactions are important poor soils and low input agro-ecosystems due to critical interspecific competition for plant growth factors (Nielson and Jensen, 2005). The techniques of intercropping induced changes in rhizosphere have been reported in peanut plants by intercropping them with maize (Inal *et al*., 2007). In the intercropping of peanut/maize, the acid phosphatese activity of the rhizosphere and bulk soil and root secreted acid phosphatese were higher than that of monocultured peanut and maize. Our result clearly show that Mn rhizosphere was modified by the roots of inter genotypes of rice plants, PSL1 and KDML105. This modified improved Mn availability. This study demonstrates that interspecific root interactions and rhizosphere effects between genotype PSL1 and KDML105 were associated with the release of root exudates of KDML105 roots, contributing to increase chemical availability of Mn to PSL1 when grown together. As also reported by Wasaki *et al*. (2003), interspecific root interactions affecedt nutrient mobilization in the rhizosphere and contribute efficiently to nutrient acquisition by intercropping.

The relative of YEB-1 chlorophyll content, number of leaves, number of tillers, shoot dry weight, root dry weight and total dry weight of PSL1 increased when grown together with KDML105. In addition, relative Mn uptake efficiency of PSL1 increased when compared to their sole growing. According to white lupin improved P-uptake when it was intercropped with wheat (Gardner and Boundy, 1983; Horst and Waschkies, 1987). Pigeonpea improved P-uptake of sorghum (Ae *et al*., 1990), and chickpea contributed to P-uptake by maize and wheat (Li *et al*., 2003; Li *et al*., 2004).

Possibly, these effects on P-acquisition are related to the release or activation of enzymes (e.g. like acid phosphates) and root exudation of carboxylates, which improve solubility and uptake of P in rhizosphere (Neumann and Romheld, 1999; Rengel, 2002). Our result may be attributable mainly to release of root exudates by plant roots.

Therefore, other experiment tried to explain the mechanism of Mn acquisition efficiency genotypes were the release of root exudates from roots, it can be reduced Mn unavailable (Mn(IV)) to Mn available (Mn(II)). KDML105 can be reduced Mn(IV) to Mn(II) consequently to similar YEB-1 chlorophyll content, number of leaves, number of tillers and Mn uptake efficiency in all status of Mn. In contrast, PSL1 may be not release root exudates for reducing Mn(IV) whereas, YEB-1 chlorophyll content number of leaves, number of tillers and Mn uptake efficiency in 0.5 Mn(IV) lower than in 0.5 Mn(II). Although, PSL1 and KDML105 were similar in number of leaves, number of tillers and Mn uptake efficiency in 0.5 Mn(IV) may be contribution of rhizosphere microbial was negligible. Uren (1981) and Marschner (1988) found that the reduction of Mn oxide was directly induced by the reducing activity of the roots and microbial. In this case, microbial is the important factor in the reduction of Mn(IV) to Mn(II).

However, KDML105 indeed reduced MnIV oxide on filter paper when grown in Mn deficiency condition and reduced pH in rhizosphere while PSL1 could not reduced MnIV oxide on filter paper and pH in rhizosphere. According to Dinkelaker (1993a, 1993b), white zones on the filter paper indicated reduction of MnIV in the rhizosphere of chickpea. It is known that chickpea roots excrete organic acids into the rhizosphere (Ohwaki and Hirata, 1990). Mn can form complexes in the rhizosphere

with organic legends of plant and microbial origin, which may increase the mobility of Mn in the rhizosphere (facilitating diffusion up to the root-cell plasma membrane) (Marschner, 1988). It has been reported that rhizosphere of wheat genotypes contained an increased proportion of Mn reducers under Mn deficiency compared to Mn sufficiency conditions (Rengel, 1997). When grown on a soil with low Mn availability, some wheat Mn efficient genotypes (like cv. Aroona) had a greater ratio of Mn reducers to Mn oxidizers in the rhizosphere compared to the Mn inefficient genotypes (Rengel, 1997). Exudation of root exudates shown to lower the pH of the rhizosphere, it clearly established that KDML105 indeed could be release root exudates from root plant.

The comparison for oxygen supply in nutrient solution with or without air bubbling suggested that in absence of Mn. Insalud (2006) has been report that the root length was an indicator for adequate oxygen supply in culture solution, when grown in aerated with air bubbling was increased roots length. However, the difference in oxygen supply did not affect to differently response of YEB-1 chlorophyll content, root and shoot length when grow in Mn deficiency. However, when grown in Mn deficiency condition with and without oxygen supply was not affected to the different responses in YEB-1 chlorophyll content, root and shoot length. Therefore, plant grown in still condition or aerated without oxygen supply was chosen for evaluating to Mn efficiency in rice.

The nutrient solution technique could be used for evaluating Mn efficiency in rice. The presence of root exudates is strongly suggested as the Mn acquisition efficiency. The mechanisms of acquisition efficiency for tolerance to Mn deficiency

showed in KDML105. However, exuded compounds from root plant should be confirmed with HPLC is highly recommended.