Chapter 4

Response to external manganese levels in Mn efficient and inefficient rice genotypes

4.1 Introduction

The physiological machanisms controling Mn efficiency in plants is not fully understood, but candidates are improved acquisition of Mn from soil via root exudation of plants (Gherardi and Rengel, 2004; Graham, 1988; Huang *et al*., 1994; Pearson and Rengel, 1997; Rengel, 1999) and differences in uptake and compartmentation (Huang *et al*., 1994). Manganese efficient genotypes can absorb more Mn from soils and thus can survive and yield better in a soil with low Mn availability than Mn inefficient genotypes (Bansal *et al*., 1991; Graham, 1988; Huang and Graham, 1997). Accrodingly nutrient efficiency genotypes may have a higher rate of uptake and translocation nutrient (Marschner, 1995), such as, Mn efficient barley genotype took up more Mn independent of Mn supply levels both in solution (Huang *et al*., 1994) and in the soil (Huang and Graham, 1997), suggesting that efficient Mn uptake in barley is a constitutive system.

Moreover, Mn efficient genotypes had Mn net uptake rate higher than Mn inefficient genotypes. When growing together in a mixed hydroponics system with a continuously low Mn concentration (10–50 nM) similar to that occurring in soil solution, the Mn efficient genotype had a competitive advantage and contained 55%

to 75% more Mn in the shoots than the Mn inefficient genotype in barley (Pedas *et al*., 2005).

From the results in chapter 3, KDML105 is mostly efficient in Mn acquisition that may also be efficient in Mn uptake. Therefore, this studies set out to further examine Mn uptake efficiency in KDML105, comparing with those inefficiency Mn accquisition, PSL1.

4.2 Materials and Methods

Two rice genotypes, PSL1 (Mn inefficiency) and KDML105 (Mn efficiency) were grown in the solution culture. The nutrient solution used was developed for rice growth in nutrient solution by Insalud (2006) (Table 2.3), containing the following Mn concentrations: 0 , 0.250 and 0.50 ppm (Mn_0, $Mn_{0.25}$ and $Mn_{0.5}$) (applied from Lindon et al., 2004). The seeds were placed on a moistened paper in petri dish until germinated. Ten days-old rice plants were transplanted in each pot at six seedlings per pot. Each plastic pot contained 10 L of nutrient solution cultures. The solution was renewed every week and pH values were adjusted daily to 5.5$\pm$0.05 with 1N HCl or 1N NaoH.

Data measurement

Data recorded were chlorophyll content in YEB-1, number of leaves plant^{-1} and tillers plant^{-1} at 30 and 60 day after transplanting. Then, dry wiegh of shoot and root (g plant^{-1}) were measured.

Plant analysis

Plant samples of YEB, Shoot and root were kept separately before oven-dried at 80 ºC for 48 hours. The samples were analysed for Mn concentration by using dry-ashed at 500 ºC for 8 hours and dissolved in 0.1 N HCl before determined Mn concentration by atomic absorption spectrometry (Delhaize *et al*., 1984). Nutrient content, Mn uptake efficiency (Mn uptake per unit root dry weight) and relative Mn uptake efficiency were calculated as follow:

Mn concentration = % concentration of Mn x plant dry weight

$$\text{Mn uptake efficiency} = \frac{\text{Mn content in root + Mn content in shoot}}{\text{Root dry weight}}$$

$$\text{Relative Mn uptake efficiency} = \frac{\text{Mn uptake efficiency in plant without Mn}}{\text{Mn uptake efficiency in plant with Mn}} \times 100$$

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 genotype, Mn treatment. The comparison of mean was used with Least Significant Difference (LSD) at $P<0.05$.

4.3 Results

Chlorophyll content

Rice genotypes grown in different levels of Mn were significantly different in their YEB-1 chlorophyll content at all harvests (Table 4.1). At 30 days after transplanting, YEB-1 chlorophyll content of PSL1 decreased at $Mn_{0.25}$ and Mn_0 whereas, it was similar between $Mn_{0.25}$ and Mn_0 in KDML105 but increased when compared to $Mn_{0.5}$. In all Mn levels, YEB-1 chlorophyll content of KDML105 was the highest compare with the others. At 60 days after transplanting, significant differences of YEB-1 chlorophyll content were found between Mn levels likely in 30 days after transplanting. KDML105 had lower YEB-1 chlorophyll content in $Mn_{0.5}$ than PSL1, whereas in other Mn levels, KDML105 was the highest.

Number of leaves

Number of leaves was significantly different between genotypes and Mn levels at 30 and 60 days after transplanting (Table 4.2). At 30 days after transplanting, Mn levels were not difference in KDML105 but for PSL1 it was decreased when grown in $Mn_{0.25}$ and Mn_0. In Mn_0, number of leaves in KDML105 was higher than PSL1. Number of leaves of KDML105 and PSL1 did not differ in $Mn_{0.25}$ and $Mn_{0.5}$. At 60 days after transplanting, number of leaves of KDML105 and PSL1 were not differed between $Mn_{0.25}$ and Mn_0 but it increased when grown in $Mn_{0.5}$.

Number of tillers

At 30 and 60 days after transplanting, number of tillers of KDML105 was not significantly different in all Mn levels, whereas it was decreased in PSL1 when grown in Mn_0 and $Mn_{0.25}$. Beside, KDML105 had the highest number of tillers in Mn_0 and $Mn_{0.25}$ whereas in $Mn_{0.5}$, KDML105 and PSL1 were similar (Table 4.3).

Dry weight

At all harvests, shoot dry weight was not significant difference between genotypes and Mn levels (Table 4.4) but it was significant difference in root dry weight. At 30 days after transplanting, root dry weight of KDML105 was not significantly different in all Mn levels but PSL1 was increased when grown in Mn_0. In $Mn_{0.25}$ and $Mn_{0.5}$, root dry weight of KDML105 and PSL were similar whereas KDML105 was lower than PSL1 in Mn_0. At 60 days after transplanting, root dry weight in Mn_0 of KDML105 and PSL1 were increased when compared to $Mn_{0.25}$ and $Mn_{0.5}$. KDML105 had lower root dry weight than PSL1 in Mn_0 and $Mn_{0.25}$ (Table 4.5).

Manganese concentration

YEB

At 30 days after transplanting, Mn concentration in YEB of all genotypes was decreased when grown in Mn_0. However, YEB Mn concentration of KDML105 was higher than PSL1 in Mn_0 and $Mn_{0.25}$ (Table 4.6). According to YEB Mn concentration of KDML105 at 60 days after transplanting was higher than PSL1 in Mn_0 and $Mn_{0.25}$.

Shoot

At 30 days after transplanting, shoot Mn concentration of all genotypes in $Mn_{0.5}$ were decreased when grown in Mn_0 and $Mn_{0.25}$. In Mn_0 and $Mn_{0.25}$, KDML105 had Mn concentration in the shoot higher than PSL1. At 60 day after transplanting, PSL1 increased Mn concentration in the shoot when grown in $Mn_{0.5}$, whereas

KDML105 was similar. Beside, in Mn_0 and $Mn_{0.5}$, shoot Mn concentration of KDML105 was higher than PSL1 (Table 4.7).

The concentration of Mn in root was not significantly difference in all genotypes and Mn levels at all harvests (Table 5.8).

Manganese content

Shoot

At 30 days after transplanting, shoot Mn content of KDML105 did not differ in all Mn levels but in PSL1 it was decreased when grown in Mn_0 and $Mn_{0.25}$. In Mn_0 and $Mn_{0.25}$, shoot Mn content of KDML105 was higher than PSL1. At 60 days after transplanting, Mn content in the shoot of KDML105 and PSL1 were decreased when grown in Mn_0. However, shoot Mn content of KDML105 was higher than PSL1 in all Mn levels (Table 5.9).

Root

Manganese contents in root of all genotypes increased when grown in $Mn_{0.5}$ at all harvests. At 30 days after transplanting, root Mn content in Mn_0 and $Mn_{0.25}$ of KDML105 was higher than PSL1. At 60 days after transplanting, root Mn content of KDML105 was higher than PSL1 in Mn_0 (Table 4.10).

Whole plant

At 30 days after transplanting, whole plant Mn content of KDML105 did not differ between Mn levels, whereas PSL1 was decreased when grown in Mn_0. Beside, Mn content in whole plant of KDML105 was higher than PSL1 in Mn_0 and $Mn_{0.25}$. At 60 days after transplanting, the content of Mn in whole plant of KDML105 was significantly different between genotypes at all Mn levels. Although, Mn content in whole plant of KDML105 and PSL1 were decreased when grown in Mn_0 and $Mn_{0.25}$.

The content of Mn in whole plant of KDML105 was higher than PSL1 in all Mn levels (Table 4.11).

Manganese uptake efficiency

Manganese uptake efficiency ((Mn content in shoot + Mn content in root)/root dry weight) (mg Mn/g root dry weight) of KDML105 and PSL1 decreased when grown in Mn_0 at all harvests. However, at 30 days after transplanting, Mn uptake efficiency of KDML105 was higher than PSL1 in Mn_0. At 60 days after transplanting, Mn uptake efficiency of KDML was the highest in all Mn levels (Table 4.12)

At 30 days after transplanting, in Mn_0, relative Mn uptake efficiency ((Mn uptake efficiency in Mn_0, $Mn_{0.25}/Mn_{0.5}$) X 100) of KDMlL105 was higher than PSL1 was 32.78% (Figure 4.1 (a)). At 60 days after transplanting, KDML105 was higher than PSL1, 44.36 and 19.62% in Mn_0 and $Mn_{0.25}$, respectively (Figure 4.1 (b)).

Table 4.1 Response to Mn levels of YEB-1 chlorophyll content (SPAD unit) at 30 and 60 days after transplanting.

Variety	Mn level (ppm)			Mean
	0	0.25	0.5	
30 DAT				
PSL 1	21.31cB	24.13bB	28.68aB	24.71
KDML105	30.03bA	30.04bA	31.07aA	30.38
Mean	25.67	27.09	29.87	27.54
F-test	V***	Mn***	VxMn***	
LSD(0.05)			0.882	
60 DAT				
PSL 1	29.60cB	32.66bB	43.87aA	35.38
KDML105	35.55bA	36.92bA	41.25aB	37.91
Mean	32.58	34.79	42.56	36.64
F-test	V***	Mn***	VxMn***	
LSD(0.05)			1.3706	

*** 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 level in the same row is indicated by lower case letters.

Table 4.2 Response to Mn of number of leaves (plant^{-1}) at 30 and 60 days after transplanting.

Variety	Mn level (ppm)			Mean
	0	0.25	0.5	
30 DAT				
PSL 1	8.92bB	9.2667bA	10.92aA	9.70
KDML105	10.39aA	10.067aA	10.25aA	10.24
Mean	9.65	9.67	10.58	9.97
F-test	V*	Mn**	VxMn**	
LSD(0.05)			0.8496	
60 DAT				
PSL 1	14.50bB	16bB	23.00aA	17.83
KDML105	19.00bA	19.417bA	21.33aA	19.92
Mean	16.75	17.71	22.17	18.88
F-test	V***	Mn***	VxMn***	
$LSD_{(0.05)}$			1.7097	

*, ** 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 level in the same row is indicated by lower case letters.

Table 4.3 Response to Mn of number of tillers (plant^{-1}) at 30 and 60 days after transplanting.

Variety	Mn level (ppm)			Mean
	0	0.25	0.5	
30 DAT				
PSL 1	1.67bB	1.67bB	2.25aA	1.86
KDML105	2.33aA	2.33aA	2.33aA	2.33
Mean	2.00	2.00	2.29	2.10
F-test	V***	Mn*	VxMn*	
LSD(0.05)			0.2834	
60 DAT				
PSL 1	3.67bB	4.0267bB	5.17aA	4.29
KDML105	4.67aA	4.9167aA	5.17aA	4.92
Mean	4.17	4.47	5.17	4.60
F-test	V***	Mn***	VxMn*	
LSD(0.05)			0.5142	

* 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 level in the same row is indicated by lower case letters.

Table 4.4 Response to Mn of shoot dry weight (g plant^{-1}) at 30 and 60 days after transplanting.

Variety	Mn level (ppm)			Mean
	0	0.25	0.5	
30 DAT				
PSL 1	0.98	0.81	0.98	0.92
KDML105	0.83	0.86	0.98	0.89
Mean	0.90	0.84	0.98	0.91
F-test	V^{ns}	Mn*	$VxMn^{ns}$	
LSD(0.05)		0.0844	0.1194	
60 DAT				
PSL 1	4.567	4.257	4.330	4.384
KDML105	4.630	3.787	4.057	4.158
Mean	4.598	4.022	4.193	4.271
F-test	V^{ns}	Mn**	$VxMn^{ns}$	
$LSD_{(0.05)}$			0.5184	

^{ns}, * and ** Non significant, significant at P < 0.05vand 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 level in the same row is indicated by lower case letters.

Table 4.5 Response to Mn of root dry weight (g plant^{-1}) at 30 and 60 days after transplanting.

Variety	Mn level (ppm)			Mean
	0	0.25	0.5	
30 DAT				
PSL 1	0.183aA	0.167bA	0.160bA	0.170
KDML105	0.170aB	0.170aA	0.167aA	0.169
Mean	0.177	0.168	0.163	0.169
F-test	V^{ns}	Mn***	VxMn**	
LSD(0.05)			0.0073	
60 DAT				
PSL 1	0.780aA	0.503bA	0.447cA	0.577
KDML105	0.447aB	0.437bB	0.407bA	0.430
Mean	0.613	0.470	0.427	0.503
F-test	V***	Mn***	VxMn***	
$LSD_{(0.05)}$			0.0421	

ns, ** and *** Non significant, significant at $P < 0.01$ *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 level in the same row is indicated by lower case letters.

Table 4.6 Response to Mn of YEB Mn concentration (mg Mn kg^{-1}) at 30 and 60 days after transplanting.

Variety	Mn level (ppm)			Mean
	0	0.25	0.5	
30 DAT				
PSL 1	23.19cB	29.02bB	46.74aA	32.98
KDML105	43.37bA	45.60aA	47.60aA	45.52
Mean	33.28	37.31	47.17	39.25
F-test	V***	Mn***	VxMn**	
LSD(0.05)			2.1616	
60 DAT				
PSL 1	40.53bB	30.523cB	45.57aA	38.88
KDML105	53.37aA	45.03bA	43.83bA	47.41
Mean	46.95	37.78	44.70	43.14
F-test	V***	Mn***	VxMn***	
$LSD_{(0.05)}$			4.8127	

** and *** Significant at *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 level in the same row is indicated by lower case letters.

Table 4.7 Response to Mn of shoot Mn concentration (mg Mn kg^{-1}) at 30 and 60 days after transplanting.

Variety	Mn level (ppm)			Mean
	0	0.25	0.5	
30 DAT				
PSL 1	61.17cB	73.41bB	97.81aA	77.46
KDML105	85.84bA	89.197bA	101.19aA	92.08
Mean	73.51	81.30	99.50	84.77
F-test	V***	Mn***	VxMn***	
LSD(0.05)			5.0913	
60 DAT				
PSL 1	170.15bB	153.97bA	260.15aB	194.76
KDML105	293.01aA	153.97bA	303.54aA	250.17
Mean	231.58	153.97	281.85	222.47
F-test	V***	Mn***	VxMn***	
$LSD_{(0.05)}$			20.001	

*** 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 level in the same row is indicated by lower case letters.

Table 4.8 Response to Mn of root Mn concentration (mg Mn kg^{-1}) at 30 and 60 days after transplanting.

Variety	Mn level (ppm)			Mean
	0	0.25	0.5	
30 DAT				
PSL 1	17.13	18.737	21.84	19.24
KDML105	18.43	19.567	21.83	19.94
Mean	17.78c	19.15b	21.84a	19.59
F-test	V^{ns}	Mn***	$VxMn^{ns}$	
LSD(0.05)			1.5092	
60 DAT				
PSL 1	36.83	34.52	57.49	42.95
KDML105	41.49	38.63	55.79	45.30
Mean	39.16	36.58	56.64	44.12
F-test	V^{ns}	Mn***	$VxMn^{ns}$	
$LSD_{(0.05)}$			6.3142	

^{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 level in the same row is indicated by lower case letters.

Table 4.9 Response to Mn of shoot Mn content (mg Mn $plant^{-1}$) at 30 and 60 days after transplanting.

Variety	Mn level (ppm)			Mean
	0	0.25	0.5	
30 DAT				
PSL 1	0.053bB	0.090aB	0.100aA	0.081
KDML105	0.105aA	0.103aA	0.103aA	0.104
Mean	0.079	0.097	0.101	0.092
F-test	V***	Mn***	VxMn***	
LSD(0.05)			0.0124	
60 DAT				
PSL 1	0.028cB	0.036bB	0.060aB	0.041
KDML105	0.064bA	0.068abA	0.075aA	0.069
Mean	0.046	0.052	0.068	0.055
F-test	V***	Mn***	VxMn**	
$LSD_{(0.05)}$			0.00704	

, * Significant at $P < 0.01$, $P < 0.001$, 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 level in the same row is indicated by lower case letters.

Table 4.10 Response to Mn of root Mn content (mg Mn $plant^{-1}$) at 30 and 60 days after transplanting.

Variety	Mn level (ppm)			Mean
	0	0.25	0.5	
30 DAT				
PSL 1	0.094cB	0.113bB	0.134aA	0.114
KDML105	0.108bA	0.117bA	0.130aA	0.118
Mean	0.101	0.115	0.132	0.116
F-test	V^{ns}	Mn***	VxMn*	
LSD(0.05)			0.0091	
60 DAT				
PSL 1	0.094cB	0.113bA	0.134aA	0.114
KDML105	0.108bA	0.117bA	0.130aA	0.118
Mean	0.101	0.115	0.132	0.116
F-test	V^{ns}	Mn***	VxMn*	
$LSD_{(0.05)}$			0.0091	

^{ns}, * and *** Non significant, 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 level in the same row is indicated by lower case letters.

Table 4.11 Response to Mn of whole plant Mn content (mg Mn plant^{-1}) at 30 and 60 days after transplanting.

Variety	Mn level (ppm)			Mean
	0	0.25	0.5	
30 DAT				
PSL 1	0.059bB	0.094aB	0.105aA	0.086
KDML105	0.105aA	0.106aA	0.107aA	0.106
Mean	0.082	0.100	0.106	0.096
F-test	V***	Mn***	VxMn***	
LSD(0.05)			0.0107	
60 DAT				
PSL 1	0.0297cB	0.0397bB	0.0667aB	0.0454
KDML105	0.0657bA	0.0707bA	0.0807aA	0.0724
Mean	0.0477	0.0552	0.0737	0.0589
F-test	V***	Mn***	VxMn**	
$LSD_{(0.05)}$			0.0070	

, * Significant at $P < 0.01$, $P < 0.001$, 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 level in the same row is indicated by lower case letters.

Table 4.12 Response to Mn of Mn uptake efficiency (mg Mn g^{-1} root dry weight) at 30 and 60 days after transplanting.

Variety	Mn level (ppm)			Mean
	0	0.25	0.5	
30 DAT				
PSL 1	0.811cB	1.225bA	1.433aA	1.156
KDML105	1.241bA	1.315abA	1.391aA	1.316
Mean	1.026	1.270	1.412	1.236
F-test	V***	Mn***	VxMn***	
LSD(0.05)			0.095	
60 DAT				
PSL 1	0.096cB	0.211bB	0.423aB	0.243
KDML105	0.350bA	0.362bA	0.523aA	0.412
Mean	0.223	0.287	0.473	0.328
F-test	V***	Mn***	VxMn***	
$LSD_{(0.05)}$			0.0488	

*** 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 level in the same row is indicated by lower case letters.

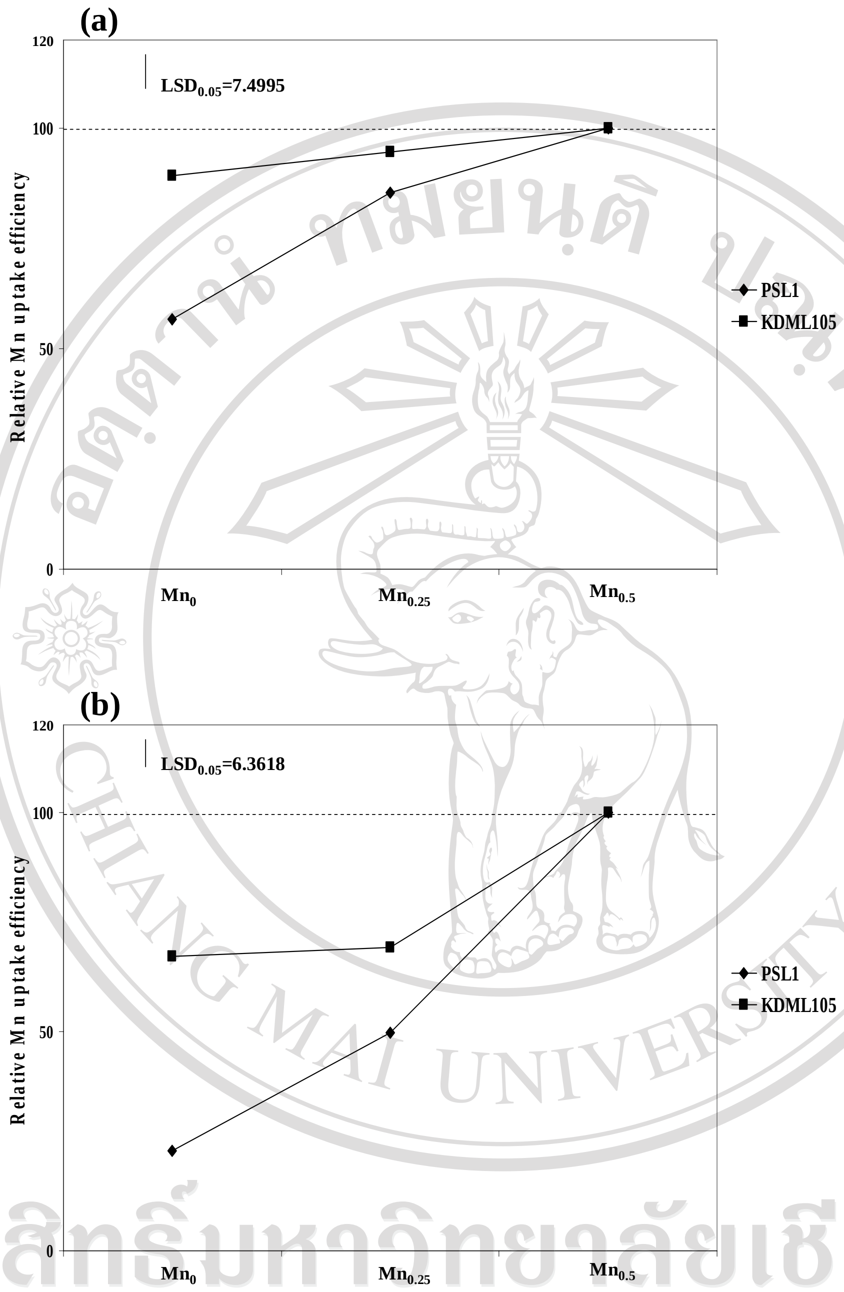

Figure 4.1 Relative Mn uptake efficiency ((Mn uptake efficiency in Mn_0, $Mn_{0.25}$/$Mn_{0.5}$) x 100) of KDML105 and PSL1 in 3 levels of Mn (0, 0.25 and 0.5 ppm; Mn_0, $Mn_{0.25}$ and $Mn_{0.5}$, respectively) at 30 days after transplanting (a) and 60 days after transplanting (b). At $Mn_{0.5}$=100%.

4.4 Discussion

The presence of a clear Mn uptake efficiency trait in KDML105 was the higher content of Mn in shoot, root and whole plant than PSL1. Accordingly, it has been observed over a wide genetic range in crops including in wheat, that the crop genotypes known to be most Mn-efficient also absorbed more Mn and had a higher tissue Mn concentration (Gladstones and Loneragan 1970; Graham *et al*. 1983; Marcar and Graham 1987b; Graham 1988; Huang *et al*. 1994; Bansal and Nayyar 1998; Marschner *et al*. 2003). Such as, the results of wheat (cv. C8MM) accumulated a higher Mn concentration in conferring its Mn efficiency reported here is consistent with the general observations that an Mn efficient genotype has a higher ability of uptake and accumulation of Mn while grown under Mn deficiency.

Furthermore, KDML105 had the highest Mn uptake efficiency and relative Mn uptake efficiency. This suggests that Mn efficiency in KDML105 more likely to be related to a higher Mn uptake efficiency such as a more efficient take up more Mn from external root to plant under Mn deficiency. Rengel (2000) and Rengel (2001) also reported that Mn efficient genotypes take up more Mn from soils with limited Mn availability.

The result demonstrated that KDML105 performs better than PSL1 under Mn deficiency in terms of chlorophyll content, number of leaves and number of tillers. These reveal Mn uptake efficiency of KDML105 that were sufficient for chlorophyll synthesis, leave and tiller production and/or also Mn use efficiency. Where, KDML105 displayed Mn efficiency in trait of internal Mn use efficiency is consistent with a previous observation by Jiang (2008) that Mn efficiency in UK wheat had highest relative photosynthetic quantum yield and relative chlorophyll a fluorescence ratio.

The lack of significant differences in shoot and root dry weight between KDML105 and PSL1 under Mn deficiency is reflective of Mn inefficient genotypes PSL1 to increase root growth for increase the absorptive root surface area to take up amount of Mn but in shoot is unclear. However, KDML105 was not affected in shoot and root dry weight from Mn deficiency.

Therefore, it was clearly in Mn uptake efficiency genotype, KDML105 should be studied more in utilization efficiency mechanism to confirm the most Mn efficiency genotype.