Chapter 2

Genotypic variation in Mn efficiency in Thai rice genotypes

2.1 Introduction

Great genotypic differences in Mn efficiency in plants have been widely recognized since Mn deficiency was first identified in the 1920s, and has been reported in wheat, durum wheat (*Triticum turgidium* L.), oat and barley (Bansal *et al*., 1991; Graham, 1988; Huang and Graham, 1997; Kaur *et al*., 1989; Marcar and Graham, 1987a, b; Nyborg, 1970; Saberi *et al*., 1997). Efficiency in published papers was defined in different ways. Genotypic variation in Mn efficiency in barley reported was based on grain yield (Hebbern *et al*., 2005). A wheat cultivar (cv. Maris Butler) was reported to have better Mn use efficiency that was associated to its superior internal utilization of Mn (Jiang and Ireland, 2005). A lucerne variety (Salado) has been reported to tolerate conditions of low Mn availability better than other genotypes by root exudation that releases organic compounds into the rhizosphere that helped to make Mn more available for uptake (Gherardi and Rengel, 2004). This considerable variability among plant species and different genotypes of the same species in their ability to grow in soil with low Mn availability has been described as differential Mn efficiency (Hebbern *et al*., 2005).

Although, genotypic variation in Mn efficiency has been reported in many plant species, so far no information is available in rice. Although wetland rice growing in flooded soils is less likely to suffer from Mn deficiency, growth and yield

of upland rice and rice grown in aerobic soil can be adversely affected by Mn deficiency. The availability of Mn is low and may be inadequate for rice grown in these conditions. In Thailand, 75% of rice cultivating area lacks irrigation and water control to keep the soil submerged through out the growing season, so that there is a risk that Mn deficiency may occur. Besides, availability of Mn is especially low in calcareous soils such as Lopburi, Banmi, Saraburi, Takhli soil series in Thailand. These soil series are wide spread in many areas in Thailand especially, in soils derived from limestone in central and northern Thailand. Farmers on soils prone to Mn deficiency in Thailand have, however, developed upland and rainfed rice cropping systems based on adapted local varieties. Therefore, these studies aim to evaluate Thai rice genotypes for Mn efficiency.

2.2 Materials and Methods

Experiment 2.2.1 Genotypic variation among improved Thai rice varieties

Five rice genotypes, PSL1, RD10, SKN, Jao-Leaung11 and KDML105 were grown in sand culture with nutrient solution to evaluate for Mn efficiency. The genotypes were identified in a previous studied (Yanaphan *et al*., 2005) that found them to differ in iron (Fe) efficiency. As Fe and Mn deficiency often occur together in upland rice on alkaline and calcareous soils, Mn efficiency of rice genotypes could mirror their Fe efficiency. The seeds were placed on a moistened paper in petri dish until germinated (approximately seven days). Germinated seeds were transplanted to the prepared pots (0.30 m diameter and 0.30 m deep) filled with washed river quartz sand at 5 seedlings per pot. Pots were supplied twice daily with otherwise complete nutrient solution with two levels of added Mn (0 and 0.5 mg Mn/L; Mn_0 and $Mn_{0.5}$). The nutrient solution was 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 (youngest emerged leaf blade) (a chlorophyll meter SPAD 502), number of leaves plant^{-1} and tillers plant^{-1}. Then, harvest measurements including: shoot dry weight (g plant^{-1}), root dry weight (g plant^{-1}) after oven drying at 70ºC for 48 hours. The samples were determined for Mn concentration in all plant parts by dry-ashing and atomic absorption spectrometry (Delhaize *et al*., 1984). The relative Mn efficiency calculated by (Mn deficiency / Mn sufficiency) X 100 and Mn

uptake efficiency calculated by (Mn content in root + Mn content in shoot)/ Root dry weight.

Table 2.1 The composition of nutrient solution for rice grown in solution culture (modified from Yoshida *et al*., 1976)

Stock solution	Element	Concentration of element (ppm)	Chemical	Preparation (g/1L.)	ml stock solution/100 L. culture solution
1	N	40	NH_4NO_3	91.4	125
2	P	10	$NaH_2PO_4 .2H_2O$	40.3	125
3	K	40	K_2SO_4	71.4	125
4	Ca	40	$CaCl_2.2H_2O$	88.6	125
5	Mg	40	$MgSO_4.7H_2O$	324	125
6	Mo B Zn Cu	0.05 0.2 0.01 0.01	$(NH_4)_6Mo_7O_{24}.4H_2O$ H_3BO_3 $ZnSO_4.7H_2O$ $CuSO_4.5H_2O$ H_2SO_4	0.074 0.934 0.035 0.031 50 (ml/1L.)	125
7	Fe	5	Fe-EDTA	38.506	100
8	Mn	0.5	$MnCl_2.4H_2O$	1.5	125

Experiment 2.2.2 Genotypic variation among upland rice from calcareous soil area in northern Thailand

Seed of 11 accessions with 7 named genotypes of upland rice were collected from lime stone area (calcareous soil) at Pang-Ma-Pha district, Mae Hong Son province, northern of Thailand. Upland rice in this study was provided by Mae Hong Son Rice Research Center (Table 2.2). Each seed accession was divided into 3 sub samples (SS1, SS2 and SS3).

Sub experiment 1 **Variation between upland rice genotypes and seed lots with the same name**

One sub-sample (SS1) was used in this experiment for preliminary evaluating variation between genotypes and seed lots with the same name, SS2 characterized for morphological variation in the seed and SS3 was used in sub experiment 2. These were evaluated against the standard Mn efficienct, KDML105, and Mn inefficient, PSL1, checks, form experiment 2.2.1. The seeds were placed on a moistened paper in petri dish until germinated (approximately seven days). Ten days-old rice plants were transplanted in each plastic pot at 10 seedlings per pot and 2 pots per seed sample. There were twenty plants in each treatment. Each plastic pot contained 10 L of nutrient solution cultures without added Mn. The solution was modified by Insalud (2006) for used in solution culture (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. Data were recorded for individual plants at 30 and 60 days after transplanting including: chlorophyll content in YEB-1 (next youngest leaf blade below YEB), number of leaves plant^{-1} and tillers plant^{-1}.

Sub experiment 2 **Characterization in seed morphology between and within some of upland rice genotype**

One hundred seeds of SS2 of 3 named genotypes, Sam Lern, Haw (3) and Haw Kaw, identified from sub-experiment 1, were evaluated for variation in seed morphology.

Seed characters

Seed characteristics determined included hull color, length, width, thickness and weight of unhusk rice. The differentiation within population in colors of husk was analyzed by Shannon-Weaver index (H'). The variation of seed size was evaluated by standard deviation (SD) and coefficient of variance (CV, %).

Data analysis

For seed characters, differentiation within population was analyzed by Shannon-Weaver index (H') (Shannon and Weaver, 1949 cited by Power and McSorley, 2000) that can be calculated as follow:

$$H' = -\sum_{i=1}^{s} pi \ln pi$$

When **s** = total number of type were found

pi = proportion of the number of type *i* divided by total number of plant in each sample

Sub experiment 3 **Responses of 3 upland rice genotypes to Mn levels**

Three upland rice genotypes, Sam Lern, Haw (3) and Haw Kaw, determined to be Mn efficient and Mn inefficient in sub experiment 1, were evaluated for response

to Mn. Each genotype was multiplied from seed in SS3 and seed from each plant was kept separately 20 seed from each accession, one seed from each line. One upland rice variety; Bue Bang (BB) identified as tolerant to Al toxicity (Phattarakul, 2008) were evaluated for Mn efficiency with two standard checks, KDML105 (Mn efficient) and PSL1 (Mn inefficient). BB, KDML105 and PSL1 were pure line seeds. Ten days after germination, twenty plants of each genotype were transplanted to 2 plastic pots containing nutrient solution (10 L). The composition of nutrient solution was the same as previously described in experiment 2.2.1. There were two Mn levels (0 and 0.5 mg Mn L^{-1}). The solution was renewed every week and pH values were adjusted daily to 5.5$\pm$0.05 with 1N HCl or 1N NaOH.

Measurement and plant analysis

Data were recorded 30 days after transplanting including: chlorophyll content in YEB-1, shoot and root dry weight after oven drying at 70ºC for 48 hours. Individual plant samples were bulked and analysed for Mn concentration in different plant parts by dry-ashing and atomic absorption spectrometry (Delhaize *et al*., 1984). Relative response to Mn was calculated as

[performance in Mn deficiency / performance in Mn sufficiency] X 100

Table 2.2 Seed samples from Pang-Ma-Pha district, Mae Hong Son province in year 2005.

Variety name	Rice type	Accession number
Sam Lern	Non-glutinous	PMPC44
Mon Kong (1)	Non-glutinous	PMPC79
Mon Kong (2)	Non-glutinous	PMPC85
Haw (1)	Glutinous	PMPC124
Haw (2)	Glutinous	PMPC136
Haw (3)	Glutinous	PMPC190
Haw (4)	Glutinous	PMPC193
Phee	Non-glutinous	PMPC184
Ja Nor Moey	Glutinous	PMPC194
Haw Kaw	Glutinous	PMPC197
Jee Dao	Glutinous	PMPC198

Table 2.3 The composition of nutrient solution for rice grown in solution culture (modified by Insalud, 2006)

Stock solution	Element	Concentration of element (ppm)	Chemical	Preparation (g/1L.)	ml stc./10 L. culture solution
1	N	40	NH_4NO_3	50.027	10
2	P	10	KH_2PO_4	27.217	10
3	K	40	KNO_3	379.133	10
4	Ca	40	$CaCl_2.2H_2O$	88.6	10
5	Mg	9.7	$MgSO_4.7H_2O$	98.588	10
6	Mo B Zn Cu	0.05 0.28 0.01 0.01	$(NH_4)_6Mo_7O_{24}.4H_2O$ H_3BO_3 $ZnSO_4.7H_2O$ $CuSO_4.5H_2O$ H_2SO_4	0.074 0.934 0.035 0.031 50 (ml/1L.)	10
7	Fe	5	Fe-EDTA	38.506	10
8	Mn	0.5	$MnSO_4$ Na-EDTA	1.53 3.388	10
9	Ni		NiCl	0.94	10
10	Si		SiO_2	6.007	10
11	Na		NaCl	2.922	10

2.2.3 Statistic analysis

Analysis of variance was conducted based on a factorial model with treatment arranged in a Randomized Complete Block Design (RCBD). 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 $P<0.05$.

Sub experiment 1, mean, range (minimum and maximum), standard deviation (SD) and coefficient of variation (CV, %) were calculated to indicate genetic variation occurred by Mn deficiency in each seed sample.

2.3 Results

Experiment 2.2.1 Genotypic variation among improved Thai rice varieties

YEB chlorophyll content, number of leaves and tillers

Rice genotypes grown in different levels of Mn differed in their number of leaves, tillers but did not differ in YEB chlorophyll content. Relative number of leaves (number of leaves in $Mn_0/Mn_{0.5}$) was significantly different between genotypes at the 2nd week after transplanting. It was found that KDML105 was the highest compared with the other genotypes (Figure 2.1). Relative number of tillers (number of tillers in $Mn_0/Mn_{0.5}$) was significantly different between genotypes at 3rd week after transplanting. KDML105 had the relative number of tillers higher than other genotypes (Figure 2.2). On the other hand, relative number of leaves and tillers of KDML105 increased every week. Relative YEB chlorophyll content did not differ in all genotypes (Figure 2.3).

Dry weight

Relative shoot dry weight (shoot dry weight in $Mn_0/Mn_{0.5}$) did not differ between genotypes (Figure 2.4) but relative root dry weight (root dry weight in $Mn_0/Mn_{0.5}$) was significantly different among genotypes at 8 weeks after transplanting. KDML105 had the highest relative root dry weight followed by JL11, SKN and RD10, whereas PSL1 was less than other genotypes (Figure 2.5). Relative total dry weight was not significantly different between genotypes (Figure 2.6)

Manganese concentration

YEB

KDML105 had the highest Mn concentration in YEB followed by RD10, PSL1 and SKN, whereas JL11 was less than the other genotypes in Mn deficiency condition (Table 2.4). PSL1, RD10, SKN and JL11 had higher Mn concentration in YEB than Mn sufficiency condition but KDML105 did not differ in all conditions.

Shoot

In Mn_0, shoot Mn concentration of all genotypes increased, excepted in KDML105 when compared to $Mn_{0.5}$. In Mn_0, Mn concentration in shoot was the highest in KDML105, followed by SKN, JL 11 and RD10, whereas PSL1 was the lowest (Table 2.5).

Root

Manganese concentration in root was significantly different between genotypes and Mn levels. In Mn_0, root Mn concentration of KDML105 increased, whereas RD10 decreased when compared to $Mn_{0.5}$. In Mn_0, the concentration of Mn in root of KDML105, SKN and JL11 were higher than PSL1 and RD10 (Table 2.6).

Manganese content

Shoot

At Mn_0, shoot Mn content of PSL1, RD10, SKN and JL11 increased, excepted in KDML105 when compared to $Mn_{0.5}$. Mn content in shoot in Mn_0 of KDML105, JL11 and SKN were the highest, whereas PSL1 and RD10 were the lowest (Table 2.7).

Root

In Mn_0, root Mn content of PSL1 and RD10 decreased, excepted in KDML105, SKN and JL11 when compared to $Mn_{0.5}$. In Mn_0, root Mn content in KDML105, SKN and JL11 were higher than RD10 and PSL1 (Table 2.8).

Whole plant

In Mn_0, whole plant Mn content of KDML105 did not differ when compared to $Mn_{0.5}$, whereas other genotypes increased when grown in $Mn_{0.5}$. In Mn_0, Mn content of the whole plant was the highest in KDML105 and SKN but it was the lowest in PSL1 (Table 2.9).

Manganese uptake efficiency and Mn uptake

Manganese deficiency was not affected on relative Mn uptake efficiency (Mn uptake efficiency in $Mn_0/Mn_{0.5}$) (Figure 2.7). Relative Mn uptake (whole plant Mn content in $Mn_0/Mn_{0.5}$) was significantly different between genotypes. KDML105 had the highest relative Mn uptake when compared with other genotypes (Figure 2.8).

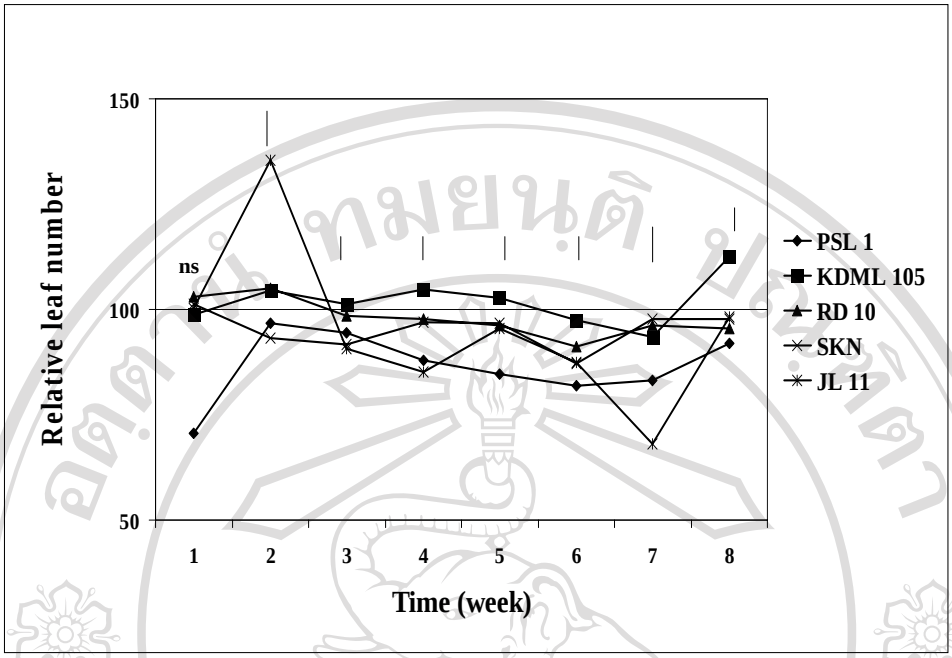

Figure 2.1 Relative number of leaves (number of leaves in $Mn_0/Mn_{0.5}$) of five rice genotypes.

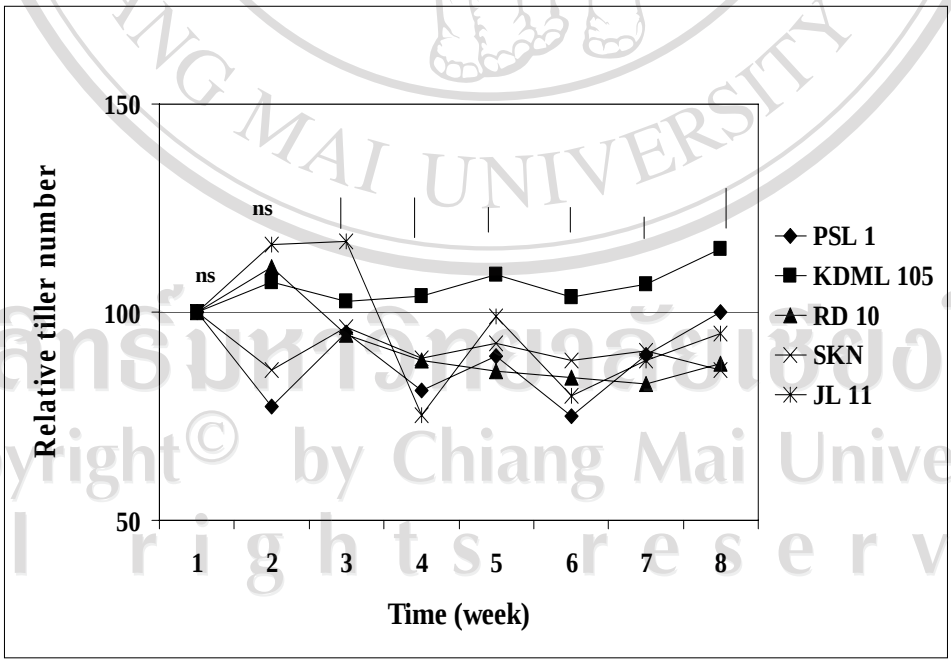

Figure 2.2 Relative number of tillers (number of tillers in $Mn_0/Mn_{0.5}$) of five rice genotypes.

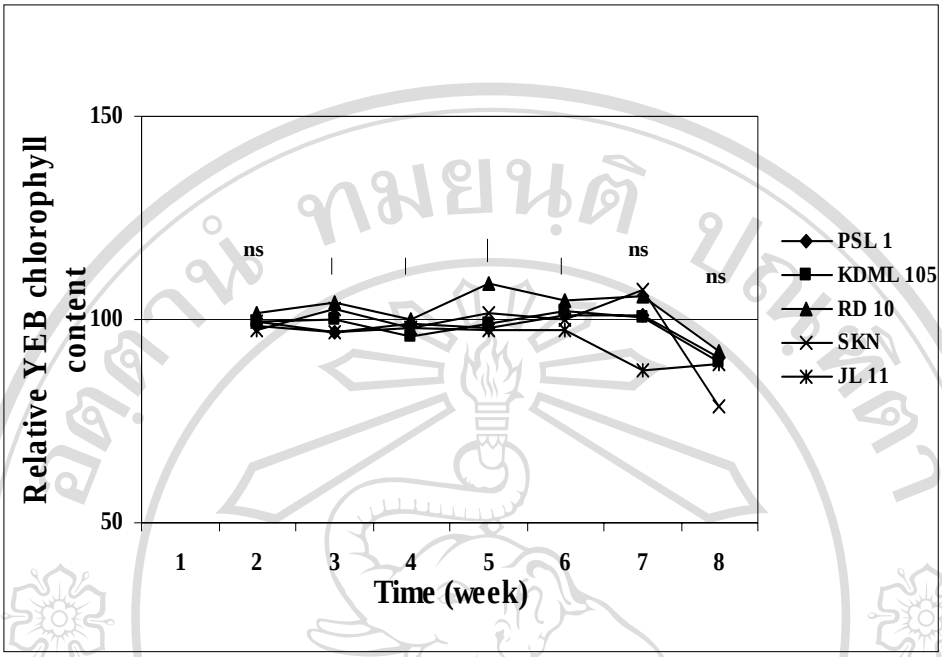

Figure 2.3 Relative YEB chlorophyll content (YEB chlorophyll in $Mn_0/Mn_{0.5}$) of five rice genotypes.

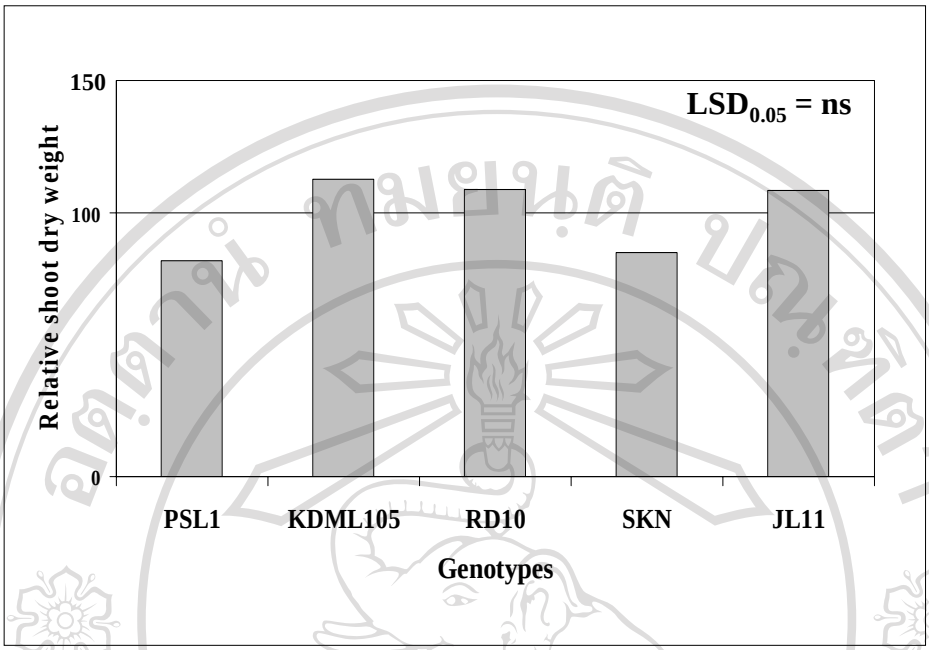

Figure 2.4 Relative shoot dry weight (dry weight in $Mn_0/Mn_{0.5}$) of five rice genotypes at 8 weeks after transplanting.

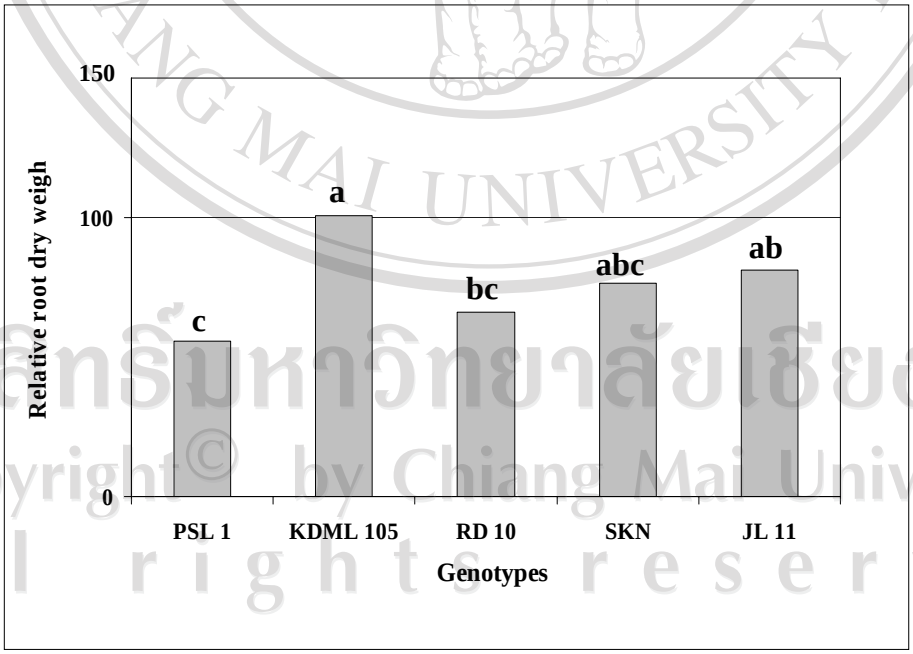

Figure 2.5 Relative root dry weight (dry weight in $Mn_0/Mn_{0.5}$) of five rice genotypes at 8 weeks after transplanting.

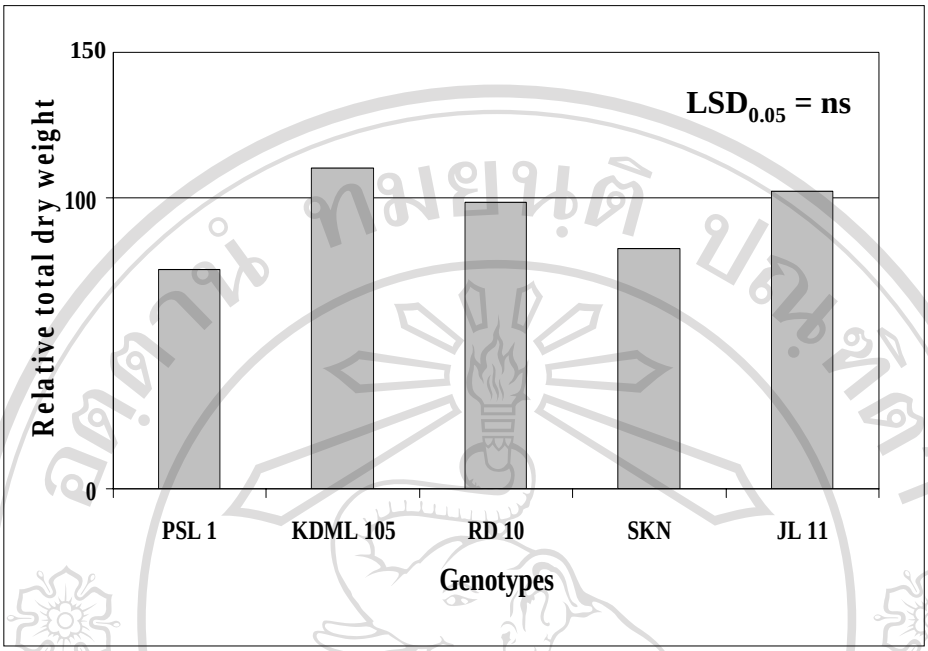

Figure 2.6 Relative total dry weight (dry weight in $Mn_0/Mn_{0.5}$) of five rice genotypes at 8 weeks after transplanting.

Table 2.4 Mn concentration in YEB (mg Mn kg^{-1}) of five rice genotypes grown in sand culture with two levels of Mn at 8 weeks after transplanting**.**

Variety	Mn level (ppm)		
	0	0.5	Mean
PSL1	18.03bA	157.10aA	87.57
KDML105	17.93bA	154.53aA	86.23
RD10	18.75bA	142.33aB	80.54
SKN	17.59bA	154.82aA	86.21
JL11	14.88bA	134.16aC	74.52
Mean	17.44	148.59	83.01
F-test	V***	Mn***	VxMn**
$LSD_{(0.05)}$			6.721

, * 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 colume is indicated by upper case letters. The difference between Mn level in the same row is indicated by lower case letters.

Table 2.5 Mn concentration in shoot (mg Mn kg^{-1}) of five rice genotypes grown in sand culture with two levels of Mn at 8 weeks after transplanting**.**

Variety	Mn level (ppm)		
	0	0.5	Mean
PSL1	345.94bBC	480.98aA	413.46
KDML105	420.96aA	425.95aBC	423.46
RD10	365.12bB	477.48aA	421.30
SKN	349.05bBC	438.69aB	393.87
JL11	326.42bC	404.36aC	365.39
Mean	361.50	445.49	403.50
F-test	V***	Mn***	VxMn***
$LSD_{(0.05)}$			24.890

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

Table 2.6 Mn concentration in root (mg Mn kg^{-1}) of five rice genotypes grown in sand culture with two levels of Mn at 8 weeks after transplanting**.**

Variety	Mn level (ppm)		
	0	0.5	Mean
PSL1	2.71bC	4.68aB	3.69
KDML105	4.86aA	4.39aB	4.63
RD10	3.86bB	4.66aB	4.26
SKN	4.21bAB	6.29aA	5.25
JL11	4.24bAB	5.03aB	4.64
Mean	3.98	5.01	4.49
F-test	V***	Mn***	VxMn***
$LSD_{(0.05)}$			0.766

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

Table 2.7 Mn content in shoot (mg Mn plant^{-1}) of five rice genotypes grown in sand culture with two levels of Mn at 8 weeks after transplanting**.**

Variety	Mn level (ppm)		
	0	0.5	Mean
PSL1	39.81aB	46.81aB	43.31
KDML105	67.54aA	43.95bB	55.74
RD10	56.44aA	46.65bB	51.54
SKN	61.28aA	62.87aA	62.08
JL11	59.75aA	50.31aB	55.03
Mean	56.96	50.12	53.54
F-test	V***	Mn***	VxMn***
$LSD_{(0.05)}$			9.5425

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

Table 2.8 Mn content in root (mg Mn plant^{-1}) of five rice genotypes grown in sand culture at 8 weeks after transplanting**.**

Variety	Mn level (ppm)		
	0	0.5	Mean
PSL1	0.74bB	1.62aBC	1.18
KDML105	1.49aA	1.21aC	1.35
RD10	0.90bB	2.07aA	1.48
SKN	1.84aA	2.18aA	2.01
JL11	1.43aA	1.77aAB	1.60
Mean	1.28	1.77	1.52
F-test	V***	Mn***	VxMn***
$LSD_{(0.05)}$			0.5192

*** 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 colume is indicated by upper case letters. The difference between Mn level in the same row is indicated by lower case letters.

Table 2.9 Mn content in whole plant (mg Mn plant^{-1}) of five rice genotypes grown in sand culture with two levels of Mn at 8 weeks after transplanting**.**

Variety	Mn level (ppm)		
	0	0.5	Mean
PSL1	3.45bC	6.30aBC	4.87
KDML105	6.35aA	5.60aC	5.97
RD10	4.76bB	6.73aB	5.74
SKN	6.05bA	8.47aA	7.26
JL11	5.68bAB	6.80aB	6.24
Mean	5.26	6.78	6.02
F-test	V***	Mn***	VxMn***
$LSD_{(0.05)}$			1.079

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

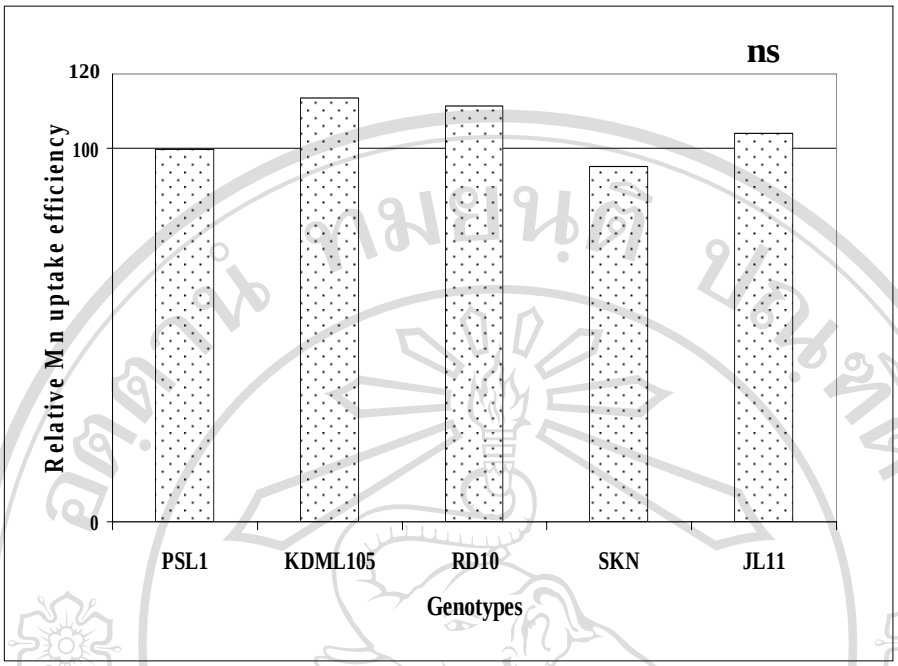

Figure 2.7 Relative Mn uptake efficiency (Mn uptake efficiency in $Mn_0/Mn_{0.5}$) at Mn_0 compared with $Mn_{0.5}$ in sand culture at 8 weeks after transplanting.

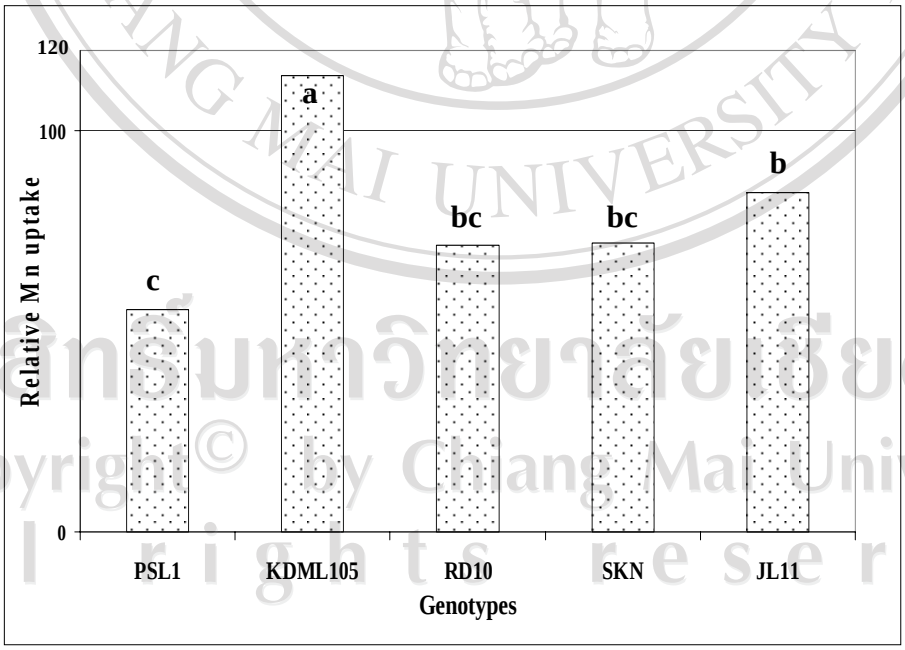

Figure 2.8 Relative Mn uptake (whole plant content in $Mn_0/Mn_{0.5)}$ at Mn_0 compared with $Mn_{0.5}$ in sand culture at 8 weeks after transplanting.

Experiment 2.2.2 Genotypic variation among upland rice from calcareous soil area in northern Thailand

Sub experiment 1 **Variation between upland rice genotypes and seed lots with the same name**

YEB-1 chlorophyll content

YEB-1 chlorophyll content varied between 13 genotypes from 20.93–31.36 SPAD units. Haw (3), KDML105, Sam Lern, Haw (4) and Mon Kong (1) had the highest YEB-1 chlorophyll content (28.47-31.36 SPAD units), but it was the lowest in Jee Dao (20.93 SPAD unit) at 30 days after transplanting (DAT). Coefficient of variation (CV, %) in YEB-1 chlorophyll content of each genotypes varied from 12.03-26.07%, Haw (3) was the highest and Mon Kong (2) was the lowest when compared with KDML105 and PSL1 which are pure line (Table 2.10).

At 60 DAT, chlorophyll content in YEB-1 was higher in KDML105, Sam Lern, Haw (2) and Haw (3) compared with other genotypes (28.19-32.34 SPAD units). Jee Dao, Phee, Haw (1), Ja Nor Moey and PSL1 were the lowest, ranging from 25.18-26.83 SPAD unit. CV in YEB-1 chlorophyll content of each genotypes varied from 11.33-17.65%, Haw Kaw was the highest and Haw (3) was the lowest compared with the others (Table 2.11).

Genotypes in the same name also varied in YEB-1 chlorophyll content. In YEB-1, Haw (3) had the highest in variation of chlorophyll content, with coefficient of variation (CV) higher than Haw (1), Haw (2) and Haw (4). Whereas, Mon Kong (1) and Mon Kong (2) were not widely varied, that CV did not differ within the genotypes. At 60 DAT, these results were confirmed that Mon Kong was not varied within the genotypes, which had similar in CV. CV of Haw was not significant

difference within genotypes. However, CV of Haw and Mon Kong was higher than KDML105 and PSL1 which are pure line.

Number of leaves

At 30 DAT, number of leaves of KDML105, Jee Dao and Sam Lern (13.27-9.44 plant^{-1}) was the highest, whereas Phee, Haw (2), Haw (4) (7.36-7.61 plant^{-1}) were the lowest. The variation of number of leaves differed between 7.36-13.27 plant^{-1} (Table 2.12). Phee was the highest in variation, but Jee Dao was the lowest, each genotypes varied from 9.57-40.81.

At 60 DAT, number of leaves of Sam Lern and KDML105 were the highest (18.15-22.00 plant^{-1}), whereas Ja Nor Moey, Haw Kaw and Haw (2) were the lowest (8.89-9.84). CV in number of leaves of each genotypes varied from 12.47-43.11, Haw (4) was the highest but Jee Dao was the lowest (Table 2.13).

CV of Mong Kong (1) was higher than Mon Kong (2). CV of Haw (4) was higher than Haw (1), Haw (2) and Haw (3) at 30 DAT (Table 2.12). In addition, at 60 DAT, these CV in Mon Kong and Haw were likely in 30 DAT (Table 2.13) and higher than KDML105 and PSL1.

Number of tillers

At 30 DAT, average number of tillers varied between genotypes from 1.62-2.90 plant^{-1}. Number of tillers of KDML105 and Sam Lern (2.44-2.91 plant^{-1}) were higher compared with the other genotypes, whereas Ja Nor Moey, Haw Kaw and Haw (2) were the lowest (1.62-1.78 plant^{-1}). CV in number of tillers of each genotypes varied from 21.35-49.40, Haw (4) was the highest but it was the lowest in Jee Dao (Table 2.14).

At 60 DAT, number of tillers of Haw (3), KDML105, Haw (1), Sam Lern and Jee Doa were higher than other genotypes (3.56-3.82 plant^{-1}). Haw (2) and Ja Nor Moey were the lowest, varied from 2.44-2.95 plant^{-1}. CV in number of leaves of each genotypes varied from 12.47-43.11, Haw (4) was the highest but in Jee Dao, it was the lowest (Table 2.15).

CV of Mon Kong (1) was higher than Mon Kong (2). CV of Haw (4) was higher than Haw (1), Haw (2) and Haw (3) at 30 DAT (Table 2.14). These results of Mon Kong and Haw at 60 DAT was similar in 30 DAT (Table 2.15).

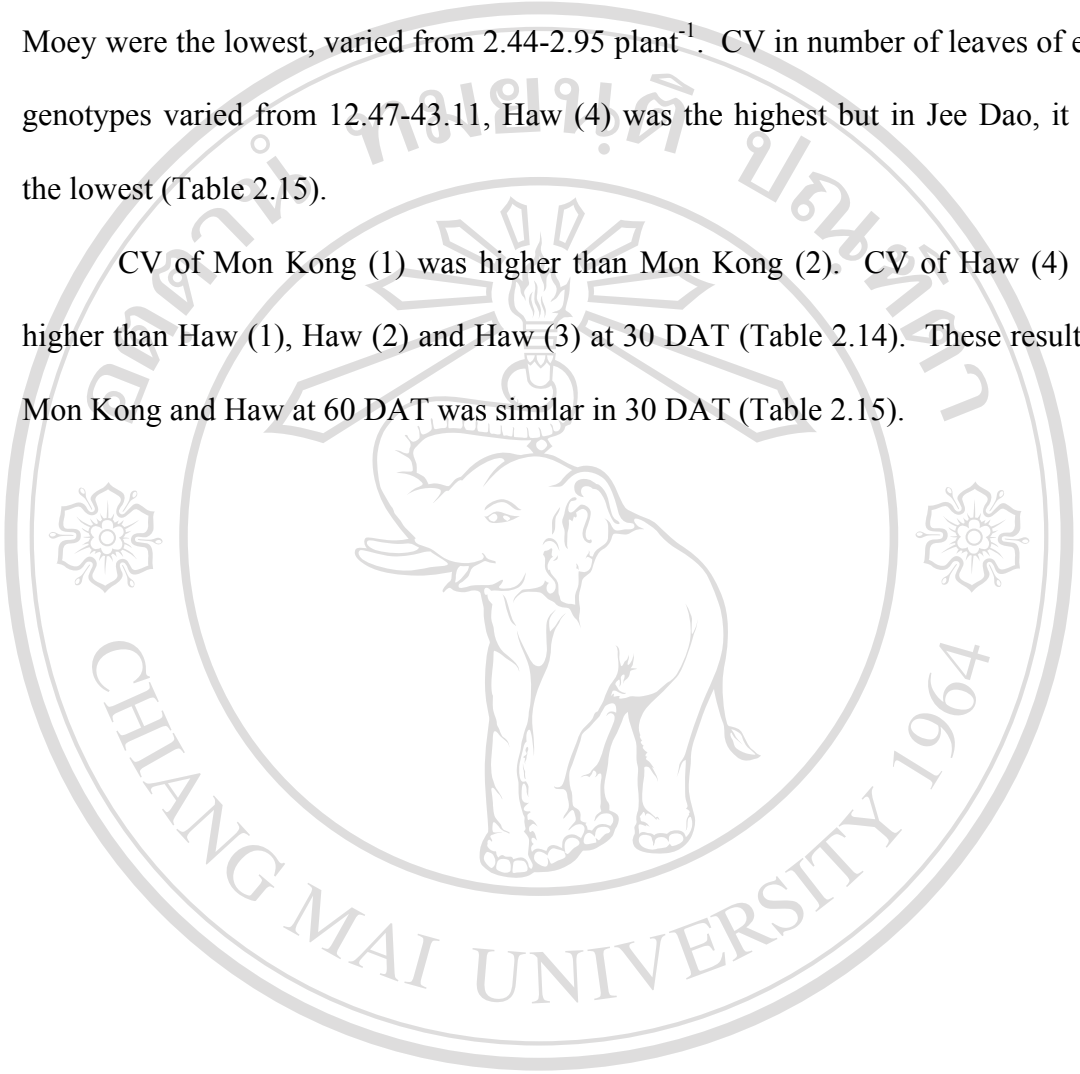

Table 2.10 YEB-1 chlorophyll content of 11 seed accessions and 2 rice local checks (KDML105 and PSL1) at 30 days after transplanting (DAT) in Mn_0.

Genotypes	**YEB-1 chlorophyll content (SPAD unit)**			
	Mean	SD	CV (%)	n
Sam Lern	28.91	5.08	17.58	9
Mon Kong (1)	27.96	3.57	12.76	14
Mon Kong (2)	28.47	3.43	12.03	7
Haw (1)	26.96	5.30	19.67	14
Haw (2)	25.38	4.79	18.87	13
Haw (3)	31.36	8.17	26.07	17
Haw (4)	28.67	5.86	20.42	19
Phee	21.27	4.69	22.05	14
Ja Nor Moey	25.41	6.42	25.26	17
Haw Kaw	26.76	4.41	16.49	13
Jee Dao	20.93	4.88	23.30	12
PSL1	22.64	1.62	7.16	15
KDML105	29.65	1.75	5.91	11

Table 2.11 YEB-1 chlorophyll content of 11 seed accessions and 2 rice local checks (KDML105 and PSL1) at 60 days after transplanting (DAT) in Mn_0.

Genotypes	**YEB-1 chlorophyll content (SPAD unit)**			
	Mean	SD	CV (%)	n
Sam Lern	30.34	4.81	15.84	9
Mon Kong (1)	27.19	3.16	11.63	11
Mon Kong (2)	27.05	4.05	14.97	6
Haw (1)	25.62	3.57	13.93	12
Haw (2)	28.59	3.61	12.65	7
Haw (3)	28.19	3.19	11.33	17
Haw (4)	27.62	3.59	12.99	18
Phee	25.22	4.27	16.95	11
Ja Nor Moey	25.88	4.01	15.49	20
Haw Kaw	27.78	4.90	17.65	12
Jee Dao	25.18	3.38	13.43	9
PSL1	26.83	2.36	8.78	15
KDML105	32.69	1.81	5.55	8

Table 2.12 Number of leaves of 11 seed accessions and 2 rice local checks (KDML105 and PSL1) at 30 days after transplanting (DAT) in Mn_0.

Genotypes	**Number of leaves (plant^{-1})**			
	Mean	SD	CV (%)	n
Sam Lern	9.44	2.74	29.05	9
Mon Kong (1)	8.67	2.44	28.15	14
Mon Kong (2)	8.43	1.51	17.94	7
Haw (1)	8.67	3.11	35.87	14
Haw (2)	7.38	1.94	26.25	13
Haw (3)	8.88	2.09	23.51	17
Haw (4)	7.61	2.77	36.37	19
Phee	7.36	3.00	40.81	14
Ja Nor Moey	8.11	2.72	33.53	17
Haw Kaw	7.69	2.18	28.28	13
Jee Dao	10.91	1.04	9.57	12
PSL1	11.47	0.92	7.98	15
KDML105	13.27	1.79	13.52	11

Table 2.13 Number of leaves of 11 seed accessions and 2 rice local checks (KDML105 and PSL1) at 60 days after transplanting (DAT) in Mn_0.

Genotypes	**Number of leave (plant^{-1})**			
	Mean	SD	CV (%)	n
Sam Lern	15.22	3.27	21.48	9
Mon Kong (1)	12.08	3.82	31.61	11
Mon Kong (2)	12.67	2.25	17.77	6
Haw (1)	13.33	5.21	39.08	12
Haw (2)	8.89	2.15	24.16	7
Haw (3)	11.06	3.21	29.03	17
Haw (4)	10.53	4.54	43.11	18
Phee	10.36	3.61	34.86	11
Ja Nor Moey	9.84	2.48	25.18	20
Haw Kaw	9.67	2.93	30.35	12
Jee Dao	15.00	1.87	12.47	9
PSL1	15.07	2.19	14.51	15
KDML105	18.13	2.47	13.65	8

Table 2.14 Number of tillers of 11 seed accessions and 2 rice local checks (KDML105 and PSL1) at 30 days after transplanting (DAT) in Mn_0.

Genotypes	**Number of tiller (plant^{-1})**			
	Mean	SD	CV (%)	n
Sam Lern	2.44	0.73	29.72	9
Mon Kong (1)	2.13	0.83	39.08	14
Mon Kong (2)	2.29	0.76	33.07	7
Haw (1)	2.13	0.83	39.08	14
Haw (2)	1.62	0.77	47.54	13
Haw (3)	2.29	0.59	25.62	17
Haw (4)	1.78	0.88	49.40	19
Phee	2.00	0.78	39.22	14
Ja Nor Moey	1.78	0.88	49.40	17
Haw Kaw	1.77	0.83	47.03	13
Jee Dao	2.36	0.50	21.35	12
PSL1	2.40	0.51	21.13	15
KDML105	2.91	0.54	18.54	11

Table 2.15 Number of tillers of 11 seed accessions and 2 rice local checks (KDML105 and PSL1) at 60 days after transplanting (DAT) in Mn_0.

Genotypes	**Number of tiller (plant^{-1})**			
	Mean	SD	CV (%)	n
Sam Lern	3.56	1.01	28.51	9
Mon Kong (1)	3.54	1.33	37.59	11
Mon Kong (2)	3.33	1.03	30.98	6
Haw (1)	3.58	1.24	34.61	12
Haw (2)	2.44	0.88	36.08	7
Haw (3)	3.82	1.13	29.58	17
Haw (4)	3.42	1.30	38.13	18
Phee	3.09	1.38	44.49	11
Ja Nor Moey	2.95	0.85	28.78	20
Haw Kaw	3.17	0.94	29.60	12
Jee Dao	3.56	0.53	14.82	9
PSL1	3.33	1.11	33.38	15
KDML105	3.75	0.71	18.86	8

Sub experiment 2 **Characterization in seed morphology between and within some of upland rice genotypes**

Seed characters

Hull color

Genetic variation was found in the hull color in both within and between 3 genotypes. Straw hull was mixed in other straw/brown hull in Haw (3) (H´ = 0.641) whereas Sam Lern and Haw Kaw was similarly in straw/brown and brown, respectively (Table 2.16).

Seed Shape: length, width, thick and weight

Seed length of all 3 genotypes ranged from 7.8 to 11.1 mm, average seed length of Sam Lern was the highest (9.84 mm) but Haw (3) was the lowest (8.95 mm). CV in seed length of each genotype varied from 5.68-6.62, Sam Lern was the highest but it was the lowest in Haw (3) (Table 2.17).

Seed width of all 3 genotypes distributed from 2.0-4.0 mm, average seed width of Haw Kaw was the lowest (2.85 mm) but Sam Lern was the highest (3.24 mm). CV in seed width of each genotype varied from 4.95-7.42, Haw (3) was the lowest but in Haw Kaw, it was the highest (Table 2.17).

Seed thickness of all 3 genotypes distributed from 1.5-2.7 mm, average seed thickness of Sam Lern was the highest (2.29 mm) but Haw (3) was the lowest (1.97 mm). CV in seed thickness of each genotype varied from 5.86-8.30, Haw (3) was the highest but Haw Kaw was the lowest (Table 2.17).

Seed weight of all 3 genotypes distributed from 0.01-0.05 mg grain^{-1}, average seed weight of Sam Lern was the highest (0.033 mm), whereas Haw (3) and Haw

Kaw were the lowest (1.97 mm). CV in seed weight of each genotype varied from 18.87-19.62, Haw (3) was the highest but it was the lowest in Haw Kaw (Table 2.17).

Table 2.16 Seed characters and Shannon's Index of 3 upland rice genotypes.

Variety	**Hull color***			
	Straw	Brown	Straw/Brown	H'
Sam Lern	0	0	100	0
Haw (3)	34	0	66	0.641
Haw Kaw	0	100	0	0

*= 100 seeds per variety

Table 2.17 Grain length, width, thickness and weight between and within 3 upland rice genotypes.

Variety	**Grain length (mm)**			
	Range	Mean	SD	CV (%)
Sam Lern	8.2-11.1	9.84	0.65	6.62
Haw (3)	7.9-9.9	8.95	0.51	5.68
Haw Kaw	7.8-10.7	9.25	0.57	6.12
	Grain width (mm)			
Sam Lern	2.8-4.0	3.24	0.22	6.79
Haw (3)	2.6-3.4	3.08	0.15	4.95
Haw Kaw	2.0-3.8	2.85	0.21	7.42
	Grain thickness (mm)			
Sam Lern	1.7-2.7	2.29	0.15	6.65
Haw (3)	1.5-2.6	2.13	0.18	8.30
Haw Kaw	1.5-2.3	1.97	0.12	5.86
	Grain weight (g grain^{-1})			
Sam Lern	0.02-0.05	0.033	0.01	19.46
Haw (3)	0.01-0.03	0.027	0.01	19.62
Haw Kaw	0.02-0.04	0.027	0.01	18.87

*= 100 seed samples per genotypes

Sub experiment 3 **Responses of upland rice genotype to Mn levels**

YEB-1 chlorophyll content

YEB-1 chlorophyll content was significantly different between genotypes and Mn levels. In Mn_0, YEB-1 chlorophyll content of BB increased, whereas PSL1 decreased when compared to $Mn_{0.5}$. In Mn_0, BB was the highest YEB-1 chlorophyll content followed by Sam Lern, Haw (3), Haw Kaw and KDML105, whereas PSL1 was the lowest (Table 2.18).

The relative chlorophyll content in YEB-1 of BB, Sam Lern and KDML105 were higher than Haw (3), Haw Kaw and PSL1 (Figure 2.9).

Dry weight

Relative shoot dry weight of Sam Lern was the highest followed by BB and KDML105, whereas Haw (3), Haw Kaw and PSL1 were the lowest (Figure 2.10).

Relative root dry weight was the highest in Sam Lern, followed by KDML105 Haw Kaw, BB and Haw (3) whereas in PSL1, it was the lowest (Figure 2.11).

Relative total dry weight was the highest in Sam Lern, followed by KDML105 Haw Kaw, BB and PSL1 whereas it was the lowest in Haw (3) (Figure 2.12).

Manganese concentration

YEB

YEB Mn concentration was significantly different between genotypes and Mn levels. In Mn_0, YEB Mn concentration of Sam Lern, Haw Kaw, PSL1 and KDML105 decreased, excepted in Haw (3) and BB when compared to $Mn_{0.5}$. In Mn_0, YEB Mn

concentration was the highest in KDML105, followed by Sam Lern and BB, whereas Haw Kaw and PSL1 were the lowest (Table 2.19).

Shoot

In Mn_0, shoot Mn concentration of all genotypes decreased when compared to $Mn_{0.5}$. In Mn_0, shoot Mn concentration was the highest in KDML105, follwed by Sam Lern and BB, whereas Haw (3), Haw Kaw and PSL1 were the lowest (Table 2.20).

Root

Root Mn concentration in Mn_0 of Haw Kaw and PSL1 decreased, excepted in Sam Lern, Haw (3), BB and KDML105. In Mn_0, root Mn concentration of KDML105 was the highest whereas PSL1 was the lowest (Table 2.21).

Manganese content

Shoot

Shoot Mn content was significantly different between genotypes and Mn levels. In Mn_0, shoot Mn content of Haw (3) and PSL1 decreased, excepted in Sam Lern, Haw Kaw, BB and KDML105 when compared to $Mn_{0.5}$. In Mn_0, shoot Mn content of PSL1 was the highest whereas Sam Lern, Haw (3), Haw Kaw, BB and KDML105 was the lowest (Table 2.22).

Root

Root Mn content was significantly different between genotypes and Mn levels. In Mn_0, root Mn content of Sam Lern, Haw Kaw and PSL1 decreased, excepted in Haw (3), BB and KDML105 when compared to $Mn_{0.5}$. In Mn_0, root Mn content was the highest in Haw (3), followed by Haw Kaw, Sam Lern and BB whereas PSL1 and KDML105 were the lowest (Table 2.23).

Manganese uptake efficiency

Relative Mn uptake efficiency was the highest in BB, followed by Sam Lern, Haw (3) and KDML105 whereas PSL1 was the lowest (Figure 2.13).

Table 2.18 YEB-1 chlorophyll content (SPAD unit) of 4 upland rice and 2 rice local checks (KDML105 and PSL1) grown in solution culture at 30 days after transplanting.

Variety	Mn level (ppm)		
	0	0.5	Mean
Sam Lern	28.88aB	28.17aAB	28.53
Haw (3)	28.67aB	28.09aAB	28.38
Haw Kaw	28.44aB	27.43aB	27.93
BB	32.28aA	29.23bA	30.75
PSL1	21.05bC	28.38aAB	24.71
KDML105	28.21aB	29.46aA	28.83
mean	27.92	28.46	28.19
F-test	V***	Mn^{ns}	VxMn***
$LSD_{(0.05)}$			1.4855

^{ns}, *** 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 colume is indicated by upper case letters. The difference between Mn level in the same row is indicated by lower case letters.

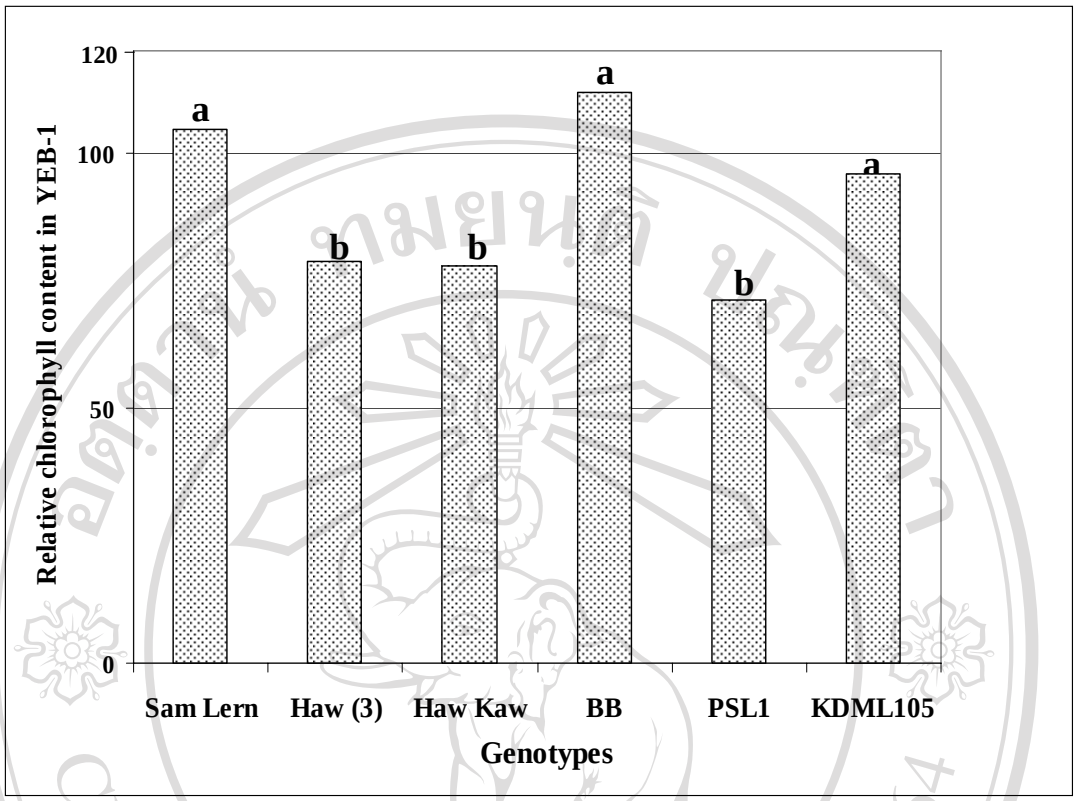

Figure 2.9 Relative chlorophyll content in YEB-1 of 4 upland rice genotypes and 2 local rice checks (KDML105 and PSL1) grown in solution culture at 30 days after transplanting.

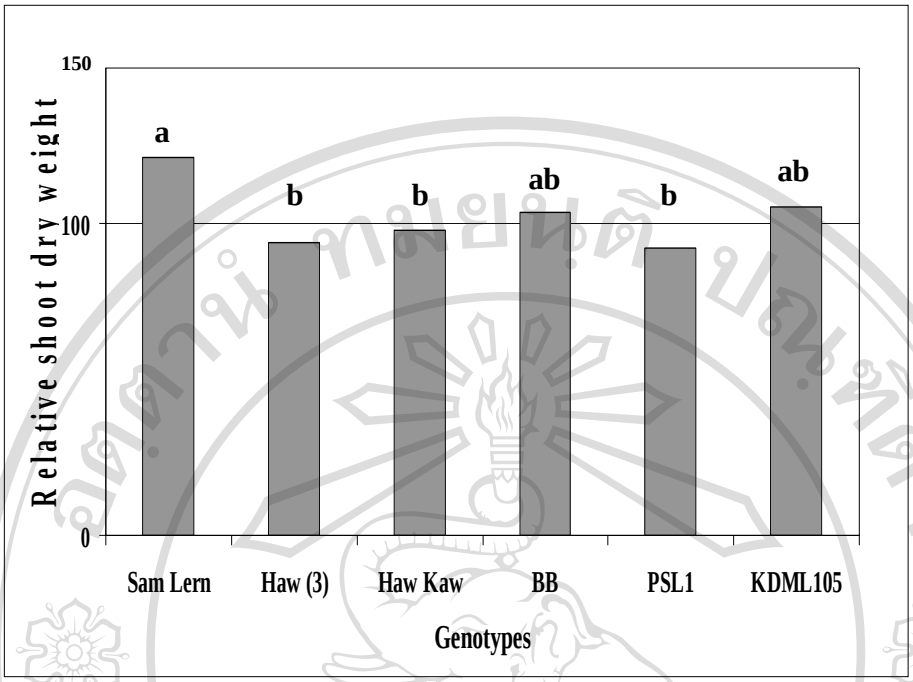

Figure 2.10 Relative shoot dry weight of 4 upland rice and 2 rice local checks (KDML105 and PSL1) grown in solution culture at 30 days after transplanting.

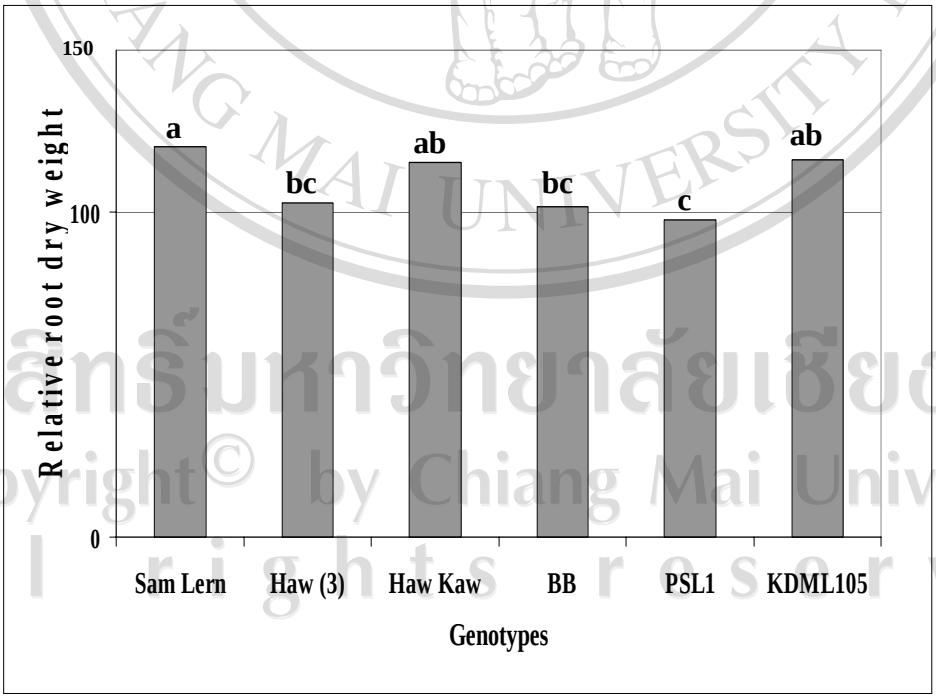

Figure 2.11 Relative root dry weight of 4 upland rice and 2 rice local checks (KDML105 and PSL1) grown in solution culture at 30 days after transplanting.

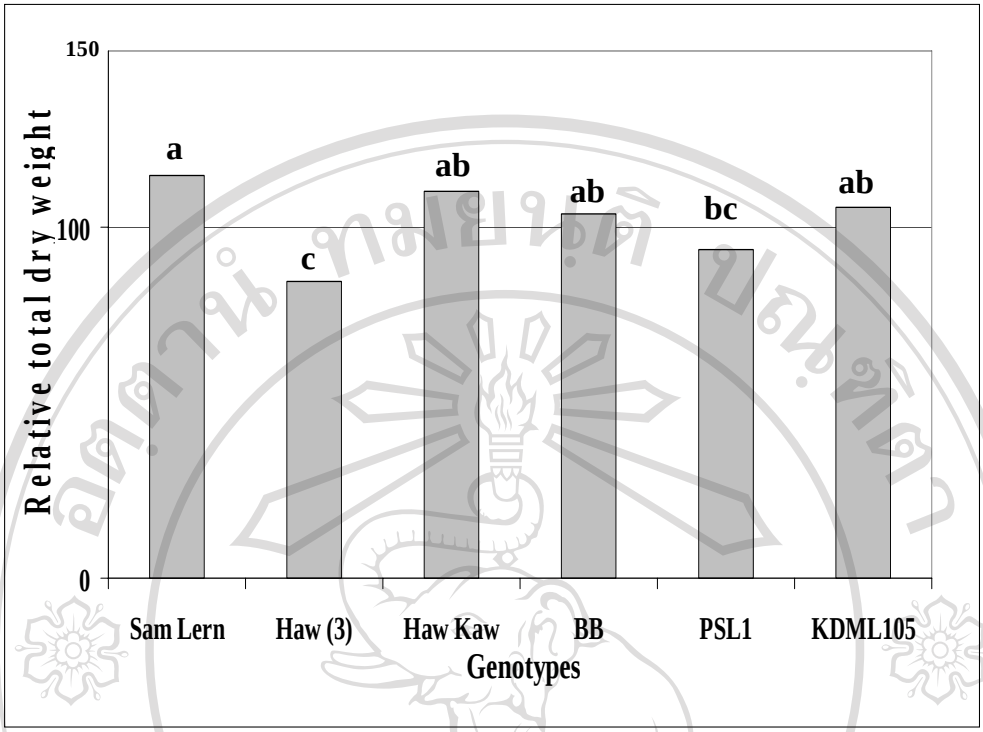

Figure 2.12 Relative total dry weight of 4 upland rice and 2 rice local checks (KDML105 and PSL1) grown in solution culture at 30 days after transplanting.

Table 2.19 Concentration in YEB (mg Mn kg^{-1}) of 4 upland rice and 2 rice local checks (KDML105 and PSL1) grown in solution culture at 30 days after transplanting.

Variety	Mn level (ppm)		
	0	0.5	Mean
Sam Lern	24.67aB	24.52aC	24.60
Haw (3)	20.25aC	22.25aD	21.25
Haw Kaw	23.86aB	20.64bD	22.25
BB	29.08bA	32.84aA	30.96
PSL1	22.84bB	29.71Ba	26.27
KDML105	27.61aA	28.49aB	28.05
mean	24.72	26.41	25.56
F-test	V***	Mn***	VxMn***
$LSD_{(0.05)}$			2.05

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

Table 2.20 Concentration in shoot (mg Mn kg^{-1}) of 4 upland rice and 2 rice local checks (KDML105 and PSL1) grown in solution culture at 30 days after transplanting.

Variety	Mn level (ppm)		
	0	0.5	Mean
Sam Lern	200.81bB	257.70aBC	229.26
Haw (3)	164.67bC	243.44aC	204.06
Haw Kaw	173.03bC	207.79aD	190.41
BB	200.77bB	243.42aC	222.10
PSL1	162.51bC	289.26aA	225.89
KDML105	249.03bA	263.15aB	256.09
mean	191.80	250.79	221.30
F-test	V***	Mn***	VxMn***
$LSD_{(0.05)}$			15.06

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

Table 2.21 Concentration in root (mg Mn kg^{-1}) of 4 upland rice and 2 rice local checks (KDML105 and PSL1) grown in solution culture at 30 days after transplanting.

Variety	Mn level (ppm)		
	0	0.5	Mean
Sam Lern	132.23aBC	151.26aC	141.75
Haw (3)	120.56aC	117.80aE	119.18
Haw Kaw	127.66bC	161.91aC	144.79
BB	142.07aB	137.49aD	139.78
PSL1	85.97bD	218.12aA	152.05
KDML105	206.65aA	201.43aB	204.04
mean	135.86	164.67	150.27
F-test	V***	Mn***	VxMn***
$LSD_{(0.05)}$			13.19

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

Table 2.22 Content in shoot (mg Mn plant^{-1}) of 4 upland rice and 2 rice local checks (KDML105 and PSL1) grown in solution culture at 30 days after transplanting.

Variety	Mn level (ppm)		
	0	0.5	Mean
Sam Lern	0.32aB	0.38aB	0.35
Haw (3)	0.26bB	0.38aB	0.32
Haw Kaw	0.27aB	0.32aB	0.29
BB	0.27aB	0.32aB	0.29
PSL1	0.52bA	0.78aA	0.65
KDML105	0.29aB	0.30aB	0.29
mean	0.32	0.41	0.37
F-test	V***	Mn***	VxMn**
$LSD_{(0.05)}$			0.09

, * 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 colume is indicated by upper case letters. The difference between Mn level in the same row is indicated by lower case letters.

Table 2.23 Content in root (mg Mn plant^{-1}) of 4 upland rice and 2 rice local checks (KDML105 and PSL1) grown in solution culture at 30 days after transplanting.

Variety	Mn level (ppm)		
	0	0.5	Mean
Sam Lern	1.08bB	1.34aAB	1.21
Haw (3)	1.36aA	1.15aBC	1.26
Haw Kaw	1.14bAB	1.50aA	1.32
BB	1.06aB	1.03aC	1.04
PSL1	0.60bC	1.49aA	1.04
KDML105	0.55aC	0.63aD	0.59
mean	0.96	1.19	1.08
F-test	V***	Mn***	VxMn***
$LSD_{(0.05)}$			0.24

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

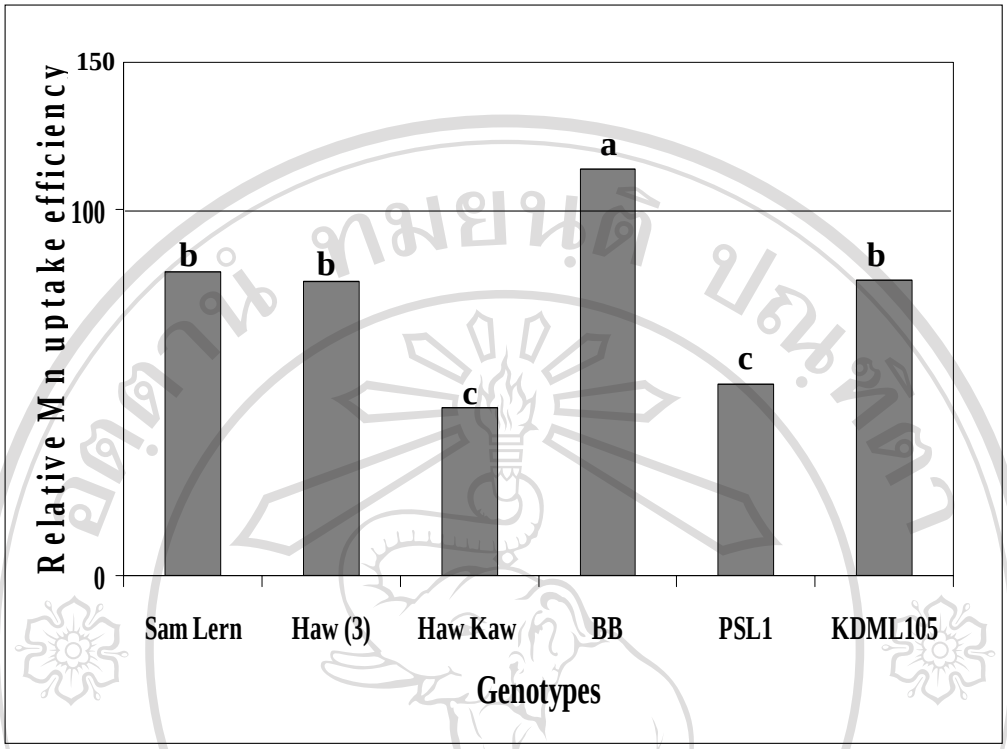

Figure 2.13 Relative Mn uptake efficiency of 4 upland rice and 2 rice local checks (KDML105 and PSL1) grown in solution culture at 30 days after transplanting.

2.4 Discussion

Yamasaki (1964) reported the results of Tokuoka and Gyo (1940) that showed the addition of manganese increased the height of the stem, and accelerated the tillering number. While, deficient plants were shorter, have fewer leaves and weigh less at tillering stage (Dobermann and Fairhurst, 2000). The concentration of Mn affected on number of leaves, tiller and root dry weight but not on YEB chlorophyll content and shoot dry weight of five rice genotypes. It was found that KDML105 had high relative number of leaves, tillers and relative root dry weight, when grew in Mn deficiency compared with Mn sufficiency. Manganese efficiency has been defined as a genotype's ability to produce high yield in a soil whose Mn content is limiting for a standard genotype (Ascher-Ellis *et al.*, 2001). These results indicated that most efficiency genotype was KDML105, whereas inefficiency genotype was PSL1.

There was apparent variation in Mn concentration and content between genotypes. A previous report indicated that the critical manganese level for the occurrence of deficiency symptoms in the rice genotypes was 40 mg Mn kg^{-1} in Y-leaf was suggested during both the tillering and panicle differentiation growth stages (Bell and Kovar, 2000). In the present study, serious Mn deficiency occurred in all genotypes when the tissue Mn concentration of the YEB was 14.87 - 18.74 mg Mn kg^{-1}. The reason for differences between the critical Mn level observed in different solution culture studies are unclear, but may be related to the use of different genotypes and culture conditions.

However, the highly significant difference between genotypes was observed for Mn uptake. Considerable genotypic variation in Mn efficiency was observed based on relative Mn uptake and Mn uptake efficiency. The results showed that Mn

uptake in Mn_0 of Mn efficient KDML105 exceeded 110% of that in $Mn_{0.5}$. So far reports variation in Mn uptake efficiency have been made over a wide range of genotypes of the other crops than rice, e.g. Gladstones and Loneragan (1970); Graham *et al*. (1983); Marcar and Graham (1987b); Graham (1988); Huang *et al*. (1994); Bansal and Nayyar (1998) and Marschner *et al*. (2003). The Mn-efficient absorbed more Mn and had higher tissue Mn concentration. Thus, the results of KDML105 accumulated a higher Mn concentration (Mn uptake) in conferring its Mn efficiency reported here is consistent with the general observations that Mn-efficient genotype has a higher ability of uptake and accumulation of Mn while grown under Mn deficiency. The classification of Mn efficiency in this study was also correlated with greater number of leaves, tillers, root dry weight and Mn uptake.

Manganese is not a constituent of chlorophyll, but it is thought to relate to chlorophyll formation and a large quantity of Mn is found in the chloroplast (Yamasaki, 1964). As, chlorophyll content were affected primary by Mn deficiency, that decreases in leaf chlorophyll content and photosynthetic activity of plant growing under Mn deficiency has been observed by Ohki (1985); Kreidemann *et al*. (1985). Therefore, YEB-1 chlorophyll content can be used to criteria in Mn efficiency genotype. Therefore, Sam Lern, BB and KDML105 were most Mn efficiency genotypes based on relative YEB-1 chlorophyll content, correlated well with their relative shoot and root dry weight.

Genetic variation in Mn efficiency was also found in seeds of upland rice form an area wild calcareous soil. The variation of Mn efficiency was found among individual plants of each seed accession and seed accession sharing the same name. Seed accession of upland rice like Sam Lern and Haw (3) which classified to Mn

efficiency genotypes were high variation (high CV) within and between genotypes based on their number of leaves, tillers and YEB-1 chlorophyll content. Sam Lern and Haw (3) were more Mn efficient than Mn inefficient check PSL1 and similar to Mn efficient check KDML105. The externally grain characters of Sam Lern, Haw (3) and Haw kaw showed small variation (low CV). However, number of leaves, tillers and chlorophyll content of Sam Lern and Haw (3) was highly varied in CV (% compared with pure lined checks).

Similar genetic variation between seed lots and wihin seed lots has been reported in BB in tolerance to Al toxic variety (Phattarakul, 2008). BB has been described with high variation between progeny lines within each seed lot and between seed lots in tolerance to Al based on relative root length. Similarly, the report of Prom-u-thai *et al*. (2004) and Pintasen *et al*. (2007) found that the levels of variation in grain iron concentration between the seed of BB within individual seed lots collected from different farmers, was the same as those found between high and low Fe varieties. According to Meesin (2004) and Supamongkol (2006) genetic variation within local rice varieties recognized by the same name has been found by molecular markers as well as morphological characteristics. However, genetic variation within and among seed lots with the same name was detected by DNA analysis although there may or may not be obvious variation in external appearance. Phattarakul (2008) suggested that analysis with molecular markers revealed more genetic differentiation than by visual characteristics

Manganese efficiency in rice appears to be complexed. The next chapters will attempt to explain some of this complexity, by examining the mechanism of Mn

acquisition, uptake and utilization by Mn efficiency and inefficiency in rice genotypes.