Chapter 3

Genotypic variation in tolerance to Al toxicity in Thai rice germplasm

3.1 Introduction

There is extensive area of acid soils in cultivated area of Thailand, the main region is in the Central Plain of Thailand which is the country's main rice producing region (Kheoruenromne and Kesawapitak, 1989; von Uexküll and Bosshart, 1989). It has been reported that rice crops in these soils showed symptoms of Al toxicity and P deficiency (Attanandana *et al.*, 1982). Although much of acid soils improvement has been reported for rice production, it is not always economical and practical for small farmers. Therefore, selection for acid-soils tolerant rice varieties should offer a practical alternative.

Generally, Al toxicity is the major factor limiting the growth of plant on acid soils with pH below 5 (Fageria *et al.*, 1988; Foy, 1984). Therefore, plant selection in tolerance to Al toxicity should be a basic screening for plant adapted to highly acid soils. Different screening methods have been used to evaluate Al tolerance such as nutrient solution culture, cell and tissue culture and soil bioassays, etc. However, the screening in acid soils is often influenced by other environmental factors such as nutrient availability, diseases and pests (Howeler and Cadavid, 1976). Much work on

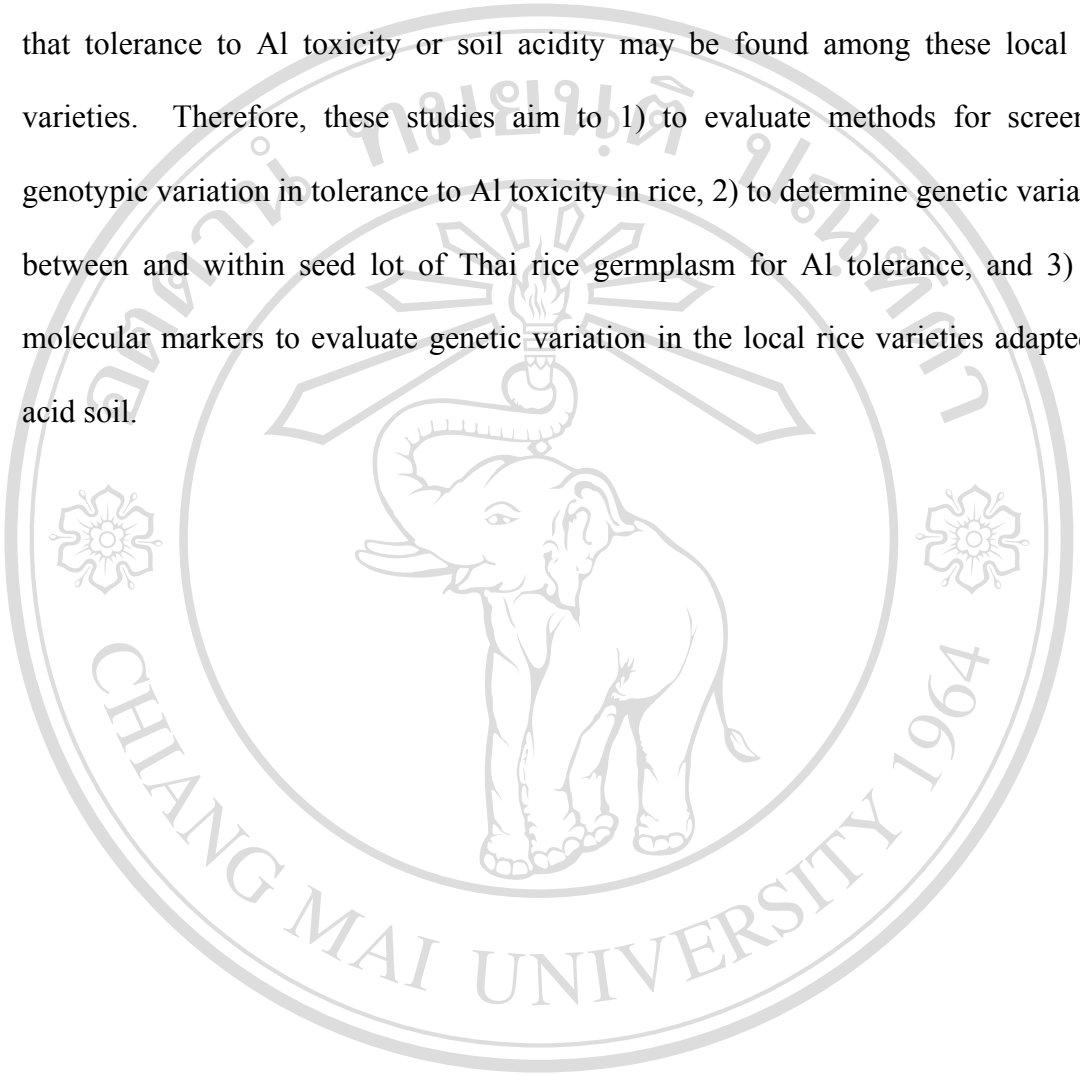
Al tolerant screening has been conducted using nutrient solution technique due to easily observed root inhibition, fast and strict control over nutrient availability and pH (Khatiwada *et al.*, 1996; Nguyen *et al.*, 2001).

The parameters of root growth inhibition and relative root growth (root growth under Al stress compared to root growth without Al stress) are widely used to identify Al tolerant and Al sensitive genotypes (Hede *et al.*, 2000). Previous studies have suggested relative root length (RRL) as an effective parameter for Al tolerance screening in rice genotype (Khatiwada *et al.*, 1996; Vasconcelos *et al.*, 2002). This parameter is not only identified genotypic variation between varieties but also useful for segregating populations for Al tolerance in rice breeding (Nyuyen *et al.*, 2002).

Thailand has been designated as part of the centers of diversity of *Oryza sativa* (Chang, 1976). Local varieties are generally considered to be a rich source of genetic variation for varieties development. There has been genetic variation not only among population that recognized as the same varieties but also found between individual plants within population (Meesin, 2003; Pintasen *et al.*, 2007). Molecular markers can reveal genetic differentiation and provide a more direct, reliable and efficient technique for evaluating genetic diversity over selection base on phenotype (Ni *et al.*, 2002; Thanh *et al.*, 1999). Microsatellites or simple sequence repeats (SSRs) have been effectively applied to identify genetic variation among rice varieties (Ni *et al.*, 2002; Zeng *et al.*, 2004).

An earlier study of Yimyam (2006) and our survey (Chapter 2) at Tee Cha village in northern Thailand found some local upland rice varieties that are growing and yield well in highly acidic soil with pH as low as 4.5. Pintasen *et al.* (2007) suggested that variation of local upland rice varieties from Tee Cha village was not

found only between varieties but also between seed lots and within individual seed lot that recognized as the same name in term of grain iron concentration. It is suspected that tolerance to Al toxicity or soil acidity may be found among these local rice varieties. Therefore, these studies aim to 1) to evaluate methods for screening genotypic variation in tolerance to Al toxicity in rice, 2) to determine genetic variation between and within seed lot of Thai rice germplasm for Al tolerance, and 3) use molecular markers to evaluate genetic variation in the local rice varieties adapted to acid soil.



3.2 Materials and methods

3.2.1 Genotypic variation among rice varieties to soil acidity and Al toxicity

Experiment 3.2.1.1 Germination of upland rice in acid soil

Five upland rice varieties; Bue Bang (BB), Bue Goa (BG), Bue Mue Ta Bong (BM), Bue Paw Low (BP) and Pa Ai Khu Phae (PA) were developed into single seed descent lines from seed collected from farmers in Tee Cha village, Mae Hong Son province, Thailand. These were evaluated against the standard check, KDML105, a popular Thai jasmine rice. Germination of six varieties were evaluated in soils with two pH levels, 5.8 (control) and 3.5. The soil for this experiment was collected from Mae Hia research station, Chiang Mai university with pH 5.8 and then acidified to pH 3.5 with $Al_2(SO_4)_3.18H_2O$ at 12 g kg^{-1} soil. Fifty seeds of each variety were germinated in a plastic pot containing 12 g soil in a completely randomized design, with three replicates. The germinated seeds were counted at 4, 7, 10, 13, 16 and 19 days after sowing.

Experiment 3.2.1.2 Responses of rice to Al levels in nutrient solution

Sub experiment 1 ***Variation among upland rice varieties***

Culture solution

The six varieties as in Experiment 3.2.1.1 were evaluated in tolerance to Al toxicity in nutrient solution. The seeds were germinated for five days. Five plants of each variety were transplanted to 10 L plastic pot containing nutrient solution in a completely randomized design, with three replicates. The culture solution was modified from Kimura B solution, the composition was described in Table 3.1 and Table 3.2. There were three treatments: nil Al in pH 7 (Al_0-pH 7); nil Al in pH 4

(Al_0-pH 4) and with 30 mg Al L^{-1} [added as $Al_2(SO_4)_3.18H_2O$] in pH 4 (Al_{30}-pH 4). The solution was renewed every week and pH values were adjusted daily to 7.0 or 4.0 ± 0.05 with 1N HCl or 1N NaOH. Plants were harvested at 14 days after treatments.

Sampling and measurement

At harvest, maximum root length, maximum shoot length, root and shoot dry weights were assessed. Maximum root length was measured in the longest root. Maximum shoot length was measured from base of stem to the tip of the top most leaf terminal. Root and shoot dry weights were measured after oven drying at 70°C for 48 hours. Relative value of root length (RRL) was calculated by dividing the root length of seedling grown with stress, Al_0-pH4 or Al_{30}-pH4, with that grown without stress, Al_0-pH7. Relative value of shoot length (RSL), root dry weight (RRW), shoot dry weight (RSW) and total dry weight (RTW) were computed in the similar way.

Sub experiment 2 Comparing the responses of rice to Al made from different aluminum salts

KDML105 was used for comparing response to different Al salts. Twelve 5 day-old seedlings was transplanted to 10 L plastic pot containing nutrient solution in a completely randomized design, with three replicates. The composition of nutrient solution was the same as previously described in sub experiment 1. There were three Al levels; 0, 10 and 20 mg L^{-1}, and two Al forms; $Al_2(SO_4)_3.18H_20$ and $AlCl_3$. The nutrient solution was renewed every week and pH value was adjusted daily to 4.0 ± 0.05 with 1N HCl or 1N NaOH. Plants were harvested at 21 days after treatments.

Maximum root length, maximum shoot length, root and shoot dry weights were measured in the same as previously described in sub experiment 1.

Sub experiment 3 ***Comparing the responses of rice to Al with and without air bubbling in nutrient solution***

Two upland rice varieties, BB and PA, and three improved varieties, Suphan Buri1 (SPR1), Chainat1 (CNT1) and KDML105 were grown for comparing response in different culture condition. Five days after germination, five plants of each variety were transplanted to 10 L plastic pot containing nutrient solution in a completely randomized design, with three replicates. The composition of nutrient solution was the same as previously described in sub experiment 1. There were two conditions; without and with oxygen supply by air bubbling in nutrient solution, and two Al levels; 0 and 30 mg L^{-1} [add as $Al_2(SO_4)_3.18H_20$]. The nutrient solution was renewed every week and pH value adjusted daily to 4.0 ± 0.05 with 1N HCl or 1N NaOH. Plants were harvested at 21 days after treatments. Maximum root length, maximum shoot length, root and shoot dry weights were measured in the same as previously described in sub experiment 1.

Sub experiment 4 ***Variation among improved Thai rice varieties***

Nine improved, popular varieties in Thailand (Table 3.3) were compared with a local upland rice BB for Al tolerance in nutrient solution. Five days after germination, five plants of each variety were transplanted to 10 L plastic pot containing nutrient solution in a completely randomized design, with three replicates. There were separated pots by growing five varieties per pot. The composition of

nutrient solution was the same as previously described in sub experiment 1. There were two Al levels; 0 and 30 mg L^{-1} [add as $Al_2(SO_4)_3.18H_20$]. Plants were grown in still nutrient solution; without oxygen supply by air bubbling. The nutrient solution was renewed every week and pH value adjusted daily to 4.0 ± 0.05 with 1N HCl or 1N NaOH. Plants were harvested at 21 days after treatments. Maximum root length, maximum shoot length, root and shoot dry weights were measured in the same as previously described in sub experiment 1.

Statistical analysis

Analysis of variance was conducted using a factorial treatment combination arranged in Completely Randomized Design (CRD). Data were analyzed using analysis of variance (ANOVA) to determine the effects of variety, Al level and interaction between variety and Al level. Means were compared by least significant difference (LSD) at $P < 0.05$.

Table 3.1 Nutrient concentration of Kimura B solution.

Element	Concentration of element in nutrient solution (ppm)
N	23.0
P	5.6
K	21.4
Ca	14.6
Mg	13.3
Fe	7.0

Source: Yoshida *et al.* (1976)

Table 3.2 Preparation of Kimura B solution.

Reagent	Preparation (mg L^{-1} of culture solution)
$(NH_4)_2SO_4$	48.2
KH_2PO_4	24.8
KNO_3	18.5
K_2SO_4	15.9
$Ca(NO_3)_2$	59.9
$MgSO_4$	65.9
Fe-EDTA	40.0

Source: Yoshida *et al.* (1976)

Table 3.3 Characteristic of popular improved Thai rice varieties.

Variety	Cross	Characteristic	Yield (kg rai^{-1})
1. Suphan Buri 1 (SPR1)	IR25393-57-2-3 / RD23 // IR27316-96-3-2-2 /// SPRLR77205-3-2-1-1 / SPRLR79134-51-2-2	Tolerant to ragged stunt disease, blast disease, bacterial leaf blight, brown plant hopper and whitebacked plant hopper	806
2. Suphan Buri 2 (SPR2)	RD23 / IR60	Tolerant to ragged stunt disease, blast disease, bacterial leaf blight, yellow orange leaf disease and brown plant hopper	700
3. Chainat 1 (CNT1)	IR13146-158-1 / IR15314-43-2-3-3 // BKN6995-16-1-1-2	Tolerant to ragged stunt disease, blast disease, brown plant hopper and whitebacked plant hopper	740
4. Chainat 2 (CNT2)	Hom Myanmar (หอมพม่า) (GS.No.3780) / IR11418-19-2-3	Aromatic rice Tolerant to brown plant hopper	657
5. Chainat 80 (CNT80)	IR29692-99-3-2-1 / IR11418-19-2-3 // SPR60	High grain iron Tolerant to bacterial leaf blight and brown plant hopper	876
6. Ayutthaya 1 (AUT1)	Au Tha Pao (อู่ตะเภา) / KDML105	Tolerance to deep water, acid sulphate soil (pH 4.6-5.1), brown plant hopper and green leaf hopper	842 (25 cm depth) 546 (100 cm depth)
7. Prachin Buri 2 (PCR2)	BKNFR80086 / HTAFR80038	Tolerance to deep water and acid sulphate soil and blast disease	846 (25 cm depth) 590 (100 cm depth)
8. Pathum Tani 1 (PTT1)	BKNA6-18-3-2 / PTT85061-86-3-2-1	Tolerant to blast disease, bacterial leaf blight, brown plant hopper and whitebacked plant hopper	650-774
9. Khao Dawk Mali 105 (KDML105)	Pure line selection	Good cooking Quality and aromatic, tolerance to acid-sulfate soil, saline soil and drought	363

Source: Rice Department, Thailand (http://www.ricethailand.go.th/rkb/index.html)

3.2.2 Genotypic variation within local rice varieties recognized by the same name

Experiment 3.2.2.1 Variation between and within seed lots of local upland rice varieties

Morphological characters

Each two seed lots of upland rice varieties that recognized as BB, BM and PA obtained from different farmers (one of each of these same as in 3.2.1.1) were grown in pots (20 plants each) until maturity. Morphological characteristics were recorded individually using the method of IRRI-IBPGR (1980). Plants were recorded at different plant parts including, colors of leaf blade, leaf sheath, auricle, ligule, node, internode, apiculus, hull and pericarp, and ligule shape, plant type and awning. At harvest, seeds were separated and collected individual plants that used for Al tolerance screening.

Response to Al toxicity

Progeny seeds of each two seed lots of BB, BM and PA(containing 20 individual progeny lines each) were evaluated for Al tolerance with two standard checks, Koshihikari (Al-tolerant; Ma *et al.*, 2002) and KDML105 (Al-sensitive). Five days after germination, five plants of each progeny line were transplanted to 30 L plastic container containing nutrient solution in a completely randomized design, with three replicates. The composition of nutrient solution was the same as previously described in Experiment 3.2.1.2. There were two Al levels; 0 and 30 mg L^{-1} [add as $Al_2(SO_4)_3.18H_20$]. The solution was renewed every five days and pH value adjusted daily to 4.0 ± 0.05 with 1N HCl or 1N NaOH. Plants were harvested at 21 days after

treatment. Maximum root length, maximum shoot length, root and shoot dry weights were measured in the same as previously described in Experiment 3.2.1.2. A plant of each line was collected and silica-dried and then kept at -20 ºC until used for DNA analysis.

DNA analysis

Genomic DNA individually was extracted from dry leaf sample using CTAB method and the PCR reactions were performed following the description of Panaud *et al*. (1996). Many of microsatellite markers were used for screening along 12 chromosomes. After screening, six SSR primer pairs, RM1, RM48, RM149, RM164, RM241 and RM335 (Table 3.4), that showed polymorphisms were selected for evaluating genetic variation within and between population of BB and BM. Amplification of DNA were performed in 20 μl reaction consisted of 20-50 ng DNA, 0.25 mM of each dNTP, 2% formamide, 0.2 μM of each primers and 0.5 unit of Taq DNA polymerase in reaction buffer [10 mM of Tris-HCl pH 8.5, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1mM EDTA, 50% (v/v) glycerol]. Amplified products were mixed with loading dye and separated in 10% Polyacrylamide Gel Electrophoresis (PAGE). Gel was stained with ethidium bromide and photographed under UV transilluminators.

Data analysis

For morphological 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 plot

For DNA analysis, an estimate of the genetic diversity index (H_e) was calculated for each rice population according to Nei (1973) as;

$$H_e = 1 - \sum P_i^2$$

Where P_i is the allele frequency. The distribution of variability between and within populations was calculated according to Nei *et al.* (1983) for each microsatellite locus. The total diversity estimate (H_T) was partitioned into within population diversity (H_S) and between population diversity (D_{ST}) components, where $H_T = H_S + D_{ST}$. Gene diversity between populations was expressed as relative to total population diversity or genetic differentiation index (F_{ST}), where $F_{ST} = D_{ST}/H_T$, according to Nei *et al.* (2000). Analyses of genetic diversity indices were performed using POPGENE version 3.2. (Yeh *et al*., 1999).

Table 3.4 Chromosomal locations, primer sequences, repeat motif and annealing temperature of six microsatellite primers.

Chromosomal location	Name	Primer sequences (5′→3′)	Repeat Motif	Annealing temperature (°C)
1	RM1	GCGAAAACACAATGCAAAAA GCGTTGGTTGGACCTGAC	(AG)26	55
2	RM48	TGTCCCACTGCTTTCAAGC CGAGAATGAGGGACAAATAACC	(GA)17	55
4	RM241	GAGCCAAATAAGATCGCTGA TGCAAGCAGCAGATTTAGTG	(CT)31	55
4	RM335	GTACACACCCACATCGAGAAG GCTCTATGCGAGTATCCATGG	(CTT)25	55
5	RM164	TCTTGCCCGTCACTGCAGATATCC GCAGCCCTAATGCTACAATTCTTC	(GT)16TT(GT)4	58
8	RM149	GCTGACCAACGAACCTAGGCCG GTTGGAAGCCTTTCCTCGTAACACG	(AT)10	55

Source: www.gramene.org

Experiment 3.2.2.2 Variation between and within seed lots of deep water rice

Seed characters

Fifteen seed lots of deep water rice variety recognized as Leung Yai were kept by different farmers at Nakorn Nayok, region problem for acid-sulphate soils in Thailand. Seed characters including hull color, pericarp color, seed length, seed width and seed weight were recorded. The differentiation within population in colors of hull and pericarp were analyzed by Shannon-Weaver index (H') as previously describe in Experiment 3.2.2.1. The variation of seed sizes was evaluated by standard deviation (SD) and coefficient of variance (CV).

Response to Al toxicity

Fifteen seed lots of Leung Yai were screened for Al tolerance with standard check, KDML105 (Al-sensitive). Five days after germination, 40 plants of each seed lot were transplanted to 10 L plastic container containing nutrient solution in a completely randomized design, with three replicates. There were separated pots by growing 20 plants each. The composition of nutrient solution was the same as previously described in Experiment 3.2.1.2. There were two Al levels; 0 and 30 mg L^{-1} [add as $Al_2(SO_4)_3.18H_20$]. The solution was renewed every week and pH value adjusted daily to 4.0 ± 0.05 with 1N HCl or 1N NaOH. Plants were harvested at 21 days after treatment. Maximum root and shoot length were measured in the same as previously described in Experiment 3.2.1.2. The variation of root and shoot length was analyzed by standard deviation (SD) and coefficient of variance (CV).

3.3 Results

3.3.1 Genotypic variation among rice varieties to soil acidity and Al toxicity

Experiment 3.3.1.1 Germination of upland rice in acid soil

Seed germination of all five upland rice varieties and check KDML105 was continuously increased and more than 80% at 7 days after germinated in soil pH 5.8. Percent germination of BP was the lowest at all times whereas the others were upper than 90% after 10 days. As decreasing soil pH to 3.5 by adding Al, percent of germination and survival seedling were depressed irrespective varieties. However, the germination was still increased until 10 days of BM and KDML105, and to 13 days of BG, BB, PA and BP. After that, the survival seedlings were declined, alive seedlings of BG and PA were highly depressed as compared with other varieties, by decreasing 40% after 13 days. At 19 days, survival seedlings of BB and BM were the highest (64 and 60%, respectively) whereas KDML105 and PA were the lowest (44 and 41%, respectively) (Figure 3.1).

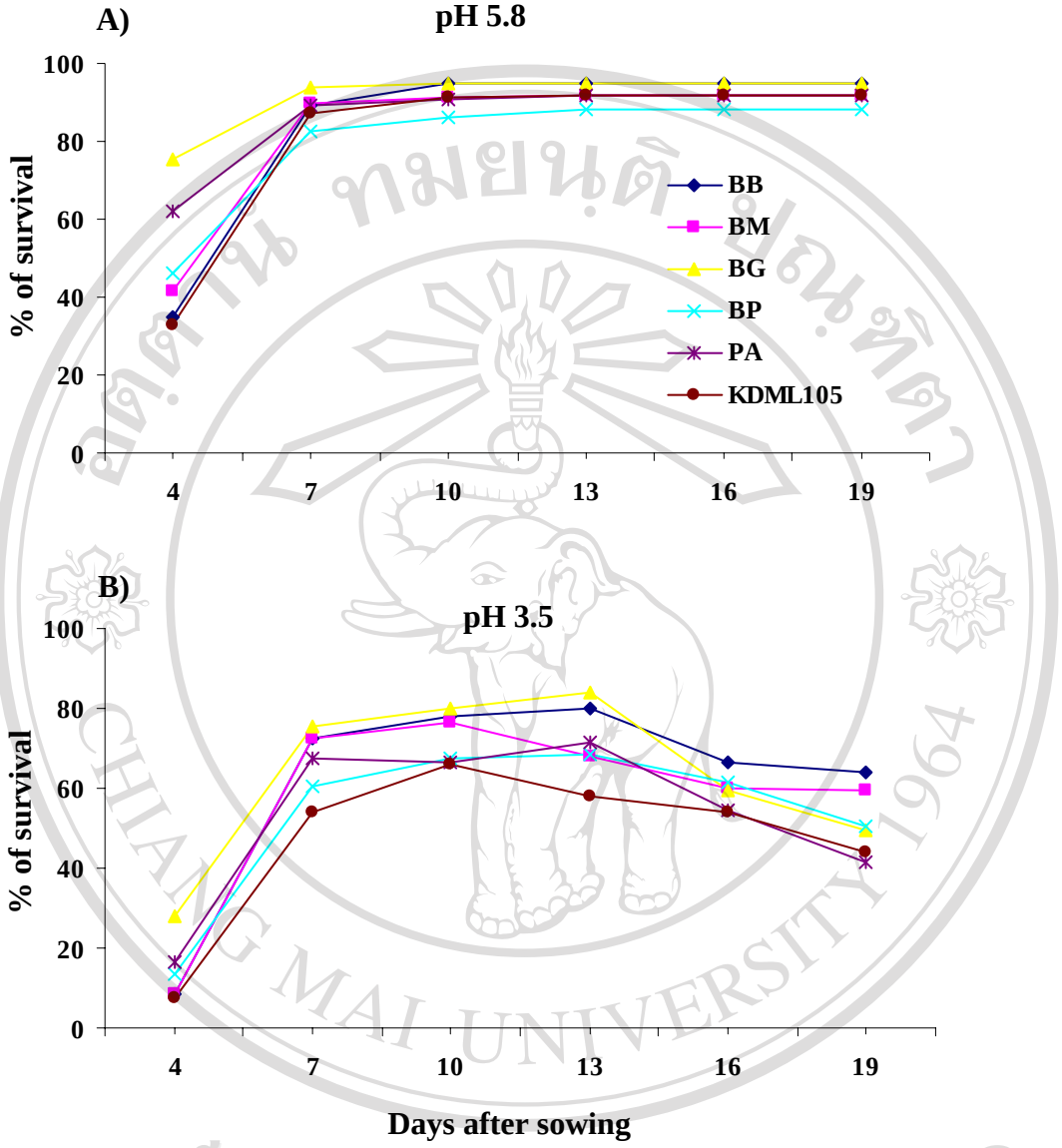


Figure 3.1 Percentage of survived seedlings when grown in soil with two pH levels; 5.8 (A) and 3.5 (B).

Experiment 3.3.1.2 Responses of rice in tolerance to Al toxicity in nutrient solution

Sub experiment 1 ***Variation among upland rice varieties***

Response to H^+ toxicity

Plant growth of rice varieties was inhibited differently with decreasing pH of nutrient solution from pH 7 to pH 4. Root length of KDML105 was shortened more at the lower pH than others, with a depression of 47% in KDML105 compared with 30% in BG and BM. Shoot length, root and shoot dry weights also inhibited the adverse effect of pH 4. Root and shoot dry weights were more severely inhibited at pH 4 than root length. The shoot and root dry weight at pH 4 were about half those at pH 7 in BB and BG, and only 30% in PA and 20% in KDML105 (Table 3.5). Relative root length by pH effect (root length at Al_0-pH4 as % of root length at Al_0-pH7) varied from 53% (KDML105) to 69% (BG). Even RRL of BG, BM, BB and PA was almost similar, relative values of root dry weight (RRW) and shoot dry weight (RSW) of BB and BG were both clearly higher than BM and PA (Table 3.6). However, the effect of pH on RRL correlated significantly with the effect on root length and root and shoot dry weight, but not with shoot length (Table 3.7).

Response to Al toxicity

The inhibition of plant growth by acidity was accentuated in the presence of Al. Root length inhibition at Al_{30} showed more differentiation among the varieties than that by just H^+. At Al_{30}, root length of PA and KDML105 was shortened more than the other varieties. Their root length at Al_{30}-pH4 depressed about 60% as compared with Al_0-pH4, and 80% as compared with Al_0-pH7. In contrast, root length

of BB and BM was the highest and several times higher than sensitive PA and KDML105 at Al_{30}. Their root length at Al_{30}-pH4 was inhibited about 30% as compared with Al_0-pH4, and 55% as compared with Al_0-pH7. By effect of Al, growths of all varieties at Al_{30}-pH4 were inhibited as compared with Al_0-pH4 except in root dry weight of BB and KDML105 that were not depressed at Al_{30}. However, root and shoot dry weights of BB were twice as much as KDML105 in Al_{30}-pH4 (Table 3.5). Relative root length by Al effect (root length at Al_{30}-pH4 as % of root length at Al_0-pH4) significantly correlated with RRL by pH + Al effect (root length at Al_{30}-pH4 as % of root length at Al_0-pH7; $r = 0.968$; $P < 0.001$) but no correlation with RRL by pH effect. In addition, RRL by Al effect and pH + Al effect was significantly correlated with parameters of root length, root and shoot dry weight but no correlation with shoot length (Table 3.7).

Five upland rice varieties could be classified in their Al tolerance into 3 groups based on their RRL by Al effect and pH + Al effect. The most tolerant were BB and BG, moderately tolerant were BP and BM, and sensitive was PA, which was about the same as check KDML105 (Table 3.6 and Figure 3.2).

Moreover, plant showed visible symptoms of Al toxicity in leaves and roots. The necrosis was found in older leaves, and roots were short, thick, turn brown and inefficient fine branching, particularly in root tips. The root symptom of Al sensitive varieties was more severe than in Al tolerant varieties (Figure 3.3).

Sub experiment 2 ***Comparing the responses of rice to Al made from different aluminum salts***

There was little difference between $Al_2(SO_4)_3.18H_2O$ and $AlCl_3$ on growth of KDML105. Root length of KDML105 that grown in Al_{10} made with $Al_2(SO_4)_3.18H_2O$ was slightly higher than in $AlCl_3$, but there was no difference between the Al forms at Al_{20}. Root length of KDML105 was decreased 15 - 20% at Al_{10} and more than 30% at Al_{20}. While shoot length, root and shoot dry weights were not inhibited at Al_{10}, and decreased 12%, 27% and 14% at Al_{20}, respectively (Table 3.8). The response in root length was positively correlated with shoot length ($r = 0.853$; $P < 0.001$), root dry weight ($r = 0.837$; $P < 0.001$) and shoot dry weight ($r = 0.762$; $P < 0.01$).

Sub experiment 3 ***Comparing the responses of rice to Al with and without air bubbling in nutrient solution***

Root and shoot growth of all varieties increased when grown in nutrient solution with oxygen supply by air bubbling in absence of Al. At Al_0, root length, root and shoot dry weights of all varieties in aerated were higher than without air bubbling about 58%, 33% and 48%, respectively. However, at Al_{30} growth of all varieties was unaffected by oxygen supply like in Al_0. At Al_{30}, root length was differently inhibited between varieties, BB was the highest which two and three times higher than CNT1 and KDML105, respectively (Table 3.9). BB was also two times higher than other varieties in root and shoot dry weight at Al_{30}. Although root dry weight of KDML105 was little inhibited by Al (15% depressed), it was 50% depressed in shoot dry weight at Al_{30} which was about the same as other improved

varieties (Table 3.10 and 3.11). The response in root length of all varieties was positively correlated with root dry weight ($r = 0.767$; $P < 0.001$) and shoot dry weight ($r = 0.890$; $P < 0.001$).

Sub experiment 4 ***Variation among improved Thai rice varieties***

Nine improved Thai rice varieties showed considerable differentiation in their tolerance to Al toxicity. Root length was differently inhibited among varieties at Al_{30}, by depressing 54% of SPR1 and up to 78% of CNT2 as compared with Al_0, whereas Al tolerant upland rice variety BB was depressed only 26%. The differential response of RRL could be classified 10 rice varieties into four groups; more than 50% (BB), 41-50% (SPR1 and AUT1), 31-40% (PGR2, PTT1, SPR2 and KDML105) and less than 30% (CNT80, CNT1 and CNT2) (Table 3.12). To considerate all 10 varieties together, RRL had significant correlation with parameters of root and shoot length and root and shoot dry weight at Al_{30}.

Table 3.5 Root and shoot growth of six rice varieties at three treatments in nutrient solution at 14 days after treatments.

Variety	Root length (cm)			Shoot length (cm)			Root dry weight (mg plant^{-1})			Shoot dry weight (mg plant^{-1})		
	Al_0-pH7	Al_0-pH4	Al_{30}-pH4	Al_0-pH7	Al_0-pH4	Al_{30}-pH4	Al_0-pH7	Al_0-pH4	Al_{30}-pH4	Al_0-pH7	Al_0-pH4	Al_{30}-pH4
BB	25.0	15.4	11.3	42.8	31.1	22.8	19.2	8.2	7.7	71.6	31.7	18.9
	aA	bA	cA	aAB	bA	cB	aBC	bAB	bA	aC	bA	cA
BG	25.9	17.9	11.4	45.0	34.1	26.0	21.9	9.0	7.3	76.9	35.1	19.1
	aA	bA	cA	aAB	bA	cA	aB	bA	cAB	aBC	bA	cA
BM	25.4	17.2	9.0	44.8	31.0	26.3	24.3	7.4	5.7	93.1	29.2	18.3
	aA	bA	cB	aAB	bA	cA	aAB	bAB	cB	aAB	bA	cA
BP	24.0	14.3	8.8	41.5	27.6	23.1	18.5	7.0	4.5	78.0	22.1	15.5
	aA	bB	cB	aB	bB	cB	aC	bB	cC	aBC	bB	cAB
PA	24.1	15.1	5.5	47.8	32.5	28.5	28.7	7.8	5.1	101.2	31.7	13.5
	aA	bAB	cC	aA	bA	cA	aA	bAB	cC	aA	bA	cB
KDML105	18.8	10.0	3.8	46.8	24.8	22.1	16.0	3.5	3.3	70.6	15.3	10.7
	aB	bC	cD	aA	bB	cB	aC	bC	bD	aC	bC	cC
V x T	*			*			*			*		

Data were transformed for statistical analysis by log10.

* Significant at $P < 0.05$. V x T indicated F-test for variety and treatment interaction effects. The difference between varieties in the same column is indicated by upper case letters. The difference between treatments in the same row is indicated by lower case letters.

Table 3.6 Relative values of root and shoot growth of six rice varieties that grown in Al_0-pH4 compared with Al_0-pH7 (pH effect), Al_{30}-pH4 compared with Al_0-pH4 (Al effect) and Al_{30}-pH4 compared with Al_0-pH7 (pH + Al effect).

Variety	RRL (%)			RSL (%)			RRW (%)			RSW (%)		
	pH effect	Al effect	pH + Al effect	pH effect	Al effect	pH + Al effect	pH effect	Al effect	pH + Al effect	pH effect	Al effect	pH + Al effect
BB	61.5	73.6	45.2	72.6	73.4	53.3	43.0	93.9	40.4	44.2	59.7	59.7
	AB	A	A	AB	C	AB	A	A	A	A	B	B
BG	69.2	63.7	44.1	75.7	76.2	57.8	41.2	81.2	33.4	45.7	54.3	54.3
	A	AB	A	A	BC	A	A	AB	B	A	B	B
BM	67.8	52.4	35.5	69.1	84.9	58.7	30.4	77.8	23.6	31.4	62.8	62.8
	A	C	B	AB	AB	A	BC	BC	C	B	AB	AB
BP	59.7	61.1	36.5	66.5	83.6	55.6	37.9	64.8	24.5	28.3	70.3	70.3
	AB	BC	B	B	AB	A	AB	C	C	B	A	A
PA	62.4	36.5	22.8	68.0	87.7	59.6	27.1	65.7	17.8	31.3	42.5	42.5
	AB	D	C	B	A	A	C	C	D	B	C	C
KDML105	53.2	37.9	20.2	53.0	89.1	47.2	22.1	93.4	20.7	21.6	69.9	69.9
	B	D	C	C	A	B	C	A	CD	B	A	A
V	*	*	*	*	*	*	*	*	*	*	*	*
$LSD_{0.05}$	10.5	10.3	6.4	6.9	9.4	6.5	8.9	13.6	5.0	9.83	9.07	9.07

* Significant at $P < 0.05$. V indicated F-test for variety. The difference between varieties in the same column is indicated by upper case letters.

Table 3.7 Correlation coefficients between relative root length (RRL) with root and shoot lengths, and plant dry weights by pH effect, Al effect and pH + Al effect.

Character	RRL		
	pH effect	Al effect	pH + Al effect
Root length	0.495*	0.992***	0.923***
Shoot length	0.448^{ns}	-0.010^{ns}	-0.174^{ns}
Root dry weight	0.553*	0.809***	0.720***
Shoot dry weight	0.481*	0.889***	0.789***
Total dry weight	0.605**	0.900***	0.785***

^{ns}, *, ** and *** non significant at $P < 0.05$, significant at $P < 0.05$, 0.01 and 0.001, respectively.

Figure 3.2 Root growth of six rice varieties at 14 days after treatments that grown in Al_0 (left) and Al_{30} (right) in nutrient solution with pH4.

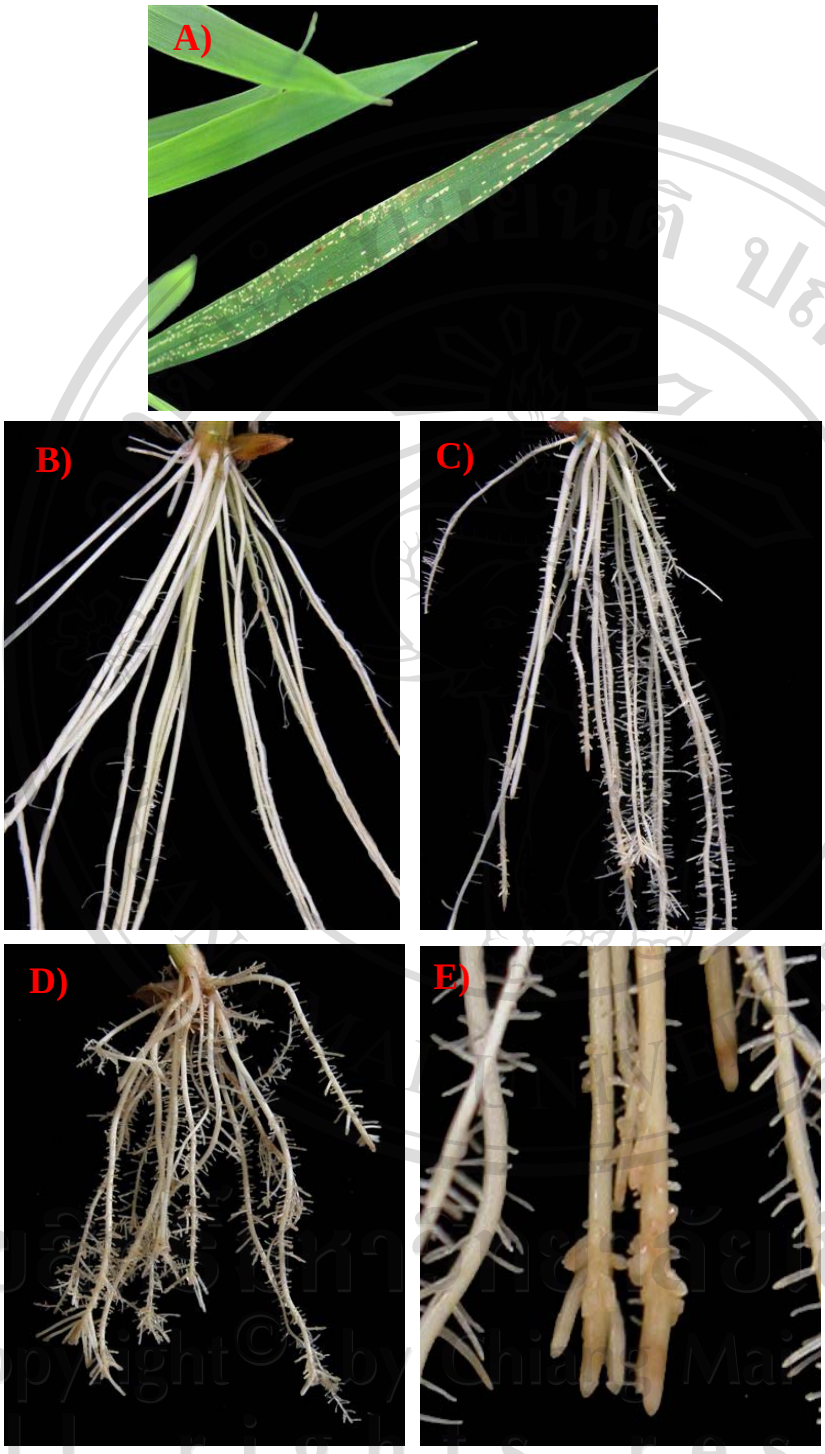


Figure 3.3 The symptoms of Al toxicity in rice; A) leaf symptom at Al_{30}, B) root growth at Al_0, C) root growth of Al-tolerant variety at Al_{30}, D) and E) root growth of Al-sensitive variety at Al_{30}.

Table 3.8 Root length, shoot length, root dry weight and shoot dry weight of KDML105 growing in 2 Al-form in nutrient solution at 21 days after treatments.

Al level	Al-form	Length (cm)		Dry weight (mg plant^{-1})	
		Root	Shoot	Root	Shoot
0	-	14.4 A	35.7 A	15.3 A	71.8 A
10	$Al_2(SO_4)_3.18H_2O$	12.2 B	35.3 A	14.8 A	72.6 A
	$AlCl_3$	11.1 C	34.8 A	14.6 A	69.0 AB
20	$Al_2(SO_4)_2.18H_2O$	9.0 D	30.8 B	10.6 B	59.8 C
	$AlCl_3$	9.8 D	31.3 B	11.3 B	61.8 BC
F-test		T*	T*	T*	T*
$LSD_{0.05}$		0.84	1.41	2.05	9.04

* Significant at $P < 0.05$. T indicated F-test for treatment. The difference between treatments in the same column is indicated by upper case letters.

Table 3.9 Root length (cm) of five varieties growing in nutrient solution without and with oxygen supply by air bubbling (B0 and B+) in Al_0 and Al_{30} at 21 days after treatment.

Air bubbling	Al level	Variety					Mean
		BB	SPR1	CNT1	KDML105	PA	
B0	Al_0	19.4	16.0	15.9	12.0	12.3	15.1 b
	Al_{30}	12.6	7.2	4.6	3.2	6.9	6.9 c
B+	Al_0	28.3	24.8	23.6	20.5	21.9	23.8 a
	Al_{30}	12.0	10.9	5.3	4.4	7.7	8.1 c
B0		16.0	11.6	10.3	7.6	9.6	11.0 b
B+		20.2	17.9	14.4	12.5	14.8	15.9 a
	Al_0	23.8 aA	20.4 aB	19.7 aB	16.3 aC	17.1 aC	19.5 a
	Al_{30}	12.3 bA	9.0 bB	4.9 bC	3.8 bC	7.3 bB	7.5 b
Mean		18.1 A	14.7 B	12.3 C	10.0 D	12.2 C	13.5
F-test	V*	Al*	B*	VxAl*	VxB^{ns}	AlxB*	$VxAlxB^{ns}$
$LSD_{0.05}$	1.4	0.9	0.9	2.0	-	1.3	-

ns and * non significant and significant at $P < 0.05$. V, Al and B indicated F-test for variety, Al level and air bubbling in nutrient condition. The difference between varieties in the same row is indicated by upper case letters. The difference between Al levels or air bubbling in the same column is indicated by lower case letters.

Table 3.10 Root dry weight (mg plant^{-1})of five varieties growing in nutrient solution without and with oxygen supply by air bubbling (B0 and B+) in Al_0 and Al_{30} at 21 days after treatment.

Air bubbling	Al level	Variety					Mean
		BB	SPR1	CNT1	KDML105	PA	
B0	Al_0	28.6	15.6	15.9	12.7	14.4	17.4 b
	Al_{30}	22.9	11.3	11.7	12.0	11.9	14.0 c
B+	Al_0	36.3	23.1	21.6	14.8	19.7	23.1 a
	Al_{30}	22.2	10.5	12.2	11.6	10.3	13.3 c
B0		25.7	13.5	13.8	12.3	13.2	15.7 b
B+		29.2	16.8	16.9	13.2	15.0	18.2 a
	Al_0	32.4 aA	19.4 aB	18.8 aB	13.7 aC	17.1 aB	20.3 a
	Al_{30}	22.5 bA	10.9 bB	11.9 bB	11.8 bB	11.1 bB	13.6 b
Mean		27.5 A	15.1 B	15.4 B	12.8 C	14.1 BC	17.0
F-test	V*	Al*	B*	VxAl*	VxB^{ns}	AlxB*	VxAlxB^{ns}
$LSD_{0.05}$	2.1	1.3	1.3	2.9	-	1.9	-

ns and * non significant and significant at $P < 0.05$. V, Al and B indicated F-test for variety, Al level and air bubbling in nutrient condition. The difference between varieties in the same row is indicated by upper case letters. The difference between Al levels or air bubbling in the same column is indicated by lower case letters.

Table 3.11 Shoot dry weight (mg plant^{-1}) of five varieties growing in nutrient solution without and with oxygen supply by air bubbling (B0 and B+) in Al_0 and Al_{30} at 21 days after treatment.

Air bubbling	Al level	Variety					Mean
		BB	SPR1	CNT1	KDML105	PA	
B0	Al_0	90.4	58.8	60.3	58.7	42.9	62.2 b
	Al_{30}	71.0	30.8	35.7	36.9	30.7	41.0 c
B+	Al_0	133.5	99.7	82.5	70.5	75.7	92.4 a
	Al_{30}	71.2	34.0	38.3	36.3	30.7	42.1 c
B0		80.7	44.8	48.0	47.8	36.8	51.6 b
B+		102.3	66.9	60.4	53.4	53.2	67.2 a
	Al_0	111.9 aA	79.3 aB	71.4 aBC	64.6 aCD	59.3 aD	77.3 a
	Al_{30}	71.1 bA	32.4 bB	37.0 bB	36.6 bB	30.7 bB	41.6 b
Mean		91.5 A	55.8 B	54.2 B	50.6 BC	45.0 C	59.4
F-test	V*	Al*	B*	VxAl*	VxB^{ns}	AlxB*	VxAlxB^{ns}
$LSD_{0.05}$	6.88	4.35	4.35	9.73	-	6.15	-

ns and * non significant and significant at $P < 0.05$. V, Al and B indicated F-test for variety, Al level and air bubbling in nutrient condition. The difference between varieties in the same row is indicated by upper case letters. The difference between Al levels or air bubbling in the same column is indicated by lower case letters.

Table 3.12 Root length and relative root length (RRL) of 10 rice varieties to Al toxicity in nutrient solution at 21 days after treatments.

Variety	Root length (cm)		RRL
	Al_0	Al_{30}	
BB	28.2	20.9	74.0 a
SPR1	17.9	8.2	45.7 b
AUT1	16.1	7.0	43.4 bc
PGR2	17.7	6.9	38.8 cd
PTT1	16.7	6.4	38.1 cd
SPR2	16.4	6.0	36.2 d
KDML105	17.5	5.8	33.0 de
CNT80	17.0	4.9	28.8 ef
CNT1	20.5	4.8	23.6 fg
CNT2	15.2	3.3	21.9 g
F-test	VxAl*		V*
$LSD_{0.05}$	1.77		5.9

* Significant at $P < 0.05$. V x Al indicated F-test for variety and Al level interaction effect. V indicated F-test for variety by RRL. The difference between varieties in the same column is indicated by lower case letters.

3.3.2 Genotypic variation within local rice as recognized as the same variety name

Experiment 3.3.2.1 Variation between and within seed lots of upland rice varieties

Morphology

The morphological characters were determined within and between seed lot of local rice variety that recognized as the same names of BB, BM and PA. From 12 characters observed, only three characters including plant type, awning and pericarp color were found to vary within seed lots of these varieties. The variation in plant type (erect and open) was observed in both seed lots of BB (H' = 0.279 and 0.168 of BB1 and BB2, respectively) and BM1 (H' = 0.551) but not in BM2, PA1 and PA2. The variation of awning was found in BM1 (H' = 0.163) and PA1 (H' = 0.500). In addition, pericarp color of BB2 was observed in white and red pericarp (H' = 0.423), and PA2 had light brown and red pericarp (H' = 0.394) (Table 3.13).

Tolerance to Al toxicity

Comparing between seed lots

Average root length of 20 individual lines of each seed lot showed that seed lots of BB were less inhibited at Al_{30} by depressing 29% and 35% of BB1 and BB2, respectively. While two seed lots of each BM and PA were similarly inhibited and depressed by more than half at Al_{30} (Table 3.14). For standard checks, Koshihikari (Al-tolerant) and KDML105 (Al-sensitive) produced root length at 8.3 ± 0.3 and 7.1 ± 0.2 cm in Al_{30}, respectively, that became the shortest as compared with the local upland rice varieties at Al_{30} (Table 3.14).

Variation within seed lots

The variation in tolerance to Al was also found among 20 individual lines within each seed lot. Relative root length at Al_{30} compared with Al_0 could identify variation within seed lot and between seed lots recognized as the same variety. Relative root length among 20 lines of BB1 was distributed from 50-80%, more than three quarter lines were higher than 70% whereas BB2 varied from 57-75%, three quarter were ranged in 60-70%. On the other hand, variation in RRL within seed lots of BM1 and BM2 were not much different, more than half of each seed lot were ranged from 40-50%. However, there was slightly difference in two seed lots of PA. Variation among progeny lines of PA1 was larger than that in PA2, ranged from 37-54% and 36-44%, respectively. Fourteen lines of PA1 were grouped in 40-50% whereas more than half of PA2 were less than 40% (Table 3.15).

For standard checks, RRL of Al tolerant Koshihikari was 19% higher than Al sensitive KDML105. More than three quarter lines within seed lots of BB1 and BB2 were higher RRL than Koshihikari, and all of them were higher than KDML105. Almost all lines of BM had lower RRL than Koshihikari, and only six and nine lines of BM1 and BM2 were higher than KDML105, respectively. However, almost all lines of PA1 and PA2 had lower RRL than KDML105 (Table 3.15).

Genetic variation detected by microsatellite markers

As local upland rice varieties have been found to be genetically diverse in morphological characters, this study evaluated genetic variation that detected by microsatellite markers. Six microsatellite loci were used for evaluating 20 sub-populations of each 2 populations of BB and BM. The largest number of alleles was

detected for RM48 (11), followed by RM335 (8) and the lowest for RM164 (2) (Table 3.16). The sample of polymorphic bands within and between populations of BB was shown in Figure 3.4.

Gene diversity index (H_e) was determined for all populations of each locus (Table 3.17). Gene diversity index of BB1 and BB2 was expressed across all loci at 0.33 and 0.37, respectively, and those of BM1 and BM2 were 0.37 and 0.23, respectively.

For each locus, genetic variation within population (H_S) of BB was observed in range of 0.09 to 0.60 and total genetic variation (H_T) ranged from 0.37 to 0.76. The highest and lowest H_S were observed when detected by RM48 (0.60) and RM241 (0.09), respectively. For H_T, the highest and lowest values were observed when detected by RM48 (0.76) and RM1 (0.37), respectively. In BM population, genetic variation within population was the highest when detected by RM241 (H_S = 0.49) but no variation when detected by RM164 (H_S = 0). The highest total genetic variation of BM was observed by RM48 (H_T = 0.60) that the same as in BB population, and followed by RM241 (H_T = 0.52) (Table 3.18).

The overall distribution of variability showed that there was genetic differentiation within and between populations that detected by different loci. In BB population, both variation within population (mean H_S = 0.35) and between population (mean D_{ST} = 0.22) were found with made mean total genetic variation (H_T) = 0.57. The genetic differentiation (F_{ST}) between BB populations was 0.39. It suggested that approximately 60% of the genetic variation in BB was found within individual populations, genetic differentiation between populations accounted for less than half. In the similar way of BM population, genetic variation within and between

populations were average 0.34 and 0.05 which was made to 0.39 of total genetic variation. The genetic differentiation (F_{ST}) between BM populations was 0.14. Therefore, genetic differentiation within individual populations of BM was up to 85% and the remainder was found between populations (Table 3.18).

The differentiation between seed lots of the same variety name BM was only 14% whereas those of BB1 and BB2 were higher to 40% (Table 3.9). The genetic of BB1 was near to BM1 (23%) and BM2 (24%) than to BB2. It was suggested that BB1 and BB2 should be classified to the different genotype that detected by DNA analysis (Figure 3.5).

Table 3.13 Morphological characters and genetic variation between and within seed lot as recognized as the same variety names of BB, BM and PA that obtained 2 seed lots of each variety.

Character	Seed lots of upland rice variety					
	BB1	BB2	BM1	BM2	PA1	PA2
Blade color	green	green	green	green	green	green
	0*	0	0	0	0	0
Sheath color	green	green	green	green	green	green
	0	0	0	0	0	0
Auricle color	white	white	white	white	white	white
	0	0	0	0	0	0
Ligule color	white	white	white	white	white	white
	0	0	0	0	0	0
Ligule shape	2 - cleft	2 - cleft	2 - cleft	2 - cleft	2 - cleft	2 - cleft
	0	0	0	0	0	0
Plant type	erect, open	erect, open	erect	erect, open	erect	erect
	0.279	0.168	0	0.551	0	0
Node color	green	green	green	green	green	green
	0	0	0	0	0	0
Internode color	green	green	green	green	green	green
	0	0	0	0	0	0
Apiculus color	white	white	white	white	white	white
	0	0	0	0	0	0
Awning	absent	absent	absent, short awn	absent	absent, short awn	absent
	0	0	0.163	0	0.500	0
Hull color	straw	straw	straw	straw	straw	straw
	0	0	0	0	0	0
Pericarp color	white	white, red	white	white	white	light brown, red
	0	0.423	0	0	0	0.394

* = value of Shannon's Index (H')

Table 3.14 Root length of each two seed lots (obtained 20 progeny lines each) recognized as the same variety names of BB, BM and PA with 2 Al levels, 0 and 30 mg Al L^{-1}, in nutrient solution for 21 days.

Lines	Root length (cm)											
	BB1		BB2		BM1		BM2		PA1		PA2	
	Al_0	Al_{30}	Al_0	Al_{30}	Al_0	Al_{30}	Al_0	Al_{30}	Al_0	Al_{30}	Al_0	Al_{30}
1	25.7	20.7	21.9	16.4	23.1	14.2	25.2	13.9	20.1	10.8	27.1	11.9
2	25.8	20.6	24.1	15.4	25.5	13.4	26.8	13.4	20.5	10.6	26.1	10.8
3	27.5	20.1	26.0	14.9	27.9	12.7	25.4	13.3	21.5	10.4	24.8	10.8
4	27.3	19.8	20.1	14.7	28.0	12.3	23.8	13.2	22.7	10.4	24.4	10.7
5	24.9	19.7	21.3	14.5	20.6	12.2	26.3	13.2	20.5	10.4	26.1	10.7
6	26.0	19.6	22.3	14.5	26.6	12.1	25.8	13.0	21.3	9.9	27.2	10.7
7	26.4	19.5	22.2	14.4	25.4	12.1	25.6	12.8	23.5	9.4	27.5	10.6
8	25.4	19.2	23.2	14.2	25.8	12.0	22.5	12.2	20.4	9.4	28.3	10.6
9	25.7	19.1	21.6	14.1	22.8	12.0	28.2	11.9	21.2	9.3	27.1	10.6
10	24.8	18.9	22.2	14.0	25.7	11.9	27.1	11.8	21.9	9.1	25.0	10.5
11	26.0	18.7	22.3	14.0	23.6	11.8	26.0	11.7	22.8	9.0	26.6	10.4
12	25.3	18.7	21.7	13.9	23.5	11.5	24.8	11.6	20.0	9.0	26.0	10.4
13	26.3	18.5	19.6	13.8	23.9	11.3	22.9	11.4	21.8	8.9	26.8	10.3
14	25.1	18.3	22.2	13.7	26.8	11.3	24.8	11.1	19.5	8.9	26.5	10.3
15	23.9	18.1	19.3	13.6	24.5	11.1	24.3	10.8	21.6	8.8	22.8	10.1
16	23.3	17.4	20.7	13.6	21.2	11.0	24.6	10.8	23.6	8.6	25.0	10.0
17	26.2	15.3	20.7	13.4	25.7	10.9	24.8	10.5	20.4	8.6	26.0	9.8
18	25.6	13.9	21.5	13.0	25.0	10.9	21.8	10.5	21.7	8.3	23.0	9.7
19	22.7	13.5	20.3	12.7	19.8	8.6	23.0	10.1	18.4	8.1	27.0	9.6
20	24.4	12.3	20.7	12.4	20.4	8.3	23.7	9.8	18.0	7.4	26.8	9.5
mean	25.4	18.1	21.7	14.1	24.3	11.6	24.9	11.8	21.1	9.3	26.0	10.4
L x Al	*		*		*		*		ns		*	
$LSD_{0.05}$	1.975		1.852		2.101		2.234		-		1.948	

ns and * non significant and significant at $P < 0.05$, respectively. L x Al indicated F-test for progeny line and Al treatment interaction effect.

Table 3.15 Relative root length of each two seed lots (obtained 20 progeny lines each) recognized as the same variety names of BB, BM and PA in response to Al toxicity.

RRL	Seed lots of upland rice varieties					
	BB1	BB2	BM1	BM2	PA1	PA2
> 70	16	4	0	0	0	0
60 – 70	0	15	1	0	0	0
50 – 60	4	1	5	8	3	0
40 – 50	0	0	14	12	14	9
< 40	0	0	0	0	3	11
Max	80.8	74.9	61.3	55.3	53.7	44.1
Min	50.6	57.2	40.7	41.1	36.6	35.5
Mean	71.1	65.0	47.8	47.7	44.1	40.0
SD	8.5	4.6	5.4	4.4	4.6	2.6
CV (%)	11.9	7.0	11.4	9.3	10.4	6.5
Mean ± SE of standard checks						
Koshihikari (Al tolerant)				60.4 ± 0.5		
KDML105 (Al sensitive)				49.2 ± 1.4		

Table 3.16 Allele frequencies of two upland rice varieties.

Locus	Population			
	BB1	BB2	BM1	BM2
RM1				
Allele A	1	0.55	0.65	0.95
Allele B	0	0.30	0.35	0
Allele C	0	0.10	0	0
Allele D	0	0.05	0	0
Allele E	0	0	0	0.05
RM48				
Allele A	0.15	0	0	0
Allele B	0.35	0	0	0
Allele C	0.20	0.05	0	0
Allele D	0.10	0.75	0.25	0
Allele E	0.10	0	0	0
Allele F	0.10	0.05	0.05	0
Allele G	0	0	0.50	0.05
Allele H	0	0	0.20	0.90
Allele I	0	0.10	0	0
Allele J	0	0	0	0.05
Allele K	0	0.05	0	0
RM149				
Allele A	0.05	0	0	0
Allele B	0.45	0.90	0.70	0.75
Allele C	0.30	0.05	0	0.05
Allele D	0.20	0	0.20	0.20
Allele E	0	0.05	0.05	0
Allele F	0	0	0.05	0
RM164				
Allele A	0.95	0.1	1	1
Allele B	0.05	0.9	0	0
RM241				
Allele A	0	0	0.10	0.10
Allele B	0	0.05	0.15	0.05
Allele C	1	0.05	0.55	0.80
Allele D	0	0	0.15	0
Allele E	0	0.90	0.05	0
Allele F	0	0	0	0.05
RM335				
Allele A	0.75	0	0	0.15
Allele B	0.05	0.05	0	0
Allele C	0.05	0	0	0
Allele D	0.15	0.1	1	0.80
Allele E	0	0.55	0	0
Allele F	0	0.10	0	0
Allele G	0	0.20	0	0
Allele H	0	0	0	0.05

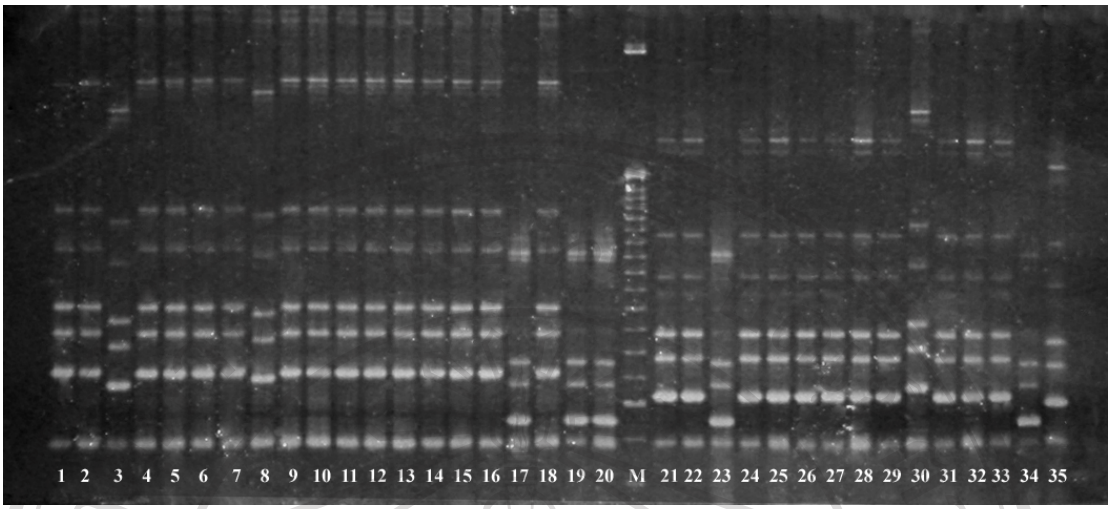


Figure 3.4 Microsatellite amplification products of BB population that detected by primer RM335. *Lane 1-20 population of BB1, M 25 bp ladder, Lane 21-35 population of BB2.*

Table 3.17 Gene diversity (H_e) of BB and BM populations based on six microsatellite loci.

Locus	Population			
	BB1	BB2	BM1	BM2
RM1	0	0.595	0.455	0.095
RM48	0.785	0.420	0.645	0.185
RM149	0.665	0.185	0.465	0.395
RM164	0.095	0.180	0	0
RM241	0	0.185	0.640	0.345
RM335	0.410	0.635	0	0.335
Mean	0.326	0.367	0.368	0.226
(sd)	(0.346)	(0.213)	(0.296)	(0.158)

H_e = gene diversity index according to Nei (1973)

Table 3.18 Partition of genetic diversity within and between population of upland rice BB and BM based on six microsatellite loci.

Locus	H_T		H_S		D_{ST}		F_{ST}	
	BB	BM	BB	BM	BB	BM	BB	BM
RM1	0.374	0.329	0.298	0.275	0.076	0.054	0.204	0.164
RM48	0.756	0.605	0.603	0.415	0.154	0.190	0.203	0.314
RM149	0.503	0.433	0.425	0.430	0.077	0.002	0.154	0.006
RM164	0.499	0	0.138	0	0.361	0	0.724	0
RM241	0.521	0.518	0.093	0.493	0.429	0.025	0.823	0.048
RM335	0.753	0.184	0.523	0.168	0.230	0.016	0.306	0.088
Mean	0.568	0.345	0.346	0.297	0.221	0.048	0.390	0.139
Total mean	**0.541**		**0.322**		**0.219**		**0.405**	

H_S = gene diversity within population, D_{ST} = gene diversity between population, H_T = total gene diversity, F_{ST} = genetic differentiation among population.

Table 3.19 Genetic differentiation among population (F_{ST}) matrix values determined across six microsatellite loci of local upland rice BB and BM.

Population	BB1	BB2	BM1	BM2
BB1				
BB2	0.390			
BM1	0.229	0.336		
BM2	0.236	0.441	0.139	

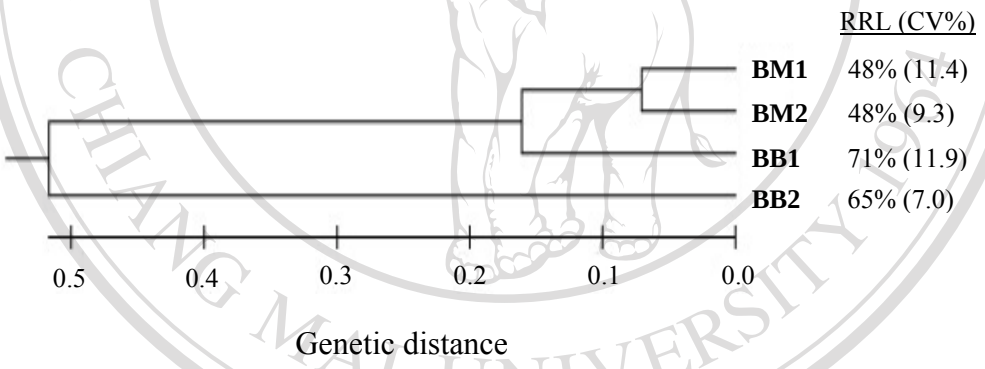


Figure 3.5 Genetic distance among population determined across six microsatellite loci of local upland rice BB and BM by UPGMA methods using the MEGA2 program.

Experiment 3.3.2.2 Variation between and within seed lots of deep water rice "Leung Yai"

Seed characters

Hull color and Pericarp color

There was no differentiation in hull color in both within and between 15 seed lots of Leung Yai that kept by different farmers while genetic differentiation was found in pericarp color. Few of red pericarp were mixed in others white pericarp in seed lot number 1, 3 and 4 (H' = 0.056, 0.098 and 0.035, respectively) whereas others seed lots were similarly in white pericarp (Table 3.20).

Seed shape: length, width and weight

Seed length of all 15 seed lots distributed from 8.1 – 11.3 mm, average grain length of seed lot 11 was the lowest (9.47 mm) and seed lot 13 was the highest (9.87 mm). Coefficient of variation in seed length of each seed lot varied from 3.7-6.5, seed lot 11 was the lowest and seed lot 6 was the highest (Table 3.21).

Seed width of all 15 seed lots distributed from 2.0-3.0 mm, average seed width among seed lots was not much different, seed lot 3 was the lowest (2.48 mm) and seed lot 5 was the highest (2.63 mm). Coefficient of variation in seed width of each seed lot varied from 4.4-6.7, seed lot 15 was the lowest and seed lot 2 was the highest (Table 3.21).

Seed weight of all 15 seed lots distributed from 15-35 mg seed^{-1}, average seed weight among seed lots was not much different, seed lot 15 was the lowest (24.6 mg) and seed lot 7 was the highest (26.9 mg). Coefficient of variation in seed weight of

each seed lot varied from 8.7-12.9, seed lot 4 was the lowest and seed lot 8 was the highest (Table 3.21).

Response to Al toxicity

There was differential response in root growth in both within and between seed lots of Leung Yai that kept by different farmers. At Al_0, root length of all 15 seed lots was distributed from 10.5-25.5 cm, average root length was the highest in seed lot 3 (20.5 cm) and the lowest in seed lot 7 (16.2 cm). Coefficient of variation in root length of each seed lot varied from 9.0-16.6, seed lot 3 was the lowest and seed lot 11 was the highest (Table 3.22).

In addition, the differential root length between and within seed lots was accentuated in presence of Al (Figure 3.6A). At Al_{30}, root length of all 15 seed lots was distributed from 2.5-18.8 cm, average root length of seed lot 2 was depressed about 50% and severely to 70% of seed lot 6 as compared with Al_0. Some seed lots suggested that, the differential root length was more variation between individual plants within seed lot than that found in average between seed lots. Coefficient of variation in root length at Al_{30} of each seed lot varied from 19.3-33.6, seed lot 15 was the lowest (Figure 3.6B) and seed lot 12 was the highest (Figure 3.6C). For standard check KDML105, its root length was depressed about 60% at Al_{30} compared with Al_0, and it was quite low as compared with 15 seed lots of Leung Yai at Al_{30} (Table 3.22).

Table 3.20 Seed characters and Shannon's Index of 15 seed lots of a variety rcognized as Leung Yai that kept by different farmers.

Seed lots	Husk color*		Pericarp color*		
	Straw	H'	White	Red	H'
1	100	0	99	1	0.056
2	100	0	100	0	0
3	100	0	98	2	0.098
4	100	0	97	3	0.135
5	100	0	100	0	0
6	100	0	100	0	0
7	100	0	100	0	0
8	100	0	100	0	0
9	100	0	100	0	0
10	100	0	100	0	0
11	100	0	100	0	0
12	100	0	100	0	0
13	100	0	100	0	0
14	100	0	100	0	0
15	100	0	100	0	0

* = 100 samples observation per seed lot.

Table 3.21 Grain length between and within seed lots recognized as the same variety name as Leung Yai collected from 15 farmers' seed lots.

Seed lots	Grain length (mm)*				Grain width (mm)*				Grain weight (mg grain^{-1})*			
	Range	Mean	SD	CV (%)	Range	Mean	SD	CV (%)	Range	Mean	SD	CV (%)
1	10.9-8.8	9.73	0.49	5.07	3.0-2.3	2.57	0.16	6.27	31-18	26.03	2.69	10.3
2	10.6-8.4	9.71	0.48	4.91	2.9-2.0	2.51	0.17	6.72	32-15	25.02	2.80	11.2
3	10.8-8.1	9.71	0.60	6.22	2.8-2.1	2.48	0.14	5.66	33-19	25.98	2.80	10.8
4	11.0-8.3	9.81	0.55	5.63	2.9-2.2	2.55	0.16	6.16	34-22	26.52	2.31	8.7
5	10.5-8.5	9.66	0.44	4.51	3.0-2.3	2.63	0.15	5.53	31-18	25.92	2.65	10.2
6	11.0-8.3	9.52	0.62	6.50	2.9-2.3	2.60	0.15	5.88	33-19	26.76	3.04	11.4
7	10.5-8.2	9.61	0.50	5.21	2.9-2.3	2.59	0.13	5.16	34-17	26.85	3.20	11.9
8	10.7-8.5	9.73	0.44	4.54	2.8-2.2	2.56	0.15	5.74	34-17	25.98	3.36	12.9
9	10.8-8.2	9.63	0.56	5.80	2.8-2.2	2.50	0.14	5.45	33-19	25.53	2.51	9.8
10	10.9-8.8	9.76	0.46	4.73	2.9-2.3	2.54	0.16	6.26	34-17	26.07	2.98	11.4
11	10.3-8.8	9.47	0.35	3.70	2.9-2.2	2.58	0.15	5.77	32-18	24.72	2.64	10.7
12	10.4-8.9	9.65	0.37	3.84	2.9-2.2	2.55	0.13	4.94	32-16	25.63	2.76	10.8
13	11.1-8.7	9.87	0.52	5.30	2.8-2.3	2.54	0.14	5.63	35-16	25.50	3.21	12.6
14	10.7-8.4	9.63	0.51	5.25	2.7-2.2	2.49	0.11	4.48	31-18	25.47	2.62	10.3
15	11.3-8.4	9.55	0.55	5.80	2.7-2.3	2.53	0.11	4.42	32-16	24.61	2.85	11.6

* = 100 seed samples per seed lot.

Table 3.22 Root length of 15 seed lots of deep water rice "Leung Yai" that grown in nutrient solution with 2 Al levels, 0 and 30 mg L^{-1} at 21 days after treatments.

Seed lot	Al_0					Al_{30}				
	Plant number	Range	Mean	SD	CV (%)	Plant number	Range	Mean	SD	CV (%)
1	38	22.5 - 14.5	18.1	2.2	12.0	40	13.0 - 4.0	7.8	2.0	26.3
2	39	24.5 - 14.5	19.5	2.3	11.8	40	15.5 - 6.0	10.1	2.2	21.3
3	40	25.5 - 17.5	20.5	1.9	9.0	40	12.0 - 5.0	8.4	2.0	23.5
4	37	25.5 - 14.5	19.8	2.7	13.8	39	12.5 - 5.5	7.6	1.6	21.0
5	35	23.5 - 15.5	18.4	2.0	11.1	34	12.0 - 5.0	7.0	1.5	21.2
6	17	24.0 - 14.5	18.0	2.6	14.2	12	11.0 - 4.5	5.2	1.4	26.7
7	28	20.0 - 10.5	16.2	2.4	14.6	30	10.0 - 2.5	6.0	1.3	21.1
8	37	22.5 - 12.5	18.0	2.4	13.2	37	12.5 - 5.5	6.2	1.7	26.5
9	39	23.0 - 15.0	19.3	2.0	10.4	39	18.0 - 4.5	8.6	1.7	20.2
10	37	23.5 - 14.0	19.3	2.4	12.7	37	11.5 - 2.5	7.9	2.3	29.5
11	33	22.0 - 12.0	16.3	2.7	16.6	24	11.0 - 4.0	6.6	2.0	29.8
12	32	21.5 - 10.0	17.0	2.6	15.0	25	8.0 - 4.0	6.0	2.0	33.6
13	40	23.5 - 13.0	19.0	2.3	12.3	39	9.5 - 3.0	6.9	2.1	31.0
14	39	24.5 - 14.0	19.3	2.3	12.0	40	10.5 - 3.0	7.9	2.0	25.1
15	39	23.0 - 14.5	18.5	2.1	11.3	40	8.5 - 3.0	7.8	1.5	19.3
KDML105			15.6					6.3		

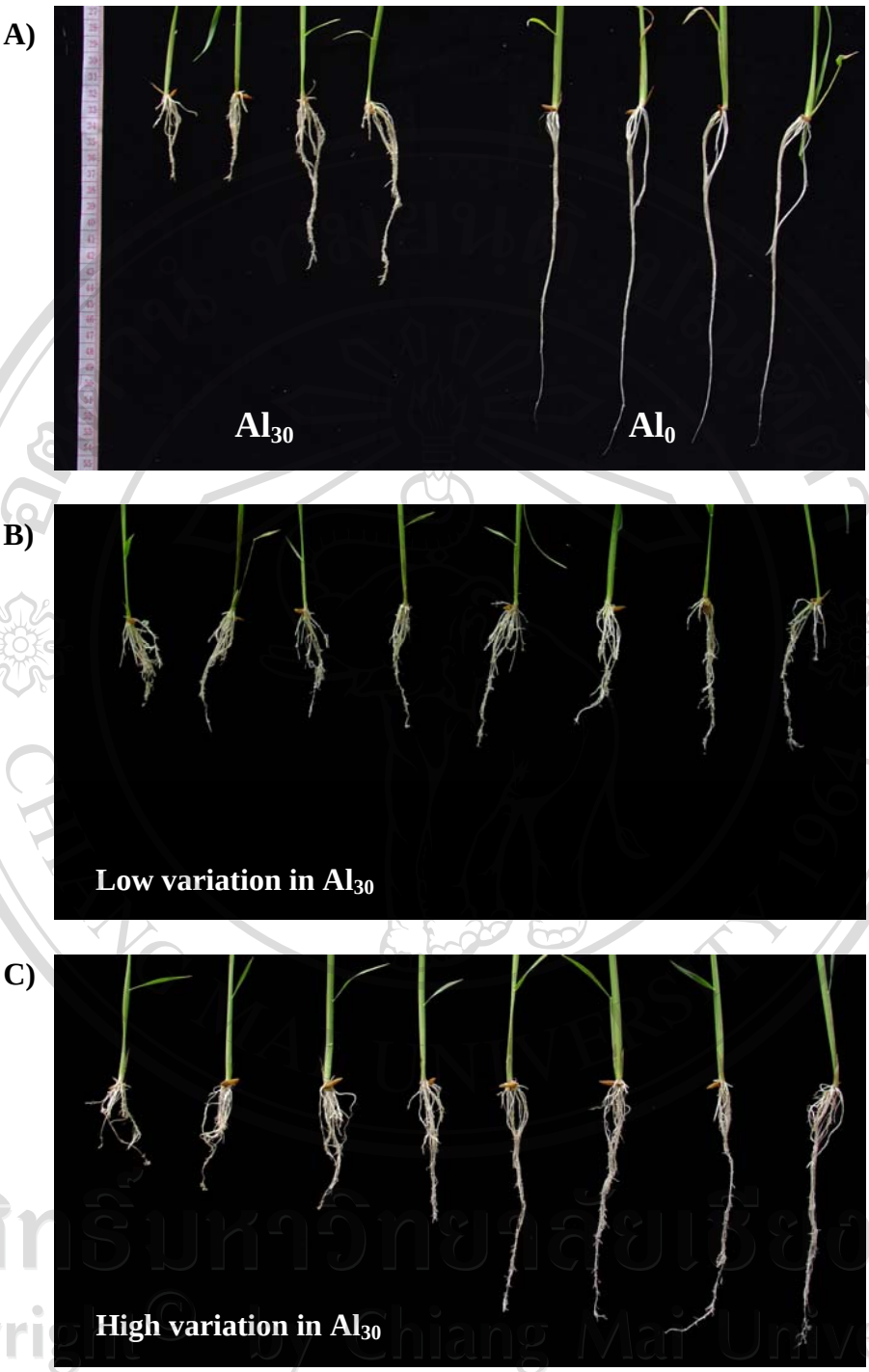


Figure 3.6 Root growth of Leung Yai seed lots in response to Al toxicity in nutrient solution; A) comparing root growth between Al_0 and Al_{30}; B) and C) variation among individual plants within seed lot at Al_{30}.

3.4 Discussion

Different screening methods have been used to evaluate the tolerant varieties for acid soils with high Al toxicity. This chapter evaluated the response of rice varieties by germination in acid soils and root growth in nutrient solution. Acid soils (pH 3.5) affected not only the germination of rice varieties, young seedlings was also susceptible to soil acidity and dead after emergence. Others suggested that Al may not interfere with seed germination, but impair the growth of new roots and seedling establishment. Root growth inhibition was detected after 2-4 days after the initial of seed germination (Mossor-Pietraszewska, 2001; Rout *et al.*, 2001). The evaluation of response to acidity in upland rice varieties has been successfully conducted in nutrient solution, as others have shown (Fageria *et al.*, 1988b; Jan and Pettersson, 1989; Khatiwada *et al.*, 1996). The suggestion that plant growth in acid soils is probably more often limited by Al toxicity than by H^+ toxicity (Foy, 1988) has been confirmed by the adverse effect of H^+ on root growth that was accentuated in presence of Al. Genotypic variation in the Al effect was much more pronounced than the pH effect, with only limited correlation between the two effects. Genotypic variation of survived seedling in acid soils was correlated with tolerance to Al in nutrient solution ($r = 0.762$; $P < 0.05$). Screening to Al toxicity in nutrient solution is therefore appropriate for evaluation of rice germplasm for adaptation to soil acidity.

It is well known that Al toxicity symptom was observed in root growth inhibition particularly in root tips and in lateral roots (i.e. Figure 3.3). Relative root length (root length with Al as % of root length without Al; RRL), provided an effectively parameter for Al tolerance in rice and Al stress at 30 mg L^{-1} was optimal for differentiating among rice varieties as previously reported (Khatiwada *et al.*, 1996;

Nguyen *et al.*, 2001). Aluminum made from different Al salts such as $Al_2(SO_4)_3.18H_2O$ and $AlCl_3$ were widely used for screening in many works. This study confirmed that both Al forms were similar to limit the growth in rice. The comparison for oxygen supply in nutrient solution with or without air bubbling suggested that in absence of Al, plant growth was enhanced when grown in aerated with air bubbling. Previous report suggested that the length of root was indicator for adequate oxygen supply in culture solution, roots were longer when grown in aerated with air bubbling (Insalud, 2006). However, the difference in oxygen supply was unaffected to differently response when grow in Al toxicity. Roots were similarly inhibited in different oxygen supplies. Due to it was difficult to control the level of oxygen in the solution and need to reduce the gap of external effect of oxygen with air bubbling, plant that grown in still condition or aerated without air bubbling was chosen for screening to Al tolerance in rice.

In this study, five local upland rice varieties were classified into three Al tolerance groups based on their RRL in Al_{30}. PA was the most sensitive which was similar to improved variety KDML105 whereas the other upland varieties were more tolerant. The sensitive of PA and tolerance of BB to Al in nutrient solution were agreement with plant growth and final yield in the acid soil field, grain yield of PA produced less than half in comparison with BB (see in Chapter 2). In the similar ways, other improved varieties, popular varieties in Thailand, were also classified as Al sensitive varieties in comparison with local upland rice BB. These results associated with previous reports that some local rice varieties often possess higher levels of tolerance to Al than introduced improved varieties (Khatiwada *et al*., 1996; Vasconcelos *et al.*, 2002).

Although the value of RRL was positively correlated with root and shoot dry weight, shorten root may be sometimes uncorrelated with inhibition root dry weight like as KDML105. KDML105 was dramatically depressed in root length by Al_{30} but root dry weight was almost unchanged. However, unchanged root dry weight at Al_{30} was ineffective to produce shoot growth to the same level as in Al_0 due to roots were already damaged by Al, abnormal branching and swollen roots (Figure 3.3). These may bring them to high weight but less efficient to take up water and nutrient because of cellular damage. In this case, shoot dry weight should be more considerable than root dry weight that correlate well with root length, however, they need more study to investigation for efficient in nutrient uptake.

The present results have also shown that genetic variation for Al tolerance can be expected in farmers' seed lots of local rice variety. The variation of Al tolerance was found among individual plants of the same seed lot and seed lots sharing the same name that came from different farmers. In the case study of Leung Yai, a popular local deep water rice variety in acid-sulphate soil problem in Nakorn Nayok and Prachin Buri provinces, the externally grain characters were small variation (low % of CV) but those of them showed much more variation of root length in response to Al toxicity (CV = 19-34%) than their performance in non toxicity (CV = 9-17%). Similarly, local upland rice variety like BB which classified to Al tolerance variety was high variation between progeny lines within seed lot and between seed lots in tolerance to Al based on their RRL. Almost of the BB lines were more tolerant to Al than standard check Koshihikari, previously reported as Al tolerant variety (Ma *et al.*, 2002). Similar genetic variation between seed lots and within seed lots has been reported in BB in grain iron concentration. The levels of variation in grain iron

concentration between the seed of BB kept by different farmers and within individual seed lots were about the same as those found between low and high Fe varieties (Prom-u-thai *et al.*, 2004; Pintasen *et al.*, 2007).

Genetic variation within local rice varieties recognized by the same name has been evaluated by morphological characteristic as previously reported (i.e. Meesin, 2004; Supamongkol, 2006). The genetic differentiation among seed lots within the same variety name may or may not detect by visual observation. In this study, genetic variation in two seed lots of BB was found only 2 (plant type and pericarp color) from 12 characters whereas the variation was up to 5-6 characters by other seed lots that collected from the same village (Pintasen *et al.*, 2007). However, genetic variation was much more revealing when detected by DNA analysis. The variation was found both within seed lot and between seed lots of BB and BM. Genetic differentiation between seed lot of BB (F_{ST} = 0.39) was much higher than in BM (F_{ST} = 0.14). It could be suggested that DNA technique was classified genetic differentiation between seed lot of BB than by visual characters, DNA of those was high genetic distance and grouped them to be a different genotype. Meesin (2004) suggested that genetic variation within population of a local variety that detected by DNA analysis may or may not show obvious variation in external appearance. The genetic differentiation of local upland rice has been found by many causes. In Tee Cha village, individual farmers grow 2-5 varieties in each time, different varieties are sometimes mixed together by agronomic practice in the field or seed collection from season to season. Karen farmers sometimes called a name of variety by seed character, therefore, the same seed character may be from different genotypes. In addition, seeds may be mixed by exchanging between farmers in the village and also between villages

(Sirabanchongkran *et al.*, 2004). The high variation in tolerance to Al within seed lot of BB may be due to the difference on their genotype within seed lot. The closely genetic of BB1 to BM1 and BM2 was not associated with the response to Al tolerance Therefore, this work was clearly information only the genetic variation of local varieties in DNA level, DNA markers that associated with Al tolerance trait should be need more study.

The nutrient solution is an effective technique screening in a large rice germplasm. The variation for Al tolerance may be found between different seed lots recognized as a same name and within individual seed lot as well as between varieties. For anyone wishing to use local rice variety like BB to study Al tolerance or as a donor of the Al tolerance trait in rice breeding will have to make sure it is the right genotype that they are dealing with. The mechanism for Al tolerance should be more clear understanding in rice.