Chapter 5

General Discussion

5.1 Genotypic variation of rice in tolerance to soil acidity

Rice is one of the most tolerant to acid soil among small grain cereals (Fageria, 2002; Keltjens and Tan, 1993). There is widely genotypic variation among rice varieties for adaptation to high Al in acidic soils (Howeler and Cadavid, 1976; Jan and Pettersson, 1989). Previous studies suggested that many of local rice varieties should be better adapted to soil acidity than improved tolerant varieties (Khatiwada *et al.*, 1996; Vasconcelos *et al.*, 2002). This has been confirmed by the present study. Some local upland rice varieties collected from acid soil areas (pH 4-5) in Northern Thailand have been found to be more tolerant to Al toxicity than improved Thai rice varieties (Chapter 3). The comparison between local variety BB and pure line selection KDML105 showed differential responses to acid soils. BB produced more roots and distributed them into subsoil than KDML105. When root penetration into the subsoil was decreased, uptake and utilization of mineral soil nutrients and water were limited (Marschner, 1995). The deeper root of BB is not only benefit for rice adapted to acid soils, but it is also useful character for rice grown on aerated condition which sometimes affected by drought stress particular on rainfed areas.

Severity of inhibition of root growth is a suitable indicator of genotypic difference to Al toxicity (Marschner, 1995). Root inhibition among varieties grown in acid soils may be difficult to identify. Nutrient solution is an effective method for evaluating the different of these varieties. It is simple, fast, cheap and strict control over nutrient availability and pH. Nutrient solution with Al levels at 30 mg Al L^{-1} (Al_{30}) compared with 0 mg Al L^{-1} (Al_0) is suitable to evaluate genotypic variation of rice in this study as corresponding with other reports (Khatiwada *et al.*, 1996; Nyugen *et al.*, 2001). Relative root length (RRL; root length with Al relative to without Al), is a reliable parameter to classify genotypic ranking which showed high correlation with other plant growth such as root and shoot dry weight. The results showed a range of genotypic variation to Al tolerance in Thai rice germplasm. At Al_{30}, the difference among local upland rice varieties covered a wider range (RRL = 36-74%) than that in improved varieties (RRL = 22–46%). Although improved Thai rice varieties contain many good characteristics such as high yield, high grain quality, and tolerance to pests or diseases, etc., none of them have been found to be as tolerant to Al as with the more tolerant among local upland rice varieties, especially BB which had an RRL of 74%.

It is well known that seed of local rice varieties maintained by farmers are high in genetic diversity (Chang, 1976). Different varieties can be given similar names and different names can be applied to the same varieties. Our results suggested that although local upland rice BB was classified to Al tolerant group, there was still high genotypic variation among individuals plant within each seed lot maintained by a farmer and between seed lots of the same varieties kept by different farmers. Farmers may call their seed lots BB or other variety names because of similarity in external observable characteristics such as shape, size and pigmentation of plant parts and uniformity of plant development in the field. Our study suggested that seed lots of BB found to be small genetic variation based on morphological and physiological characteristics. However, diversity analysis with molecular markers has revealed that seed lots of BB can be genetically highly diverse (Experiment 3.3.2). Two seed lots of BB which were almost the same morphologically are indeed different genotypes at the DNA levels. The progeny test of these seed lots also found a high range of Al tolerance, with RRL variation between 50-80% in BB1 and 57–75% in BB2. Similar within seed lot variation has also been reported for grain Fe contents (Pintasen *et al.*, 2007).

The information of BB, it is not only a source for tolerance to Al or high acidic soils but it may contain high grain nutrition as previously reports. Pintasen *et al.* (2007) suggested that some seed lots of BB accumulated higher grain Fe content than standard check IR68144, a high grain Fe variety from IRRI. In the similar way, BB was also reported to have about twice as much zinc (Zn) in the grain than KDML105, which produces the famous Thai Jasmine rice (Wongmo, 2008). It should be noted that local rice varieties like BB may enable small farmers to maintain reasonable rice productivity on acid soils as well as having a rice-based diet enriched with Fe and Zn.

5.2 Physiological mechanism for Al tolerance in rice

5.2.1 Effect of Al on germination

Acid soils with high Al toxicity reduced seed germination in rice. The present study showed that percentage of survived seedlings declined 3-4 days after rice germinated in acid soils (Experiment 3.2.1). Previous study suggested that Al toxicity does not interfere to rice seed germination, but Al is direct affected to inhibit root growth after emergence (Kikui *et al.*, 2005). Therefore, after emergence growth of younger roots may be depressed by Al in the rhizosphere, so leading to death of the roots and finally of the plant. However, there was genotypic variation for seedling survival in acid soils that was closely correlated with the plant response. Acid-soil tolerant BB and BM also survived acidity as seedlings better than sensitive PA and KDML105. These results suggest two things. Firstly, root growth of BB and BM may be more tolerant to Al damaged. Secondly, the early sensitivity to Al may be a good and early indicator of effects of Al on a genotype's later response to Al from seedling vigor to subsequent growth and yield.

5.2.2 Effect of Al on root growth

The primary target of Al is inhibition of root growth. This study showed that root symptom of Al toxicity in rice was easier and readily observed than the shoot. Roots adversely affected by Al toxicity were short, particularly root tips and lateral roots become thickened, turn brown with insufficient fine branching (Figure 3.3), similar to previously described in rice other crops (Jan and Pettersson, 1995; Foy, 1984; Delhaize and Ryan, 1995). The root tip is the most sensitivity part to Al toxicity and it has been reported to accumulate more Al than other sections of the root (Kikui *et al.*, 2005). When Al enters to the roots, it may bind to DNA of the root cap cells in particular, inhibition of root cell elongation is faster response to Al and inhibition of cell division is presumably an indirect effect of Al that follows the effect on elongation (Marschner, 1995). Our results have shown that Al sensitive varieties such as PA and KDML105 not only had shorter roots but were observed to sustain more roots damaged by Al toxicity than tolerant varieties such as BB and BM (Figure 3.3).

5.2.3 Inhibited nutrient uptake and induced deficiency

Aluminum toxicity is often expressed simultaneously in two ways, inhibition in root elongation and induced deficiency of mineral nutrients. Since root growth is restricted, the ability of plant to absorb nutrients and water is much reduced. As the result, plants suffering from Al toxicity may exhibit symptoms in the shoot and leaves in similarly as nutrient disorder such as P, Ca or Mg deficiency (Foy *et al.*, 1978; Rout *et al.*, 2001). In Chapter 4, P accumulation of the plant tissue was linearly depressed with increasing Al levels. Plants appeared to be P deficient especially in Al sensitive varieties. The concentration of P on the plant top at Al_{30} (0.20-0.46%) was much lower than P critical value of rice at tillering (0.7%; reported by Reuter and Robinson, 1997). Similarly, Ca concentration of all rice varieties at Al_{30} (0.15-0.19%) was lower than optimum levels (0.2-0.6%; reported by Dobermann and Fairhurst, 2000; Reuter and Robinson, 1997), particularly in Al sensitive PA which was close to the critical level of Ca deficiency (<0.15%). Therefore, rice suffering from Al toxicity is closely associated with nutrient deficiency. The foliar symptom of rice suffering from Al toxicity may be not easily identifiable because of often combination of one or more of nutrient deficiencies.

The ability of roots to absorb and accumulated mineral nutrients in plant parts is one of the tolerance mechanisms in rice. Although Al tolerant BB and BM were not similar in their root length response to Al, the shorter root length of BM appeared to be compensated by root dry weight and presumably surface area to take up essential nutrients in the same amount of BB. The varieties showed clear difference between Al tolerant groups based on nutrient accumulation. In the presence of toxic Al the sensitive PA and KDML105 accumulated much less nutrients than the Al tolerant varieties. At Al_{30}, Al sensitive group accumulated essential nutrients (i.e. P, K, Ca and Mg) less than 10% of the control (Al_0) whereas Al tolerant group was about 20–30%. In the field where nutrient supply may be marginal, as is the case in most acidic upland soils, less nutrient acquired and accumulated would mean less growth in Al sensitive varieties. Therefore, nutrient acquisition and accumulation is an important component in tolerance to Al which will help to maintain plant growth and grain yield in Al stress conditions.

5.2.4 Al accumulation in plant part

Since organic acid secretion from the roots is not a key mechanism for Al tolerance in rice (Ishikawa *et al.*, 2000; Ma *et al.*, 2002), the tolerant mechanism in rice may be grouped according to where Al accumulated within plant tissues. The results suggested that the efficient retention of Al in roots and less transportation from the roots to the shoots may play a key role in Al tolerance mechanism in rice. While Al tolerant BB and sensitive KDML105 had about the same Al accumulation in their roots, BB appeared to transport less Al from roots to shoot than KDML105. In Al_{30}, Al sensitive KDML105 with 280 mg Al kg^{-1} in shoot was very close to critical Al toxicity in rice which ranged from 100 mg Al kg^{-1} (Doberman and Fairhurst, 2000) to 300 mg Al kg^{-1} (Yoshida, 1981) while Al tolerant BB had only half as much Al in the shoot as KDML105. This evidence is in agreement with other studies, further confirming Al partitioning between roots and shoot as a key mechanism for Al tolerance. The low accumulation of Al in the roots of sensitive varieties is caused by rapid transportation of Al to the shoots and its accumulation there (Hai *et al.*, 1989; Howeler and Cadavid, 1976; Jan and Pettersson, 1993). Foy (1984) noted that high

Al content in the roots and low content in the shoot is one of the tolerance mechanisms to Al toxicity. Some pieces of evidence suggested that after Al enters the roots, it may be bound in some Al-ligand complexes (i.e. organic acids), leading to Al detoxification inside the plants (Kochian *et al.*, 2005, Ma, 2005).

5.3 Acid tolerance for upland rice production on acidic soils in the highlands

5.3.1 Farmer's management on upland field

Upland rice is the major staple crop for many upland production systems. Soils of the uplands are generally acidic and infertile, such soil amendments are neither practical nor economical. Small farmers, particular ethnic minority groups (e.g. Karen, Hmong, Lua and Akha, etc.) who make a living on the slash-and-burn system of shifting cultivation in the mountainous areas in Southeast Asia are unable to afford lime and fertilizers. A case study at Tee Cha village, upland rice crop managed on shifting cultivation is often suffered by soil acidity. Grain yields were generally low and variable, 1.0-3.0 ton ha^{-1} (Chapter 2). The variation in grain yield of upland rice is associated with the tolerance of upland rice varieties to soil acidity and spatial variation of soil fertility. These soils were suggested to be low available P, 3-4 mg P kg^{-1} (Yimyam, 2006), and high exchangeable Al, 40-100 mg Al kg^{-1} on the top soils and more than 200 mg Al kg^{-1} in the subsoil (Chapter 2). The lower Al in the top soils is related to the effect of ash from biomass burning in the slash-and-burn system. Ash acts as a liming agent by increasing soil pH as well as depressing exchangeable Al (Saarsalmi *et al.*, 2001). However, ash affects only the top soil and does not remove Al toxicity in the sub soil. The study of Yimyam (2006) suggested that soil pH and mineral soil nutrients after burning in acid-soil fields were higher in the top soil (0-15 cm) than in the sub soil (15-30 cm). The soil pH increased from 4.3 before biomass burning to 4.8 after burning. The nutrient concentrations in soils were also increases in this similar ways.

In the slash-and-burn system, the nutrients stored in the above ground biomass is released by burning into the soil surface has been shown to contribute significantly to crop yield. The amount of nutrients released by burning is associated with many factors such as plant species in fallow period, rotational duration, density of biomass, soil capacity to absorb nutrients, etc (Juo and Manu, 1996). In this village, *Macaranga denticulata* has been recognized by farmers as the main fallow enriching species and shown by vegetation sampling to significantly increase nutrient accumulation in the above ground and below grown biomass of the fallow and upland rice yield (Yimyam *et al.*, 2003). Fallow enrichment property of *M. denticulata* was, on the other hand, shown to be highly dependent on its associated arbuscular mycorrhizal fungi (Younpensuk *et al.*, 2004; Yimyam *et al.*, 2008). This thesis has shown that another important component of this productive and sustainable system of upland rice production is tolerance to soil acidity of local rice varieties (Experiment 2.3.1).

The benefit of ash to support plant growth was confirmed experimentally (Experiment 2.3.3). The ash from the village field at the rate equivalent to that returned to the soil in the fallow patches with dense stand of fallow enriching *Macaranga* contained high amounts of nutrient elements particular Ca, K and Mg. Application of the ash increased rice growth and nutrient uptake. Watanabe and

Okada (2005) suggested that increasing external Ca content seem to alleviate Al toxicity by less inhibition of root growth in rice. Thus Ca in the ash could have a direct effect in lessening the toxic effect of Al on the roots. This evidence may be associated with this result, suggesting that the high amount of Ca can improve soil acidity, increasing soil pH from 4.0 to 4.5, as well as supported the growth of upland rice. However, the liming effect of ash that increased pH in this and other studies (e.g. Yimyam, 2006) may also decrease toxicity of Al by precipitating the Al^{3+} to the less soluble Al_2O_3.

5.3.2 Performance of upland rice varieties on acidic soils

A previous study in the same village of Yimyam (2006) investigated benefit of *Macaranga* density to support rice growth but less information of differentiation among upland rice varieties. Genotypic difference of upland rice varieties in their adaptation to acid soil has been confirmed in farmers' fields. Differential response between varieties was shown in the same farmer's fields. Soil acidity sensitive upland rice variety PA grew and yielded more poorly than the tolerant varieties, BB or BM, that were grown in the same field (Experiment 2.3.2). That PA is the most popular variety in the village (Sirabanchongkran *et al.*, 2004) with mostly acidic soils seems to contradict with the finding that PA has been shown to be most sensitive to soil acidity, both in controlled experiment and in farmers' fields. However, PA is not a staple variety in this Karen village but a glutinous rice variety which is grown in a small amount by each household mainly for the purpose of brewing rice wine. In such case the brewing quality of the rice is more important consideration than yield.

In these soils, higher yield of BB and BM were associated with Al in the shoot at tillering of 178 to 241 mg Al kg^{-1}, which were in the same range as the 184 -231 mg Al kg^{-1} found in PA. This suggests that the relationship between shoot Al and plant growth and yield may not be so direct and straight forward, and response to Al in the shoot is still poorly understood. The tolerance to acid soils of BB and BM appeared to be associated with higher root efficiency in the uptake and utilization of mineral nutrients to promote plant growth and final grain yields.

The performance of upland rice varieties on acid soil fields was in close agreement with their response in tolerance to Al toxicity in nutrient solution. Even with some variations such as exchangeable Al, soil fertility and farmer's management were found in acid soils, the Al tolerant BB and BM produced above ground biomass and took up essential nutrients twice as much as Al sensitive PA in the field. These differences are likely to have contributed towards yield difference, in which grain yield of PA was only one quarter of BM or one half of BB in the same field. This study has shown that tolerance to soil acidity can contribute to the solution to upland rice production on acidic upland soils.

5.4 General conclusion

Rice varieties differ markedly in their tolerance to soil acidity. Aluminum tolerant varieties have great potential for increasing dry matter at high levels of Al toxicity because they grew more roots that are able to take up more nutrients in the presence of Al. They may also retain more Al in the roots, and so prevent Al to accumulate in the shoots. Nutrient solution method is successful for screening rice for Al tolerance and could predict final crop yield in acidic soils. Local rice varieties tolerant to Al like BB and BM are useful to upland farmers like those in Tee Cha village who must grow their rice on the soils with extreme acidity without inputs of lime and fertilizers. The acid soil tolerant local rice varieties are valuable sources for breeding programs that serve acidic soils. However, as local rice germplasm carries a great amount of genetic diversity within individual populations, anyone wishing to use local rice varieties like BB to study Al tolerance or use 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.

5.5 Further research

Since rice has a wide range of genotypic variation in tolerance to Al toxicity particularly in local varieties, a large number of rice germplasm should be more screened for rice adapted to acid soils region. The Al tolerant varieties will be necessary for future rice breeding programs and need more understanding in genetic control of Al tolerance trait. Since our study suggested that Al tolerance in rice is associated with retaining Al in the roots and less transported to shoots, the tolerance mechanism of roots to maintain Al in the cellular level should be clearly understood in future work. As local rice BB is containing many great characteristics that reported by different works; Al tolerance (this thesis), high grain Fe (Pintasen *et al.*, 2007) and high grain Zn (Wongmo, 2008). The selection BB lines combining with these characters should be interesting and useful for rice farming (a) on unsaturated acidic soils, including upland rice, aerobic rice, dry seeded rice and rainfed rice during dry spells, and (b) in extreme acidity of acid-sulphate soil that is not neutralized by flooding.