Chapter 1

Literature review

1.1 Acid soil problems

1.1.1 Extension of acid soils

Regional distribution of acid soils

Soil acidity is a common yield limiting constraint for crop production in various parts of the world. Acid soils with pH below 5.5 occupy approximately 30% or 4 billion ha of the world arable land. The largest area of acid soils is found in America, and the second is in Asia (Figure 1.1). More than 200 million ha of major crops, cereals and legumes, are grown on acidic soils. World-wide, it has been estimated that 35% soybean, 20% maize and 13% rice are grown on acid soils (von Uexküll and Mutert, 1995). In Asia, 90 million ha of acid soils are arable and permanent cropped, almost of these soils, commonly Oxisols and Ultisols, are distributed throughout Southeast Asia (von Uexküll and Bosshart, 1989).

The extent of acid soils is continuing to increase worldwide, primarily because of continuous leaching by heavy rains. The rainfall leaching and the harvesting of crops remove the soluble nutrients out of the top layers of soil. In addition, crop productions accelerate soil acidification by application of fertilizers (Helyar, 1991; Samac and Tesfaye, 2003). Urea fertilizer reduced soil pH because of nitrification,

and potassium fertilizers exchange Al at the exchange site of clay minerals and increase Al concentration in soil solution (Okada and Fischer, 2001).
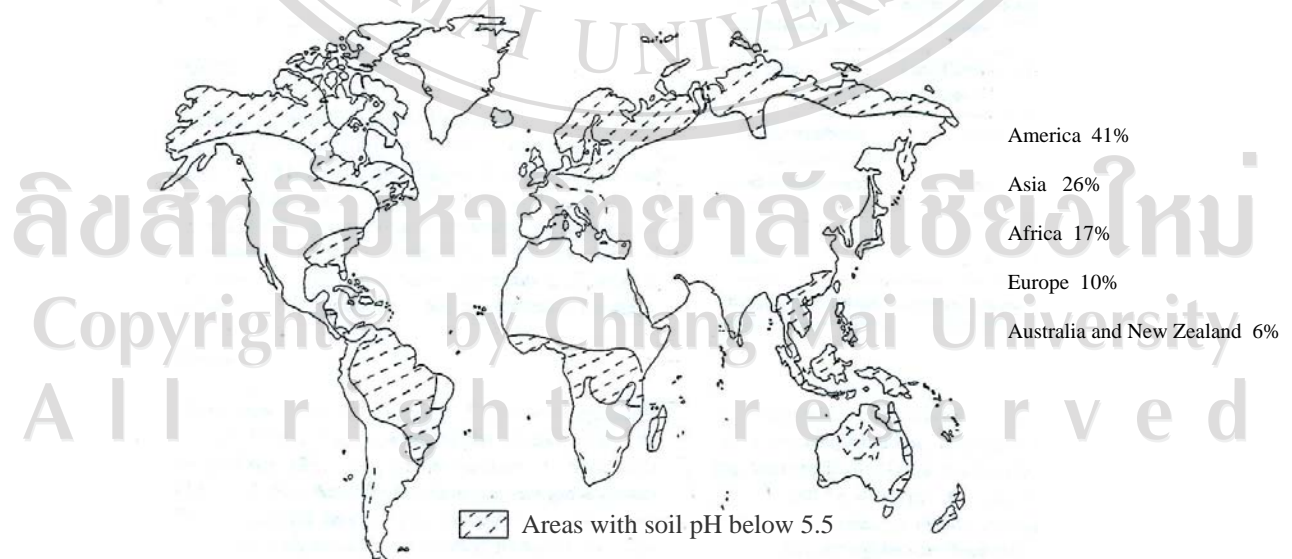
Soil chemical and plant response to soil acidity are different between dry and wet lands. In rice system, upland rice cultivation that is grown on 19 million ha worldwide (60% in Asia) is often found in various degrees of soil acidity because of deep weathering and leaching of cation (Okada and Wissuwa, 2004). In wetland rice, transplanted and wet seeded rice are normally not affected by soil acidity because flooded conditions increase the availability of the nutrients and stabilize soil pH closer to neutral (Ponnamperuma, 1994; Savant and Kibe, 1971). Recently, acidity can be a problem for dry seeded rice, which is becoming common in rainfed rice areas because of rising labor cost, in the first 4-8 weeks before there is sufficient rain to flood the soil. The normalization of pH also does not happen in acid-sulphate soils, which cover approximately 12 million ha worldwide, mainly in coastal lowland of Southeast Asia (Indonesia, Vietnam, Thailand) (FAO, 2000). As soil chemical of dry and wet lands are different, the description of acid soils in dry land or aerate condition is emphasized in this review.

Distribution of acid soils and crops in Thailand

Acid soils with pH below 5.5 are extensively distributed in all parts of the country, covering about 45% of total land area or about 23 million ha (Kheoruenromne and Kesawapitak, 1989). The extent and distribution of acid soils in Thailand are shown in Figure 1.2. Much of these soils are Ultisols which can be found both in upland and lowland areas of the country. Low plant nutrients (especially P) and a high degree of acidity including aluminum (Al) and manganese

(Mn) toxicities are the most common chemical constraints to crop production on most acid soils in Thailand (Parkpian *et al.*, 1991). Crops grown on these soils are quite diverse, including rice, cassava, corn, sorghum, peanut, soybean and sugarcane (Kheoruenromne and Kesawapitak, 1989).

Approximately 3 million ha of acid soils are utilized for rice production, these cover both in upland and lowland rice. On upland acid soils, field crops accounted for 1.4 million ha (Parkpian *et al.*, 1991), in some of these areas small farmers grow upland rice on soils with pH as low as 4.0 (Yimyam, 2006). In the lowlands, acid-sulphate soils are the major problem that covers quite a large area in the Central Plain of Thailand, which is an important paddy field region of the country. Strongly acidic (pH below 4.0) and low fertility of these soils are causes of low crop production, only 1.2-1.8 ton ha^{-1} (Charoenchamratcheep and Tantisira, 1982; Maneewan and Sa-nguansubpayakorn, 2007).

America 41%

Asia 26%

Africa 17%

Europe 10%

Australia and New Zealand 6%

Areas with soil pH below 5.5

Figure 1.1 World acid soils (FAO, 1991 cited by von Uexkül and Mutert, 1995).

Figure 1.2 Extent and distribution of Ultisols and Alfisols in Thailand (Kheoruenromne and Kesawapitak, 1989).

9

1.1.2 Soil acidity and nutrient deficiency

Acid soil stress is not a single factor but a combination of many factors such as hydrogen ion (H^+), aluminum (Al) and manganese (Mn) toxicity, and deficiency or unavailability of essential elements, particularly Ca, Mg, P and K (Foy, 1988; Marschner, 1991). The cation exchange sites vacated by leached nutrients are gradually replaced by Al^{3+}, Mn^{2+} and H^+ (Foy, 1984; Rao *et al.*, 1993). However, acid soils having similar pH values may cause different mineral stress problems in a given plant genotypes. The specific causes of poor plant growth on acid soils may vary with soil pH, clay mineral types and amounts, organic matter contents and kinds, levels of salts, and particularly, with plant species or genotypes (Foy, 1984).

Although poor fertility in acid soils is due to many stress factors, Al toxicity is generally the main factor that limits plant growth and yield in acid soils with pH below 5.0 (Fageria *et al.*, 1988a; Foy, 1988; Samac and Tesfaye, 2003). In the topsoil where the organic matter content is higher, H^+ toxicity may dominate, but in the subsoil root growth may be depressed by Al toxicity (Marschner, 1991). In the absence of Al, rice roots have been reported to be unaffected by H^+ ion concentrations up to pH 3.5 (Thawornwong and Diest, 1974). Okada *et al.* (2003) also suggested that rice growth was not differently affected by H^+ in the range of pH between 3.5 and 5.5, and that other native plant species were more adversely affected by Al than H^+ in the solution. Others have reached the same conclusion that on many acid soils it is not so much the high H^+ concentration in the soil solution as the high Al concentration which is harmful to plants and especially to root growth (Mengel and Kirkby, 1987). Therefore, it would be safe to generalize Al toxicity rather than H^+ toxicity is the main factor limiting crop growth and yield in acid soils.

Generally solubility of Al increases with decreasing pH, but at the same pH the Al concentration in soil solution may differ widely among soils (Adams, 1984). Plant growth inhibition is not only related to the quantity of Al in soil solution but also the species of soluble Al present. The Al released from soil minerals under acid conditions occurs as $Al(OH)_2^+$, $Al(OH)^{2+}$ and the latter commonly referred to as Al^{3+} (Figure 1.3). When the soil pH drops below 5.0, Al^{3+} is solubilized into the soil solution and this is the most important rhizotoxic Al species (Kinraide, 1991; Delhaize and Ryan, 1995). However, other evidence suggested that $Al(OH)^{2+}$ or $Al(OH)_2^+$ were the primary toxic species and Al^{3+} appeared to be considerably less toxic in dicot species (Kochian, 1995).
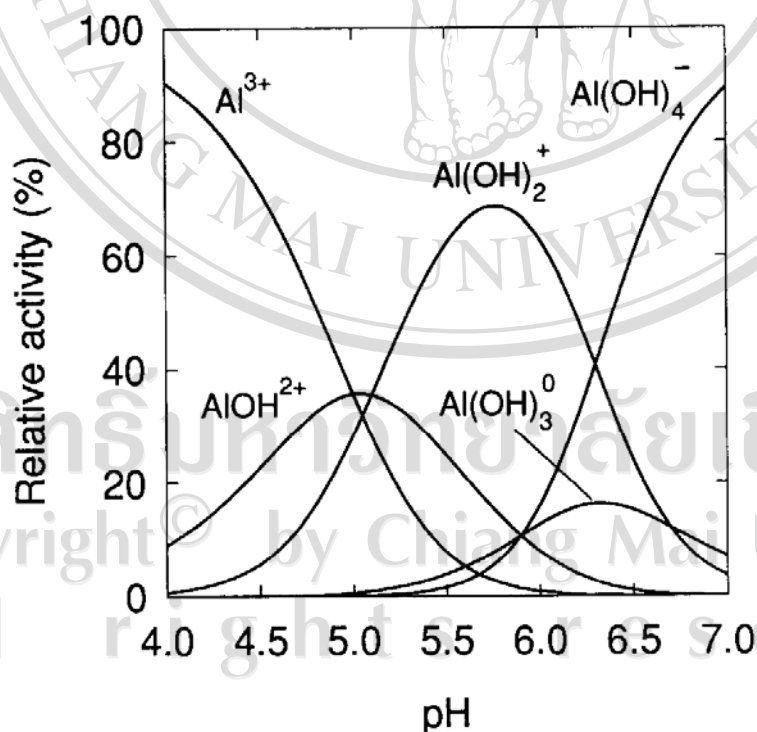
Figure 1.3 Relative activities of mononuclear Al species of soluble Al as a function of pH (Kinraide, 1991).

After Al, Mn toxicity is the second most important growth limiting factor in many acid soils (Foy, 1988). Manganese is rapidly transported from the roots to shoots and symptoms first occur on the shoots (Rao *et al.*, 1993), but it is not re-translocated in the phloem (Marschner, 1995). Plant symptoms of Mn toxicity include marginal chlorosis and necrosis of leaves, crinkle leaf in young leaves and brown spots on older leaves (Foy, 1984; Marschner, 1995). In rice, grain yield is markedly depressed because of high sterility (Dobermann and Fairhurst, 2000). Because Al toxicity is generally recognized as the major limiting factor of crop growth and yield in acid soils worldwide than Mn toxicity, Al toxicity and its mechanisms will be emphasized in this review.

Acid soils increased the solubility of Al and Fe as well as decreased the availability of P and other essential elements. Phosphorus deficiency is particularly problematic under Al-toxic soils because of its fixation as aluminum phosphate that contributed to low P availability (Fageria *et al.*, 1988a). Usually, critical level of soil P deficiency in rice occurs at $P < 5$ mg kg^{-1} (Dobermann and Fairhurst, 2000). Previous reports in Northern Thailand suggested that upland rice that grown in acid soils (pH < 5.0) have been often suffered from low soil P, only 3-4 mg P kg^{-1} (Yimyam, 2006). Among the soil acidity in Brazil, two main reasons of P deficiency in these soils are low natural level of soil P and capacity to fix high levels of added P. The amount of P fixed increased from 45 to 268 kg P ha^{-1} as the P application rate increased from 50 to 400 kg P ha^{-1} (Fageria and Baligar, 2001). Rao *et al.* (1993) suggested that the ability of a soil to supply nutrients to growing plants depends on its capacity to maintain adequate nutrient concentrations in the soil solution at the root surface. This ability depends on the plant uptake rate, the amount of nutrient in rapid

equilibrium with the soil solution, the soil buffering power for the available form of the nutrient in solution, and the rate of resupply of available nutrient from solid phase such as organic matter and inorganic minerals. However, soil acidity affects most of these soil properties.

1.2 Impact of Al toxicity on plants

1.2.1 Growth responses to Al toxicity

It is well known that high Al concentration in soil solution is the main factor in restricting plant growht in acid soils. In general, symptoms of Al toxicity include inhibition of root growth, especially localized at the root apex which suffers more injury than in shoot growth (Foy, 1988; Marschner, 1991; Samac and Tesfaye, 2003). Root elongation is inhibited within hours of Al exposure. The rapidity of this response indicated that Al first inhibits root cell expansion and elongation, over the long term, cell division is also inhibited (Kochian, 1995). Roots are stubby, brittle and have no fine branching. Root tips and lateral roots become thickened and may turn brown (Foy, 1984). Since root growth is restricted, the ability of plant to absorb nutrients and water is much reduced. As a result, nutrient and water stresses are common in plants suffering from Al toxicity (Fageria *et al.*, 1988a; Marschner, 1991).

The symptoms of Al toxicity on leaves are not easily identifiable and are different among plant species. Rice that was suffering from Al toxicity showed interveinal white to yellow discoloration of the tips of older leaves, which may later turn necrotic. Plant growth is stunted, but tillering may be normal (Dobermann and Fairhurst, 2000). As Al toxicity causes deficiency of essential nutrient elements (i.e. Ca, Mg, P and K), plants suffering Al toxicity may exhibit symptoms in the shoot and

leaves that are similar to deficiency in these nutrients (Foy *et al.*, 1978; Rout *et al.*, 2001).

Although Al is not regarded as an essential nutrient, Al has sometimes been reported have a beneficial effect on plant growth. The application of Al enhanced plant growth and increased nutrient concentration in the tissues of some native plant species that adapted to low pH soils in tropics of Thailand and Malaysia, those species were designated as Al-tolerant and Al-stimulated plants (Osaki *et al.*, 1997). In rice, low levels of Al also stimulated plant growth which Al tolerant varieties produced more root and shoot dry weight than Al sensitive varieties (Howeler and Cadavid, 1976; Jan and Pettersson, 1993). Clark (1977) suggested that low Al level enhanced the maize growth and nutrient uptakes of Al tolerant varieties as compared with no Al but inhibited in Al sensitive one.

1.2.2 Nutrient uptake efficiency and Al toxicity

Soil acidity is not only increase ionic strength of Al, but also affected to plant nutrient availability. At soil pH range 6 to 7 seems to promote the most availability of plant nutrition (i.e. N, P, K, Ca and Mg). When the soil pH is lower, the amount of Al, Fe and Mn are soluble and may become toxic to plants. At the same time, the solubility of various salts and compounds of essential elements are declined (Brady, 1974). Therefore, Al-toxic soil induces the deficiency of these essential elements (Foy, 1984; Marschner, 1991). Plant nutrient deficiency could be associated with acquisition (availability uptake and transport across membranes) of nutrient on the soil, distribution and redistribution within the plants (mobilization), or utilization during metabolism (Rao *et al.*, 1993). Keltjens and Tan (1993) noted that plants

suffering from Al toxicity generally have impaired root development often in combination with a deficiency of one or more of the essential nutrients.

Aluminum toxicity of plants grown in acid soils is generally considered to be closely related to P nutrition, partly because of Al-phosphate complex (Foy *et al.*, 1978). Acid soils may have high total P but are often low in available P (Rao *et al.*, 1993). As Al tend to increased P concentration in roots and decreased those in shoots (Foy, 1988), in some plants the foliar symptoms of Al toxicity resemble those of P deficiency, plants are stunted with dark green leaves (Haynes and Mokolobate, 2001). In sorghum, Al-induced P deficiency was suffered more at very low P supply, while increasing P supply was a beneficial effect to eliminate Al toxicity, probably by reducing Al-inhibited root development (Tan and Keltjens, 1990).

Accumulation of P in plants were reduced by Al application, but differently inhibited depend on genotypes. In cowpea, P accumulation was reduced by 28% in Al tolerant and 95% in Al sensitive varieties. In this case, the tolerant variety was not only better adapted to Al-toxic but tend to adapted to P deficiency in acid soils than Al sensitive variety (Jemo *et al.*, 2007). Aluminum tolerant rice variety also maintained higher concentration of P in the shoots than Al sensitive variety (Howeler and Cadavid, 1976). In the similar, P accumulation in shoots of Al tolerant rice variety was slightly affected by increasing Al, while Al sensitive variety linearly decreased P concentration in shoots by Al from 0 to 140 μM. At the same time, root P was linearly increased by increasing Al, irrespective of varieties (Jan and Pettersson, 1993). It was suggested that precipitation of Al-phosphate complexes in the free space of roots may inactivate part of the available P and lead to less P being available for metabolic reactions and transport to the shoots (Jan and Pettersson, 1995).

Therefore, it appears ability to deliver more P from root to shoot in the presence of Al could be an important mechanism for Al tolerance, especially in soils with low available P.

Aluminum toxicity also appears as induced Ca deficiency or reduced Ca transport within plant. Calcium transport into the root is greatest near the root apex which is also the primary site of Al accumulation and Al-induced toxicity. Therefore, Al may displace Ca^{2+} from critical sites in the root apoplast (Rengel, 1992), or Al may be blocking Ca^{2+} channels in the plasma membrane (Kochian, 1995). Exposure to Al inhibited Ca uptake across plasma membrane of wheat root tips which was much more pronounced in Al sensitive varieties (Huang *et al.*, 1992). Genotypic differences between upland rice varieties in tolerance to acid-soil conditions were also investigated in terms of the contents of Ca and Al in the apoplast of root tips. Apoplastic Ca and Al contents are assumed to be directly related to the inhibition of root cell elongation. Calcium content of acid-soil sensitive variety was found to be depressed much further at higher Al levels but it did not decrease much in tolerant variety (Okada *et al.*, 2003).

As Al induced nutrient deficiency differently among genotypes, one of Al tolerance mechanism could be associated with more efficient nutrient uptake (Fageria, 1985; Foy, 1988). Marschner (1995) suggested that Al tolerant varieties tended to increase rhizosphere pH more than in Al sensitive varieties. The increasing in rhizosphere pH not only decreased concentration of Al^{3+}, H^+ toxicity can be eliminated and the binding of Ca^{2+} and Mg^{2+} in the root apoplast can be increased.

1.2.3 Difference in Al tolerance among crop species and genotypes

Difference in adaptation to high Al in acidic soils can be viewed from the differences among crop species and among genotypes within crop species. The range of tolerance to Al among crop species is shown in Table 1.1. These reports agreed with Al tolerance among cereals by Keltjens and Tan (1993) which showed that rye > rice > maize > oat > wheat > barley. Genotypic ranking in tolerance to acid soils of tested crops in Brazil also defined in the order of rice > maize > soybean > common bean > wheat (Fageria, 2002). Kikui *et al.* (2005) indicated that a higher level of Al tolerance in rice than in wheat is based on Al tolerance mechanism operating at germination stage, because of the loss of plasma membrane integrity and cell death in wheat, but not in rice.

Much of works on Al tolerance evaluated genotypic variation among genotypes within species in Al tolerance or adaptation to soil acidity. The difference in Al tolerance among genotypes has been reported from a wide range of crops, including rice (Balakumar *et al.*, 1992), wheat and rye (Kim *et al.*, 2001), soybean (Villagarcia *et al.*, 2001) and maize (Baligar *et al.*, 1997), and many more. For examples, eighteen soybean genotypes exhibited a range of Al tolerance based on their root elongation with Al relative to without Al, from 29% to 85% (Nian *et al.*, 2004). Twenty-two maize genotypes from different origins showed genetic diversity for growth and nutrient uptake efficiency in acid soil conditions. At 41% soil Al saturation, shoot dry weight of all maize genotypes ranged from 70 to 270 mg plant^{-1}, and nutrient efficiency ratio for P (mg of shoot dry weight / mg of P in shoot) were 288 of Al sensitive varieties and up to 437 of Al tolerant varieties (Baligar *et al.*, 1997). The screening of 250 accessions of wheat collected from 21 countries found

most to be sensitive to Al, only 35 accessions were classified to Al tolerant, 21 were

intermediate in tolerance (Stodart *et al.*, 2007). Therefore, the wide range of

genotypic variation in Al tolerance in crops offers opportunities for identification of

the most tolerant and for breeding for crop varieties that are tolerant to Al toxicity for

regions with soil acidity problem.

Table 1.1 The range of Al tolerance in field crops (Adapted from Rao *et al.*, 1993).

Field crop	Tolerance to Al (% Al saturation)		
	Low (0-40)	Moderate (40-70)	High (70-100)
Maize	+		
Cotton	+		
Mungbean	+		
Soybean	+	+	
Sorghum	+	+	
Wheat	+	+	
Groundnut		+	
Cowpea		+	+
Millet		+	+
Rice		+	+
Cassava			+

1.3 Tolerance mechanisms to Al toxicity in plants

The understanding of the physiological mechanisms of plant to tolerate Al

toxicity is essential for further improvement of the adapted genotypes. Several

mechanisms have been advanced to explain differences in tolerance to Al toxicity. In

general, two basic mechanisms of Al tolerance have been proposed. One is the ability

to maintain Al outside the root (exclusion mechanism or external detoxification), and

the latter is tolerance to accumulate Al in roots and shoots (internal tolerance mechanism or internal detoxification) (Ryan *et al.*, 2001; Kochian *et al.*, 2004; Ma, 2005).

1.3.1 External detoxification of Al

Many reports suggested that organic acids play an important role in mechanism of Al detoxification and may be a key of Al tolerant mechanism in plants (Ma *et al.*, 2001; Ryan *et al.*, 2001; Kochian *et al.*, 2005). The secretion of organic acids is highly specific to Al and the site of secretion is localized to the root apex (Ma, 2000). There is considerable evidence implicating a role for organic acids in the Al tolerant mechanisms of a range of plant species. Some organic acids are able to complex Al^{3+} into forms that are not toxic to plants. Organic acids with hydroxyl and carboxyl groups able to form stable ring structures with Al^{3+} that consisted of 5- or 6-bonds conferred the greatest protection from Al toxicity (Delhaize *et al.*, 1993; Figure 1.4). The root of tolerant genotypes could secrete more specific organic acid than in sensitive ones (Figure 1.5). The application of citric acid in culture solution appeared to ameliorate the Al toxicity in soybean genotypes by promote plant growth (Abdullahi *et al.*, 2004).

The kind of organic acids involved in Al tolerance is different among plant species. For examples, malate is released from wheat (Delhaize *et al.*, 1993), citrate from maize (Pellet *et al.*, 1995) and soybean (Yang *et al.*, 2001). Some plant species such as triticale (Ma *et al.*, 2000) and rye (Li *et al.*, 2002) released both malate and citrate. These reports also suggested that the amount of organic acid released from the root apices under Al stress was positively correlated with the level of tolerance to

Al toxicity. However, it has also been reported that although increasing of malate increased resistance of some wheat genotypes to Al, it might not necessarily decrease Al accumulation in root apices correspondingly (Rengel, 1996).

However, for other crops such as rice and sorghum no correlation between Al tolerance and the amount of organic acid exudation has been found (Ishikawa *et al.*, 2000; Ma *et al.*, 2002). Nian *et al.* (2004) also indicated that citrate secretion induced by Al stress may not be a key mechanism responsible for the differential Al tolerance in soybean because some of Al sensitive genotypes secreted more citrate than tolerant ones. Therefore, these supported the view that exudation of organic acids may not be the only mechanism of Al tolerance.

Figure 1.4 Organic acids able to form 5- or 6-membered ring structures with Al^{3+} protect plants from Al toxicity (adapted from Delhaize E. in website; www.plantstress.com).
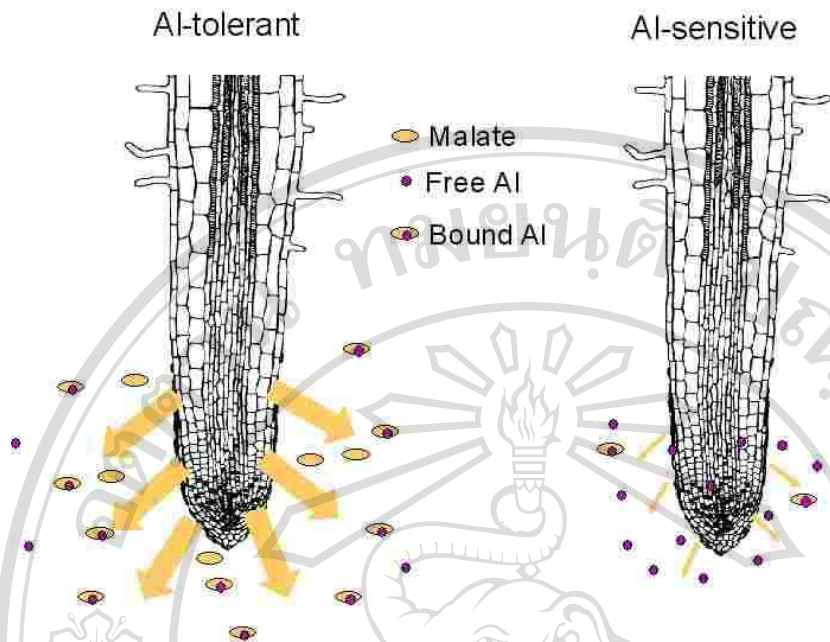
Figure 1.5 A model showing how Al-activated malate efflux protects wheat root tips from Al toxicity (adapted from Delhaize E. in website; www.plantstress.com).

1.3.2 Internal detoxification of Al

Several researchers have recently identified a second Al tolerance mechanism that is based on the detoxification of Al after it enters the plant. This discovery has come from research focusing on plants that can accumulate Al to high levels in shoot without showing Al toxicity, these plants are called "Al accumulator" (Barceló and Poschenrieder, 2002; Ma, 2005). Al accumulator species are supposed to create an Al-ligand (mainly organic acids) complex for translocation from roots to shoots, and for accumulation in the leaves (Ma *et al.*, 2001; Watanabe and Osaki, 2002), mostly accumulates in the cytosol and subsequent storage of the Al-carboxylate complex in the vacuole (Kochian *et al.*, 2005).

There are numerous species that tolerate to high Al concentrations in plant tissue. Many reports suggested that plant species classified as Al accumulators should

accumulate Al more than 1,000 mg Al kg^{-1} in the leaves (Foy, 1984; Geoghegan and Sprent, 1996). For examples, tea plants can accumulate Al up to 30,000 mg Al kg^{-1} in older leaves (Watanabe and Osaki, 2002). Buckwheat (*Fagopyrum esculentum*), highly tolerant to Al, accumulated Al in the leaves at high concentration by secretion oxalic acid forms a complex with external Al and then Al-complex is taken up by the roots (Ma and Hiradate, 2000). Old leaves of buckwheat have been reported to accumulate as much as 10,000 mg Al kg^{-1} and about 4,500 mg Al kg^{-1} in the seed. However, Al concentration was decreased when grown in limed soil, with Al concentrations in leaves immediately adjacent to seeds dropped to about 1,500 mg Al kg^{-1} (Shen *et al.*, 2006).

1.3.3 Mechanisms of Al tolerance in rice

Rice is known as an Al-tolerant crop, although its tolerance varies widely among varieties (Khatiwada *et al.*, 1996; Ishikawa *et al.*, 2000). The mechanism of Al tolerance in rice is still debated. There have been contrasting pieces of evidence of Al accumulation for tolerant mechanism in rice. Several researchers showed that Al tolerant rice varieties accumulated less Al in root than in Al sensitive varieties (Hai *et al.*, 1993; Xu *et al.*, 2004; Watanabe and Okada, 2005). This point may be associated to Al exclusion mechanism that excluded Al from the root of tolerant varieties. However, although citric acid from rice roots were increased in the presence of Al, there was no correlation in the amount of organic acid secretion and the tolerance Al genotypes (Ishikawa *et al.*, 2000; Ma *et al.*, 2002). Therefore, organic acid secretion may not be considered for tolerance mechanism in rice.

In contrast, some other pieces of evidence suggested that an Al tolerant rice variety had higher Al concentration in its roots than Al sensitive variety, but it accumulated in the shoots less than Al sensitive variety (Howeler and Cadavid, 1976). The low Al accumulation in roots of Al sensitive variety has been suggested to have been caused by rapid transportation of the Al from roots to shoots and accumulated there (Jan and Pettersson, 1993; Jan and Pettersson, 1995). Therefore, this evidence may involve to internal Al tolerant mechanism that efficient retention of Al in root is one of the characteristics of Al tolerance in rice (Howeler, 1991).

On the other hand, Al tolerance mechanism in rice varieties has also been reported to be associated with more efficient nutrients uptake. An Al tolerant rice variety showed less inhibition of the nutrient uptake than sensitive variety (Fageria, 1985; Jan and Pettersson, 1993). For example, Al tolerant varieties took up and utilized more Ca and P while Al sensitive and intermediate varieties exhibited less Ca and P uptake and utilization in the presence of Al (Sivaguru and Palival, 1993). The tolerance to Al in rice was also related to production of proteins and peroxidase activities. The decrease in protein content in Al sensitive variety was related to increases in peroxidase activity under Al stress, and opposite in Al tolerant variety (Jan *et al.*, 2001).

1.4 Management of Al toxicity and soil acidity

1.4.1 Amelioration of acid and Al-toxic soils

In acid soil, application of lime and P fertilizers are common and effective practice for reducing soil acidity which increase soil pH and consequently reduced the amount of Al in the soil solution which could improve crop production (Fageria and

Baligar, 2001; Ludwig *et al.*, 2002). Fageria *et al.* (1991) suggested that after lime application, soil pH, exchangeable Ca and Mg were increased and exchangeable Al decreased at all depth intervals of root plants. However, it is necessary to know not only minimum rates of lime for maximum crop production, but also what residual value of lime may be expected. Haynes and Mokolobate (2001) also suggested that the rise in soil pH varies depending on the type of residue, its rate of application and the buffering capacity of the soil. The comparing between lime and fused magnesium phosphate (FMP; a slow-release phosphate fertilizer) suggested that both of them increased yields of rice and peanut by reducing soil acidity and increasing P and Ca uptake. Particularly, FMP seemed to be more advantageous than lime because it is a source of P to support plant growth (Chang and Sung, 2004). Nakagawa *et al.* (2003) also suggested that phosphate application could ameliorate the toxicity of Al when it enters and account for the uninhibited shoot growth in presence of Al in rice plants.

The plant symptoms of severe Al toxicity in the field resembled Ca deficiency and that application of Ca as gypsum ($CaSO_4$) or lime ($CaCO_3$) alleviated Al stress (Delhaize and Ryan, 1995). Rengel (1992) suggested that the amelioration of Al toxicity by Ca^{2+} may be seen in Ca-efficient genotypes of various crop plants. With increasing external Ca and Mg supplies seem to protect the plant against Al toxicity by improving the Ca or Mg nutrition and by alleviating the toxic effect of Al on root development. Mg appeared to be more effective than Ca in alleviating Al toxicity with the monocots, whereas Ca is more effective for the dicots (Keltjens and Tan, 1993).

Although lime is used to reduce Al toxicity but it is often too expensive or impractical in many part of the tropics. Because lime leaching is very small, liming

affects only the top soil and does not remove Al toxicity in the subsoil (Adams, 1984). Moreover, application of high rates of lime is often detrimental to soil structure as well as to the availability of P and several micronutrients, especially zinc (Rao *et al.*, 1993).

Wood ash is a source of nutrient and liming agent for crop production in acid soils (Ohno, 1992). Wood ash was found to be a rich source of macronutrients: the concentration of K, Ca, Mg and P was ten times higher than in soil, as well as micronutrients (Górecka *et al.*, 2006). Park *et al.* (2004) suggested that wood ash application could be useful to counteract nutrient deficiencies and imbalances in soils, and to replace nutrients removed by harvesting. Plants growth on ash-amended soils showed higher biomass production than plants grown on lime and control (pH 4.8) treatments, and also reduced Al and Mn toxicity (Nkana *et al.*, 1998). The ash-induced pH increase of 0.6-1.0 pH units and exchangeable acidity decrease of 58-83% were detected after ash application (Saarsalmi *et al.*, 2001).

In developing countries, small farmers on upland fields have been cropped in slash-and-burn agriculture, particularly upland rice is an important crop which is often depressed by soil acidity (Gupta and O'toole, 1986). In shifting cultivation, burning of biomass releases nutrients previously stored in the forest biomass onto the soil surface and makes it available for the crops during cultivation period. Ash present in sparse to dense succeed in increasing the rice grain yield from 0.7 to 1.1 ton ha^{-1}. Applying fertilizers had about the same effect on grain and dry matter yield of rice as the ash application (Yimyam, 2006). However, the quantities of nutrient elements gained by the soil after burning depend not only upon the amount of each element in

the ash but also the capacity of the soil to retain and store these nutrient elements in forms that are readily available to the plant (Juo and Manu, 1996).

1.4.2 Selection for acid-soil or Al tolerant plants

Various techniques have been developed to evaluate the acid-soil or Al tolerance of germplasm, either in nutrient solutions, in greenhouse soil or in the fields. Each of these approaches has advantages and disadvantages.

Nutrient solution techniques

Nutrient solution techniques could be subdivided into short term (hours to days) and long term (several weeks). For short term screening, hematoxylin staining method is widely to identify Al tolerant and Al sensitive genotype. The hematoxylin dye forms complexes with Al on the root tips. As the intensity of staining increases, reflecting a higher level of Al uptake, the level of tolerance decreases (Hede *et al.*, 2000). Cançado *et al.* (1999) suggested that hematoxylin staining is an easier, rapid and more reliable method than any other known method for discerning among Al tolerant and Al sensitive tropical maize genotypes. This method was highly suitable for screening large segregating populations derived from improved germplasm of barley (Echart *et al.*, 2002) and rye (Hede *et al.*, 2002), etc.

The root growth in nutrient solution is another screening method for Al tolerance in longer period of times. The Al sensitivity of genotypes could be determined from root or shoot growth at two Al concentrations, one being a zero or low-Al level, at which near maximum growth is attained, and the other being a high-Al level, at which the growth of Al sensitive genotypes are seriously impaired, while

those of Al tolerant genotypes are little affected (Howeler, 1991). Therefore, it is important to choose the levels of Al concentration to be able to separate genotypes according to their Al tolerance. For example, the screening of rice germplasm is widely using 0 and 30 mg Al L^{-1} (Khatiwada *et al.*, 1996; Nguyen *et al.*, 2001). Moreover, seed age is also very important for plant and root vigor. Seed should be regenerated before evaluating for Al tolerance. This ensured that differences in root growth are not due to differences in vigor caused by seed age (Hede *et al.*, 2000).

Usually, Al tolerance parameter is considered from root growth under Al stress and relative root growth, root growth under Al stress compared to root growth without stress. Several studies demonstrated that relative root length (RRL) is more convenient parameter and better indicator of Al tolerance because it can eliminate genotype specific differences in root growth and standardize comparisons between genotypes (Khatiwada *et al.*, 1996; Hede *et al.*, 2000). Howeler and Cadavid (1976) suggested that RRL was also used as index of Al tolerance in rice cultivars, and RRL value corresponded to field screening of relative tolerance to acid soils.

The advantage of nutrient solution techniques is that they are fast, allowed the evaluation of many germplasm accessions in a short time. They are often non-destructive and can later be transplanted to the field to evaluate for other factors and to produce seed. Moreover, the concentration of Al, pH and other nutrients could be controlled. The disadvantages are that they measure only the initial response to the stress factor. This method does not take into account the effect of other soil factors, such as organic matter and mycorrhizal fungi. Therefore, the results in nutrient solution do not always correspond with those obtained in the field.

Field screening techniques

Since nutrient solutions require a very careful control and are not very representative of real acid soil condition, some researchers prefer to screen in Al-toxic soil in the fields. This evaluation is normally conducted in two soil condition, one in an un-amended and naturally Al-toxic soil and the other in lime-amended (Howeler, 1991). The ultimate and most direct method of evaluating for Al tolerance is by measuring economic yield. The zero or low lime level is chosen that growth and yield of Al sensitive varieties are markedly reduced while Al tolerant varieties are little affected. At the high level of lime all varieties should be free of Al toxicity symptoms and close to maximum yield (Hede *et al.*, 2000).

Field screening techniques have an advantage over nutrient solution in that they screen varieties under natural soil and climatic conditions. The final yield data measure the integrated effect of acidity over the entire growth cycle (Howeler, 1991). The disadvantage is that they take a long time (usually one growing season), require a lot of area and may be more cost. Moreover, they are not always successful because of other environmental factors such as problems of soil variability, confounding effects of differential resistances to diseases and pests, susceptible to environmental hazards such as drought and flooding (Howeler and Cadavid, 1976; Rao *et al.*, 1993). Therefore, plant growths or final yields may be limited because of factors other than Al toxicity.

1.5 Genetic variation for Al tolerance in rice

There are widely genotypic differences in rice tolerance to acid soil or Al-toxic conditions. Much of work on Al tolerance in rice focused on differentiation

among rice varieties. For examples, evaluating of 22 rice varieties in South India showed a wide range for Al tolerance, only two varieties were the most tolerant which performed extremely in the presence of Al (Sivaguru and Paliwal, 1993). The comparing between *japonica* and *indica* types suggested that *japonica* type was higher tolerance to Al than *indica* type (Ma *et al.*, 2002). In addition, tolerant rice genotypes belonged to upland and the sensitive ones to irrigated groups (de Freitas *et al.*, 2006). The screening among 62 local lowland rice varieties which originate from acid-sulphate soil areas of Asia and West Africa found that 11 varieties were higher levels of Al tolerance than improved tolerant check (Khatiwada *et al.*, 1996).

It is well known that local rice varieties are generally considered to be a rich source of useful traits. Those varieties are not uniformed, there are genetic variations not only between accessions within a variety but also between individuals within accession (Olufowote *et al.*, 1997). A case study in Thailand, a local variety name *Bue Chomee* which collected by different farmers in Northern Thailand had genetic variation in morphological characters and grain quality (Meesin, 2003). There was high genetic diversity in resistance to gall midge infestation between and within populations in local variety name *Muey Nawng* collected from 8 locations in Northern Thailand (Supamongkol, 2006). Pintasen *et al.* (2007) also suggested that variation in grain Fe concentration in local Thai rice was found between different seed lots bearing the same name and within individual seed lot as well as between varieties. However, difference in Al tolerance has not yet been detected in local rice varieties.

Besides visual characters, molecular markers are useful tool for revealing differences among accessions at DNA level. They provide a more direct, reliable and efficient technique for evaluating genetic diversity over selection based on phenotype

(Thanh *et al.*, 1999; Ni *et al.*, 2002). Meesin (2004) indicated that genetic variation within population of same local variety was detected by DNA analysis which may or may not show obvious variation in external appearance.

Several types of molecular markers are available for evaluating the genetic variation in rice. Microsatellites or simple sequence repeats (SSRs) which is the region of short, tandemly repeated DNA sequences that exhibit repeat units of less than 6 bp in length have been effectively applied to identify genetic variation between varieties (Panaud *et al.*, 1996; Zeng *et al.*, 2004) and between accessions within a variety (Olufowote *et al.*, 1997; Supamongkol, 2006). The genetic variation identified by microsatellites to be useful in evaluating upland rice accessions from Vietnam for drought tolerance related morphology (Thanh *et al.*, 1999).

As rice in Thailand is genetically diverse I expect the Thai rice germplasm, especially the part that are grown on highly acidic soils in the uplands, to be a rich source of Al tolerance. Findings from this study should explain how some farmers are able to grow upland rice successfully on soils with pH as low as 4. Varieties and genotypes most tolerant to Al identified should be useful for upland rice farmers on acid soils in Northern Thailand, and also for breeding programs with Al tolerance and adaptation to acid soils as a breeding objective.