CHAPTER 2

LITERATURE REVIEW

2.1 Distribution and causes of acid soil

Three thousand nine hundred and fifty million hectare or 30% of the ice-free land in the world is acidic (von Uexküll and Mutret, 1995). The percentage may appear not so high but when arable land for cultivation in the world is considered the problem is quite serious. From the FAO report (FAO, 1969) the world arable land for cultivation is around only 3,190 million hectare and about 2,500 million hectare is acidic. Acidic soils, which cover four-fifths of the world's arable land, are distribute in all continents around the world, from including Australasia, America, Asia, Africa, and Europe (Figure 2.1).

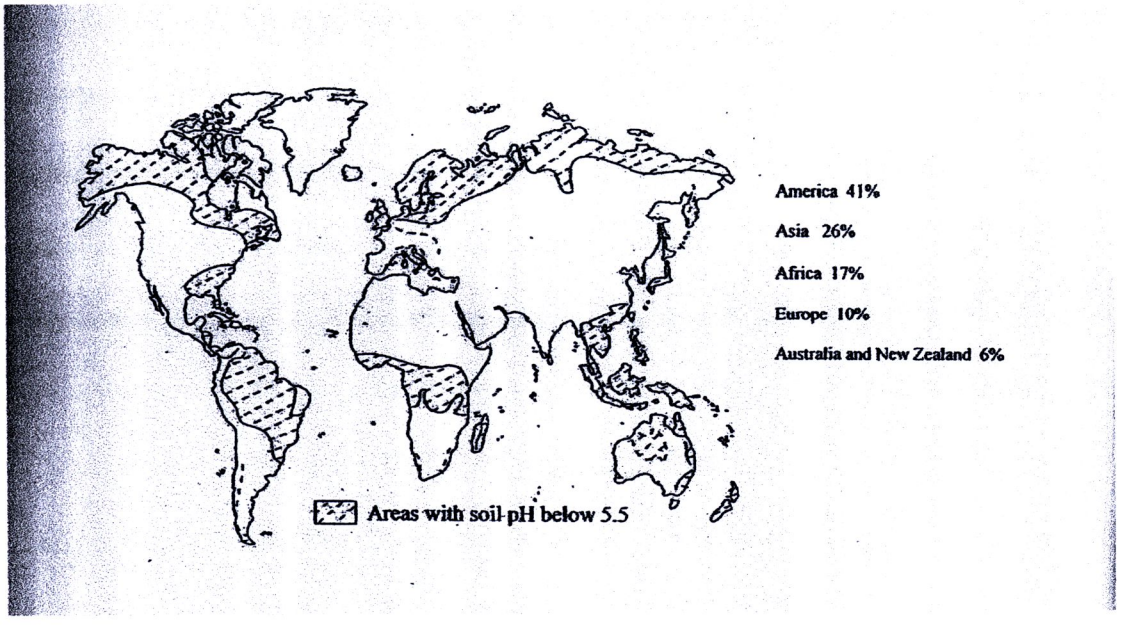

Figure 2.1 Distribution of acid soil in the world, with percentage of total land area with acidic soil in each continent (FAO,1991 cited by Uexkül and Mutert, 1995).

Soils become acid because leaching of non-acid metal cations (Ca^{2+}, Mg^{2+}, K^{+} and Na^{+}) and input of acidic cations (Al^{3+}, Mn^{2+}, H^{+}) from soil mineralization (Singer and Munns, 1987). Acid soils are usually found in high rainfall and free drained area because non-acid cations are easily leached from soil. Hydrogen ion and acid metal cations such as Al^{3+} are absorbed more strongly by soil colloids than non-acid metal cations (Ca^{2+}, Mg^{2+}, K^{+} and Na^{+}). Acid rain has been a concern as a cause of acid soil, but almost all rains are acidic (pH lower than 7) because of hydrolysis of carbon dioxide (CO_2) in atmosphere. Concentration of carbon dioxide in soil is a lot higher than in atmosphere because respiration of plant roots and soil organisms releases CO_2 as a byproduct. Hydrolysis of CO_2 has an acidification effect in the soil, as dissolution one molecule of CO_2 in water results in one molecule of carbonic acid and releasing 2 H^{+} atoms in to soil solution (Harter, 2002).

$$H_2O + CO_2 = H_2CO_3 = H^{+} + HCO_3^{-} = 2H^{+} + CO_3$$

Fertilizer application is another major cause of soil acidification. Ammonium is commonly used as nitrogen source in modern fertilizer. In normal healthy, well drained soils, ammonium ions are oxidized into nitrate in 2 steps of nitrification by 2 successive groups of soil bacteria, *Nitrosomonas* sp. and *Nitrobactor* sp.

$$2NH_4^{+} + 3O_2 = 2NO_2^{-} + 2H_2O + 4H^{+} + \text{energy (by } \textit{Nitrosomonas} \text{ sp.)}$$

$$2NO_2^{-} + O_2 = 2NO_3^{-} + \text{energy (by } \textit{Nitrobactor} \text{ sp.)}$$

Oxidation of 2 molecules of nitrate releases 4 hydrogen ions (Singer and Munns, 1987).

Monocalcium phosphate is commonly used as source of P in fertilizer. Hydrolysis of monocalcium phosphate gives dicalcium phosphate and phosphoric acid.

$$Ca(H_2PO_4)_2 + H_2O = CaHPO_4 + H_3PO_4$$

One molecule of phosphoric acid can dissociates up to 3 times as pH rising from 3 to 12 each time of dissociation phosphoric acid releasing a H^+.

$$H_3PO_4 = H^+ + H_2PO_4^- = 2H^+ + HPO_4^{2-} = 3H^+ + PO_4^{3-}$$

But the third H^+ will be released only in alkaline soil. However 2 H^+ of phosphoric acid can be released in acid soil range (pH rang 3 to 7) (Tan, 1993).

Every oxidation reaction can acidify soil because it gives proton as a byproduct. The most effective acidifying reaction is oxidation of pyrite (FeS_2) a sulfur mineral. The process is normally found in soils from mine spoils and mangrove reclamation areas. Oxidation of 2 molecules of pyrite produces 8 hydrogen ions and strongly decreases soil pH.

$$2FeS_2 + 6H_2O + 7O_2 = 4SO_4^{2-} + 8H_+ + 2Fe(OH)_2$$

The reaction not only produces hydrogen ion but also sulfur dioxide gas, which causes acid rain (Tan, 1993).

Decomposition of plant and animal residues is another cause of acid soil because the process give carbon dioxide as a by product. The final product of the process is organic matter and many forms of organic matter can acidify soil. The effect of this process depends on composition of plant and animal residues. Some residues contain a lot of organic acids. When they are decomposed the organic acids will be released and acidify the soil. Moreover in the decomposition process by microorganism the organism need some nonacid metal cations (Ca^{2+}, Ma^{2+}, K^+ and Na^+) as nutrients for their growth. If the residues contain insufficient amount of these nutrients for their growth, they must remove the cations from soil in the decomposition process and this immobilization of the cations can also make the soil more acidic (Harter, 2002).

Plant nutrient uptake is another cause of acid soil. To take up ions form soil solution across plasma membrane of root cell, plant uses electrical potential gradient from "*proton pump*" (Marschner, 1995) to drive this process. ATPase enzyme in plasma membrane of root cell will excretes H^+ from cytoplasm to apoplasm and create the electrical gradient between two sides of plasma membrane (Marschner,1995). The excretion of proton in to the apoplasm increases concentration of proton or hydrogen ion in soil solution, which acidifies the soil (Harter, 2002). Moreover plants normally take up non-acid cations such as Ca^{2+}, Mg^{2+}, K^+, NH_4^+ and Na^+ from soil in greater total amount than acid cations ($Fe^{2+}$and Mn^{2+}), so the balance of this greater removal of non-acid cations is another cause of soil acidification (Singer and Munns, 1987). Soil acidification is also affected by the form of combined N taken up by plants. Uptake of NH_4^+ tends to increase soil acidity uptake of NO_3^- tends to make the soil less acidic. Soil acidification through the imbalance of cation/anion uptake is a special effect of growing legumes, when acquiring N from the atmosphere by microbial N fixation and take up less nitrate than non-legume plants that must get their N supply from the soil as NO_3^-. The lower uptake of anions in legume causes higher H^+ excretion to apoplast for maintaining the electrical potential to drive nutrient uptake process (Harter, 2002).

2.2 Nutrient disorders in acid soil

High concentration of hydrogen ion (H^+) in acid soil can limit plant growth directly but it is not commonly a major problem in acid soil. Although Rohyadi et al (2004) found that H-ion toxicity can depress cowpeas growth at pH medium 4.7 (in sand culture), but growth of cowpea in soil is already depressed at soil pH 5.5. The

growth depression at pH 5.5 should be influenced by toxicity and deficiency of some elements that are influenced by H^+. When soil is acidified by any causes described above, soil still had buffering capacity to resist pH changing. At pH higher than 5.5 buffering capacity of soil to resist acidifying reaction depends on amount of available calcium (Ca) adsorbed on soil colloid.

$$\mathbf{Ca\text{-}Soil + 2H+ = 2H\text{-}Soil + Ca^{2+}}$$

But the amount of Ca would be depleted when soil pH is below 5.5. In this situation dissociation of aluminum (Al) hydroxide ($Al(OH)_3$) and iron (FeIII) hydroxide ($Fe(OH)_3$) will take over the role.

$$\mathbf{Al(OH)_3 + H^+ = Al(OH)_2^+ + H_2O}$$

$$\mathbf{Al(OH)_2^+ + H^+ = AlOH^{2+} + H_2O}$$

$$\mathbf{AlOH^{2+} + H^+ = Al^{3+} + H_2O}$$

$$\mathbf{Fe(OH)_3 + 3H^+ = Fe^{2+} + 3H_2O}$$

Dissociation a molecule of $Al(OH)_3$ or Fe(III) hydroxide consumes 3 hydrogen ions. This reaction is very effective to resist pH change comparing with acidification reaction (Harter 2002). Especially the $Al(OH)_3$ that soil have high reserve as solid form phase of normal part of every soil. But releasing of Al ion is a major problem of plant growth in acid soil. The solid from is not available for plant. When soil is acidified hydrogen ion dissociate the solid form of $Al(OH)_3$ to Al ion. This soluble form of Al can strongly impact plant growth (Harter 2002), directly and indirectly. The toxicity of Al ion its self normally show in inhibiting cell elongation and cell division in root. Inhibition of cell division in root apical meristems is a rapid response to Al toxicity. Aluminum may inhibit cell division by disturbing nucleic acid metabolism. Because nuclear DNA synthesis was inhibit after 4 hours by Al treating

(Sampson *et al.*, 1965). In Al toxic condition, double strands of DNA are captured by Al polymer and are unable to separate and function as a template for transcription. Consequently, cell division is block and elongation of root thereby inhibited (Matsumoto, 1991). Aluminum toxicity can affect many mechanisms at plant cellular level such as binding of Al to cell membranes which result in both function and structure alterations (Matsumoto, 1989). Because Al alters the activity of root meristem cells and cytokinin is plant hormone that stimulate cell division in shoot meristem and shoot morphogenesis (Pan *et al.*, 1989) that are synthesized in root meristem (Van Staden and Davey, 1979). Al could alter plant shoot morphogenesis by altering the rate of cytokinin supply to the site of action. Pan *et al.* (1989) found that Al toxicity inhibits shoot-lateral branching in soybean but the effect of Al on shoots branching could be removed by applying exogenous cytokinin. More over acid cation like Al^{3+} can depress availability of other nutrients. Because in acid soil Al and hydrogen ion will replaces another cation (Ca^{2+}, $Mg^{2+}$and K^{+}) in cation exchange sites of clay minerals. It the main factor responsible for leaching of Ca^{2+}, $Mg^{2+}$and K^{+} from soil. Moreover the Al^{3+} and H^{+} can restrict loading of Ca^{2+} and Mg^{2+} into apoplast. Apoplasmic loading of Ca^{2+} and Mg^{2+} strongly enhance uptake rate of these cations into the symplasm. In acid soil H^{+}, mono- and polyvalent Al species will compete with Ca^{2+} and Mg^{2+} to bind at cation exchange sites in apoplast. It is a cause of depression of Mg and Ca uptake and appearance of Mg and Ca deficiency (Marschner, 1991). Aluminum may also inhibit Ca uptake by blocking Ca^{+2} channel in plasma membrane (Huang *et al.*, 1992). And also inhibit Mg uptake by blocking binding sites of transport proteins in plasma membrane (Rengel and Robinson, 1989).

Bonding between phosphate ions and Al and Fe hydroxide is responsible for phosphate fixation in acid soil (Bolt, 1979). Therefore legumes crop that need high P supply have always been under P difficiency (Haynes and Ludecke, 1981; Hafner *et al.* 1992). the bonding between oxide of Al and Fe and phosphate ion make phosphorus becoming to low soluble from as variscite and strengite.

$Al(OH)3 + H_2PO_4^- = Al(OH)_2H_2PO_4 + 2OH^+$

(variscite)

$Fe(OH)3 + H_2PO_4^- = Fe(OH)_2H_2PO_4 + 2OH^+$

(strengite)

Because these reactions produce OH^- the reactions are speeded up when soil is more acidic. Therefore availability of phosphorus is very low in acid soil.

The Al and Fe are also causes for immobilization of molybdenum (Mo) in acid soil. An available form of Mo is molybdate ion (MoO_4^{2-}). At pH below 6.0, molybdenum's availability is rapidly diminished because Mo is easily "fixed" in the soil by free $Fe(OH)_3$, $Al(OH)_3$ and Fe_2O_3.

Furthermore the dissociation of molybdic acid wish is a weak acid in soil solution, is decreased when soil pH decreased.

$$MoO_4^{2-} \Rightarrow HMoO_4^- \Rightarrow H_2MoO_4$$

It is the cause to form plymolybdate (molybdate⇒tri-⇒hexa-molybdate) that are less available for plant uptake. Molybdenum plays important roles in N metabolism because it is a component of nitrogenase and nitrate reductase enzymes. Molybdenum deficiency symptoms especially legumes normally appear as N deficiency symptoms. Molybdenum deficiency is widespread in acid soil especially in legume (Marscher, 1995).

Manganese (Mn) toxicity is another factor affecting plant growth in acid soil. In non-acid soil most of Mn is in form of MnO_2 that is in solid from and low in solubility and thus low in potential toxicity. When soil pH is below 5.5 MnO_2 is reduced to become Mn^{2+}that is soluble from. The high Mn^{2+} concentration in soil solution causes toxicity of Mn in plant (Marscher,1995).

$$MnO_2 + 4H+ + 2e^- = Mn^{2+} + 2H_2O$$

Manganese toxicity symptoms normally appear in the shoot, contrasting with Al toxicity symptom that first occurs in roots. The brown speckle on mature leaf is an Mn toxicity symptom. In bean plants growing with excessive Mn supply, they had 'crinkle leaf' which is a symptom of Ca deficiency (Maracher, 1995). It mean Mn toxicity may relate with Ca deficiency. Excessive Mn induce Ca deficiency by enhance degradation of IAA. Because IAA plays an important role in plant Ca transport (Allan and Rubery, 1991). Excessive Mn can depress magnesium uptake by blocking binding sites of magnesium in plasma membrane of root cell (Le Bot *et al.*, 1990). Goss and Carvalho (1992) found that the major cause of depressing wheat growth in high Mn soil is Mg deficiency because the effect of excessive Mn on wheat growth can be alleviated by Mg supplying.

2.3 Acid soil constraints for legume growth and nitrogen fixation

Soil acidity limits legume growth and yield in many areas around the world. For example in Acrisols (Ustults) soil, Cambisol (Tropept) soil and Phaeozem (Ustoll) soil in the Southern Mexican State of Chiapas Mexico (original soil pH 4.6 to 5.0) Piedra and Munns (1990) reported that liming to increase soil pH for 0.4 to 1.3 units increased grain yield of common bean for 76 to 313 %. This lifting soil pH resulted in

40% greater shoot and 18% greater root dry weight, and also improved nodule weight per plant by 110% at early flowering. In a Hythe clay loam soil in Beaverlodge, Alberta, Canada (soil pH around 5), increasing soil pH to 6.3 by liming increased field pea's grain yield and above ground dry matter for 20 and 26% respectively (Arshad and Gill, 1996). Edwards *et al.* (1981) conducted experiment in acid Ultisol (Typic Paleudult) soil from southeastern Nigeria. They found that growing cowpea in this soil need liming to up soil pH from 4.25 to around 7. The liming increased cowpea dry matter yield for 500 kg/ha. Munns and Fox (1997) conducted an experiment in Wahiawa silty clay soil (Typic Eutrustox in the New Taxonomy, original pH 4.7) in north of Wahiawa on the island of Oahu. They tested the growth response of some tropical and temperate legumes to liming. They found that increasing soil pH by $CaCO_3$ application increased yield of all tested legumes especially *Coronilla varia* that very sensitive to acid soil. It need 16 tons lime/ha to lift soil pH from 4.7 to 6.9 for eliminating acid soil stress (get 90% maximum yield). In un-limed soil (pH 4.7) its yield was just around 13% of maximum yield (maximum was yield at pH 7.1). In Australia Peoples *et al.* (1995) studied the responding to lime and P application of subterranean clover growth and N fixation at Bungendore in N.S.W. They found that increasing soil pH form 4.2 to 4.9 by 2.5 tons/ha $CaCO_3$ application increased clover biomass yield for 45%. And applying 10 kg P/ha with 2.5 tons lime/ha increased N yield of cover for 200%. But liming or P application alone could not increase N yield. This indicates the interaction between soil pH and P level on N fixation. Munns (1965) tested the response to liming of Lucerne in several acid soils of pH 5.1-6 from A.C.T. and N.S.W. Australia. He found that applying 1 ton $CaCO_3$/acre to lift 0.7-1.1 soil pH unit increased Lucerne growth and nodulation in all

soil types. Cline and Kaul (1990) presented the effect of acidified N deficient soil (by adding S to Lowell silt loam soil: fine, mixed, mesic Typic Hapludalfs) on growth of soybean at Kentucky State University, showing that decreasing soil pH from 6.7 to 4.6 decreased shoot dry weight, nodule number and nodule dry weight by 80%.

Many more reports may be found along the line of those cited above on how soil acidity is a serious constraint for legume crops. However it is difficult to draw a general conclusion about the limiting factors in acid soil for legume growth, because of the complexity and variation amongst both nodule bacteria and the legumes in their tolerance to the complex of factors implicit in acid soil. Munns *et al.* (1981) concluded that soy bean was more sensitive to high Al level than N fixation but Alva *et al.* (1987) report in opposite way that the symbiosis is more sensitive to high level of Al. But Cline and Kaul (1990) reported that H-ion toxicity was probably the most limiting factor for nodulation of soybean in acid soil and effects of Al appeared less likely. From these evidences it is difficult to conclude what is the limiting factor for legume growth and what is the phase of symbiosis that is most sensitive. It depend on legume genotype, the rhizobium strain, interaction between plant and bacteria and condition of acid soil that legume confront. The adverse effect for plant growth in acid soil was described in 2.2. This section will look at the factors that are constraint to the symbiotic process in acid soil. Many factors in acid soil can depress N_2 fixing in legumes such as high concentration of proton and Al, low concentration of Ca, low availability of phosphorus and molybdenum. These factors effect N_2 fixation in various process including the growth of the host plant independently of N fixation, growth and multiplication of rhizobium in rhizosphere, root infection and nodule formation and function in the fixation of N_2 from the atmosphere.

The process of N fixing symbiotic development in legumes begins with survival and multiplication of rhizobium in rhizosphere prior to infection. Soil acidity can depresses growth and survival of soil bacteria such as cowpea rhizobia (Rerkasem, 1977) bean rhizobium (Aarons and Graham, 1991), Lotus rhizobia (Wood *et al.*, 1988). Depressing of growth and survival of rhizobium in soil may be caused by many factors such as proton and Al toxicity that often interact with Ca deficiency (Glenn and Dilworth, 1991). But Rerkasem (1977) found there was no effect of Ca application on growth of cowpea rhizobia in acid soil. Calcium deficiency may not affect the symbiosis by limiting growth of rhizobium in acid soil. Lowther and. Loneragan (1968) concluded that nodule initiation is the most sensitive process for Ca deficiency.

Nodulation of legume plants is a process that affected by soil acidity. The motility of bacteria is very sensitive to pH. Low pH in acid soil could limit motility of rhizobium in soil. Bowra and Dilworth (1981) found that the motility of *Rhizobium leguminosarum* bv *viciae* WU 235 was completely inhibited when pH below 5. Because the rhizobial motility is important for infection of rhizobia to legume root (Catlow *et al.*, 1990), soil acidity may restrict the infection process by inhibit bacterial motility. Moreover soil acidity not only effect bacterial mobility but also effect adsorption of rhizobia to legume root surface. Absorption of rhizobia to legume root surface is a very important process of legume and rhizobia symbiosis. The adsorption was depressed when soil pH decreased. This may relate with low Ca availability in acid soil because adding Ca into growth medium can enhance the adsorption (Caetano-Anollés et al,1989). Lowther and loneragan (1968) found that growth of nodule after nodule initiation was not affected by low concentration of Ca, it mean

just first step of infection is sensitive to low Ca supply. Marschner (1995) conclude that at low Ca concentrations, particularly in combination with high proton concentrations, the adsorption of rhizobia at host plant root surface is impair. Waluyo *et al.* (2004) demonstrated that Ca is important for the establishment of nodules, whilst P is essential for the development of the formed nodules.

Low phosphorus availability is one of important factors that constraint nitrogen fixation in acid soil (Haynes and Ludecke, 1981). Nodulation need high phosphorus supply. The requirement of phosphorus for nodulation might be higher than the requirement for root and shoot growth (Cassman *et al.*, 1980). The phosphorus concentration is normally higher in nodules than in root or shoot, especially in low external phosphorus supply condition (Hart, 1989). The N fixation process need energy source for bacteria growth and transform N_2 to NH_3. Plant photosynthesis produces high energy sugar. The translocation of photosynthate from leaves to roots and the movement of N containing compounds from nodules to other plant parts are vital to an efficient symbiotic system. Phosphorus is an integral part of the compounds needed to drive the system (Marschner, 1995). Phosphorus plays an important role as the energy storage in from of adenosine triphosphate (ATP). This compound transfer energy to fuel plant function such as N fixation. Sixteen molecules of (ATP) are converted to adenosine diphosphate (ADP) as each molecule of N_2 is reduced to NH_3. Therefore legume that realize on N_2 fixation need more phosphorus than the legumes applied with N fertilizer (Dadson and Acquaah, 1984).

Soil acidity decrease bioavailability of molybdenum and molybdenum play important roles in nitrogen metabolism in plant including N fixation (Marschner, 1995). Nitrogen fixation of legume plants in acid soil might be limited by molybdenum

deficiency. Molybdenum is a metal component of nitogenase enzyme. Therefore every N_2 fixing system requires high molybdenum supply. Nitrogen deficiency can be caused by molybdenum deficiency in legume plants relying on N_2 fixation especially in acid soil that molybdenum is low availability (Quaggio *et al.*, 2004).

The legume growth and symbiosis of nodule bacteria and legumes could be affected by many factors in acid soil that was descried above. But it is difficult to draw general conclusion, because of the variation amongst both the root nodule bacteria and legumes in their tolerance to complex adverts effects in acid soil.

The responses of legumes to soil acidity are different between species. Such as the experiment of Munns and Fox (1977), they compare lime requirements in 18 species of tropical and temperate legumes when they grown in acid soil (pH 5). The lime requirement of individual species varied. The species ranked as follows according to the amount of lime needed for 90% of the maximum attained yield : *Coronilla varia* (16 tons/ha) > *Leucaena leucocephala* (11) > *Phaseolus vulgaris, Medicago sativa* (9-10) > *Glycine max* var. Kanrich (7) > *Glycine wightii* var. Cooper, *Lotus corniculatus* (6) > *Glycine wightii* var. Tinaroo, *Tri/olium repens, Tri/olium subterraneum* (5) > *Desmodium canum, Dolichos axillaris, Glycine max* var. Kahala (4) > *Arachis hypogea, Desmodium intortum, Vigna sinensis* (1-2) > *Stylosanthes /ruticosa, Stylosanthes gu yanensis* (0.1). The experiment showed variation of acid soil tolerance between species. But in same species may have variation between cultivar such as the variation of acid soil tolerance for biomass production and seed yield between common bean genotype (Goenaga and Smith, 2002; Singh *et al.*,2003) root growth between soybean cultivars (Liao, et al 2006). The adverse factors for plant growth in acid soil are complicate and interact between factors. The variation of

Al tolerance between soybean genotype depends on P level in solution culture. The differential between Al tolerance and Al sensitive genotypes was most distinct when solution contain 80 μM P (inform KH2PO4), higher P level make all genotype tolerate to Al while lower P level reduce variation by making all genotype sensitive to Al (Liao, et al 2006). Plant species that can grow well in one acid stress condition may fail to growth in another acid condition because the limiting factors vary from one location to another (Marscher, 1991). So that the Al tolerance cowpea genotype that screened in nutrient solution may fail to adapt to acid soil that have another limiting factors more than only Al toxicity (Horst, 1985). Moreover in same acid soil the limiting factor may be different between legume species. Adrew (1976) found that applying Ca can alleviate acid stress in *Medicago sativa* while it was not effective for *Macroptilium lathyroides* and depress growth of *Stylosanthes humilis*. It means growth of *Medicago sativa* in this acid soil was depressed by Ca deficiency but growth of *M. lathyroides* and *S. humilis* was limited by another factor(s). Plant species may use different mechanism to cope the adverse effect in acid soil and may used more than one mechanism. For example Poolpipatana and Hue (1994) evaluate acidity tolerance of 4 green manure legumes. They found *Cajanus cajan* and *Sesbania aculeate* are more tolerant than *S. rostrata* and *S. speciosa* because *C. cajan* and *S. aculeate* uptake mush more Ca but much less Fe and Mn than the other species. Releasing some organic acid from root to complex with Al ion in soil solution, is a mechanism that plant use for cope acid soil stress (Marschner, 1991). The variation of root exudation between species may be the cause of variation in acid soil tolerance. Acid tolerance soybean cultivars excreta much more malate from tap root than sensitive cultivars when they were treated by Al (Liao *et al.*, 2006). The difference

architecture of root makes variation of acid tolerance between legume genotypes. Normally Al toxicity and P deficiency are stronger in deep soil (Marschner, 1991). Some soybean genotypes can grow in acid mineral soil because they have greater growth of shallow roots in upper soil horizon that have less effect of Al toxicity and P deficiency (Liao ey al., 2006; Zhao *et al.*, 2004).

Nitrogen acquisition of legume generally realize on the N fixation symbiosis. Therefore tolerance to acid soil of root nodule bacteria should be considered. There is a variation between nodule bacterial species in their tolerance to acid soil (Glenn and Dilworth, 1991). *Rhizobium loti* can multiply in acid medium (pH 4.5) but *Bradyrhizobium* strains failed to multiply (Cooper *et al.*, 1985). Barnet (1991) reported that *Rhizobium leguminosarum* bv *phaseoli* is much more tolerant to acid soil than *R. meliloti.* Loowendorf *et al.* (1981) tested survival of rhizobiums in acid soil. Population of commercial stain *Rhizobium meliloti* 411 distinctly declined at 21 days after inoculating in Mardin channery silt loam (pH 4.4) while *Bradyrhizobium sp. cowpea stain 13B* (isolated from cowpea in an acid soil pH 4.7) could maintain their population in the acid soil. The variation of acid tolerance is not only found between different species but al so within species of nodule bacteria. Howison *et al.* (1991) screened for acid soil tolerance in *Rhizobium meliloti* poppulation. Strain WSM688 that isolated from acid soil (pH 4.2) had more ability to nodulate and promote medicago growth in acid soil (pH4.7) than CC169 commercial stain. Moreover the tolerance to acid soil highly depends on many environment factors. The priority of acid tolerant between bacterial stain might be different in different acid conditions. Hartel and Alexander (1983) determined survival of 3 Rhizobium stains nodulating cowpeas in 4 different acid soils. In Windsor soil (pH 4.9), stain IRc80 growth faster

than stain IR190 and IRc130. But in Mardin soil (pH 4.7) stain IRc109 maintained their population in soil better than IRc80 which is better than IRc130. Although two soils had a litter bit different of pH but the result was so different. The authors suggested that this is the result of different exchangeable Al ion in the soil (0.93 meq/100g in Winsor soil and 1.78 meq/100g in Mardin soil).

Aluminum toxicity is always concerned as a main impact of rhizobium survival in acid soil (Wood *et al.* 1988; Rogers *et al.*, 2001; Alva *et al.*, 1990). Wood et al. (1988) test response to acidity and Al of fast (*Rhizobium loti*) and slow growing (*Bradyrhizobium* sp.) rhizobia.for the legume *Lotus*. In culture medium, the fast growing rhizobium was more tolerant to Al and acidity than slow growing. But when the two genera were inoculated to *Lotus pedunculatus* in acid soil (pH 4.1) the slow growing rhizobium was more effective in nodulating lotus than the fast growing rhizobium. This result indicated that the variation of acid tolerance might change when the acid condition change.

2.4 Role and benefit of Arbuscular mycorrhiza fungi (AMF) in legumes

Mycorrhizas are the most widespread association between fungus and higher plants. Mycorrhiza literally mean "fungus root" and the most common mycorrhiza association is arbuscular mycorrhiza which produces fungal structure in the cortex region of plant root. Arbuscular mycorrhiza fungi (AMF) association is found in most plant families (Powell and Bagyaraj, 1984). The fungus is strongly dependent on the higher plant, whereas the plant may or may not benefit (Marschner, 1995). The plants partner benefit from improving supply of mineral nutrition of low mobility in the soil solution, predominantly phosphorus especially in low fertility soil. Moreover AMF

association can enhance plant tolerance to heavy metal such as Al in acid soil (Kelly *et al.* 2005; Koslowsky and Beorner, 1989) and protect plant from soil born pathogen (Marschner, 1995).

It is normal for legume growing in both uncultivated and agricultural soil to form mycorrhizae. Many legumes are extensively colonized by mycorrhizal fungi and some of them are really need AMF under natural condition (Hayman, 1986). Legume requires adequate P for satisfactory nodulation and nitrogen fixation (Marschner, 1995). In low P soil, AMF are known to improve P acquisition of host plant. The benefit of mycorrhiza to enhancing P acquisition may fill the gap of high P requirement for nodulation and N_2 fixation in legumes. There are a lot of works that study about three ways symbiosis between AMF legume and nodule bacteria. Many of them presented mycorrhiza association enhance legume growth and nitrogen fixation (Badr el-din and Moawad, 1988; Rajapakse, 1989; Ganry *et al.* 1985). But a few experiment showed that in some condition AMF had no benefit to legume growth and N_2 fixing (Grant et al, 2005). These evident show that mycorrhiza do not always enhance legume growth and N_2 fixation.

The benefit of AMF in legume depends on many factors such as soil P concentration, soil pH, plant species or cultivar and AMF genotype. Phosphorus has strong effect on AMF effectiveness. In the three ways symbiosis plant has to spend photosynthate for root growth nodule bacteria and AMF (Hayman, 1986). The question is "is it worth to spend more photosynthate to mycorrhizal fungi for get more P by mycorrhiza symbiosis". Normally plants get benefit form AMF when P is the limiting factor. But when P is adequate for plant, AMF may have negative effect on plant growth (Peng *et al.*, 1993). In contrast very low P could depress the benefit of

mycorrhiza association too (Janos, 2007). Koide (1991) found that very high or low P can depress mycorrhiza colonization in plant root. The benefit of mycorrhiza depends on plant species. Researchers try to compare the benefit of mycorrhiza between plant species or genotype by some indicators such as mycorrhiza dependency (Menge *et al.*, 1978; Habte and Manjunath, 1991), Relative Field Mycorrhiza Dependency (RFMD: Linderman and Hen drix,1982), Mycorrhiza responsiveness (Janos, 1988), Mycorrhiza Dependence (Janos, 2007). These indexes were used in difference meaning. And the same indexes referred by difference authors, may be calculated from variety formulas. And the indexes are strongly depend on soil property especially soil P, the index value is changed when soil P change (Janos, 2007). So that, it have to been careful for comparing the benefit of mycorrhiza from difference papers. The effective of AMF association is not only influenced by plant genotype but also AMF genotype. Green (1983) reported that biomass of clover was increased when inoculated with *Glomus fasciculatus* but it was not affected when inoculated with *G. moseiae.* Soil pH is an important factor that determines survival and effectiveness of mycorrhizal fungi. Soil acidity restricted the variation of AMF spore (Porter *et al.*,1987) and hyphe growth (van Aarle *et al.*, 2002). And the effective of AMF may depend on soil pH. For example, in soil pH 6.2 *Gigaspora gigantean* and *Glomus mosseae* enhanced soybean growth but when pH was reduced to 5.1, *Glomus mosseae* lost their effectiveness only *Gigaspora gigantean* was still effective (Skipper and Smith, 1979).

2.5 Management of soil acidity for legume crop

2.5.1 Liming

The common solution to acid soil is adding agricultural lime such as $CaCO_3$ or more effective but more expensive lime like CaO and $Ca(OH)_2$. These limes normally react with CO2 in soil and convert to bicarbonate (HCO_3^-).

$CaCO_3 + H_2O + CO_2 = 2HCO_3^- + Ca^{2+}$

$Ca(OH)_2 + 2CO_2 = 2HCO_3^- + Ca^{2+}$

The hydrogen ions in soil are neutralized by the bicarbonate giving water and carbon dioxide. Soil pH will be lifted up by this reaction.

$HCO_3^- + H^+ = H_2O + CO_2$

The Ca^{2+} ions will repress Al^{3+} and H^+ at cation exchange side of soil colloid. Both Al^{3+} and H^+ will be released to soil solution

$$Al^{3+} + \boxed{\overset{H^+}{\text{Colloid}}} + 2Ca^{2+} = \boxed{\overset{2Ca^{2+}}{\text{Colloid}}} + H^+ + Al^{3+}$$

The Al^{3+} and H^+ in soil solution will react with the bicarbonate. Aluminum will be transformed to. $Al(OH)_3$

$Al^{3+} + H^+ + 4HCO_3^- = Al(OH)_3 + H_2O + 4CO_2$

Aluminum hydroxide is solid stage and unavailable for plant, so alleviating the toxic effect of Al.

2.5.2 Fertilizer application

As described before soil acidity causes nutrient disorders in soil for both the legume plant and nodule bacteria. Therefore applying nutrients is a choice to overcome acidity problem, but the first step is to identify the nutrient that is the limiting factor. In many acid soils P deficiency is the primary factor that limits legume growth (see 2.2.5). Therefore improving legume growth and N fixation by P application has been reported from many acid soils. In Katrine silt loam soil of New Zealand (pH 4.2) applying 350 kg P/ha (in form of $Ca(H_2PO_4)_2$) doubled biomass yield of lotus *(Lotus pedunculatus* Cav.). The P application can compensate 3,000 kg $Ca(OH)_2$ /ha to neutralize this soil (Haynes and Ludecke, 1981). Applying 16 kg P/ha as single super-phosphate to peanut growing in Psammentic Paleustalf acid soil (pH 4.9) of Niger increased biomass yield and pod yield for 22% and 13% respectively (Hafner *et al.* 1992). Low availability of Mo in acid soil is well known. Because Mo plays important role in N fixation process, the symptom of Mo deficiency normally show as N deficiency (see 2.2.6). Applying very small amounts of Mo to acid soil or coating legume seed with Mo fertilizer are efficient methods to improved N fixing and legume growth in many acid soils. In Brazil an application of 100 g Mo/ha to acid soil of pH 4.2 increased N concentration in peanut leaves by 22% and increased seed yield by 600 kg/ha, same results as an application 6 ton limestone /ha (Quaggio *et al.*, 2004). In acid soil pH 5.5 (in H_2O) of Eungella Queensland Australia coating tick clover seed *(Desmodium intortum)* with molybdenum trioxide (20g/kg seed) increased biomass yield nearly 20 times (Kerridge *et al.*, 1973).

2.5.3 Use acid tolerant legume cultivars and rhizobium strains

Acid tolerant legumes

Using tolerant cultivar is a common way to solve stress problem in crop production. Many works of screening for acid tolerance legumes were conducted in different regions. Edward *et al.* (1981) recommended TVu4557 cowpea line for growing in acid soil (Uitisol pH 4.25) in Onne, River State, Nigeria because this line can maintain high yield in the acid soil (77% of maximum yield). While TVu4552 a sensitive line produced only 47% of maximum yield in this soil. In brown sandy clay loam acid soil (pH 4.8) of Dunedoo in New South Wales, Australia, high in Mn (50 mg/kg) and Fe (Fe 53 mg/kg), Hunterfield and PL55 lucerne varieties performed well. They gave high yield and had long leaf duration in the acid soil with out liming (Grewal and Williams, 2001). Tolerant cultivars use different mechanisms to cope with adverse effects of soil acidity depending on the limiting factor in each location. Some tolerant genotypes produce large root surface area for better P uptake (Marschner, 1991). Velvet bean (*Mucuna pruriens*) avoids Al toxicity in sub soil by distributing root system in top soil (Hairiah *et al.*, 1993). Therefore the acid tolerant genotypes screened from one site might not be tolerance in another site that had different limiting factor. This is the limitation of using tolerance varieties. It is hard to breed a tolerance genotype which can tolerate many adverse factors in different acid soils.

Acid tolerant rhizobium

Many author reported that using acid tolerance rhizobium stain improving nodulation, N fixing and legume growth in acid soil. Humphries *et al.* (2009) recommended *Sinorhizobium meliloti* stain SRDI675 (isolate from soil pH 5.5 of Bookham New South Well) for inoculating to lucerne growing in acid condition. The ability to nodulate Lucerne of the stain SRDI675 was 10 and 2 folds higher than commercial stain in solution culture (pH4.8) and acid soil respectively. An acid tolerance *Bradyrhizobium* strains PSR011 screened by Appunu and Dhar (2006) successfully nodulated and improved N fixing of soybean growing in acid soil (pH 4.5) of Madhya Pradesh, India. Acid tolerance rhizobium normally isolate from acid soil (Loowendorf *et al.*, 1981, Howison *et al.*, 1991). But not all of rhizobiums that found in acid soil are suitable to improve legume growth in acid soil. Some stain can survive in acid condition but are not effective to nodulate legume root and fix N (Wright and Zeto, 1991). The problem of using tolerance rhizobium is the maintenance of the acid tolerance. The tolerance race screed in one acid condition might not effective in another acid condition (Hartel and Alexander, 1983).

2.5.4 Mycorrhizal symbiosis

There are many ways that AMF can reduce acid soil problem both in enhancing uptake of nutrient that deficient and reduce toxicity of some elements in acid soil. The most well know case is P uptake. As described before, P defficiency is a major limiting factor of legume growth in acid soil. Mycorrhiza is a well known

mutualistic between plant and fungi to improve plant nutrient acquisition especially for un-mobile nutrients in soil such as P. The small diameter and higher growth rate of hyphe comparing with plant root is the advantage point of AMF to uptake P (Marschner, 1995). For example biomass yield of sweet potato growing in acid soil (pH 4.2) was doubled by *Gigaspora margarita* inoculation because P status in sweet potato was improved by mycorrhiza (Yano and Takaki, 2005). Releasing of siderophore to soil solution is an effective mechanism to prevent uptake of toxic element and enhancing uptake of P. Mycorrhizal fungi normally release siderophores to soil solution (Haselwandter, 2008). The siderophore are chelating agent that coordinate covalent bond with acid cations such as Fe^{3+}, Mn^{2+} and Al^{3+} and makes these acid cations less active (Klugh-Stewart and Cumming, 2009; Hu and Boyer, 1996). This mechanism not only prevent phytotoxic of Fe^{3+}, Mn^{2+} and Al^{3+} (Marschner, 1991) but also lifts availability of P because it prevents bonding between phosphate ion (H_2PO4^-, $HPO4^{2-}$) and Al^{3+} that responsible for phosphate fixation in acid soil (see 2.2).

Reduce toxicity of acid cations like Mn^+, Fe^{2+} and Al^{3+} in acid by AMF was confirmed by many reports. Biomass yield of switchgrass *(Panicum virgatum)* growing in soil pH 4 was increased 17-fold by *Glomus etunicatum*. The *G. etunicatum* not only improve P status in switchgrass but also decreased the concentration of toxic elements such as Mn, Fe and Al in switchgrass tissue (Clark *et al.*, 1999). Biomass yield of *Vaccinium macrocarpon* growing in Mn rich solution (1 mg Mn/ml) was doubled by AMF inoculation. While shoot Mn concentration of inoculated plant was a half of un-inoculated plant (Hashem, 1995).

To reduce toxicity of some element AMF prevent uptake of these elements by several mechanisms. Availability of Al, Fe and Mn is reduced by siderophore releasing form infected root as describe before. Moreover as describe in 2.2 the reduction of Mn^{4+} to Mn^{2+} is the key factor of Mn toxicity in acid soil. AMF depresses availability of Mn in acid soil by decreasing amount of Mn reducer bacteria in rhizosphere and Mn-solubilizing excretion from host root (Posta *et al.*, 1994). Containing toxic ions in the AMF structure instead of transferring them to host plant is another mechanism to decrease phytotoxicity of acid cations. Cuenca *et al.* (2001) used a histochemical technique to determine the distribution of Al in inoculated root of *Clusia multiflora,* growing in acid soil. Most of Al in root was bond to AMF structure. The bounding of Al decreased Al toxicity in root showing in higher root growth of inoculated plant.

The performance of AMF association to alleviate acid soil stress varies between AMF species. Kelly *et al.* (2005) test effectiveness of 3 AMF species to alleviate acid stress (rich Al) in broomsedge (Andropogon virginicus) in sand culture. *Glomus clarum* was more effective than *Scutellospora heterogama* and *Acaulospora morrowiae* was the least effective species. But they found the variation between stains within each species. The stains that isolated from neutral soil normally less effective than stains from acid soil. And a study of Cuenca *et al.* (2001) confirmed the importain of stain source. They found that AMF isolated from acid soil have higher performance to stimulate *Clusia multiflora* growth in acid solution than the AMF from neutral soil.

Not only the source of stain that influence effectiveness of AMF but also the condition for maintaining AMF stain too. Malcova *et al.* (2003) test effectiveness of

Glomus sp. isolated from Mn rich acid soil but multiplied in 2 different conditions. One was multiplied in original soil and the other in non-acid stress condition. The last one lost it ability to heavily colonized maize root in Mn rich acid soil. These report indicated that variation of AMF effectiveness was caused by edaphic factors in place that they distribute or have been maintained.

From the above review of published literature, it could be concluded that there are many advert factors depressing legume growth in acid soil. To sole the problem the limiting factors have to be identified. And difference area of acid soil or legume genotype may have difference limiting factor. It is high possibility for using AMF association to sole acid soil problem but the succession may depends on soil condition, AMF and plant genotype. The suitable management need more information abut the effect of these factor and interaction between them on AMF effectiveness in acid soil.