CHAPTER II

LITERATURE REVIEW

2.1. The cause of iron (Fe) toxicity in rice

Iron toxicity is primarily caused by the toxic effect of excessive Fe uptake due to high Fe concentrations in the soil solution. Recently transplanted rice seedlings may be affected when large amounts of Fe^{2+} accumulate immediately after flooding. In later growth stages, excessive Fe^{2+} uptake due to increased root permeability and enhanced microbial Fe reduction in the rhizosphere affects rice plants. Excessive Fe uptake results in increased polyphenol oxidase activity, leading to the production of oxidized polyphenols, the cause of leaf bronzing. Large amounts of Fe in plants can give rise to the formation of oxygen radicals, which are highly phytotoxic and responsible for protein degradation and peroxidation of membrane lipids.

Oxidation of Fe^{2+} in the rhizosphere can help to lessen Fe stress in rice (Sahrawat 2000). Root oxidation power includes the excretion of O_2 (transported from the shoot to the root through aerenchyma) from roots and oxidation mediated by enzymes such as peroxidase or catalase. (Marschner, 1995).The of Fe^{3+} hydroxide in the rhizosphere of healthy roots is indicated by reddish brown coatings on the roots also prevents excessive Fe^{2+} uptake. In strongly reduced soils containing very large amounts of Fe, however, there may be insufficient oxygen at the root surface to oxidize Fe^{2+}. In such cases, Fe uptake is excessive and roots appear black because of the presence of Fe sulfide. An inadequate supply of nutrients (K, Si, P, Ca, and Mg) and excessive amounts of toxic substances (H_2S) reduce root oxidation power (Ottow

et al., 1993). Rice varieties differ in their ability to release O_2 from roots to oxidize Fe^{2+} in the rhizosphere and protect the plant from Fe toxicity (Yamanouchi et al., 1989). Fe stress tolerance may be due to the avoidance or tolerance of toxin accumulation (Gunawardena et al 1982). Another mechanism involves the retention of Fe in root tissue (oxidation of Fe^{2+} and precipitation as Fe^{3+}) (Sahrawat, 1979). Fe toxicity is related to multiple nutritional stresses, which leads to reduced root oxidation power.

2.2. Iron in the soil

2.2.1. Distribution of Fe in soils

Apart from the seasonal changes due to the rainfall pattern (rainfed rice) or drainage and irrigation (irrigated rice), changes in Fe^{2+} concentrations are found on a small scale horizontally in the soil profile and vertically between the bulk soil and the rhizosphere (Howler and Bouldin, 1971). Highest Fe^{2+} concentration is found at 2–15 cm soil depth, it can decline in deeper layers or below the plough pan where the soil contains less organic matter than in the puddled soil layer (Revsbech et al., 1980). Horizontal variations in Fe^{2+} are linked to the oxic rhizosphere soil, which is the result of oxygen release from rice roots (Yamanouchi et al., 1989). Its extent is determined by the formation of aerenchyma (oxidation power of the rice root) and the root density (Frenzel and Bosse, 1999).

2.2.2. Conditions for Fe reduction

Iron toxicity in rice plants is linked with amount of Fe^{2+} in the soil. The occurrence of its may vary with soil types, all-purpose characteristics shared through

most Fe-toxic soils are high amounts of reducible Fe, low pH, and transferable K content (Ottow et al., 1982). Moreover, these may be linked with P and Zn deficiency and H_2S toxicity (Kirk, 2004). Most importantly, Fe toxicity is linked to water logging and only occurs under anoxic soil conditions (Ponnamperuma et al., 1967). The paddy rice soils are subjected to periodic changes between oxic and anoxic conditions. Since oxygen diffuses in air about 10^3–10^4 times faster than in water or in water-saturated soils (Armstrong, 1979). Oxygen is used up quickly by the respiration of soil microorganisms and plant roots in waterlogged soils (Prade et al., 1990). With the reduction in oxygen, NO^{3-} , Mn^{4+}, Fe^{3+}, and SO_4 can proceed as electron acceptors for microbial respiration with the result in reduced condition of flooded rice soil.

The amount of extractable Fe^{2+} increases with the quantity of decomposable organic matter, temperature, and the amount of available redox buffers (Ponnamperuma et al., 1967). It is enhanced by a low initial soil pH, a sustained supply of organic matter (Prade, 1987), and the absence of compounds with a higher oxidation state than Fe (III)-oxide (Ponnamperuma, 1972). It increases with the duration of submergence, potentially reaching peak values 2–8 weeks after soil flooding (Swarup, 1988) and remains constant later (Sadana et al., 1995). Before the harvest of rice, fields are usually exhausted which results in a re-oxidation (detoxification) of Fe^{2+} to Fe^{3+} (Sahrawat, 1979). This re-oxidation has been shown to be either an enzymatic process, involving aerobic microorganisms (Emerson and Moyer, 1997; Blake et al., 1993) or an anaerobic microbial Fe^{2+} oxidation. In addition, phototrophic and nitrate-reducing bacteria can also be caused of the re-oxidation (Straub et al., 1996).

2.3. Iron in the plant

2.3.1. Function of Fe in plant

Iron is involved in energy production and use in the plant. It is important in many redox reactions. The function of Fe is in both heme and non-heme forms. About 9% of total leaf iron is found in leaves as heme Fe (Hevitt, 1983). Non-heme Fe proteins have about 19% of the total Fe, and they are found as ferredoxin, thylakoid complexes, mitochondrial complexes, aconitase, nitrate reductase and sulfide reductase (Hevitt, 1983). Most of the residue of the Fe is found as ferritin (35%). Thus, Fe protein contains about 63% of the total Fe in leaves. Fe is situated in the chloroplasts which are the site of photosynthesis. The remainder of a plant's Fe is dispersed in the cytoplasm and other organelles which contain supplementary heme and/or Fe sulfur proteins (Miller et al., 1995).

The most important physiological functions of Fe for all higher plants are biological redox system e.g., electron transport chains in photosynthesis and respiration (Halliwell and Gutteridge, 1986), catalyst for enzyme actions (Marschner, 1995), oxygen transporter in nitrogen fixation e.g., leghaemoglobin in bacteroids of legume roots (Werner et al., 1981) and the major metabolites of Fe are heme proteins (e.g., cytochromes, cytochrome oxides, catalane, peroxides, leg hemoglobin, nitrate reductase), Fe-S proteins (e.g., feedoxin, aconites), non-heme Fe proteins (e.g., lipoxygenase), aminolerulinic acid synthetase, coproporphyrinogen oxides, various peroxides (Romheld and Marschner, 1991).

2.3.2. Iron uptake and transportation in plant

Rice plants are susceptible to absorb more iron than most other plant species. Furthermore, the ferrous ion is quite rich in paddy soils. Because the reduced iron is easily absorbed, iron oxide (Fe^{3+}) uptake mechanisms are most likely less important in flooded environments (Mengel, 1972). After absorption in the root cortex, the reduced iron (Fe^{2+}) can make the xylem after its symplastic passage through the Casparian band. The greater portion, however, of the absorbed ferrous ions can reach the xylem directly through an apoplast. This route has been verified, mostly for sodium (Yeo *et al.*, 1987; Tsuchiya *et al.*, 1995).

Iron absorption and transport is not the same for cultivated plants under aerobic conditions (rainfed rice) or non-toxic conditions. In those cases iron transport is dominated by the ferric ion (Fe^{3+}) complexed by citrate (Clark *et al.*, 1973; Schmidt, 1999) or carbon hydrate peptidic components. When it reaches the apoplastic zone of the leaf, the iron gets back into the symplasm. In the cell, too much quantities of iron can catalyze the synthesis of components with an active oxygen base such as superoxide, hydroxyl radicals, and H_2O_2 (Marschner, 1995). These reactions are extremely amplified when the iron is abundant, and the iron itself, can be involved in highly reactant perferryl radicals (Halliwell and Gutteridge, 1984) or associated with fatty acids (Peterson, 1991). Free radicals are responsible for the damages caused by iron toxicity.

Transport of Fe in the phloem follows the transport of photosynthetic carbohydrates, which is directed from photosynthetic leaves to plant tissues where carbohydrates are either consumed or stored for later use. Phloem transportation of Fe depends on the capacity of phloem loading. Fe is loaded into the phloem when

complexed with nicotianamine. Phloem loading of Fe appears to be limited by the availability of this Fe-chelator rather than by the presence of Fe (Grusak, 1994). However, the supply of Fe to the growing tissues requires a continuous uptake by the roots. Remobilisation and transfer of Fe from well supplied older tissues to deficient growing tissues does not occur before senescence of the older tissue is induced (Marschner, 1995). Therefore, visible symptoms of Fe deficiency are first apparent in young growing leaves.

2.4. Iron toxicity in rice

2.4.1. Conditions enhancing iron toxicity

As Fe is reduced to potentially toxic Fe^{2+} in submerged soil, rice is unique among the major crop plants in that it can suffer from Fe toxicity. The severity of iron-toxicity in rice has been related to soil factors especially, the content and type of clay minerals. In addition, the amount of exchangeable soil Fe and soil pH. The concentration of soil Fe^{2+} is reportedly less in clay than in sandy soils (Das et al., 1997). Clay was found to control the content and release Fe in both Alfisols and Vertisols (Rajkumar et al., 1997). Clay content affected the Fe dynamics primarily using Fe retention on clay-mineral surfaces (cation-exchange capacity CEC), explanation why in iron-toxic lowland soils with kaolinite (low CEC) as main clay mineral. Therefore, toxicity symptoms in rice occur more often than in those having mainly chay mineral (high CEC) (Prade et al., 1990). High amounts of soluble Fe^{2+} (100–1000 mg L^{-1}) may be found in acid soils (Ponnamperuma, 1972). Fe concentrations can up to 5000 mg kg^{-1} in acid sulfate soils (Harmsen and Van Breemen, 1975). Iron toxicity symptoms also occur in near neutral soils (Tadano,

1976). However, it's not for all acid sulfate soils that rice expressed the symptoms of Fe toxicity (Tinh et al., 2000). Generally, the critical of soluble Fe in lowland rice is 300 mg water-soluble Fe L^{-1} (Lantin and Neue, 1989). Potentially toxic Fe^{2+} has been previously reported in a much wider range, from 20 to 2500 mg kg^{-1}. Such factors that influence Fe toxicity in plants may include the accumulation of hydrogen sulfide, organic acids (Tadano and Yoshida, 1978) and the availability of other nutrient elements (Ottow et al., 1982). Sulfide acts as a respiratory inhibitor and strongly affects root metabolism (Tanaka et al., 1966). Organic acids may apply their toxic effects by chelating Fe and thus increasing its plant availability (Marschner, 1995). As plants develop, the toxic effect of both respiratory inhibitors and organic acids decreases (Jaqc, 1977).

2.4.2. Symptoms of iron toxicity

The characteristic Fe toxicity symptom is the "bronzing" of the rice leaves, linked mainly to the accumulation of oxidized polyphenols (Mathias, 2005). The bronzing symptom of rice suffering from Fe toxicity develops in older leaves with the occurrence of tiny brown spots that spread from the leaf tip to the base. In further development of the symptom, the leaf tips become orange-yellow and dry up in some rice varieties. These symptoms are particularly well developed in older leaves having higher transpiration rates (Yamanouchi and Yoshida, 1981). Eventually, the entire transpiring leaf becomes orange to rusty brown, or purple brown when toxicity is extremely severe (Fairhurst and Witt, 2002).

These symptoms can occur at different growth stages and may affect rice at the seedling stage, during the vegetative growth, and at the early and late reproductive

stages. Depending on the growth stage leaf bronzing occurs, other symptoms and growth effects may be associated. In the case of toxicity occurring during seedling stage, the rice plants remain stunted with extremely limited tillering (Abraham and Pandey, 1989). Toxicity during the vegetative stages is associated with depressed plant height and dry-matter accumulation (Abu et al., 1989), with the shoot biomass being more affected than the root (Fageria, 1988). Both the tiller formation and those that develop into productive tillers can be severely depressed (Cheema et al., 1990). When Fe toxicity occurs during the late vegetative or early reproductive growth phases, it is associated with fewer panicles per hill (Singh et al., 1992), an increase in spikelet sterility (Virmani, 1977), and delayed flowering and maturity by up to 20–25 days. In highly susceptible cultivars, no flowering at all will occur (Ayotade, 1979). After booting, root growth stops, and the aerenchyma starts to senescence and decompose. As a result, the oxidation power of the root breaks down, and the root surface is coated with dark brown to black precipitates of $Fe(OH)_3$, and many roots die (Morel and Machado, 1981).

2.5 Management of iron (Fe) toxicity in rice

2.5.1 Land management

Carry out dry the soil after the rice harvest to improve Fe oxidation during the unplanted period. This reduces Fe^{2+} accumulation during the subsequent flooding period, but will require machinery (tractor). Preventive management strategies (see above) should be followed because treatment of Fe toxicity during crop growth is difficult. The following are options for treating Fe toxicity:

- Applying additional K, P, and Mg fertilizers.
- Incorporating lime in the topsoil to raise pH in acid soils.
- Incorporating about 100-200 kg MnO2 ha^{-1} in the topsoil to decrease Fe^{3+} reduction.
- Carrying out midseason drainage to remove accumulated Fe^{2+}. At the midtillering stage (25-30 d after planting/sowing), drain the field and keep it free of floodwater (but moist) for about 7-10 d to improve oxygen supply during tillering.

2.5.2 Fertilizer management

Iron toxicity is a complex nutrient disorder and the deficiencies of other nutrients, especially phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and zinc (Zn), are careful in the occurrence of iron toxicity in rice (Ottow et al 1983). Other nutrients may play an important role not only in reducing the effect of iron toxicity but also in the appearance of iron tolerance by different rice variety (Sahrawat et al 1996, Sahrawat 2004). Deficiencies of P, K, Ca, Mg, and manganese (Mn) decrease the iron-excluding power of rice roots and can as a result affect the rice plant's tolerance of iron toxicity(e.g., see Yoshida 1981, Sahrawat 2004). Deficiencies of Ca, Mg, and Mn are not normally observed in lowland rice, except for perhaps on acid sulfate soils; deficiencies of P, K, and Zn therefore deserve particular attention (Yoshida 1981).

Balance the use of fertilizers (NPK or NPK + lime) to avoid nutrient stress. Apply sufficient K fertilizer. Apply lime on acid soils. Do not apply excessive

amounts of organic matter (manure, straw) on soils containing large amounts of Fe and organic matter and where drainage is poor. Use urea (less acidifying) as an alternative of ammonium sulfate (more acidifying).

2.5.3 Possible interaction with zinc (Zn)

Iron toxicity is a common problem in rice cultivation and Zn deficiency in rice is often linked to this phenomenon (Montás Ramírez et al., 2002). If dissolved ferrous iron is high in the root zone, Fe plaques form at the root-soil interface and acts as an efficient adsorbent for Zn making it unavailable for the rice plant (Sajwan & Lindsay, 1986). These plaques are formed under anaerobic conditions with low redox values when the roots release oxygen into the rhizospere causing oxidation of Fe^{2+} to Fe^{3+} and therefore precipitation of Fe^{3+}-oxide or hydroxide. In general, the plaques are most extensive about 1 cm from the root tip and are detected as a thin orange-brown put down on the roots. The plaques might protect the plant from toxic levels of elements such as Cu and Ni but also reduce the uptake of nutrients such as Zn (Greipsson and Crowder, 1991). However, it has been shown that in Fe deficient rice plants an Fe plaque can increase the Zn uptake. This is because the rice plant under these conditions releases phytosiderophores, a type of amino acid, that mobilize Zn adsorbed to plaque and therefore enhancing Zn uptake.

Another possibility is the adverse effect of Zn deficiency on membrane transport. Zinc deficiency has been shown to increase root uptake of phosphate, and translocation of the phosphate to the shoot (Cakmak and Marschner, 1996). Permeability of the plasma membrane root cells to phosphate, chloride (Welch et al, 1982) and boron (Cakmak and Marschner, 1996) is increased by Zn deficiency. The

leaky membrane caused by Zn deficiency is therefore likely to enhance Fe accumulation.

2.5.4 Tolerant varieties

Rice varieties are different in their tolerance for iron toxicity and this lection of rice variety with better iron tolerance is an important component of research for reducing iron toxicity. Genetic differences in adaptation and tolerance for iron toxic soil conditions have certainly been broken for developing rice variety with tolerance for iron toxicity (Gunawardena et al 1982). Breeding and screening efforts at the International Rice Research Institute in the Philippines and at WARDA (West Africa Rice Development Association) in Côte d'Ivoire have identified a number of rice varieties for growing in iron-toxic soils (De Datta et al 1994, Sahrawat 2004). Sahrawat et al (1996) evaluated 20 lowland rice varieties for tolerance of iron toxicity at an iron-toxic site in Korhogo, Côte d'Ivoire, under irrigated conditions. The varieties differed in tolerance of iron toxicity. Grain yields varied from 0.10 to 5.04 t ha^{-1} and iron toxicity scores, based on the degree of bronzing symptoms on foliage, ranged from 2 to 9 (1 indicates normal growth and 9 indicates that most plants are dead or dying).

Work done at WARDA in West Africa showed that some *Oryza glaberrima* varieties, adapted to lowland rice-growing conditions, have a higher tolerance for iron toxicity than their *O. sativa* counterparts. Sahrawat and Sika (2002) conducted experiments at an iron-toxic site (Korhogo, Côte d'Ivoire) during the 2000 wet and dry seasons to evaluate the performance of promising *O. sativa* (CK 4, tolerant check; Bouake 189, susceptible check) and *O. glaberrima* (CG 14) variety. At the same time

as CK 4 and Bouake 189 showed typical iron toxicity symptoms in varying degrees, CG 14 plants did not show any iron toxicity symptoms at all as measured by iron toxicity scores. Although CG 14 did not give high grain yields because of its lower harvest index, lodging of the crop, especially under the application of nutrients, and shattering of seeds at maturity, it showed notable tolerance for iron toxicity. Research shows that CG 14 has a high tolerance for iron toxicity and remains an understandable choice as a contributor for iron tolerance in breeding programs (Sahrawat and Sika 2002, Sahrawat 2004).