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Physiological mechanisms of iron toxicity tolerance in lowland rice

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Tam Aung

Myanmar

Main supervisor	**PD Dr. F. Asch**
Co-supervisor	**Prof. Dr. M. Becker**
Chair person	**Prof. Dr. M. Janssens**

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This work is dedicated to my parents!

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Abstract

Iron toxicity is one of the most important abiotic stresses limiting rice production in lowland systems. Fe toxicity during the seedling stage widely affects the yield of lowland rice in West Africa and several Asian countries. The development of rice cultivars that tolerate high Fe(II) concentrations under a range of environmental conditions is lengthy and has been hindered by the lack of standardized screening methodology. To date screening tools for Fe-toxicity tolerance in rice are based on leaf symptoms and yield, but not on actual resistance mechanisms such as exclusion or tolerance. Thus, cultivars that reportedly showed Fe(II) tolerance frequently succumb to iron toxicity. Asch *et al*., 2005 had developed a mechanistic screening method (early vegetative stage) that allows the investigation of actual tolerance mechanisms. However, the rice roots are not accessible in this screening method and it is not possible to identify the Fe exclusion potential or retention power of rice roots. The first aim of this research was to develop artificial Fe-toxicity in the soil and validate this screening tool, aiming at adapting the method for field condition. Our second aim was to adapt this screening tool in a controlled growing environment (greenhouse) that allows accessing the rice roots.

A field trial was conducted in Myanmar and a set of pot experiments was carried out in the greenhouse of the Institute of Plant Nutrition, University of Bonn, Germany with the following objectives: (1) to develop artificial Fe toxicity in the field for screening rice cultivars for iron toxicity tolerance by applying iron (II) sulphate to the soil, (2) to determine the uptake and distribution of Fe in the plant for different rice cultivars under controlled growing conditions in order to identify the tolerance mechanisms, and (3) to screen some rice cultivars from Myanmar to identify their varying degree of sensitivity or tolerance to Fe toxicity.

In the field trial, leaf symptoms were observed neither in Fe-treated nor in control plots in any genotype. We found that to create Fe-toxicity artificially in the soil was not possible. Thus, screening for Fe-toxicity tolerance in rice cultivars at the early vegetative stage, this tool was not suited for uncontrolled field condition. In the pot experiment in the greenhouse, rice seedlings were grown in a plastic box filled with half- strength Yoshida nutrient solution. Four weeks after sowing, seedlings were subjected to full-strength Yoshida solution without Fe(II) as control, and 1000 and 1500 mg L^{-1} Fe(II) (applied as $FeSO_4$) as treatments. Nitrogen gas was infiltrated to the cultural solution through porous stones for 15 minutes in every two hours to prevent oxidation of Fe(II) to Fe(III). Reliable differentiation of cultivars was possible 3 days after exposure of the rice seedlings to Fe(II) 1500 mg L^{-1} based on visual determination of leaf symptoms, root Fe plaque, iron uptake and partitioning within plant tissues (chemical analysis,

AAS method). The amount of Fe formed as plaque at the root surface was approximately three times higher in all tested genotypes than the tissue-Fe concentrations of the rice cultivars. This indicates that the oxidation power of rice root plays an important role in genotypic resistance to Fe-toxicity as an avoidance mechanism. According to the investigation of root Fe plaque (as indicator of rhizospheric oxidation power), root and stem-tissue Fe concentrations (retention power), leaf-tissue Fe concentration and toxicity symptom expressions (as indicators for leaf tissue tolerance) in the tested genotypes, it can be concluded that the retention of Fe in root tissue seems to be rather more efficient than stem retention as an avoidance mechanism. Further researches are required to elucidate the leaf tissue tolerance mechanism.

List of Abbreviation

IRRI	International Rice Research Institute
CEC	Cation Exchange Capacity
POD	Peroxidase
SOD	Superoxide Dismutase
WARDA	West Africa Rice Development Association

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Chapter I. Introduction

Lowland rice is being cultivated on approximately 128 million hectares of irrigated and rainfed land (Maclean *et al*., 2002). As many as 100 million hectares show some sort of nutritional constraints to rice growth caused by either deficiencies or toxicities (Brady, 1982). Among toxicities, iron toxicity is well recognized as the most widely distributed nutritional disorder in lowland rice production (Dobermann and Fairhurst, 2000). On acid soils, it is one of the most important constraints to rice production, and together with Zinc deficiency, it is the most commonly observed micronutrient disorder in wetland rice. (Neue *et al*., 1998).

In Asia, most rainfed lowlands are situated in the warm sub-humid tropics (eastern India, Myanmar, Thailand) and the warm humid tropics (Laos, Cambodia, Vietnam, Bangladesh, Philippines, Indonesia, Sri Lanka, Malaysia). About 11 million ha of lowland rice areas are prone to temporary submergence (Huke and Huke, 1997). Further constraints arise from the widespread incidence of problem soils. Common soil constraints in rice-based lowlands include salinity/alkalinity (˜ 1.3 million ha), Fe toxicity (˜ 7 million ha), and acid sulfate soils (˜ 2 million ha) (Garrity *et al*., 1986; Akbar *et al*., 1987; Van Bremen and Pons, 1978). Acid sulfate rice soils are widespread in Vietnam, Thailand, Bangladesh, and Indonesia. Salinity and alkalinity often induce P and/or Zn deficiency. Soil acidity often occurs together with Al and Fe toxicity, and P deficiency. (Garrity *et al.,* 1986) estimated that in northeast Thailand, Laos, and Cambodia, about two-thirds of the rainfed area is characterized by soil acidity, widespread Fe toxicity, low CEC, and low soil N, P, and K reserves.

In West Africa Fe toxicity is widespread throughout the humid forest and Savanna zones in about 30 to 40% of all cultivated lowlands (WARDA, 1998) and there, rice yields are reportedly reduced by 12-100% depending on the severity of toxicity and the tolerance of the rice cultivars (Prade *et al*., 1982; Sahrawat *et al*., 1995; Audebert *et al*., 2000).

Flooded soils are characterized by periodic changes for aerobic and anaerobic conditions. Oxygen diffuses faster in air than in water or in water saturated soils (Armstrong, 1979). Hence it is rapidly depleted by the respiration of microorganisms and plant roots. In flooded soils, after the depletion of oxygen, NO_3^-, $Mn^{4+,}$ Fe^{3+}, So_4^{2-}, act as electron acceptor for facultative anaerobic microorganisms and are subsequently reduced. The reduction of O_2 and NO_3 takes place within a couple of hours and it is followed by the reduction of Mn^{4+} and Fe^{3+}, which can occur within a few days after flooding (Ponnamperuma, 1972). Therefore, shortly after inundation of the rice field, the reduction of Fe oxides and hydroxides can result in the massive accumulation of Fe(II) in the soil solution.

This process is particularly pronounced in inland swamps and irrigated lowlands that are characterized by light textured soils with high extractable acidity (Benkiser *et al*., 1982) and with low fertility, and in the absence of compounds with higher oxidation state. Typical for these characteristics are Oxisols and Ultisols, which dominate the landscape of humid and sub-humid regions. It also occurs when large amounts of ferrous iron are mobilized *in-situ* in soil solution or when interflow brings ferrous irons from adjacent slopes (Ponnamperuma, 1972; Sahrawat, 1979). A wide range of soil types can be iron-toxic, including acid sulfate soil (Tinh, 1999), acid clay soils (Alaily, 1998), peat soils (Deturck, 1994) and valley-bottom soils receiving interflow water from adjacent slopes (Sahrawat and Diatta, 1995). Fe toxicity occurrence is also linked on the one hand to low external input use and poor water control and on the other hand to specific soils (low CEC clays, acid alluvial, or acid sulphate soils). While there are no reports on Fe toxicity from Myanmar, the conditions and soils present indicate a high likelihood for Fe toxicity to occur in Myanmar (personal contact with Dr. Nyi Nyi, Land Use Division, Ministry of Agriculture and Irrigation). The Fe^{2+} concentrations in the soil solution that reportedly affect lowland-rice yield can range from 10 to >2000 mg L^{-1}. Iron-induced yield reduction is frequently associated with poor nutrient status of the soil (Benckiser *et al*., 1983) or with accumulation of respiration inhibitors (Tanaka *et al*., 1966). Hence, iron toxicity may be described as a multiple nutritional disorder hastened by, but also increasing conditions of P, K and Zn deficiency and H_2S toxicity (Ottow *et al*., 1982).

Iron toxicity is a nutrient disorder, which is brought about by the uptake of Fe(II) in the amounts that disrupts or over expresses a number of metabolic processes, resulting in damage of the rice plant. It is characterized by rusty leaf spots (bronzing) stained leaf edges and a dark brown rigid poorly developed root system. The expression of iron-toxicity symptom requires the excessive uptake of Fe^{2+} by roots and its acropetal translocation via xylem flow into the leaves. Inside the leaf, excess amounts of Fe^{2+} cause an elevated production of radicals, which can irreversible damage cell structural components (Thompson and Ledge, 1987) and lead to an accumulation of oxidized polyphenols (Yamauchi and Peng, 1993). The typical visual symptom associated with those processes is the ``bronzing´´ of the rice leaves (Howeler, 1973). Rice-yield losses associated with the appearance of bronzing symptoms commonly range from 15% to 30%. However, in the case of severe toxicity, complete crop failure can occur (Audebert and Sahrawat, 2000).

There is no clearly established relationship between the severity of Fe toxicity, symptom expressed and yield. These relationships may vary among crop developmental stages within the cropping season as well as among seasons and years. Average reported yield losses due to Fe

toxicity are in the range of 12% to 35% (Lantin and Neue, 1989). However, Fe-induced yield losses and bronzing were more pronounced in a dry-season as compare to wet-season crop (Sahrawat and Diatta, 1995), whereas, Fe-induced yield reductions of up to 30% have been reported to occur without any foliar symptoms (Abifarin, 1988). Generally, crop damage is largest when toxicity occurs at the seedling and early vegetative stages of rice, in the worst case leading to a complete failure of the crop (Abu *et al*., 1989: Abraham and Pandey, 1989).

For Fe toxicity to occur Fe(II) must pass the oxidation barrier of the rhizosphere before it can enter and being taken up by the plant. Rice roots diffuse molecular oxygen into the root medium through aerenchyma, which increases the redox potential of the rhizosphere over that of the bulk of the growing media (Ando, 1983). If Fe(II) is able to escape the oxidative barrier and to permeate into the roots, part is retained in the roots and the remainder may be translocated to the shoots via the xylem after having pass the barrier of the Casparian strip (Marschner, 1995). There are indications that an apoplasmic pH more than of 6.5 prevents influx of Fe(II) into the symplasm (Kosegarten *et al*., 1999) which may be one mechanism explaining a high tissue tolerance describes a high levels of iron detected in the leaf tissue.

A number of resistance strategies to Fe toxicity in rice have been proposed.

1) Exclusion of Fe^{2+} at the root level, thus avoiding Fe^{2+} damage to the shoot tissue via rhizospheric oxidation and root ion selectivity (Chen *et al*., 1980a, b),
2) Inclusion and avoidance via internal compartmentation of Fe^{2+}, e.g., apoplastic immobilization or preferential storage in old leaves or photosynthetically less active leaf sheath tissue (Tadano, 1975; Smith, 1984; Audebert and Sahrawat, 2000) and
3) Inclusion and tolerance via increased thresholds to elevated Fe^{2+} within leaf cells, probably through enzymatic detoxification in the symplast (Bienfait, 1985; Gupta *et al*., 1993; Thongbai and Goodmann, 2000).

Even though some knowledge to the mechanisms underlying those strategies exists, to date screening tools for Fe-toxicity tolerance in rice are exclusively based on leaf symptoms and yield (e.g., Gunawardena *et al*., 1982; Akbar *et al*., 1987; Mahadevappa *et al*., 1991) but not on actual resistance mechanisms such as exclusion or tolerance. This may partly be due to the fact that the physiological mechanisms proposed are difficult to investigate and thus are not suited for mass-screening methods (Asch *et al*., 2005). The main objective of this research is to investigate the uptake and compartmentation of Fe^{2+} within different tissues of rice plants among reported tolerant and sensitive cultivars, and some Myanmar cultivars to study the underlying proposed tolerance mechanisms.

Chapter II. Hypothesis and Objectives

Field screenings of rice for iron toxicity tolerance were mainly conducted on soils with naturally occurring iron toxicity. In some regions, the iron toxic soils are not easily accessible to conduct field screening (e.g., Myanmar). Therefore, we have tried to artificially induce iron toxic soil conditions in the field using iron (II) sulphate ($Fe_2SO_4 . 7H_2O$).

Rice cultivars that reportedly showed Fe(II) tolerance in Asia failed to produce reliable results in Africa and *vice versa.* The understanding of the mechanisms of the uptake and tissue tolerance of Fe(II) is necessary to improve the targeting of interventions and to develop physiology-based breeding tools. Varietal improvement combined with agronomic management is the best chance to improve Fe-toxicity control. Varietal improvement needs to be based on an improved understanding of (1) the genetic diversity within the existing rice germplasm and (2) the morphological and physiological mechanisms underlying Fe-tolerance. Therefore, the objectives of this research are:

(1) To develop artificial Fe toxicity conditions in the field for screening rice cultivars for iron toxicity tolerance by applying iron (II) sulphate to the soil.

(2) To determine the uptake and distribution of Fe in the plant for different cultivars under controlled growing condition in order to identify the tolerance mechanisms.

(3) To screen some rice cultivars from Myanmar to identify their varying degree of sensitivity or tolerance to Fe toxicity.

Chapter III. Literature Review

Iron toxicity is a nutrient disorder in plants which is brought about by the uptake of Fe^{2+} to concentrations exceeding 300 mg L^{-1} (Tanaka and Yoshida, 1972; Yamauchi and Peng, 1995) that disrupt or over-express a number of metabolic processes, resulting in damage of the rice plant (e.g., Bienfait, 1985; Bode *et al*., 1995). Commonly observed symptoms are rusty leaf spots (bronzing), stained leaf edges, and a dark brown and poorly developed root system (Dobermann and Fairhurst, 2000).

3.1 Iron in soil

3.1.1 Iron toxicity in relation to soil characteristics

The presence of iron in the soil may depend on their base rocks. Iron toxicity can occur when the parent rock material is rich in iron (Abifarin, 1990; Zhao, 1992). For example, Alfisols derived from igneous material have relatively higher amounts of total iron than those derived from granite (Rajkumar *et al*., 1997). The relation between iron (II) and iron (III), however, depends on soil texture and other environmental conditions.

Several studies conducted in Asia and Africa show that Fe toxicity is a major nutrient disorder of rice grown on acid sulfate soils, Ultisols and sandy soils with low CEC, moderate to high acidity and active Fe, and low to moderately high organic matter content (Sahrawat, 2004).

Van Bremen and Moormann (1978) suggested that Fe toxicity is common in the young acid sulfate soils (Sulfaquepts) but is rare on the older, more deeply developed acid sulfate soils (Sulfic Tropaquepts) which do not produce high levels of Fe(II) upon submergence. Iron toxicity in soils other than acid sulfate soils is often associated with other nutrient disorders.

At a very low pH (< 4.0), the physiochemical activity of the rice plant decreased drastically and this weakens the root functions (Tadano and Yoshida, 1978; Rorison, 1973). Additionally, under conditions described above (low pH) lower concentration of Fe in the soil solution may cause Fe toxicity.

It has been reported that the exchangeable Fe fraction in flooded soils was related to Fe toxicity in rice. Organic matter content in soils plays an important role in mobilizing Fe, cations such as K, Ca, Mg, Al, and Mn, which in turn influence the exchangeable Fe fraction [Fe/(Ca + Mg + Fe + Mn + Al)] which was found to be related to Fe toxicity. The sum of exchangeable cations was increased with organic C more than exchangeable Fe, and this resulted in lower exchangeable Fe fractions in most organic soils. Moreover, organic soils have large CEC and their adsorbed Fe

fraction remains relatively low. It was concluded that peaty soil (> 25% organic C) exhibited a lower Fe toxicity hazard than the mineral soils with intermediate organic content (10-25%) (Genon, *et al*., 1994).

3.1.2 Conditions for Fe reduction

Iron toxicity is a syndrome expressed in rice plants that is associated with an excess amount of Fe^{2+} in the soil (Becker and Asch, 2005). While it may occur in a wide range of soil types, general characteristics most Fe-toxic soils are high amounts of reducible Fe, low pH, and low CEC and exchangeable K content (Ottow *et al*., 1982). This may be associated 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). 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 depleted rapidly by respiration of soil microorganisms and plant roots in waterlogged soil (Prade *et al*., 1990). With the depletion in oxygen, NO_3^-, Mn^{4+}, Fe^{3+}, SO_4^{2-} can act as electron acceptors for microbial respiration and sequentially become reduced in a flooded rice field. Oxygen and nitrate are used within the first hours or days of inundation. The subsequent reduction of Mn^{4+} also occurs rapidly, since the Mn content of soils is usually low (Ponnamperuma, 1977). Within a few days after inundation or at a redox potential of <180-150 mV, the reduction of Fe^{3+} begins (Patrick and Reddy, 1978).

The amount of extractable Fe^{2+} increases with the quantity of decomposable organic matter, the 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; Patra and Mohany, 1994) and remains constant thereafter (Sadana *et al*., 1995). Before the harvest of rice, fields are usually drained 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, involving both phototrophic and nitrate-reducing bacteria (Straub *et al*., 1996). Other than that, a strictly chemical (non-enzymatic) oxidation can occur in the presence of molecular oxygen or Mn(IV) oxide and nitrite (Schwertmann, 1985).

3.1.3 Distribution of Fe in soils

Apart from the seasonal changes due to the rainfall pattern (rain-fed rice) or drainage and irrigation (irrigated rice), changes in Eh and 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). Three compartments have been differentiated and include a thin oxic surface layer, the reduced puddle bulk soil and the oxic rhizosphere soil and rhizoplane (Liesack *et al*., 2000). The horizontal distribution of Eh and reduced Fe in the profile has been described by Ratering and Schnell (2000). The extent of the oxic surface layer can vary between 2 and 10 mm and is partially determined by a nitrate-dependent microbial re-oxidation of Fe^{2+}. Highest Fe^{2+} concentrations are found at 2-15 cm soil depth.

They can again decline in deeper layers or below the plough pan where the soil contains less organic matter than in the puddle soil layer (Revsbech *et al*., 1980). Horizontal variations in Fe^{2+} are linked to the oxic rhizosphere soil, which is the result of oxygen releases 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).

While the rhizosphere of rice is a potential site of Fe^{2+} oxidation, it can also act as a site of Fe reduction (Prade, 1987). Facultative anaerobic chemo-organotrophic microorganisms (e.g., Geobacter, Pseudomonas, Clostridium, or Bacillus sp.) play a key role in the reduction and mobilization of Fe-oxides (Munch and Ottow, 1977; Trolldenier, 1988). Some fungi are also hypothesized to be capable of enzymatically reducing Fe(III)-oxides (Schwertmann, 1985). As the use of oxidized Fe as an alternative electron acceptor for respiration requires energy-rich electron donors (e.g., easily mineralizable organic root exudates), the abundance of iron-reducing microorganisms can be higher in the rhizosphere than in the bulk soil (Benckiser *et al*., 1983; Wang and Liu, 1992). However, processes of iron re-oxidation dominate over the Fe reduction in the rhizosphere of most indica-type rice, leading to a considerable Fe^{3+} accumulation and the formation of iron plague around rice roots (Kirk *et al*., 1990).

3.1.4 Conditions enhancing iron toxicity

The severity of iron-toxicity expression in rice has been related to a number of soil factors. These involve most prominently (1) the content and type of the clay minerals, (2) the amount of exchangeable soil Fe, (3) the soil pH, and (4) the presence of ``stress factors´´ (Becker and Asch, 2005). 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 distribution of iron in both Alfisols and Vertisols (Rajkumar *et al*., 1997). Clay content affected the Fe dynamics primarily via Fe retention on clay-mineral surfaces (cation-exchange capacity–CEC), explaining why an iron-toxic lowland soils with kaolinite (low CEC) as predominant clay mineral, toxicity symptoms in rice occur more frequently than in those having predominantly smectites (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). In acid sulfate soils, concentrations of up to 5000 mg kg^{-1} have been reported (Harmsen and Van Breemen, 1975). However, toxicity symptoms reportedly also occur in near neutral soils (Tadano, 1976) not in all acid sulfate soils rice expresses toxicity symptoms (Tinh *et al*., 2000) A soil solution concentration of 300 mg water-soluble Fe L^{-1} is generally considered the critical limit for the cultivation of lowland rice (Latin and Neue, 1989). However, depending on the site and the cultivars used, reported critical concentrations can range from 20 to 2500 mg kg^{-1}, indicating that factors other than pH and Fe concentration influence the occurrence of Fe toxicity symptoms. Such factors may include the accumulation of hydrogen sulfide and organic acids (Tadano and Yoshida, 1978) and the availability of (nutrient) elements (Ottow *et al*., 1982). Sulfide acts as a respiratory inhibitor and strongly affects root metabolism (Tanaka *et al*., 1966). Organic acids may exert their toxic effects by chelating iron and thus increasing its plant availability (Marschner, 1995). As plants develop, the toxic effect of both respiratory inhibitor and organic acids decreases (Jaqc, 1977). Ottow *et al* (1982) described iron toxicity as a multiple nutritional disorder, which also involves other (nutrient) elements. Their chemical availability can be affected as a result of iron reduction (P, Mo, Al), or their uptake by plants can be impaired as a result of iron accumulation in the rhizosphere (K, Zn, Mn).

In the process of the reduction of Fe(III)-oxides, essential nutrients such as P and to a lesser extent Mo can be released (Ponnamperuma, 1977), but the availability toxic aluminum may also increase (Fischer, 1983). Consequently, large quantities of Al^{3+} can also be taken up along with iron by the rice plant (Prade, 1987). The amount of water-soluble P in the soil is related to the reduction of iron (Navarro *et al*., 1988a; Krairapanond *et al*., 1993) and increases with declining Eh (Satawathananont *et al*., 1991). In the absence of mineral-fertilizer-P application, the P released from iron phosphates upon Fe^{3+} reduction is the dominant source of P for rice (Liao *et al*., 1994). There is also some evidence that P- and K-deficient plants exude elevated amounts of organic metabolites, thereby stimulating the microbial reduction of iron and the subsequent P release (Prade, 1987).

Apart from increasing the availability of P, the Fe^{2+} formed by the reduction of Fe oxides shows antagonistic interactions with other cationic nutrients, mainly manganese, zinc, and potassium. Consequently, with increasing Fe concentrations, Mn, Zn and K deficiencies are likely to occur (Fageria, 1988). Thus large amounts of reduced Fe adversely affected the uptake of Mn. *Vice versa*, Mn application can suppress iron desorption, delay the reduction of Fe oxides and subsequently reduce Fe uptake by rice (Tanaka and Navasero, 1966; Howeler, 1973). Iron oxides are known to have a strong zinc-binding capacity. When soils are flooded, Zn becomes available in the process of iron oxide reduction (Mandal *et al*., 1992). On the other hand, reduced Fe can also exert a direct antagonistic effect on Zn uptake (Sajwan and Lindsay, 1988). Furthermore, the plaque formation resulting from Fe re-oxidation around the rice root can reduce the concentration of soluble Zn in the rhizosphere by forming sparingly soluble $ZnFe_2O_4$ (Sajwan and Lindsay, 1988). When the Fe plaque exceeds 25 g (kg root dry weight)$^{-1}$, the plaque becomes a physical barrier for the uptake of Zn (Zhang *et al*., 1998) and of Mn (Wang and Shuman, 1994). Similar to Zn, the uptake of K is affected by excess Fe^{2+} in the soil solution (Jugsujinda and Patrick, 1993). A large body of literature supports the ameliorative effects fertilizer (mainly, P, K and Zn) application on the performance of lowland rice grown under iron toxic conditions. Given the large diversity of soil and environmental conditions that do affect the rate of reduction and the amount of Fe^{2+} in the soil and the time of occurrence and severity of Fe stress, a systematic categorization of iron-toxic environments is required to improve the targeting of intervention strategies (Becker and Asch, 2005).

Becker and Asch (2005) categorized iron–toxic environments into three clusters as described in the following table.

Table 1: **Characteristics of site and crop parameters of major iron toxic environments.**

	Cluster 1	Cluster 2	Cluster 3
Land form	Coastal plane, river deltas	Marches, highland swamps	Inland valley swamps
Soil	Young acid sulfate soil	Clayey Ulti- and Histosols	Sandy valley-bottom soil
Soil iron (mg Fe^{2+} l^{-1})	500–2500	300–900	200–600
Leaf tissue iron (mg (kg dry matter)$^{-1}$)	500–2000	300–800	100–1500
Cropping season (most symptoms)	Dry and wet season	Mainly dry season	Mainly wet season
Rice growth stage (most symptoms)	Seedling to maturity stages	Late vegetative to reproductive stages	Early to late vegetative stages
Spatial distribution of symptoms	Whole field	Isolated patches (organic matter, H_2S)	Valley fringe (zone of upwelling)
Maximum yield losses (%)	40–100	15–50	30–70
Country examples	Vietnam (MD), Liberia, Thailand	Philippines, Indonesia (Java), Burundi, Madagascar	Guinea, Madagascar, Ivory coast, Sri Lanka

Cluster 1 refers to acid sulfate soils, in which extremely large concentrations of Fe^{2+} in the soil solution arise as a result of the soils´ peculiar mineralogy. The soil Fe^{2+} can range from 500 to over 5000 mg kg^{-1}, and tissue concentrations are generally between 500 and 2000 mg kg^{-1}. The symptoms on plant can occur throughout the rice-growing period, and yield losses reportedly range from 40%–100%. Plants suffering from iron toxicity may cover large contiguous areas such as in the Mekong Delta in Vietnam or in the coastal plains of West Africa (e.g., Liberia, Sierra Leone, Senegal) and of Thailand. **Cluster 2** associated with more clayey acid, iron-rich soils in sediments derived from highly weathered soils. Iron concentrations of 300–1000mg kg^{-1} occur usually one month or more after transplanting, and tissue Fe concentrations range from 300–800 mg kg^{-1}. Symptoms on rice appear during the late vegetative growth stage, and yield

losses rarely exceed 30%. However yield losses of 35%–50% can occur when rice is grown during the dry season. In this situation, plants suffering from iron toxicity are found in localized spots within a landscape or a field (e.g., The Philippines, Java) and are frequently associated with an extremely low redox potential, relatively high organic matter content (e.g., peat soils in Burundi and Madagascar), and high concentrations of respiration inhibitors (e.g., H_2S). **Cluster 3** refers to poorly drained sandy soils in valleys receiving interflow water from adjacent slopes with highly weathered sediments. The lowland soils tend to be kaolinitic with a low CEC and little available P. The concentrations of Fe^{2+} range from 20 to 600 mg kg^{-1} and are largely restricted to the wet season (interflow coincides with the onset of rainy season). Leaf tissue Fe at harvest is highly variable, but often below the supposedly critical level of 300 mg L^{-1}.
Symptoms usually occur very early in the rice plant´ s development and are associated with the onset of interflow from the slopes. The occurrence of toxicity symptoms is patchy and localized in the zone of upwelling of the interflow in the valley fringe. As toxicity symptoms may be observed at relatively low soil Fe^{2+} concentration and are frequently associated with relatively low tissue Fe content, it has been hypothesized that interflow is mainly aggravating a low level of *in-situ* toxicity by mechanisms other than bringing in Fe^{2+}, possibly involving the depletion of nutrients and upsetting the rice plant ´s ability to exclude Fe^{2+} (Kirk, 2004). Yield losses can range from 30% to 70%. However, when the peak of iron interflow coincides with the transplanting of rice, severe toxicity in seedling leaves can result in complete crop failure. This situation is prototypic for the highland valleys in Madagascar and Sri Lanka as well as for much of the inland valley landscape in the humid zone of West Africa.

3.2 Iron in the plant

3.2.1 Uptake and transport of iron

The rice plant has a tendency of taking up iron than most other plants, and Fe^{2+} is the iron species prevailing in paddy field. Since Fe^{2+} is easily taken up, the uptake mechanism of Fe^{2+} is probably of less importance in flooded environments (Mengel, 1972). After uptake into the root cortex, the reduced Fe^{2+} can enter the xylem after symplastic passage through the Casparian strip. However, a large share of Fe^{2+} may enter the xylem directly via an apoplastic by pass (shown for sodium by Yeo *et al*., 1987; Tsuchiya *et al*., 1995; Asch, 1997) or after root injury resulting from the pulling and transplanting of the seedling. In the xylem, Fe^{2+} follows the transpiration stream in the acropetal long-distance transport. This is in contrast to the iron

transport in upland crops or plants that do not grow under iron-toxic conditions, where the long distance transport is dominated by Fe^{3+} complexes with citrate (Clark *et al*., 1973; Schmidt, 1999) or peptide carbohydrate compounds (Höfner, 1970). Reaching the leaf apoplastic space, Fe may re-enter the symplast. The exact mechanism by which Fe is taken up by leaf cells is not yet well understood. In rice, this uptake is likely to occur in the form of Fe^{2+}, being the physiologically active form of iron in the symplast (Machold *et al*., 1968).

Within the cell, excess amounts of Fe^{2+} may catalyze the generation of active oxygen species such as superoxide, hydroxyl-radical, and H_2O_2 as follows: $O_2 + Fe^{2+}$? $O^{2-} + Fe^{2+}$ or $H_2O_2 + Fe^{2+}$? $Fe^{3+} + OH? + OH°$ (Marschner, 1995). These reactions are greatly enhanced when iron is abundant, and iron itself can be part of highly reactive perferryl-radicals (Halliwell and Gutteridge, 1984), or in combination with fatty acids, Fe^{2+} can form peroxy fatty acids (Peterson, 1991). Free radicals are responsible for the damage caused by iron toxicity (Thongbai and Goodman, 2000). They irreversibly damage membrane lipids (Thompson and Legge, 1987), proteins (Chevrier *et al*., 1988; Miyao *et al*., 1995), and nucleic acids (Elstner, 1982) and affect the membrane charge (Vladimirov, 1980). The activity of phenol oxidases increases and oxidized polyphenols can accumulate (Yamauchi and Peng, 1993). Free radicals will eventually oxidize chlorophyll and subsequently lead to a decrease in chlorophyll content (Monteiro and Winterbourn, 1988).

3.2.2 Symptoms of iron toxicity

The typical visual symptom associated with above described processes (Section 3.2.1), and particularly with the accumulation of oxidized polyphenols, is the ``bronzing´´ of the rice leaves. The bronzing symptoms start in fully developed older source leaves with the occurrence of tiny brown spots that spread from the leaf tip to the base. In the further development of the symptom, the leaf tips become orange-yellow and dry up in some rice varieties. These symptoms are particularly developed in older leaves having higher transpiration rates (Yamanouchi and Yoshida, 1981). Eventually, the entire transpired 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 stage is associated

with reduced plant height and dry-matter accumulation (Abu *et al*., 1989), with the shoot being more affected by the root biomass (Fageria, 1988). Both the tiller formation and the share of productive tillers can be severely reduced (Cheema *et al*., 1990). When iron toxicity occurs during the late vegetative or early reproductive growth phases, it is associated with fewer panicles per hill (Singh *et al*., 1992), and 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 senesce and decay. 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).

There is some correlation between the severity of iron-toxicity symptom expression and yield. This relationship can vary within the cropping season as well as between seasons and years. Average reported yield losses due to iron toxicity are in the range of 12%- 35% (Lantin and Neue, 1989). However, toxicity at seedling early vegetative stages can strongly affect plant growth and result in a complete yield loss (Abifarin, 1988). Seasonal and inter-seasonal variation in the relationship between symptom expression and yield loss are mainly related to transpiration and differences in acropetal Fe translocation. Hence, bronzing symptoms and Fe uptake were increased by a high vapor-pressure deficit in a phytotron study (Kpongor *et al*., 2003), and iron-induced yield losses and leaf bronzing were more pronounced in a dry as compared to a wet season crop (Sahrawat and Diatta, 1995). On the other hand, yield reductions of up to 30% have also been reported to occur without any foliar symptoms (Abifarin, 1988).

3.3 Mechanisms for iron toxicity

Evidently, rice plants have developed morphological and physiological avoidance and/or tolerance mechanisms to cope with and survive adverse iron-toxic soil conditions and large amounts if iron in the plant. These mechanisms are important in the selection of tolerant or adapted rice genotypes. However, problems in selection of rice genotypes for tolerance to iron toxicity still relate to the inadequacy of knowledge on physiological mechanisms of tolerance (Gunawardena, 1982).

The relative importance of the avoidance mechanisms or tolerance mechanisms to iron (toxic) resistance depends on several factors. One important factor is the duration of iron toxic stress. For short-term stress situations tolerance mechanisms are probably adequate. For long-term

stress conditions tolerance alone may not be sufficient and the plant may have to adopt avoidance mechanisms. Another distinction can be made between plants which exclude iron through oxidation of the rhizosphere or exclusion mechanisms and those which include high amounts of iron and subsequently resort to enzymatic adaptation or inactivation mechanisms of iron in the plant tissues (Marschner, 1993). The following mechanisms have been found to be relevant in rice plants in coping with excess iron concentrations.

(1) Oxidation of iron at the root surface (Ando, 1983).
(2) Exclusion of iron at the root surface (Tadano, 1976).
(3) Retention of iron in the root tissue (Tadano, 1976).

Leaf tissue tolerance to excess amount of iron (Yamanouchi and Yoshida, 1981).

Three major types of adaptation strategies can be differentiated and comprise ``includer´´ and ``excluder´´ strategies as well as ``avoidance´´ and ``tolerance´´ mechanisms (Becker and Asch, 2005). Plants employing **strategy I** (exclusion/avoidance) exclude Fe^{2+} at the root level and hence avoid of Fe^{2+} damage to the shoot tissue (rhizospheric oxidation and root iron selectivity). With **strategy II** (inclusion/avoidance), Fe^{2+} is taken up into the rice root, but tissue damage may be avoided by either compartmentation (immobilization of active iron in ``dumping sites´´, e.g., old leaves of photosynthetically less active leaf sheath tissue) or exclusion from the symplast (immobilization in the leaf apoplast). **Strategy III** plants (inclusion/tolerance) actually tolerate elevated levels of $Fe^{2+}$ within leaf cells, probably via enzymatic ``detoxification´´ in the symplast. Whereas Fe^{2+} exclusion by oxidation in the rhizosphere and the detoxification of leaf cells well established Fe-tolerance mechanisms of rice, the other mechanisms are not yet well understood and to date are not considered in rice breeding or screening for iron tolerance (Becker and Asch, 2005).

Strategy Ia: Oxidation of iron at the root surface.

Fe^{2+} formed either *in situ* in the soil or *via* interflow, must first pass the oxidation barrier in the rhizosphere before being absorbed by the root. To establish this barrier, molecular oxygen is channelled from the atmosphere through the stems into the roots *via* a gas-conducting aerenchyma. This aerenchyma forms upon the establishment of anoxic conditions induced by an increased production of ethylene (Kawase, 1981). It involves the degeneration of cortex cells and the subsequent formation of large intercellular lumina (Ando, 1983). The aerenchyma may occupy 20%- 50% of the total root volume of flooded rice (Armstrong, 1979), whereby genotypic differences in the extent of aerenchyma formation appear to be correlated with the rate of ethylene formation in the root tissues (Blake and Reid, 1981). The rate of iron exclusion

by oxidation in the root zone *via* aerenchyma depends on the phonological stage of the rice plant and on the growth stage of the root system. Young secondary roots and root tips diffuse oxygen at the highest rates into the rhizosphere (Chen *et al*., 1980a). At these growing zones, large quantities of Fe^{2+} can be microbial or chemically oxidized, resulting in the formation and accumulation of immobile $Fe(OH)_3$ deposits or iron plaque. Iron plaque is calculated to amount to some 500 kg FeOOH ha^{-1} in a crop of the wetland rice cultivar ``Brazos´´, amounting to as much as 10% of the total root dry weight (Chen *et al*., 1980b). The formation of iron plaque on rice roots not only reducing the Fe^{2+} concentrations in the soil solution, but is also thought to form a physical barrier for further influx of reduced iron (Tanaka *et al*., 1966). Flooded/ethylene-induced aerenchyma formation starts in 2-4 week-old plants, and the oxidation power of the root is highest at the maximum tillering stage (Tadano, 1975). As root senesce, the aerenchyma starts to disintegrate, thus losing its capacity for gas transport, and little Fe oxidation occurs in the root zone after flowering stage of rice. Consequently, late-season Fe-toxicity symptoms in flag leaf of rice, grown in acid sulfate soil were primarily associated with a break down of the root oxidation power (Tinh, 1999). Given the importance of the oxidation power of the root in excluding iron, vigorous and early development as well as longevity of aerenchyma are desired traits for Fe tolerance (Jayawardena *et al*., 1977).

Strategy Ib: Root membrane selectivity

Reduced iron having passed in the oxidative barrier of the rhizosphere enters the root apoplast. In plants with an undamaged root system, xylem loading requires ions to pass the root cell membranes at the endodermis due to the Casparian strip. Reduced iron can be excluded at the root cell membranes (Tadano, 1976), explaining the Fe^{3+} deposition in the apoplast of root parenchymatic cells observed by Green and Etherington (1977). Xylem-sap analysis of 2-months-old rice plants indicated that up to 87% of the Fe reaching the root apoplast by mass flow was not detected in the xylem and must hence have been ``excluded´´ at the endodermal barrier between cortex and stele (Yamanouchi and Yoshida, 1981). There was no such reduction found at the seedling stage, and reduction levels remained low through the early growth stages of rice (Tadano and Yoshida, 1978). Iron exclusion by root-cell membrane is strongly affected by respiration inhibitors, indicating an active and probably energy-consuming metabolic process (Tadano, 1975). At concentrations larger than 50 mg Fe^{2+} L^{-1}, this reduction process is first impaired and eventually no longer detectable (Tadano, 1976). It is also affected by the rice plants´ nutritional status with deficiencies in Ca and K (Yamanouchi and Yoshida, 1981) and P (Yoshida, 1981) reducing the ability of root cells to exclude iron.

Strategy IIa: Retention in root and stem tissues.

The Fe^{2+} that has entered the xylem stream will follow the transpiration-driven acropetal long-distance transport. However, some of this iron may be immobilized and deposited at specific ``dumping sites´´ within the plant. Some ``metabolic inactive´´ Fe has been found inside the root tissue (Tanaka *et al*., 1966). The rice plants ability to retain iron inside the root reportedly decreases with plant age (Tadano, 1976). During the further acropetal transport, Fe^{2+} may be immobilized and deposited in stem/leaf tissues. Iron tolerant cultivars West Africa transported less iron from the roots into leaf blades, whereas the iron content of the leaf sheaths substantially increased (Audebert and Sahrawat, 2000), possibly involving both immobilization and re-oxidation of Fe^{2+}. This ``withdrawal´´ of active Fe may involve the formation of phytoferritin in the xylem and its subsequent storage in stem tissue (Seckbach, 1982; Smith, 1984). The efficiency of this process is likely to be determined by the rate of acropetal Fe^{2+} transport and may not be sufficient to prevent iron influx into the leaves under conditions of high transpiration such as during exponential growth phase of rice or under dry-season conditions when a high vapor-pressure deficit greatly enhances crop transpiration rates (Asch *et al*., 2000, 2003). The effectiveness may also decline when the storage capacity of stem tissues is saturated, which may occur towards the end of the growth cycle of rice, when the crop was continuously exposed large Fe(II) concentrations and no more leaf tissue is produced. There is, however, no empirical evidence of either genotypic variation or the dynamics of the process in time or during the growth cycle (Becker and Asch, 2005).

Strategy IIb: Retention in the apoplast of the leaf.

The apoplastic pH is hypothesized to largely determine the mobility of iron in leaves (Kosegarten *et al*., 1999). An acid apoplastic pH will favor the uptake of Fe^{2+}. A pH increases in the apoplast reduces the mobility of Fe and possibly favors Fe^{2+} oxidation (studies on grape wine by Mengel and Kosegarten, 2000 and by Nicolic and Römheld, 2001). This is associated with the formation of non-diffusible polymers that reduce the activity of a plasma-membrane-bond ferric-chelate reductase (Schmidt, 1999; Lucena, 2000) and involve specific polysaccharides in the cell wall (Yamauchi and Peng, 1995). In plants unable to regulate apoplastic pH or reduce the activity of Fe^{3+} reductase, an uncontrolled accumulation or influx of Fe^{2+} in the leaf can occur (Welch *et al*., 1993).

Strategy lll: Symplastic tissue tolerance.

Once it has entered the symplast, Fe^{2+} will catalyse the generation of active oxygen species and of various radicals (described in Section 3.2.1). Prevention of oxidation damage and detoxification of these radicals is vital in alleviating of the damage caused by Fe^{2+} and is responsible for tissue tolerance (Yamanouchi and Yoshida, 1981; Vose, 1983). Tolerance mechanisms may involve strong binding or incorporation of Fe^{2+} into symplastic structures that allow control oxidation/reduction reactions such as phytoferritin (Bienfait, 1985). The extent of phytoferritin formation has been hypothesized to be a possible protection mechanism against high concentrations of Fe^{2+} in the symplast (Landsberg, 1996). Radical scavengers such as cytosolic ascorbate (Hu *et al*., 1999) or glutathione (Thongbai and Goodmann, 2000) have also been shown to reduce oxidative stress as induced by Fe^{2+} (Larson, 1988; Thomüson and Legge, 1987). Much of the radical scavenging inside the leaf cell of rice, however, is associated with superoxide dismutase (SOD) isoenzymes. As zinc is a component of SOD, Zn application can reportedly increase the iron tolerance of rice while Zn deficiency can produce symptoms that resemble those of iron toxicity (Ottow *et al*., 1982). The activity of SOD results in the formation of hydrogen superoxide. While this H_2O_2 is a less active oxidizing agent than the radicals, it reduces the activity of SOD (Cakmak, 1988). Hence, to effectively prevent oxidative damage, H_2O_2 needs to be further detoxified by catalases and/or peroxidase (POD) (Gupta *et al*., 1993). The combined SOD-POD enzyme activity has been established to be largely responsible for preventing Fe^{2+}-induced oxidative stress in rice leaves. This mechanism may be of particular importance in situations of seedling toxicity (poorly developed aerenchyma) and especially when rice plants have been injured during transplanting and are exposed an uncontrolled influx of Fe^{2+} (Yamauchi and Peng, 1995). The genetic variability of SOD-POD activity in the available gene pool is largely unknown to date, it has not been use as a screening tool by rice breeders (Becker and Asch, 2005).

3.4 Varietal selection and screening for resistance to Fe toxicity

Among the most prominent strategies to address Fe toxicity at field level is the use of tolerant rice cultivars. Breeders have developed a wide array of cultivars with various degrees of adaptation, using both traditional breeding methods (Akbar *et al*., 1987; Gunawardena et al., 1982; Lou *et al*., 1997; Mahadevappa *et al*., 1991) and quantitative trait loci (QTL) analysis combined with marker-assisted breeding (Bennett, 2001; Wan et al., 2003a, b; Wissuwa, 2005). Segregating populations or target genotypes were subsequently subjected to usually non-defined Fe toxic environments both in the greenhouse and in the field for cultivar screening (Becker and

Asch, 2005). Thus, more than 100 cultivars tested in farmers´ fields in Philippines out-yielded farmers´ traditional variety by an average of 1.9 Mg ha^{-1} (Neue *et al*, 1998). Greenhouse trials conducted in the Philippines, identified 479 out of 6140 tested rice cultivars to be ``relatively tolerant´´ to excess Fe in soil (Lantin and Neue, 1989). In West Africa, some 20 cultivars out-yielded the highest farmers varieties under Fe-toxic conditions in two inland valleys of Ivory Coast (WARDA, 1995; 2002). Based on tissue iron concentrations of >300 mg kg^{-1}, the majority of these cultivars were hypothesized to employ tolerance rather than avoidance or exclusion mechanisms (Yamanouchi and Yoshida, 1981).

However, a high concentration of Fe in the aboveground plant biomass without the expression of the typical damage symptom (bronzing) does not necessarily indicate symplastic tolerance. It cannot be excluded that such cultivars may have exhibited an efficient mechanism of symplastic exclusion or of stem/leaf sheath retention (Becker and Asch, 2005). In addition, cultivars that showed high tolerance to Fe^{2+} in greenhouse trials or in field environments, failed to express the same tolerance level in another environment (WARDA, 1993). The available QTL populations are all based on crosses with the stress adapted cultivars ``Niponbare´´ (Bennett, 2001) or ``Azucena´´ (Wu *et al*., 2003), and the sequencing of the genes associated with stress tolerance is on going (Wissuwa, 2001). However, the projected marker-assisted breeding for Fe-toxicity tolerance is unlikely to advance progress in the development of rice varieties suited to the diverse Fe-toxic environments as long as the mechanisms by which the supposedly tolerant parent cultivars cope with elevated Fe^{2+} concentrations are unknown (Becker and Asch, 2005).

Chapter IV. Materials and Methods

A field trial was conducted in the rice field of Yezin Agricultural University, Myanmar between June and July, 2005 to identify the varying degree of sensitivity and tolerance mechanisms of Myanmar rice cultivars to iron toxicity. Additionally, to validate the results from the field trial a set of pot experiments was carried out in the greenhouse of the Institute of Plant Nutrition at the University of Bonn between September and November 2005.

4.1 Field trial

4.1.1 Materials, experimental design and field layout

We used three check cultivars, *ITA 320* (tolerant), *Sahel 108* (tolerant) and *IR 31785-58-1-2-3-3* (sensitive) provided by the Institute of Plant Nutrition, University of Bonn and 7 cultivars of Myanmar obtained from Rice Division, Department of Agricultural Research, Ministry of Agriculture and Irrigation, Myanmar. These cultivars are listed regarding to their varying degree of sensitivity to excess amount of reduced iron (Fe^{2+}) in Table 2.

Table 2: Rice cultivars used in the experiment classified according to their sensitivity towards iron toxicity.

Unknown	Fe tolerant	Fe sensitive
Imma Yebaw	*ITA 320*	*IR 31785-58-1-2-3-3*
Manaw Thukha	*Sahel 108*	
Pawsan Baykya		
Shwethwe Yin		
Shwewar Tun		
Thukha Yin		
Yebaw Lat		

We used 2*10 factorial split plot design, two iron concentration levels as main plot treatments, 10 cultivars as sub-plot treatments and 3 replications.

Experimental design: 2x10 factorial split plot design.

Replication : 3

Main plots : 2 (2 Fe levels- 0 and 1000 mg L^{-1})

Sub-plots : 20 (10 cultivars* 2 main plots)

Planting density : 10 x10 cm

Sub-plot spacing : 20 cm

Code numbers of cultivars used in this experiment:

Yebaw Lat = C1; *Manaw Thukha* = C2*; IR 31875*-58-1-2-3-3 = C3, *Thukha Yin* = C4

Shwethwe Yin = C5; *Pawsan Baykya* = C6; *ITA 320* = C7; *Shwewar Tun* = C8;

Imma Yebaw = C9; *Sahel* 108 =C10.

Each step of random assignment of field layout is described in Figure 1, 2 and 3.

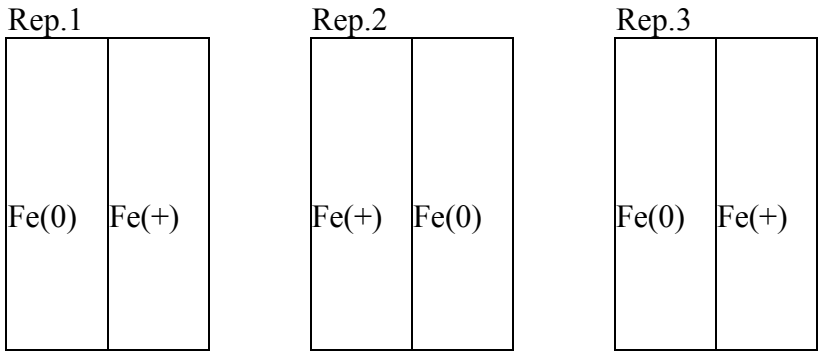

Figure 1: Random assignment of 2 iron levels (0 and 1000 mg Fe(II) L^{-1}) in three replications.

Rep.1		**Rep.2**		**Rep.3**	
Fe(0)	**Fe(+)**	**Fe(+)**	**Fe(0)**	**Fe(0)**	**Fe(+)**
C3	C9	C8	C2	C8	C6
C8	C4	C6	C1	C2	C3
C4	C2	C10	C9	C1	C1
C5	C10	C3	C10	C7	C7
C1	C5	C7	C4	C5	C9
C10	C3	C9	C3	C4	C2
C2	C8	C1	C7	C3	C4
C9	C7	C5	C8	C6	C10
C6	C6	C2	C6	C9	C5
C7	C10	C4	C5	C10	C8

Figure 2: **Random assignment of 10 cultivars in each main plot in three replications.**

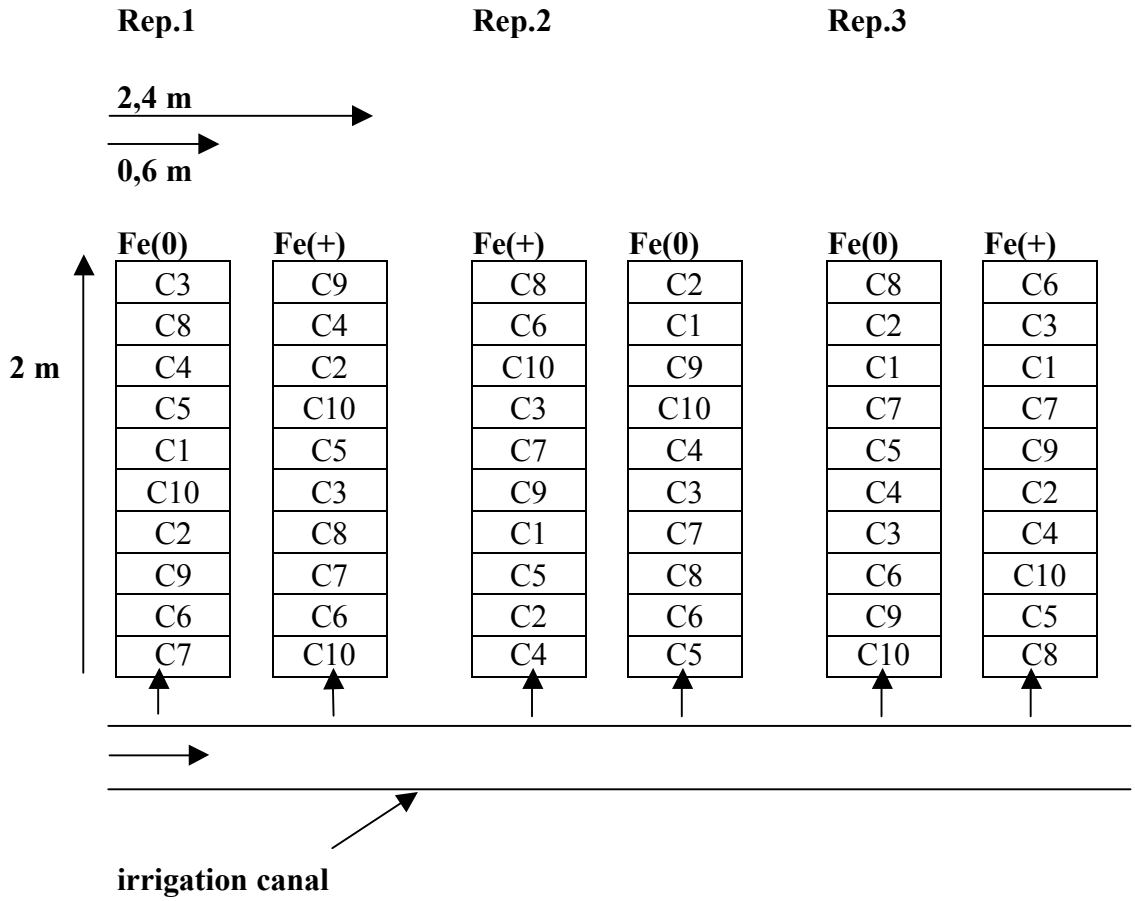

Figure 3: **Field layout of the 2 x 10 factorial experiment, including 10 cultivars (C1 to C10) and 2 iron levels (0 and 1000 mg Fe(II) L^{-1}) arranged in a split plot design with cultivars as the sub-plot treatments in three replications.**

4.1.2 Soil preparation, planting and treatment application

The following procedures were carried out to obtain the growing condition according to field layout.

- The soil was dug out to 20 cm depth for all main plots and puddled.
- Plastic sheets were placed in each main plot to prevent Fe(II) losses through percolation and interflow.
- Puddled soil was re-filled to the level of 5 cm and $FeSO_4 . 7H_2O$ (1.7 kg for each treated main plot) was applied to evenly induce at a concentration of 1000 mg L^{-1} Fe (II). Then, we re-filled the soil up to level and applied N, P and K fertilizer (200, 100, 100 kg ha^{-1}, respectively) as basal application.

- All the main plots were irrigated and kept under submerged condition to avoid oxidation of Fe(II) to Fe(III).
- Seeds of 10 cultivars were soaked for 48 hours and sown (direct seeding) in the plots according to the field layout.

Figure 4: The experimental set-up in the field of Yezin Agricultural University, Myanmar

4.1.3 Leaf scoring and sampling

Four weeks after sowing, iron-toxicity responses were scored by subjective visual assessment of Fe-toxicity symptoms on fully developed leaves (bronzing symptoms) for the entire plant and expressed as percentage leaf area affected. As scoring system, the Standard Evaluation System for leaf blast (*Pyricularia oryzae*) lesion scoring provided by the International Network for Genetic Evaluation of Rice (INGER; IRRI, 1996) was adopted for Fe toxicity as follows (percentage leaf area affected = score) (Table 2). Thereafter, we sampled three plants (above-ground biomass) of each cultivar from the sub-plots. Five weeks after sowing, leaf symptoms were scored and the remaining three plants of each cultivar were sampled.

4.1.4 Dry weight and Fe content in plant tissues

At each sampling, leaves were scored for Fe-toxicity symptoms. The above ground biomass (leaf and stem separately) of all cultivars was harvested to determine dry matter accumulation and Fe content in tissues. Samples were oven-dried at 70°C (48 hours) to constant weight, weighed and analyzed for Fe content by atomic-absorption spectrometry (Perkin-ELMER AAS 1100B, Überlingen, Germany), following a hot pressure digestion with concentrated nitric acid (HNO_3) solution (4 ml for each sample) at 180°C for 7 hours and a subsequent filtering and standard dilution to 100ml.

4.2 Pot experiment

4.2.1 Growing condition and plant materials

A set of pot experiments was conducted under climate-controlled conditions in the greenhouse of Institute of Plant Nutrition at the University of Bonn, Germany between September and November 2005. The climatic conditions in the greenhouse were adjusted to a constant temperature of 26°C, average relative humidity of 60%, 14-hours dark phase and a light intensity of 300 mmol m^{-2} s^{-1} for 10 hours. We used the same 10 cultivars that were used in the field trial. These cultivars are listed in Table 2.

4.2.2 Growth media, plant culture and experimental set-up

Rice plants were cultivated in Yoshida medium (hydroponic culture) developed specifically for rice (Yoshida, 1976). It contained in full strength 40 mg L^{-1} N (as NH_4NO_3), 10 mg L^{-1} P (as $NaH_2PO_4 . 2H_2O$), 40 mg L^{-1} K (as K_2SO_4), 40 mg L^{-1} Ca (as $CaCL_2$), 0.5 mg L^{-1} Mg (as $MgSO_4 . 7H_2O$), 0.05 mg L^{-1} Mn (as $MnCL_2 . 4H_2O$), 0.05 mg L^{-1} Mo (as $(NH_4)_6 . MO_7O_{24} . 4H_2O$), 0.2 mg L^{-1} B (as H_3BO_3), 0.01 mg L^{-1} Zn (as $ZnSO_4 . 7H_2O$), 0.01 mg L^{-1} Cu (as $CuSO_4 . 5H_2O$), 2 mg L^{-1} Fe (as $FeCL_3 . 6H_2O$ [in mono hydrate citric acid]) and buffer adjusted to pH 5.

PVC pipes (3.6 cm in diameter) were cut in 9 cm to length. Such 60 cut pipes were tied (6x10) for one box (6.5 L, 37 cm in length, 26.5 cm in width). A set of tied PVC pipes was inserted in each box. Rice seeds were soaked with water, incubated and pre-germinated on filter paper in petri dishes until the seeds were sprouting (72 hours). Sprouted seeds were transferred into a sand nursery bed. Six boxes were filled with half-strength Yoshida solution (3 L each) and

bubble stones were placed inside all boxes (2 bubble stones for each box). Such bubble stones were connected to a nitrogen gas bottle through plastic tubes in order to infiltrate nitrogen gas into the growth media (to create anaerobic condition in the growth medium). Two-week old seedlings from sand nursery beds were transplanted in the PVC pipes. Each rice plant was fixed in half-split foam plugs (3.5 cm in diameter and 3 cm in height) (stem is above the foam plug) and inserted into the PVC pipes. In such a way, only roots were in direct contact with the Yoshida solution. The set-up is shown in Figure 5.

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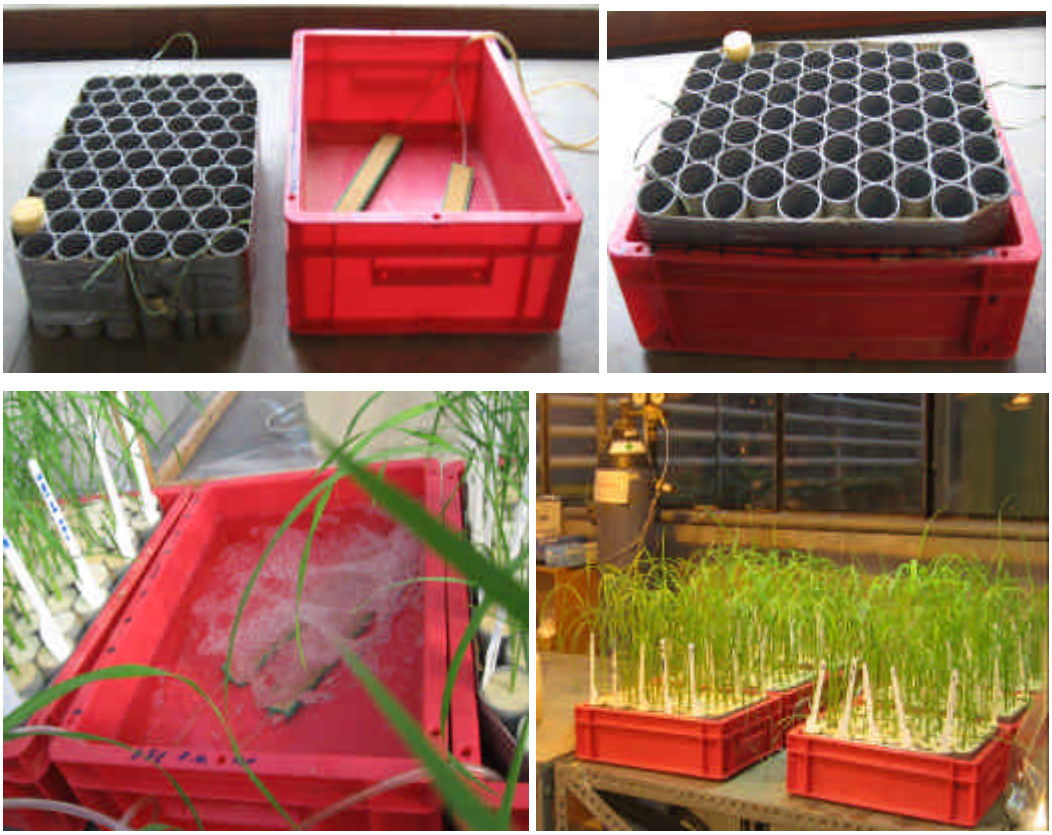

Figure 5: **Schematic diagrams of experimental set-up in the greenhouse.**

10 cultivars were randomly assigned in 10 rows of tied PVC pipes for each box. Each row contained 6 PVC pipes. In such a way, 6 plants of each cultivar were transplanted in one box.

4.2.3 Treatment application and Fe(II) addition to the growing medium

A set of pot experiments was conducted to determine the up-take of Fe(II) by plants, dry matter accumulation and root iron plague of different low land rice cultivars. Three reported check cultivars (*IR 31785-58-1-2-3-3* (sensitive), *ITA 320* and *Sahel 108* (tolerant) and 7 Myanmar

cultivars (Table 1) were used in both experiments in order to compare and determine their differential responses to Fe-toxicity followed Fe (applied as $FeSO_4$. $7H_2O$) addition in concentrations of 0, 1000, 1500 (mg L^{-1}).

At the seedling stage of 4 weeks, we changed the cultural solution (full-strength) for all boxes. Then Fe(II) solution (applied as $FeSO_4$. $7H_2O$), 1000 mg L^{-1} was added to the cultural solution of 2 boxes for treatment 1 and for treatment 2, we added Fe(II) solution (1500 mg L^{-1}) to the culture solution of 2 boxes. For control 2 boxes, no Fe(II) was added. The nitrogen gas was bubbled into all boxes through bubble stones for 15 minutes for every two hours to prevent oxygen diffusion into the root zone and maintain sufficiently low redox potential to prevent the oxidation of Fe^{2+} to Fe^{3+}. Three days after Fe(II) addition, seedling leaves were assessed visually for the expression and their severity of Fe-toxicity symptoms (bronzing). Roots from three boxes (0, 1000, 1500 mg Fe(II) treatments) were washed with 0.5 N hydrochloric acid (15 ml) for the determination of root iron plaque. Thereafter, the seedlings (stem, leaves and root) were harvested for dry biomass and the determination of tissue Fe content.

4.2.4 Sample preparation

Two sampling methods (fresh and dry) were applied. For fresh sampling, the roots were washed with 0.5 N hydrochloric acid (HCl) for 5 minutes in a scintillation vial, and stems, leaves and roots were sampled separately, wrapped in aluminium foil, transferred to liquid nitrogen and kept in a freezer at -20°C. For dry sampling, stems, leaves and roots were sampled separately, put in paper bags and oven-dried to a constant weight.

4.2.5 Methods of analysis

The responses of 10 cultivars of low land rice to added Fe (II) was determined visually (leaf scoring), gravimetrically (dry matter) and chemically (iron content in plant tissues and root iron plaque).

4.2.5.1 Leaf scoring

Table 3: Score of leaf area damaged by uptake of excess Fe (II).

Percentage leaf area affected	Score
0	0
1-9	1
10-29	3
30-49	5
50-69	7
70-89	9
90-100	10 (dead leaf)

Iron-toxicity responses were scored by subjective visual assessment of Fe-toxicity symptoms on fully developed leaves (bronzing symptoms) for the entire plant and expressed as percentage leaf area affected. As scoring system, the Standard Evaluation System for leaf blast (*Pyricularia oryzae*) lesion scoring provided by the International Network for Genetic Evaluation of Rice (INGER;IRRI, 1996) was adopted for Fe toxicity Table 3: percentage leaf area affected = score).

4.2.5.2 Dry weight and Fe content in plant tissues

At each sampling, leaves were scored for Fe-toxicity symptoms. The above ground biomass (leaf and stem separately) and root were harvested to determine dry matter accumulation and Fe content in tissues. Dry samples were oven-dried at 70°C (48 hours) to constant weight. Fresh samples were freeze-dried to a constant weight. All samples were weighed and analysed for Fe content by atomic-absorption spectrometry (Perkin-ELMER AAS 1100B, Überlingen, Germany), following a hot pressure digestion with concentrated nitric acid (HNO_3) solution (4 ml for each sample) at 180°C for 7 hours and a subsequent filtering and standard dilution to 100ml.

4.2.5.3 Root iron plaque

Roots from three boxes (0, 1000, 1500 mg L^{-1} treatments) were washed with 0.5 N hydrochloric acid (15 ml) in a scintillation vial for 5 minutes and the washing solution was filtered and analysed to determine the root iron plaque formed in the root surface.

4.2.6 Data analysis

Data analysis was carried out with all experimental data (leaf symptoms score, plant height, dry biomass, tissue Fe content and root iron plaque subjected to analysis of variance using SPSS Statistical Software for Windows (Version 11.5).

Chapter V. Results

A field trial and a set of pot experiments were carried out to validate the screening method (early vegetative stage) for rice to Fe-toxicity tolerance developed by Asch *et al*., (2005).

5.1 Effect of Fe in the soil on leaf symptoms and Fe(II) uptake

Five weeks after sowing, the leaf symptoms were scored for all cultivars grown under control and Fe(II) treated plots. Stem and leaf samples were analyzed for tissue Fe content to determine the uptake. We observed no leaf bronzing symptoms in all cultivars. In addition, we found no significant differences (p = 0.05) between tissue-Fe concentrations among the cultivars. Tissue-Fe concentrations of three cultivars are exemplarily shown in Table 4.

Table 4: **Tissue Fe concentrations (uptake) of 3 cultivars 5 weeks after exposure to 0 and 1000 mg Fe(II) L^{-1} in the soil under field condition (mean values ± standard errors).**

Cultivars	Stem Fe (mg g^{-1})	DMRT	Leaf Fe (mg g^{-1})	DMRT	Shoot Fe (mg g^{-1})	DMRT
0 mg L^{-1} Fe(II) (Control)						
Sahel 108	1.64 ± 0.24	a	0.13 ± 0.05	a	2.56 ± 0.60	a
Shwethwe Yin	2.43 ± 0.60	a	0.10 ± 0.03	a	1.92 ± 0.28	a
Yebaw Lat	1.82 ± 0.26	a	0.22 ± 0.06	a	1.87 ± 0.24	a
1500 mg L^{-1} Fe(II) (Treatment)						
Sahel 108	2.81 ± 0.63	a	0.23 ± 0.06	a	3.05 ± 0.65	a
Shwethwe Yin	1.82 ± 0.19	a	0.23 ± 0.06	a	2.05 ± 0.19	a
Yebaw Lat	1.96 ± 0.18	a	0.31 ± 0.04	a	2.27 ± 0.19	a

DMRT: Duncan's Multiple Range Test at 5% probability error. (n = 9). Means with the same letters are not significantly different**.**

5.2 Pot experiment

5.2.1 Effect of Fe(II) stress on biomass accumulation, plant height and tillering.

Three days after exposure to Fe(II) concentrations of 0, 1000 and 1500 mg L^{-1} at the seedling age of 4 weeks, only Myanmar cultivar, *Imma Yebaw* showed significant difference in shoot and total dry weight between control and Fe(II) treatment, however, no significant differences were observed in the rest nine cultivars.

Table 5: **Effect of Fe(ll) addition on above-ground dry matter accumulation, plant height and tiller formation of different rice cultivars (3 days of exposure to 0 and 1500 mg Fe(II) L^{-1} in nutrient solution at 4 week-old seedling stage).**

Cultivars	Shoot dry weight (g)			Plant height (cm)			Tiller number		
			Prob[a]			Prob[a]			Prob[a]
	T0	T1		T0	T1		T0	T1	
Imma Yebaw	0.291	0.193	*	61.50	49.33	*	1.50	1.17	*ns*
IR 31785	0.201	0.197	*ns*	42.17	40.83	*ns*	2.00	2.00	*ns*
ITA 320	0.136	0.147	*ns*	41.00	36.50	*	1.00	1.17	*ns*
Manaw Thukha	0.132	0.111	*ns*	37.67	36.83	*ns*	1.17	1.33	*ns*
Pawsan Baykya	0.197	0.181	*ns*	57.17	53.83	*ns*	1.33	1.33	*ns*
Sahel 108	0.121	0.176	*ns*	39.67	37.83	*ns*	1.67	2.17	*ns*
Shwethwe Yin	0.212	0.168	*ns*	43.17	39.17	*	2.83	3.17	*ns*
Shwewar Tun	0.254	0.234	*ns*	44.67	42.00	*ns*	2.83	2.67	*ns*
Thukha Yin	0.113	0.146	*ns*	42.33	38.50	*ns*	1.17	1.50	*ns*
Yebaw Lat	0.216	0.203	*ns*	63.33	56.33	*	1.33	1.17	*ns*

LSD test. Prob[a] level of significance for the mean differences between shoot dry weights, plant height and tiller numbers at 0 mg Fe(II) L^{-1} and 1500 mg Fe(II) L^{-1}. **ns and *** indicate not significant and significant at p = 0.05, respectively. **T0:** Control (without Fe(II) added) and **T1:** 1500 mg Fe(II) L^{-1} in nutrient solution.

We also found that no significant difference in tiller formation among the cultivars between control and Fe(II) treatment. However, *Yebaw Lat*, *Shwethwe Yin*, *Imma Yebaw* and *ITA 320* showed significant differences in plant height. Level of significance for the mean differences between shoot dry weight, total dry weight, plant height and tiller formation for 10 cultivars is presented in Table 5 and 6.

Table 6: **Effect of Fe(II) addition on total dry biomass accumulation of different rice cultivars (3 days of exposure to 0 and 1500 mg Fe(II) L^{-1} in nutrient solution at 4 week-old seedling stage).**

Cultivars	Total dry biomass (g)					
	0 mg Fe(II) L^{-1}		1000 mg Fe(II) L^{-1}		1500 mg Fe(II) L^{-1}	
	Mean ± Std. error	DMRT	Mean ± Std. error	DMRT	Mean ± Std. error	DMRT
Imma Yebaw	0.39 ± 0.04	b	0.21 ± 0.04	ab	0.25 ± 0.06	a
IR 31785	0.27 ± 0.03	a	0.25 ± 0.04	a	0.26 ± 0.04	a
ITA 320	0.18 ± 0.05	a	0.20 ± 0.02	a	0.20 ± 0.01	a
Manaw Thukha	0.18 ± 0.01	a	0.16 ± 0.02	a	0.15 ± 0.02	a
Pawsan Baykya	0.25 ± 0.03	a	0.25 ± 0.03	a	0.22 ± 0.03	a
Sahel 108	0.23 ± 0.02	a	0.21 ± 0.04	a	0.17 ± 0.05	a
Shwethwe Yin	0.28 ± 0.03	a	0.25 ± 0.03	a	0.23 ± 0.03	a
Shwewar Tun	0.34 ± 0.03	a	0.29 ± 0.03	a	0.30 ± 0.03	a
Thukha Yin	0.16 ± 0.01	a	0.18 ±0.02	a	0.20 ± 0.02	a
Yebaw Lat	0.29 ± 0.03	a	0.39 ± 0.11	a	0.27 ± 0.01	a

DMRT: Duncan's Multiple Range Test at 5% probability error (n = 6). Means with the same letters are not significantly different**.**

5.2.2 Leaf symptom score

Four week-old seedlings of 10 genotypes were exposed for 3 days to 0, 1000 and 1500 mg Fe(II) L^{-1} in the nutrient solution. Symptom scores ranged from 0.50 to 1.75 in 1000 mg L^{-1} Fe(II) treatment, and 1.33 to 5.75 in 1500 mg L^{-1} treatment. In 1000 mg L^{-1} Fe(II) treatment, significantly higher symptoms($p = 0.05$) were recorded in genotypes, *ITA 320* and *IR 31785-58-1-2-3-3* whereas the genotypes, *Shwethwe Yin* (5.75) and *Thukha Yin* (4.08) showed

significantly higher bronzing symptoms ($p = 0.05$) in 1500 mg L^{-1} Fe(II) treatment as compared to the other tested genotypes. Lower symptom scores were recorded for genotypes, *Shwewar Tu*n (1.33), *Imma Yebaw* (1.50), *Sahel 108* (1.58), *ITA 320* (1.58) and *Yebaw Lat* (1.75) as compared to sensitive check, *IR 31785-58-1-2-3-3* (3.5). With the exception of tolerant check genotypes (*ITA 320* and *Sahel 108*), Fe^{2+}-toxicity symptoms in leaves of all genotypes increased as a function of Fe(II) concentration in the nutrient solution. However, symptom scores were not very pronounced as compared to the studies of Asch *et al*., (2005) (3 days exposure to 2000 mg Fe(II) L^{-1} in nutrient solution). Mean values, standard errors and Duncan's Multiple Range Test for 10 genotypes are shown in Table 7.

Table 7: Toxicity symptom expressions of 10 cultivars (3 days after exposure to 1000 and 1500 mg Fe(II) L^{-1} at 4 week-old seedling stage).

Cultivars	Fe(II) 1000 mg L^{-1} treatment			Fe(II) 1500 mg L^{-1} treatment		
	Symptom score			Symptom score		
	Mean	Standard error (±)	DMRT	Mean	Standard error (±)	DMRT
Imma Yebaw	0.75	0.11	ab	1.50	0.18	ab
IR 31785	1.75	0.17	c	3.50	0.22	c
ITA 320	1.50	0.00	c	1.58	0.15	ab
Manaw Thukha	1.08	0.08	b	3.17	0.46	bc
Pawsan Baykya	0.50	0.00	a	2.67	0.59	abc
Sahel-108	0.58	0.08	a	1.58	0.43	ab
Shwethwe Yin	0.67	0.17	a	5.75	0.79	d
Shwewar Tun	0.58	0.08	a	1.33	0.33	a
Thukha Yin	0.83	0.21	ab	4.08	1.13	c
Yebaw Lat	1.08	0.08	b	1.75	0.38	ab

DMRT: Duncan's Multiple Range Test at 5% probability error. (n = 6). Means with the same letters are not significantly different at $p = 0.05$.

5.2.3 Total Fe uptake by the plant

Three days after exposure to external Fe(II) concentrations of 0, 1000 and 1500 mg L^{-1} at 4 week-old seedling stage, different plant organs (stem, leaf and root) were sampled and analyzed for Fe contents in plant tissues. In Fe(II) 1000 mg L^{-1} treatment, we observed no significant differences ($p = 0.05$) in total Fe concentration in plant tissues among all genotypes. Total tissue Fe concentration ranged from 26.09 mg g^{-1} to 40.76 mg g^{-1} and 35.34 mg g^{-1} to 48.06 mg g^{-1} in 1000 and 1500 mg Fe^{2+} L^{-1} treatments respectively. In the treatment with 1500 mg Fe(II) L^{-1}, *IR 31785-1-2-3-3* (sensitive check) showed significantly highest amount of total plant Fe concentration ($p = 0.05$), whereas the lowest total Fe concentration was recorded in *Shwethwe Yin*, and *ITA 320* (tolerant check), *Sahel 108* (tolerant check), *Yebaw Lat*, *Pawsan Baykya*, *Shwewar Tun* showed medium total plant-tissue Fe concentrations (Figure 6).

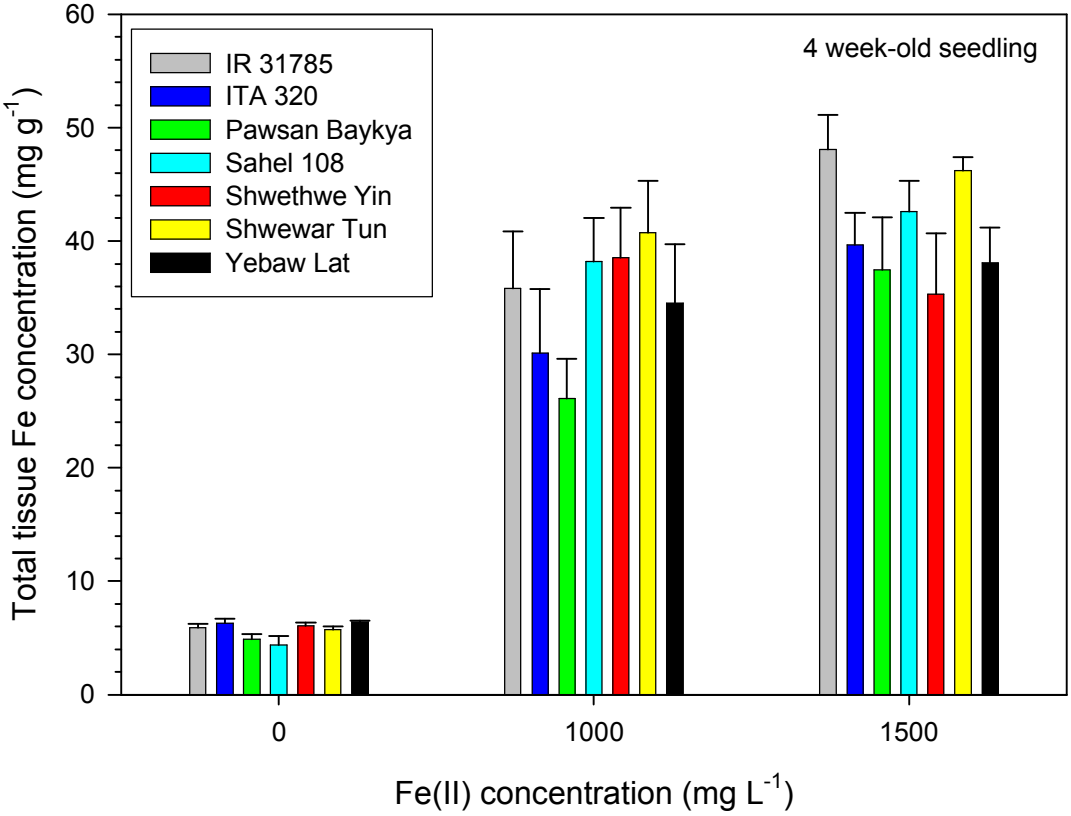

Figure 6: **Total tissue Fe concentrations of 7 rice cultivars (3 days after exposure to 0, 1000 and 1500 mg Fe(II) L^{-1} in nutrient solution at 4 week-old seedling stage). Error bars = SE (n = 6).**

In treatment 1000 mg Fe^{2+} L^{-1}, shoot Fe concentration ranged from 3.31 mg $g^{-1}$to 6.26 mg g^{-1} and from 3.77 mg g^{-1} to 7.04 mg g^{-1} in treatment 1500 mg Fe^{2+} L^{-1}. The highest shoot Fe concentration was observed in sensitive check genotype *IR 31785-1-2-3-3* and tolerant check, Sahel 108 in both treatments and the lowest shoot Fe concentrations were recorded in *Pawsan Baykya* and *Yebaw Lat* in treatment 1000 and 1500 mg Fe^{2+} L^{-1} treatments, respectively (shown in Figure 7).

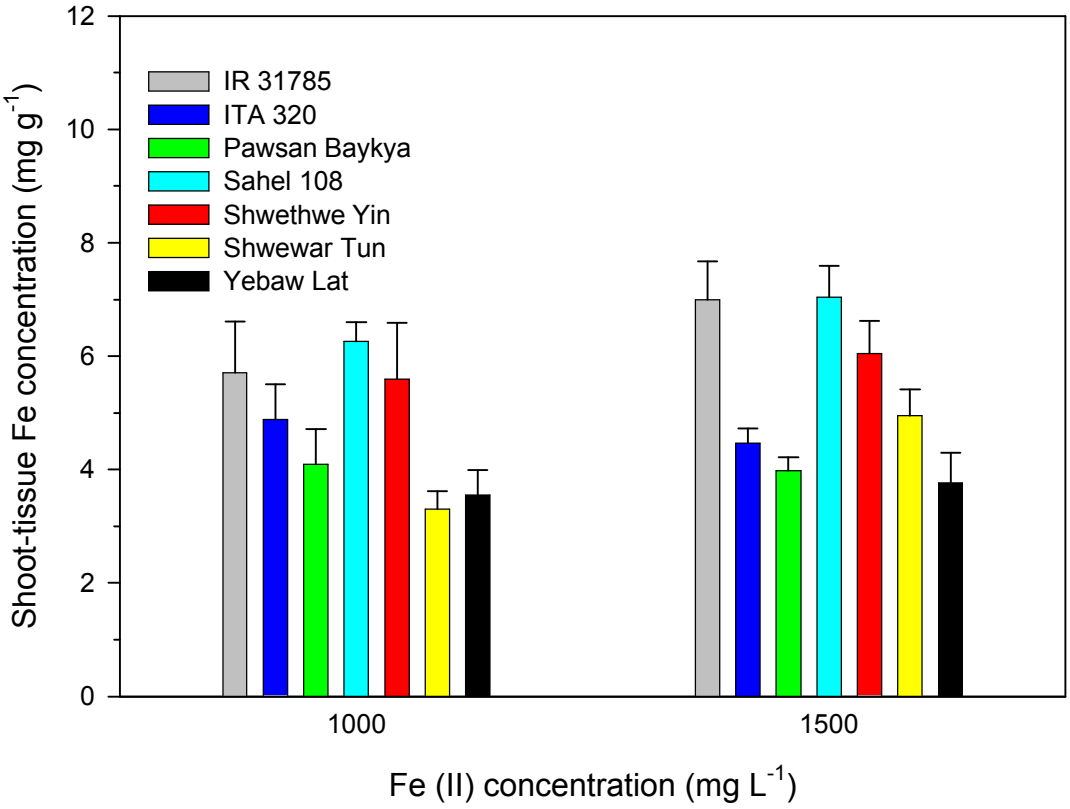

Figure 7: **Shoot Fe concentrations of 7 cultivars (3 days after exposure to 0, 1000 and 1500 mg Fe(II) L^{-1} in the nutrient solution at 4 week-old seedling stage). Error bars = SE (n = 6).**

5.2.4 Distribution of Fe within plant tissues

Four weeks after sowing, seedlings of 10 genotypes were exposed to 0, 1000 and 1500 mg L^{-1} Fe(II) in the nutrient solution for 3 days. Root, stem, and leaf of ten cultivars were sampled separately and analyzed for Fe concentrations in plant tissues to determine Fe distribution within plant tissues. In addition, roots (n = 3) were washed with 0.5 N HCl and the washing solutions

were analyzed to determine the amount of Fe excluded at the root surface (Fe plaque) and how much amount is actually retained in the root tissue.

In the treatment Fe(II) 1000 mg L^{-1} in the nutrient solution, stem tissue Fe concentrations of tested genotypes ranged from 2.16 mg g^{-1} to 4.88 mg g^{-1} and from 2.33 mg g^{-1} to 5.28 mg g^{-1} in Fe(II) 1500 mg L^{-1} treatment, respectively. Leaf tissue Fe concentrations ranged from 1.14 to 1.79 in Fe(II) 1000 mg L^{-1} treatment and 1.17 mg g^{-1} to 2.18 mg g^{-1} in Fe(II) 1500 mg L^{-1} treatment. Root tissue Fe concentrations ranged from 22.00 mg g^{-1} to 30.96 mg g^{-1} in Fe(II) 1000 mg L^{-1} treatment and from 29.14 mg g^{-1} to 41.25 mg g^{-1} in Fe(II) 1500 mg L^{-1} treatment. Data of mean values, their standard errors and Duncan's Multiple Range Test (stem, leaf and root-tissue Fe concentrations) for 3 check genotypes and 4 Myanmar genotypes are presented in Table 8.

Table 8: Fe distribution within plant tissues of 5 cultivars in relation to external Fe(II) stress (3 days after exposure to Fe(II) 1000 and 1500 mg L^{-1} at 4 week-old seedling stage).

Cultivars	Fe concentration in plant tissues (mg g^{-1})								
	Stem			Leaf			Root		
	Mean	Std. error (±)	DMRT	Mean	Std. error (±)	DMRT	Mean	Std. error (±)	DMRT
Fe(II) 1000 mg L^{-1} in nutrient solution									
IR 31785	4.02	0.88	ab	1.68	0.23	bc	3.25	0.04	ab
ITA 320	3.49	0.48	ab	1.40	0.22	abc	2.43	0.39	a
Shwethwe Yin	3.82	0.97	ab	1.79	0.09	c	2.31	0.40	a
Shwewar Tun	2.16	0.27	a	1.14	0.07	a	4.14	0.65	b
Yebaw Lat	2.26	0.38	a	1.29	0.09	ab	2.90	0.44	ab
Fe(II) 1500 mg L^{-1} in nutrient solution									
IR 31785	5.28	0.58	c	1.71	0.13	ab	6.15	0.32	ab
ITA 320	3.16	0.37	ab	1.31	0.21	a	7.29	0.19	b
Shwethwe Yin	4.52	0.68	bc	1.87	0.26	ab	4.42	0.30	a
Shwewar Tun	3.77	0.45	abc	1.18	0.08	a	7.28	0.68	b
Yebaw Lat	2.33	0.56	a	1.44	0.20	ab	9.23	1.02	c

DMRT: Duncan's Multiple Range Test. Means with the same letters are not significantly different at p = 0.05.

The concentrations of iron plaque formed at the root surface of 7 genotypes ranged from 18.23 mg g^{-1} of root to 29.03 mg g^{-1} of root in Fe(II) 1000 mg L^{-1} treatment, and from 24.60 mg g^{-1} of root to 33.89 mg g^{-1} of root in Fe(II) 1500 mg L^{-1} treatment, however, no significant differences (p = 0.05) were observed among the genotypes(Figure 8). Root-tissue Fe concentration (washed with HCl) of 5 genotypes ranged from 2.31 mg g^{-1} of root to 4.14 mg g^{-1} of root and from 4.42 mg g^{-1} of root to 9.23 mg g^{-1} of root in 1000 and 1500 mg L^{-1} Fe(II) treatments respectively. We found significant differences (p = 0.05) in root tissue Fe concentrations between genotypes in both treatments (Table 8, Figure 9 and FigureA1).

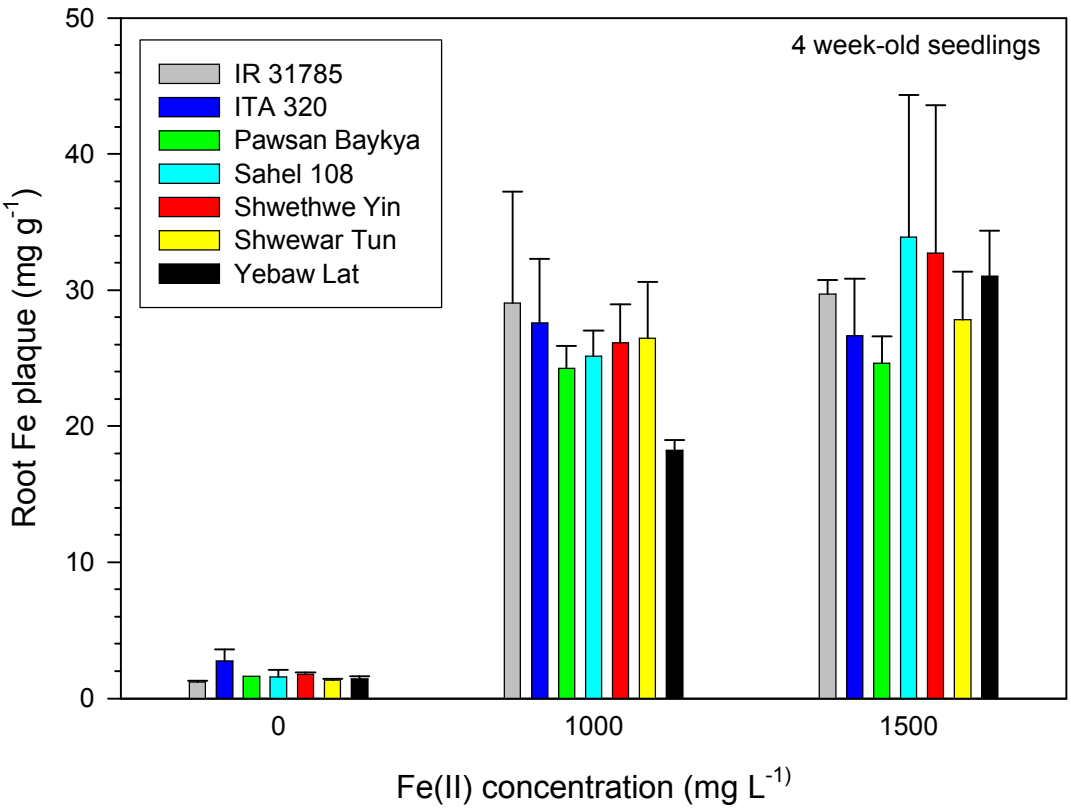

Figure 8: **Root Fe plaque of 7 cultivars (3 days after exposure to 0, 1000 and 1500 mg L^{-1} Fe(II) in the nutrient solution at 4 week-old seedling stage). Error bars = SE (n = 3).**

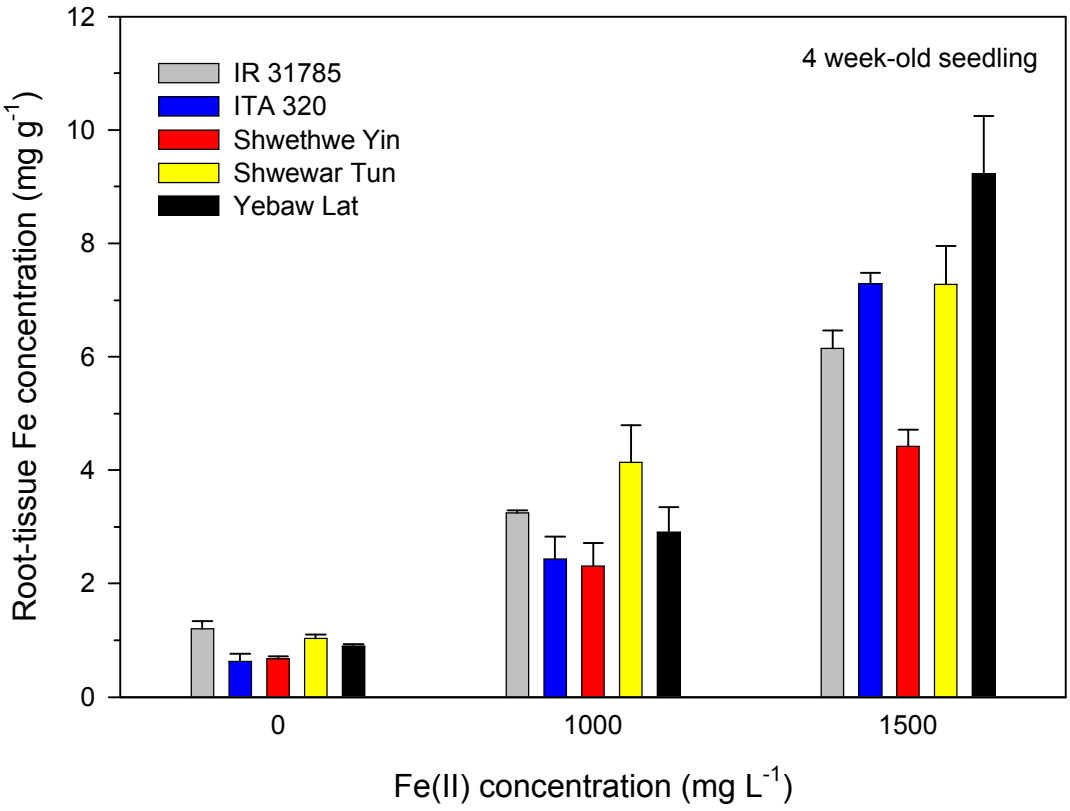

Figure 9: **Root-tissue Fe concentrations (washed with HCl) of 5 cultivars 3 days after exposure to external Fe(II) 0, 1000 and 1500 mg L^{-1} in the nutrient solution at 4 week-old seedling stage. Error bars = SE (n = 3).**

Chapter VI. Discussion

6.1 Validation of the screening tool in the field trial

To date, screening tools for Fe-toxicity tolerance in rice are based on leaf symptoms and yield but not actual resistance mechanisms such as exclusion or tolerance (e.g., Gunawardena *et al*., 1982; Akabar *et al*., 1987; Mahadevappa *et al*., 1991). Asch *et al*., (2005) have developed a standardized method (controlled condition in phytotron) to screen a large numbers of rice genotypes for their response to Fe toxicity in the early vegetative stage. In the present work a field trial was carried out to validate and aiming to adopt this screening method under a field conditions. We also aimed at developing artificially Fe-toxic conditions in the soil through the application of Fe(II) sulfate in the soil. These may allow us to screen a larger number of genotypes in the field with lower costs than that of the screening method in the phytotron.

6.1.1 Effect of Fe in the soil on leaf symptoms and Fe(II) uptake

Stem and leaf-tissue Fe concentrations of genotypes from control (without Fe (II) application) and iron treatment (1000 mg Fe(II) L^{-1} application) plots showed no significant differences and the leaf-tissue Fe concentrations of all tested genotypes were very low, ranged from 0.1 to 0.31(Section 5.1, Table 4) as to compared to the leaf-tissue Fe concentrations of the same cultivars with the same Fe(II) treatment (1000 mg L^{-1}) in the greenhouse (Section 5.2.4, Table 8). Additionally, no leaf bronzing symptoms were observed in any genotype subjected to both treatments. Leaf symptom score is the most important indicator relating leaf tissue Fe concentration and damage caused by Fe^{2+} allowing to identify the varying degree of leaf tissue tolerance to Fe^{2+}. These results suggest that this screening method applied to the early vegetative stage does not sufficiently discriminate the genotypes for the physiological mechanisms of Fe toxicity under field conditions. This may be due to the following reasons.

1) It was difficult to induce Fe-toxicity in the soil by using Fe(II) sulfate, though the use of Fe(II) sulfate, despite the easy control of Fe(II) concentration in hydroponics (Asch *et al*., 2005). The oxidation-reduction (microbial and chemical) of Fe in the soil was not well understood. Some soil fauna burrowed and turned the soil up to the surface (oxic layer) in Fe(II) sulfate treated plots so that oxygen was available to oxidize Fe^{2+} to Fe^{3+}.
2) Although the plots were kept submerged throughout the growing period, reddish-brown soil surface in treated plots indicated that the presence of Fe^{3+} deposits due to oxidation.

3) Plastic sheets were placed at 25cm soil depth (see Section 4.1.3) to prevent Fe(II) losses from percolation and interflow, however, the efficacy of plastic sheets as in question. Fe(II) losses may have occurred through percolation to the subsoil and seepage to the adjacent soil layer where the rice root cannot reach if the plastic sheets were punctured.
4) Inhomogeneity of Fe^{2+} in the soil, $FeSO_4$ was applied at a depth of 15 cm (see Section 4.1.3), where it may have formed a layer and thus was not evenly distributed in the root zone.

6.2 Validation of screening tool in control growing condition

A screening method for early vegetative growth stage developed by Asch *et al*., 2005 has a number of advantages. However, to prepare the set-up (see Section 2.1, Asch, *et al*., 2005) is quite laborious (each single plant needs a set-up) and may be limited to screen a large number of genotypes. In addition, in the set-up of this method only stems and leaves were accessible, but not to the root to analyse the Fe plaque and root Fe concentration to identify the oxidation power of rice root which seems to be one of the most important avoidance mechanisms. Thus, we adapted this screening method in a combined growing box (described in Section 4.2.2), in such a way that all plant parts were accessible for the analysis of tissue Fe concentrations as well as root Fe plaque.

6.2.1 The relationship between symptom score and leaf-tissue Fe concentration.

The symptom expressions were not very pronounced (Section 5.5.2) as to compare with the studies of Asch, *et al*., 2005 (symptom scores from 2000 mg L^{-1} Fe(II) in nutrient solution). However, the data from the 1500 mg L^{-1} Fe(II) treatment showed that there was a linear correlation between leaf symptom expressions and leaf tissue Fe concentrations (Figure A2). We found that the reportedly sensitive genotypes (*IR 31785-58-1-2-3-3*) showed the highest symptom score and leaf tissue concentrations in 1000 mg L^{-1} Fe(II) treatment. In 1500 mg L^{-1} Fe(II) treatment, tolerant check *ITA 320*, *Shwewar Tun* and *Yebaw Lat* were recorded significantly lower leaf-tissue concentrations and symptom scores than those of *Shwethwe Yin* and sensitive check *IR 31785-58-1-2-3-3*. Myanmar cultivar, *Shwethwe Yin* had the highest leaf-tissue concentration in both treatments and highest symptom score in treatment Fe(II) 1500 mg L^{-1} (Table 7 and 8; Figure 10, A1 and A2). These results suggest that *Shwethwe Yin* seems to be

more sensitive than the reportedly sensitive *IR 31785-58-1-2-3-3*. Lower leaf-tissue Fe concentrations and leaf symptom scores in *ITA 320*, *Shwewar Tun* and *Yebaw Lat* indicated that these genotypes seemed to exclude Fe quite efficiently and transport less Fe from the shoot to the leaf to compare with *IR 31785-58-1-2-3-3* (sensitive check).

In order to obtain pronounced leaf bronzing symptoms, in a relatively short period the 2000 mg L^{-1} Fe(II) should be applied to the cultural solution or the stress duration should be extended to 5 instead of 3 days.

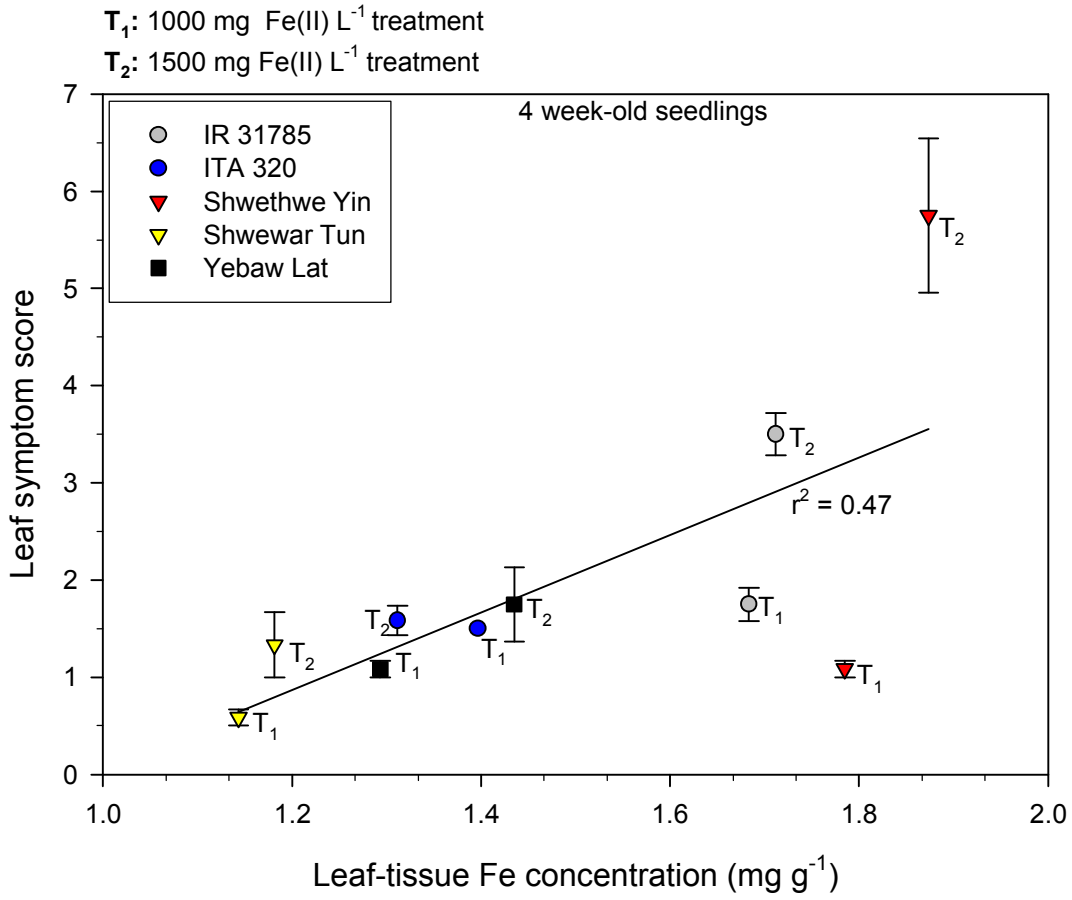

Figure 10: **The relationship between symptom scores and leaf-tissue Fe concentrations of 5 rice cultivars (3 days after exposure to external Fe(II) 1000 and 1500 mg L^{-1} in nutrient solution at 4 week-old seedling stage). Error bars = SE (n = 6).**

6.2.2 Genotype differentiation

One of the problems in field screening large numbers of genotypes for tolerance to Fe-toxic conditions is to provide sufficiently homogenous and elevated Fe levels in the soil, thus providing comparable stress levels to all materials (Audebert and Sahrawat, 2000). Pot experiments with submerged soils also faces the problem that the concentration of Fe(II) is not always distributed homogenously. The concentration of Fe(II) in solution may be lowered in the surface layers where most roots are generally concentrated in small pots (Van Breemen and Moormann, 1978).

Differentiation of genotypes according to their sensitivity or tolerance to iron toxicity was achieved with the adapted set-up. As compared to the mechanistic screening method (early vegetative stage) developed by Asch *et al*., (2005), the adapted set-up allowed us to access to the root for the analysis of Fe plaque at the root surface and consequently, we were able to analyze the root Fe Plaque and the root-tissue Fe concentrations that allowed us to differentiate the exclusion of Fe(II) at the root surface (oxidation power of rice root) and at the root cells (root retention) of all tested cultivars. We washed the roots with HCl (15 ml of 0.5 N HCl for each root) for 5 minutes and analyzed both the washing solution and washed root to differentiate root-tissue Fe concentration and Fe concentration at the root surface (Fe plaque). The stems and leaves were also analyzed separately for Fe concentrations in plant tissues. In such a way, genotype differentiation was possible based on exclusion potential for Fe on the root surface, iron partitioning in different tissues and toxicity symptom expression on the leaf.

We found no significant differences ($p = 0.05$) in root iron plaque among the tested genotypes (Figure 8 and Figure A1). This suggests that the oxidation power of tested genotypes seems to be the same. However, the amount of Fe plaque formed at the root surface in all tested genotypes was approximately three times higher than the tissue-Fe concentrations of rice plants. This indicates that the oxidation power of rice root plays an important role to cope with Fe stress as avoidance mechanism. Obviously, reduced iron having passed the oxidative barrier of the rhizosphere enters the root apoplast. Xylem loading requires ions to pass the root cell membranes at the endodermis due to the Casparian strip. Reduced iron can be excluded at the root cell membranes (Tadano, 1976). Our investigation on root-Fe concentrations (Table 8 and Figure A.1) showed that much of the reduced iron entered the root was excluded or retained in the root tissues of all tested genotypes. Significantly higher ($p = 0.05$) root tissue-Fe concentrations were recorded in tolerant check cultivar *ITA 320*, *Yebaw Lat* and *Shwewar Tun* . The root-tissue Fe concentration was relatively low in sensitive check *IR 31785-58-1-2-3-3* and

Shwethwe Yin showed the lowest root-tissue Fe concentrations. These data suggest that the genotypes vary in their root ion selectivity and root retaining power. Tolerant check *ITA 320*, *Yebaw Lat* and *Shwewar Tun* seem to have high root membrane selectivity and root retention power, whereas the sensitive check *IR 31785-58-1-2-3-3* has relatively low and *Shwethwe Yin* has extremely low root membrane selectivity and root retention power. However, it is not possible to quantify how much iron is excluded at the root apoplast and retained inside root tissues in this study.

The Fe^{2+} that has entered the xylem stream will follow the transpiration-driven acropetal long-distance transport. However, some of this iron may be immobilized and deposited at specific ``dumping sites´´ within the plant. The analytical data of stem, leaf and root-tissue Fe concentrations (Table 8, Figure 9 and Figure A1) showed significance differences ($p = 0.05$) among tested genotypes. In contrast to root-Fe concentrations of tested genotypes, lower stem and leaf tissue-Fe concentrations were recorded in *ITA 320*, *Yebaw Lat* and *Shwewar Tun*, whereas *IR 31785-58-1-2-3-3* and *Shwethwe Yin* showed higher stem and leaf tissue-Fe concentrations. These data showed that *ITA 320* (tolerant check), *Yebaw Lat and Shwewar Tun* retained greater amount of Fe in the root and transport lesser amount of Fe to the stem and leaf tissues as to compare with *IR 31785-58-1-2-3-3* and *Shwethwe Yin*. Evidently, the leaf-tissue concentrations increased as a function of increased stem-tissue concentrations in genotypes *IR 31785-58-1-2-3-3* (sensitive check) and *Shwethwe Yin*. The efficiency of ``withdrawal´´ of active Fe is likely to be determined by the rate of acropetal Fe^{2+} transport and may not be sufficient to prevent iron influx into the leaves under conditions of high transpiration such as during exponential growth phase of rice or under dry-season conditions when high vapor-pressure deficit greatly enhances crop transpiration rate (Asch *et al*., 2000, 2003). The increased in stem tissue-Fe concentration increased the leaf tissue-Fe concentration in tested genotypes in this study, indicates that the stem tissue may act as a mediator unlike to root tissues. These results suggest that the retention power of rice root play a major role in inclusion/avoidance mechanisms rather than stem tissue retention.

The leaf damage symptoms were significantly higher ($p = 0.05$) in *IR 31785-58-1-2-3-3* (sensitive check) and *Shwethwe Yin* as to compare with *ITA 320* (tolerant check), *Yebaw Lat and Shwewar Tun* (Table 7 and Figure 10). We also found that the leaf-tissue Fe concentrations reflected the symptom scores in tested genotypes. Genotypes with higher leaf-tissue Fe concentrations showed higher leaf symptom scores except *Yebaw Lat*, which had relatively high leaf-tissue Fe concentration but showed relatively low symptom score. This genotype, *Yebaw Lat* may have moderately high leaf tissue tolerance.

According to the investigation of root Fe plaque (as indicator of rhizospheric oxidation power), root and stem-tissue Fe concentrations (retention power), leaf-tissue Fe concentration and toxicity symptom expressions (as indicators for leaf tissue tolerance) in tested genotypes as described above, the tolerance or sensitivity of genotypes to iron toxicity were differentiated as follows:

- *ITA 320* and *Shwewar Tun* (tolerant- includer/avoidance).
- *Yebaw Lat* (tolerant- includer/avoidance and moderately high leaf tissue tolerance).
- *IR 31785-1-2-3-3* (sensitive) and *Shwethwe Yin* (extremely sensitive).

Chapter VII. Conclusion and outlook

The analytical data of tissue Fe concentrations in all cultivars from both Fe(II)-treated and control plots showed no significant differences in our field trial. In addition no leaf symptoms were observed in any genotype neither in control nor in Fe(II)-treated plots. These indicate that to create Fe-toxicity artificially in the field condition by applying $FeSO_4$ is not possible.

However, our adapted screening set-up under controlled growing condition based on a screening set-up developed by Asch *et al* , 2005 allowed us to determine Fe plaque at the root surface, Fe distribution within plant tissues and visual assessment of leaf symptoms.The adapted set-up is cheap and easy to handle as compared to the set-up of mechanistic screening for early vegetative stage (Asch *et al*, 2005). It required 1 m^2 to screen 60 plants (one set-up) in the greenhouse. A large number of rice genotypes can be reliably screened for their response to Fe toxicity based on the proposed tolerant mechanisms such as rhizospheric oxidation potential, stem and root retention and leaf tissue tolerance with root Fe plaque and leaf symptoms score as indicators. We have shown the possibility to differentiate and quantify the Fe content at root surface (Fe plaque) and root tissue-Fe concentration. In combination with stem and leaf-tissue Fe concentration analyses, it is possible to investigate the Fe partitioning within plant tissues, which allows for a classification of cultivars by Fe tolerance mechanisms. Only one-time fully-strength medium change is required before Fe(II) addition to the growing medium. 2000 mg Fe(II) L^{-1} should be applied to the cultural solution (Asch *et al*., 2005) or the stress duration should be extended to five days to obtain well pronounced leaf symptoms. The adapted set-up also allows regulating the required pH of the growing medium at any time. We had screened one Myanmar cultivar, Shwethwe Yin that showed higher sensitivity to Fe toxicity than the reportedly sensitive check genotype, *IR 31785-58-1-2-3-3*, and two relatively tolerant cultivars *Shwewar Tun* and *Yebaw Lat*. Myanmar cultivar, *Shwethwe Yin* may be useful as a sensitive check genotype in future studies.

We have shown that iron exclusion at the rice root (oxidation power) plays an important role in all tested genotypes. Given the importance of oxidation power of the root in excluding iron, vigorous and early development as well as longevity of aerenchyma are desired traits for Fe tolerance (Jayawardena *et al*., 1977). This aerenchyma forms upon the establishment of anoxic conditions induced by an increased production of ethylene (Kawase, 1981). Thus, ethylene production of rice plants should be investigated to estimate the vigor of aerenchyma development in future studies. Using ethylene production of rice plants as indicator for oxidation power of rice root may allow us to transfer the screening for avoidance mechanisms

to the field as an indirect method. Another research should be aimed at distinguishing between Fe(II) and Fe(III) in the plant which may help us in investigating the re-oxidation and mobility of Fe^{2+} inside plant tissues to identify root and stem retention power of rice genotypes. The analytical method for Fe in rice plant tissues (high pressure hot water digestion in autoclave and spectral-photometry- 490 nm- using Microtiter plate) developed by Ripken *et al*., 2005 may be suited to distinguish Fe(II) and Fe(III) from rice plant tissues. Research should also be directed towards developing more sophisticated techniques, like those based on the relationship between Fe toxicity and enzymatic activity such as superoxidase dismutase, peroxidase and catalase in the plant tissues. This will allow to determine the genotypic differences, and the mechanisms involved in Fe toxicity tolerance, with regards to the oxidative stresses induced by high tissue-Fe concentration that are likely to occur in genotypes with low oxidation potential in the root zone.

References

Abifarin, A. O. (1988): Grain yield loss due to iron toxicity. WARDA (West Africa Rice Development Association) Techn. Newsl. 8, 1-2.

Abifarin, A. O. (1990): Progress in breeding rice for tolerance to iron toxicity. WARDA Annual Report 1989. West Africa Rice Development Association, Bouaké, Côte d Ivoire, pp. 34 39.

Abraham, M. J., Pandey, D. K. (1989): Performance of selected varieties and advance generation genotypes in rainfed lowland iron toxic soil. Intl. Rice Res. Newsl. 14, 21-21.

Abu, M. B., Tucker, E. S., Harding S. S., Sesay, J. S. (1989): Cultural practices to reduce iron toxicity in rice. Intl. Rice Res. Newsl. 14, 19-19.

Akbar, M., Gunawardena, I., Ponnamperuma, N. (1987): Breeding for soil stresses: Progress in rainfed lowland rice. The International Rice Research Institute, Manila, The Philippines, pp. 263-272.

Alaily, F., Sib, J., Madrid, K., Soubeiga, Z., Roth, C. (1998): Paddy soils derived from basaltic colluvium in the highland of Madagascar. J. Plant Nutr. Soil Sci. 163, 633-637.

Ando, T. (1983): Nature of oxidizing power of rice roots. Plant Soil 72, 57-71.

Armstrong, W. (1979): Aeration in higher plants. Adv. Bot. Res. 7, 226-332.

Asch, F. (1997): Physiologische Faktoren der Salzresistenz bei Reis (*Oryza Sativa* L.). PhD Thesis, University of Hamburg, Germany.

Asch, F., Dingkuhn, M., Dörffling, K. (2000): Leaf K/Na ratio predicts salinity induced yield loss in irrigated rice. Euphytica 113, 109-118.

Asch, F., Dahal, K. P., Das, U. S. (2003): Is the transpiration history of rice leaves indicative for the salt load of individual leaves? In University of Göttingen and ATSAF: Technological and Institutional innovations for Sustainable Rural Development. Klartext GmbH, Göttingen, Germany, p. 124.

Asch, F. Becker, M., Kpongor, D. S. (2005): A quick and efficient screen for tolerance to iron toxicity in lowland rice. J. Plant Nutr. Soil Sci. 168, 764-773.

Audebert, A., Sahrawat, K. L. (2000): Mechanisms for iron toxicity tolerance in lowland rice. J. Plant Nutr. 23, 1877-1885.

Ayotade, K. A. (1979): Reaction of some rice varieties to iron toxicity in flooded strongly acid ferralitic soil in Nigeria. WARDA (West Africa Rice Development Association) Techn. Newsl. 1, 11-11.

Becker, M., Asch. F. (2005): Iron Toxicity – Conditions and management concepts. J. Plant Nutr. Soil Sci. 168, 558-573.

Benckiser, G. Ottow, J. C. G., Santiago, S., Watanabe, I. (1982): Physiochemical characterization of iron-toxic soils in some Asian countries. IRRI Research Paper Series No. 85, The International Rice Research Institute, Manila, The Philippines, pp. 11.

Benckiser, G. Ottow, J. C. G., Santiago, S., Watanabe, I. (1983): Iron toxicity – Effect of phosphorous, potassium, calcium and magnesium fertilization on rhizoflora, redox potential and iron uptake by different rice varieties (*Oryza sativa* L.). Landw. Forschung 36, 285-299.

Bennett, J. (2001): Status of breeding for tolerance of abiotic stresses and prospects for use of molecular techniques. TAC Secretariat, Food and Agriculture Organization-FAO, Rome.

Bienfait, H. F. (1985): Regulated redox processes at the plasma-lemma of plant root cells and their function in iron uptake. J. Pioneer. Biomembr. 17, 73-83.

Blake, D. J., Reid, D. M. (1981): Ethylene, water relations and tolerance to water logging of three Eucalyptus species. Austr. J. Plant Physiol. 8, 497-505.

Blake, R. C., Shute, E. A., Greenwood, M. M., Spencer, G. H., Ingledew, W. J. (1993): Enzymes of aerobic respiration on iron. FEMS Microbial Reviews 11, 9-18.

Bode, K., Döring, O., Lüthje, S., Neue, H. U., Böttger, M. (1995): The role of active oxygen in iron tolerance of rice (*Oryza sativa* L.). Protoplasma 184, 249-255.

Brady, N. C. (1982): Rice research strategies for the future. The International Rice Research Institute, Manila, The Philippines, pp. 13-25.

Cakmak, I. (1988): Morphologische und physiologische Veräaderungen bei Zink-Mangelpflanzen. Ph.D. Thesis, University of Hohenheim, Stuttgart, Germany.

Cheema, S. S., Chaudhary, U., Takkar, P. N., Sharma, B. D., (1990): Effect of dates of transplanting on uptake of micronutrients by rice cultivars of different growth stages. J. Res. Punjab Agri. Univ. 27, 199-206.

Chen, C. C., Dixon, J. B., Turner, F. B. (1980a): Iron coatings on rice leaves: Morphology and models of development. Soil Sci. Soc. Am. J. 44, 1113-1119.

Chen, C. C., Dixon, J. B., Turner, F. B. (1980b): Iron coatings on rice leaves: Mineralogy and quantity influencing factors. Soil Sci. Soc. Am. J. 44, 635-639.

Chevrier, N., Sarkan, F., Chung, Y. S. (1988): Oxidative damages and repair in *Euglean gracilis* exposed to ozone. Plant Cell Physiol. 29, 321-327.

Clark, R. B., Tiffin, L. O., Brown, J. C. (1973): Organic acids and iron translocation in maize genotypes. Plant Physiol. 52, 147-150.

Das, K. N., Bordoloi, P. K., Bora, N. (1997): Tolerance level of iron in irrigation water for rice crop. Int. J. Trop. Agric. 15, 59-166.

Deturck, P. (1994): Iron toxicity to rainfed lowland rice in Sri Lanka. KULFLTB, Leuven, Belgium.

Dobermann, A., Fairhurst, T. H. (2000): Nutrient disorders and nutrient management. The International Rice Research Institute, Manila, The Philippines, p. 191.

Elstner, E. F. (1982): Oxygen activation and oxygen toxicity. Ann. Rev. Plant Physiol. 33, 73-96.

Emerson, D., Moyer, C. (1997): Isolation and characterization of novel iron oxidizing bacteria that grow at circumneutral pH. Appl. Environ. Microbiol. 60, 4032-4038.

Fageria, N. K. (1988): Influence of iron on nutrient uptake by rice. Int. Rice Res. Newsl. 13, 20-21.

Fairhurst, T. H., Witt, C. (2002): Rice: A practical guide to nutrient management. The International Rice Research Institute, Manila, The Philippines.

Fischer, W. R. (1983): Theoretische Betrachtung zur reduktiven Auflösung von Fe(III)-oxiden. Z. Pflanzenmähr. Bodenkd. 146, 611-622.

Frenzel, P., Bosse, U. (1999): Rice roots and methanogenesis in a paddy soil: Ferric iron as an alternative electron acceptor in rooted soil. Soil Biol. Biochem. 31, 421- 430.

Garrity, D. P., Oldeman, L. R., Morris, R. A. Lanka, D. (1986): Rainfed lowland rice ecosystems, in IRRI: Characterization and distribution: Progress in rainfed lowland rice. The International Rice Research Institute, The Philippines, pp. 3-24.

Genon, J. G., De Hepcee, N., Dufey, J. E., Delvaux, B., Hennebert, P. A. (1994): Iron toxicity and other chemical soil constraints to rice in highland swamps of Brundi. Plant Soil 166, 109-115.

Genon, J. G., De Hepcee, N., Dufey, J. E., Delvaux, B., Hennebert, P. A. (1995): Iron toxicity and other chemical soil constraints to rice in highland swamps of Brundi. Plant Soil 171, 165-171.

Green, M. S., Etherington, J. R. (1977): Oxidation of ferrous iron by rice roots: A mechanism for water logging tolerance. J. Exp. Bot. 28, 678-690.

Gunawardena, I., Virmani, S., Sumo, F. J. (1982): Breeding rice for tolerance to iron toxicity. Oryza 19, 5-12.

Gupta, A. S., Webb, R. P., Holaday, A. S., Allen, R. D. (1993): Over expression of superoxide dismutase protects plants from oxidative stress. Plant Physiol. 103, 1067-1073.

Halliwell, B. J., Gutteridge, M. C. (1984): Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem. J. 219, 1-14.

Harmsen, K., Van Breemen, N. (1975): Translocation of iron in sulfate acid soils: II. Reduction and diffusion of dissolved ferrous iron. Soil Sci. Am. Proc. 39, 1148-1153.

Höfner, W. (1970): Eisen- und manganhaltige Verbindungen im Blutungssaft von *Helianthus annus*. Physiol. Plant 23, 673-677.

Howeler, R. H. (1973): iron-induced oranging disease of rice in relation to physiochemical changes in a flooded Oxisol. Soil Sci. Soc. Am. Proc. 37, 898-903.

Howeler, R. H., Bouldin, D. R. (1971): The diffusion and consumption of oxygen in submerge soils Soil Sci. Soc. Am. Proc. 35, 202-208.

Hu, B., Wu, P., Liao, C. Y., Jing, W. Z., Zhu, J. M. (1999): Effect of ascorbate-specific H_2O_2-scavenging on tolerance to iron toxicity in rice. Acta Physiol. Sin. 25, 43-48.

Huke, R. E., Huke, E. H. (1997): Rice area by type of culture: Southeast and East Asia. A revised and updated database. The International Rice Research Institute, Manila, The Philippines.

IRRI? INGER (1996): Standard evaluation system for rice. 4th ed., International Rice Research Institute, Manila, The Philippines.

Jacq, V. A. (1977): Senssibilité du riz aux sulfures d origine microbeinne. Cahiers Orstom, Série Biologie XII 2, 97-99.

Jayawardena, S. D. G., Watabe, T., Tanaka, K. (1977): Relation between root oxidizing power and resistance to iron toxicity in rice. Crop Sci. Plant Breed. Soc. J. 22, 38-47.

Jugsujinda, A., Patrick Jr., W. H. (1993): Evaluation of toxic conditions associated with oranging symptoms of rice in a flooded Oxisol in Sumatra, Indonesia. Plant Soil 152, 237-243.

Kawase, M. (1981): Automatical and morphological adaptation of plants to water logging. Hortsci. 16, 30-34.

Kirk, G. J. D. (2004): The biochemistry of submerged soils. John Wiley & Sons, Chichester, UK., p. 291.

Kirk, G. J. D., Nye, P. H., Ahmad, A. R. (1990): Diffusion and oxidation of ferrous iron in the rice rhizosphere. Transactions 14th International Congress of Soil Science, Kyoto, Japan, August 1990, Volume II, pp. 153-157.

Kosegarten, H., Hofmann, B., Mengel, K. (1999): Apoplastic pH and Fe^{3+}-reduction in intact sunflower leaves. Plant Physiol. 121,1069-1079.

Kpongor, D. S., Vlek, P. L. G., Becker, M. (2003): Developing a standardized procedure to screen lowland rice (*Oryza sativa*) seedlings for tolerance to iron toxicity, in University of Göttingen and ATSAF: Technological and Institutional Innovations for Sustainable Rural Development. Klartext GmbH, Göttingen, Germany, p. 103.

Krairapanond, A., Jugsujinda, A., Patrick Jr, W. H. 81993): Phosphorous sorption in acid sulfate soils of Thailand: Effect of controlled and uncontrolled redox potential (Eh) and pH. Plant soil 157, 227-237.

Landsberg, E. C. (1996): Hormonal regulation of iron-stress response in sunflower root: a microbial and cytological investigation. Protoplasm 194, 69-80.

Lantin, R. S., Neue, H. U. (1989): Iron toxicity: a nutritional disorder in wetland rice. 17th Irrigated Rice Meeting. Brazil. 26-30 Sep. 1989. Lavoura-Arrozeira 42, 3-8.

Larson, R. A. (1988): The antitoxidants of higher plants. Phytochem. 27, 969-978.

Liao, Z. W., Woodard, H. J., Hossner, L. R. (1994): The relationship of soil and leaf nutrients to rice leaf oranging. J. Plant Nutr. 17, 1781-1802.

Liesack, W., Schnell, S., Revsbech , N. P. (2000): Microbiology of flooded rice bodies. FEMS Microbiol. Rev. 24, 625-645.

Lucena, J. J. (2000): Effects of bicarbonate, nitrate and other environmental factors on iron deficiency chlorosis. A review, in Lopez, M.: Environmental Factors and Fe Deficiency Chlorosis. Marcel Dekker Inc. New York, pp. 1591-1605.

Luo, A., Jing, G., Wu, P., Ni, J., Jiang, S., Zhang, Y. (1997): Rice genotypes differences in nutrient status under excessive ferric-iron conditions. J. Plant Nutr. 20, 1361-1373.

Machold, O., Meisel, W., Schnorr, H. (1968): Bestimmung der Bindungsformen des Eisens in Blätterndurch Mössbauer-Spektrometrie. Naturwissensch. 55, 499-500.

Maclean, J. L., Dawe, D. C., Hardy, B., Hettel, G. P (2002): Rice Almanac. CABI publishing, Wallingford, UK, p. 253.

Mahadevappa, M., Bulbule, A. V., Siddaramappa, R., Kulkarni, R. S. (1991): Field screening of rice for growth, yield and nutrient uptake in acidic soil. Develop. Plant Soil Sci. 45, 959-965.

Mandal, B., Chatterjee, J., Hazra, G., Mandal, L. N. (1992): Effect of pre-flooding on transformation of applied zinc and its uptake by rice in lateritic soils. Soil Sci. 153, 250-257.

Marschner, H. (1995): Mineral nutrition of higher plants. Functions of mineral nutrients: Micronutrients: Iron. Academic Press, London, pp.313-323.

Mengel, K. (1972): Einführung in den Stoffwechsel der Pflanze. G. Fischer Verlag, Stuggart.

Mengel, K., Kosengarten, H. (2000): Iron transport through plant membrane. Jahrestagung der Deutschen Gesellschaft für Pflanzenernährung 3-5.

Miyao, M., Ikeuchi, M., Yamamoto, N., Ono, T. (1995): Specific degradation of the D 1 protein of photosystem II by treatment with hydrogen peroxide in darkness: Implications for the mechanism of degradation of the D 1 protein under illumination. Biochem. 34, 10019-10026.

Monteiro, H. P., Winterbourn, C. C. (1988): The superoxide-dependent transfer of iron from ferritin to transferritin. Biochem. J. 256, 923-928.

Morel, D. A., Machado, M. O. (1981): Identification of iron toxicity in Brazil. Int. Rice Res. Newsl. 6, 9-9.

Munch, J. C., Ottow, J. C. G. (1977): Preferential reduction of amorphous to crystalline iron oxides by bacterial activity. Science 129, 15-31.

Navarro, N., Pedroso, A., Almoguea, S. (1988a): Influence of iron-mangnese relation in a dark plastic gley concretionary soil cropped with rice. Ciencias de la Agricultura, Ciudad de la Habana, Cuba, pp. 7-14.

Neue, H. U., Quijano, C., Senadhira, D., Setter, T. (1998): Strategies for dealing with micronutrient disorders and salinity in lowland rice systems. Field Crop. Res. 56, 139-155.

Nicolic, M., Römheld, V. (2001): The role of the leaf apoplast in iron nutrition of plants, in Horst, W. J. et al.: Plant Nutrition – food security and sustainability of Agro-Ecosystems. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 274-275.

Ottow, J. C. G., Benckiser, G., Watanabe, I. (1982): Iron toxicity of rice as a multiple nutritional soil stress. In Proc. Int. Symp. On Distribution, Characteristics and Utilization of Problem soils. Tropical Agriculture Research Center, Ibaraki, pp. 167-179.

Patra, B. N., Mohany, S. K.. (1994): Effects of nutrients and liming on changes in pH, redox potential and uptake of iron and manganese by wetland rice in iron toxic soil. Biol. Fertile. Soils 17, 285-288.

Patrick Jr., W. H., Reddy, C. N. (1978): Chemical changes in rice soils, in IRRI: Soils and Rice. The International Rice Research Institute, Manila, The Philippines, pp. 361-379.

Peterson, D. A. (1991): Enhanced electron transfer by unsaturated fatty acids and superoxide dismutase. Free Radicals Res. Comm. 12/13, 161-166.

Ponnamperuma, F. N. (1972): The chemistry of submerged soils. Adv. Agron. 24, 29-96.

Ponnamperuma, F. N. (1977): Physico-chemical properties of submerged soil in relation to fertility. IRRI Research Paper Series No. 5. The International Rice Research Institute, Manila, The Philippines.

Ponnamperuma, F. N., Tianco, E. M., Loy, T. (1967): Redox equilibria in flooded soils: The iron hydroxide systems. Soil Sci. 103, 374-382.

Prade, K. (1987): Einfluß der Nährstoffversorgung auf die Eisentoxizität bei Naßreis (*Oryza sativa* L.) in der Basse Casemance/Senegal. Ph.D. Thesis, University of Gießen, Germany.

Prade, K., Ottow, J. C. G., Jacq, V. A., Malouf, G., Loyer, J. Y. (1990): Relationships between the properties of flooded rice soils and iron toxicity in Lower Casemance (Senegal). Studies, review and summary of previous work. Cahiers ORSTOM, Serie Pedologie, IRD, Montpelliers, France, pp. 453-474.

Rajkumar, G. R., Patil, C. V., Prakash, S. S., Yeledhalli, N. A. (1997): Distribution of forms of iron in relation to the properties of rice soils. J. Maharashtra Agricult. Univ. 22, 267-270.

Ratering, S., Schnell, S. (2000): Localization of iron-reducing activity in paddy soil by profile studies. Biochem. 48, 341-365.

Revsbech, N. P., Jorgensen, B. B., Blackburn, T. H. (1980): Oxygen in the sea bottom measured with a microelectrode, Science 207, 1355-1356.

Ripken, C., Asch, F., Becker, M., Wimmer, M. (2005): A low cost miniature method to determine iron content in samples suitable for small research laboratories. Poster, Tropentag, 2005, Stuttgart, Germany.

Rorison, I. H. (1973): The effect of extreme soil acidity on the nutrient and physiology of plants: Acid Sulfate Soils, Proceedings of the International Symposium on Acid Sulfate Soils. International Institute for Land Reclamation and Improvement. Wageningen, The Netherlands. Publ. 18, Vol. II, 391-406.

Sadana, U. S., Chahal, D. S., Abadia, J. (1995): Iron availability, electrochemical changes and nutrient content of rice as influenced by green manuring in a submerged soil. Iron nutrition in soils and plants. Develop. Plant Soil Sci. 59, 105-109.

Sahrawat, K. L. (1979): Iron toxicity to rice in an acid sulfate soil as influenced by water regimes. Plant Soil 51, 143-144.

Sahrawat, K. L. (2000): Elemental composition of rice plant as affected by iron toxicity under field conditions. Comm. Soil Sci. Plant Anal. 31, 2819-2827.

Sahrawat, K. L., Diatta, S. (1995): Nutrient management and season affect soil iron toxicity. Annual Report, 1994. West Africa Rice Development Association, Bouaké, Côte d Ivoire, pp. 34-35.

Sahrawat, K. L., Mulbah, C. K, Diatta, S., Delaune, R. D., Patrick Jr., W. H., Singh, B. N., Jones, M. P. (1996):The role of tolerant genotypes and plant nutrients in the management of iron toxicity in lowland rice. J. Agric. Sci. 126, 143-149.

Sajwan, K.S., Lindsay, W. L. (1988): Effects of redox on zinc deficiency in paddy rice. Soil Sci. Soc. AM. J. 50, 1264-1269.

Satawathananont, S., Patrick Jr., W. H., Moore Jr., P. A. (1991):Effect of controlled redox conditions on metal solubility in acid sulfate soils. Plant Soil 132, 281-290.

Schmidt, W. (1999): Mechanisms and regulation of reduction-based iron uptake in plants. New Phytol. 141, 1-26.

Schwertmann, U. (1985): The effect of pedogenic environments on iron oxide minerals. Adv. Soil Sci. 1, 171-200.

Seckbach, J. (1982): Ferreting out the secrets of plant ferritin – a review. J. Plant Nutr. 5, 369-394.

Singh, B. P., Das, M., Prasad, R. N., Ram, M. (1992): Characteristics of Fe-toxic soils and affected plants and their correction in acid Haplaquents of Meghalaya. Int. Rice Res. Newsl. 17, 18-19.

Smith, B. N. (1984): Iron in higher plants: Storage and metabolic role. J. Plant Nutr. 7, 759-766.

Straub, K. L., Benz, B. Widdel, F. (1996): Anaerobic nitrate-dependent microbial oxidation of ferrous iron. Appl. Environ. Microbiol. 62, 1458-1460.

Swarup, A. (1988): Effect of gypsum, farmyard manure and rice husk on the availability iron and manganese to rice in submerged sodic soil. Oryza 38, 42-42.

Tadano, T. (1975): Devices of rice roots to tolerated high iron concentration in growth media. Jpn. Agr. Res. Quart. (JARQ) 9, 34-39.

Tadano, T. (1976): Studies on the method to prevent iron toxicity in the lowland rice. Mementos of the Faculty of Agriculture, Hokkaido University 10, , 22-68.

Tadano, T., Yoshida, S. (1978): Chemical changes in submerged soils and their effect on rice growth, in IRRI: Soils and rice. The International Rice Research Institute, Manila, The Philippines, pp. 399-420.

Tanaka, A., Navasero, S. A. (1966): Manganese content of rice plant under water culture conditions. Soil Sci. Plant Nutr. 12, 67-72.

Tanaka, A., Loe, R., Navasero, S. A. (1966): Some mechanism involved in the development of iron toxicity symptoms in the rice plant. Soil Sci. Plant Nutr. 12, 158-164.

Thompson, J. E., Legge, R. L. (1987): The role of free radicals in senescence and wounding. New Phytol. 105, 317-344.

Thongbai, P., Goodmann, B. A. (2000): The radical generation and post-anoxic injury in rice grown in an iron-toxic soil. Ninth International Symposium on Iron Nutrition and Interactions in Plants, Stuttgart, Germany, pp. 1887-1900.

Tinh, T. K. (1999): Reduction chemistry of acid sulphate soils: Reduction rates and influence of rice cropping. Acta Universitatis Agriculturae Sueciae, Agraria 206, Uppsala, Sweden.

Tinh, T. K., Nilsson, I., Thu Houng, H. T. (2000): Effect of the depth of water submergence on rice performance and soil solution chemistry. J. Soil Use Manage 1, 26-31.

Trolldenier, G. (1988): Visualization of oxidizing power of rice root and possible participation of bacteria in iron deposition. Z. Pflanzenernähr. Bodenkd. 151, 117-121.

Tsuchiya, M., Miyake, M., Bonilla, P., Kumano, S. (1995): Physiological response to salinity in rice plant.: IV. Ion exclusion of the excised root exposed to NaCl stress with hydraulic pressure. Jap. J. Crop Sci. 64, 93-101.

Van Breemen, N., Moormann, F. R. (1978): Iron-toxic soils, in IRRI: Soils and Rice. The International Rice Research Institute, Manila, The Philippines, pp. 781-799.

Van Breemen, N., Pons, L. J. (1978): Acid sulfate soils and rice, in IRRI: Soils and Rice, The International Rice Research Institute, Manila, The Philippines.

Virmani, S. S. (1977): Varietal tolerance of rice to iron toxicity in Liberia. Int. Rice Res. Newsl. 2, 4-5.

Vladimirov, Y. A. (1980): Lipid peroxidation in mitochondrial membrane. Adv. Lipid Res. 17, 409-423.

Vose, P. B. (1983): Rationale of selection for specific nutritional characters in crop improvement with *Phaseolus vulgaris* L. as a case study. Plant Soil 72, 351-364.

Wang, J. Liu, Z. Y. (1992): Iron transformation in rice rhizosphere. Acta Pedol. Sin. 29, 358-364.

Wang, J., Shuman, L. M. (1994): Transformation of phosphorus in rice (*Oryza sativa* L.) rhizosphere and its influence on phosphorous nutrition of rice. J. Plant Nutr. 17, 1803-1815.

Wan, J. L., Zhai, H. Q., Wan J. M., Ikehashi, H. (2003a): Detection and analysis of QTLs for ferrous iron toxicity tolerance in rice (*Oryza sativa* L.). Rice Genet. Newsl. 19, 56-58.

Wan, J. L., Zhai, H. Q., Wan J. M., Yasui, H., Yoshimura, A. (2003b): Mapping QTL for traits associated with resistance to ferrous iron toxicity in rice (*Oryza sativa* L.), using Japonica chromosome segment substitution lines. Yi Chuan Xue Bao 30, 893-899.

WARDA (West Africa Rice Development Association) (1991): Annual Report 1990. WARDA, Bouaké, Côte d Ivoire, pp. 52-53, 68, 74-76.

WARDA (West Africa Rice Development Association) (1995): Annual Report 1994. WARDA, Bouaké, Côte d Ivoire, pp. 57-59.

WARDA (West Africa Rice Development Association) (1998): Annual Report 1997. WARDA, Bouaké, Côte d Ivoire, pp. 57-59.

WARDA (West Africa Rice Development Association) (2002): Annual Report 2001-2002. WARDA, Bouaké, Côte d Ivoire, pp. 25-37.

Welch, R. M., Norvell, W. A., Schaefer, S. C., Shaff, J. E., Kochian, L. V. (1993): Induction of iron(III) and copper(II) reduction in pea (*Pisum sativum* L.) by Fe and Cu status: Does the root-cell Fe(III)-chelate reductase perform a general role in regulating cation uptake? Planta 190, 555-561.

Wissuwa, M. (2005): Mapping nutritional traits in crop plants, in Broadley, M., White, P.: Plant Nutritional Genomics. Blackwell Publishing, UK, in press.

Wu, P., Lou, A., Zhang, L., Wu, Y., Huang, N. (2003): Molecular markers linked to genes for tolerance to ferrous toxicity. Rice Genet. Newsl. 14, 17-17.

Yamauchi, M. (1989): Rice bronzing in Nigeria caused by nutrient imbalances and its control by potassium sulphate application. Plant Soil 117, 21-28.

Yamauchi, M., Peng, X. X. (1993): Ethylene production in rice bronzing leaves induced by ferrous iron. Plant Soil 149, 227-234.

Yamauchi, M., Peng, X. X. (1995): Iron toxicity and stress-induced ethylene production in rice leaves. Plant Soil 173, 21-28.

Yamanouchi, M., Yoshida, S. (1981): Physiological mechanisms of rice's tolerance for iron toxicity. Paper presented at the IRRI Saturday Seminar, June 6, 1981. The International Rice Research Institute, Manila, The Philippines.

Yamanouchi, M., Tominaga, Y., Fujiyama, H., Nagai, T. (1989): Iron oxidizing mechanisms in nutrient solution during cultivation of rice plant. J. Faculty Agric. Tottori Univ. 25, 9-14.

Yeo, A. R., Yeo, M. E., Flowers, T. J. (1987): The contribution of an apoplastic pathway to sodium uptake by rice roots in saline conditions. J. Exp. Bot. 38, 1141-1153.

Yoshida, S. (1981): Fundamentals of rice crop science. The International Rice Research Institute, Manila, The Philippines.

Zhang, X., Zhang, F., Mao, D. (1998): Effect of iron plaque outside roots on nutrient uptake by rice (*Oryza sativa* L.): II. Zinc uptake by Fe-deficient rice. Plant Soil 202, 33- 39.

Zhao, H. (1992): Differentiation of iron oxides in paddy soils of Chengdu Plain. Acta Pedol. Sin. 17, 191-198.

Appendices

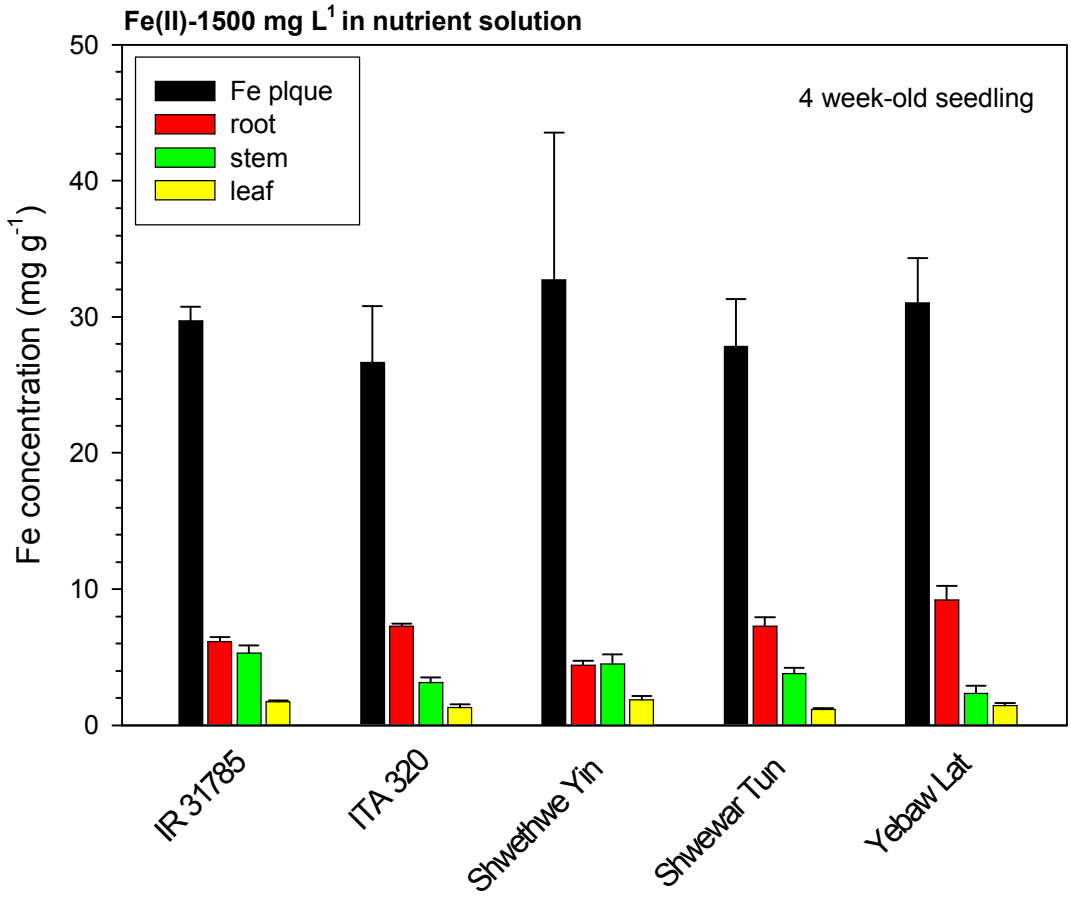

Figure A1: **Root Fe plaque and Fe partitioning within plant tissues of 5 cultivars (3 days after exposure to external Fe(II), 1500 mg L^{-1} in nutrient solution at 4 week-old seedling stage). Error bars = SE (n = 3 for root Fe plaque and root-tissue Fe concentration, and n = 6 for stem and leaf-tissue Fe concentration).**

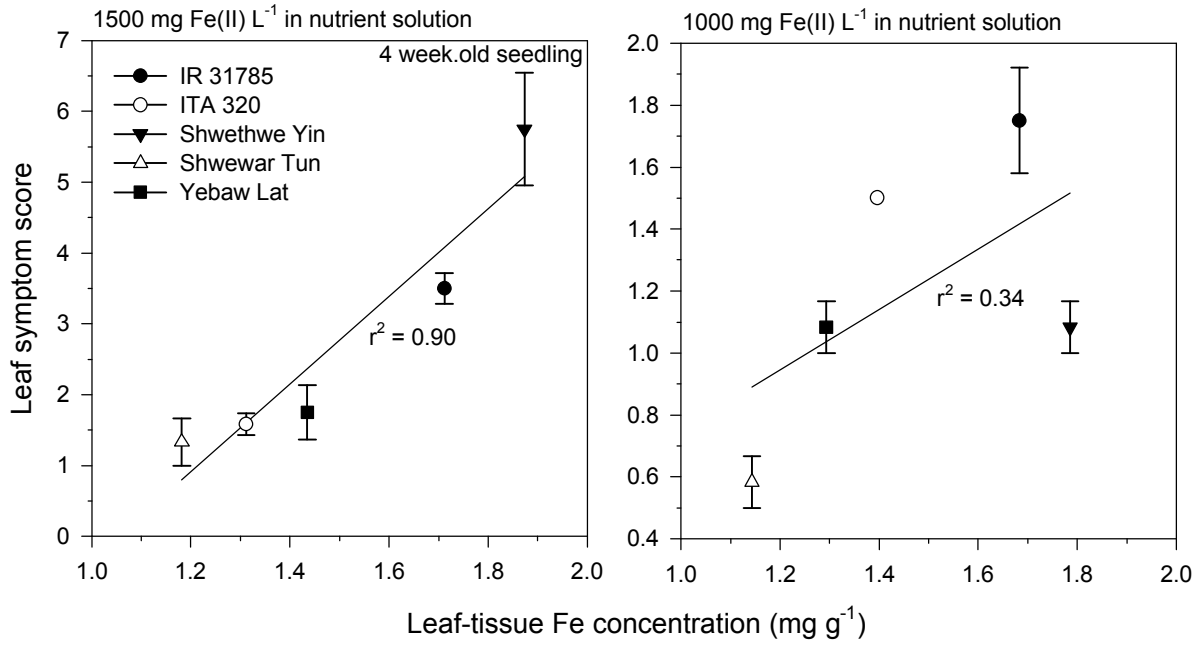

Figure A2: The relationship between symptom scores and leaf-tissue Fe concentrations of 5 rice cultivars (3 days after exposure to external Fe(II) 1000 and 1500 mg L^{-1} in nutrient solution at 4 week-old seedling stage). Error bars = SE (n = 3).

Table A1: **Statistical analysis for symptom scores of 10 cultivars (3 days after exposure to 1000 and 1500 mg Fe(II) L^{-1} in nutrient solution at 4 week-old seedling stage).**

Post Hoc Tests
Homogeneous Subset

Fe(II) 1000 mg L^{-1} treatment

symptoms

Duncan[a,b]

Cultivars	N	Subset 1	Subset 2	Subset 3
Pawsan Baykya	6	.5000		
Sahel 108	6	.5833		
Shwewar Tun	6	.5833		
Imma Yebaw	6	.7500	.7500	
Thukha Yin	6	.8333	.8333	
Shwethwe Yin	6		1.0833	
Yebaw Lat	6		1.0833	
Manaw Thukha	6		1.0833	
ITA 320	6			1.5000
IR 31785	6			1.7500
Sig.		.078	.078	.138

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = .083.

a. Uses Harmonic Mean Sample Size = 6.000.

b. Alpha = .05.

Fe(II) 1500 mg L^{-1} treatment

Symptoms

Duncan[a,b]

Cultivars	N	Subset 1	Subset 2	Subset 3	Subset 4
Shwewar Tun	6	1.3333			
Imma Yebaw	6	1.5000	1.5000		
ITA 320	6	1.5833	1.5833		
Sahel 108	6	1.5833	1.5833		
Yebaw Lat	6	1.7500	1.7500		
Pawsan Baykya	6	2.6667	2.6667	2.6667	
Manaw Thukha	6		3.1667	3.1667	
IR 31785	6			3.5000	
Thukha Yin	6			4.0833	
Shwethwe Yin	6				5.7500
Sig.		.139	.064	.101	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 1.807.

a. Uses Harmonic Mean Sample Size = 6.000.

b. Alpha = .05.

Table A2: **Analysis of variance (DMRT) for tissue Fe concentrations (stem, leaf, root-unwashed, shoot and total Fe) of 7 cultivars (3 days after exposure to 1500 mg Fe(II) L^{-1} in nutrient solution at 4 week-old seedling stage).**

Post Hoc Tests

Homogeneous Subsets

Stem Fe

Duncan[a,b,c]

Cultivars	N	Subset 1	Subset 2	Subset 3
Yebaw Lat	6	2.3300		
Pawsan Baykya	6	2.8133		
ITA 320	6	3.1567	3.1567	
Shwewar Tun	6	3.7700	3.7700	3.7700
Shwethwe Yin	6		4.5200	4.5200
Sahel 108	6			4.8533
IR 31785	6			5.2833
Sig.		.078	.085	.065

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 1.597.

a. Uses Harmonic Mean Sample Size = 6.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Leaf Fe

Duncan[a,b,c]

Cultivars	N	Subset 1	Subset 2
Pawsan Baykya	6	1.1667	
Shwewar Tun	6	1.1817	
ITA 320	6	1.3117	
Yebaw Lat	6	1.4350	1.4350
IR 31785	6	1.7117	1.7117
Shwethwe Yin	6	1.8733	1.8733
Sahel 108	6		2.1833
Sig.		.097	.068

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = .400.

a. Uses Harmonic Mean Sample Size = 6.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Table A2 continued.

Root Fe (unwashed with HCl)

Duncan[a,b,c]

Cultivars	N	Subset 1	Subset 2
Shwethwe Yin	6	29.1383	
Pawsan Baykya	6	33.4683	33.4683
Yebaw Lat	6	34.3033	34.3033
ITA 320	6	35.2200	35.2200
Sahel 108	6	36.3617	36.3617
IR 31785	6		41.0683
Shwewar Tun	6		41.2483
Sig.		.198	.173

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 72.191.

a. Uses Harmonic Mean Sample Size = 6.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Shoot Fe

Duncan[a,b,c]

Cultivars	N	Subset 1	Subset 2	Subset 3
Yebaw Lat	6	3.7633		
Pawsan Baykya	6	3.9800		
ITA 320	6	4.4683		
Shwewar Tun	6	4.9533	4.9533	
Shwethwe Yin	6		6.0517	6.0517
IR 31785	6			6.9950
Sahel 108	6			7.0400
Sig.		.128	.125	.191

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 1.466.

a. Uses Harmonic Mean Sample Size = 6.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Table A2 continued.

Plant total fe

Duncan[a,b,c]

Cultivars	N	Subset 1	Subset 2
Shwethwe Yin	6	35.3400	
Pawsan Baykya	6	37.4500	37.4500
Yebaw Lat	6	38.0700	38.0700
ITA 320	6	39.6867	39.6867
Sahel 108	6	42.5950	42.5950
Shwewar Tun	6	46.1983	46.1983
IR 31785	6		48.0633
Sig.		.061	.067

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 73.920.

a. Uses Harmonic Mean Sample Size = 6.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Table A3: **Analysis of variance (DMRT) for root Fe plaques of 7 cultivars (3 days after exposure to 1000 and 1500 mg Fe(II) L^{-1} in nutrient solution at 4 week-old seedling stage).**

Post Hoc Test

Homogeneous Subsets

Root Fe plaque

Duncan[a,b]

Cultivars	N	Subset 1
Pawsan Baykya	3	24.6043
ITA 320	3	26.6367
Shwewar Tun	3	27.8200
IR 31785	3	29.7133
Yebaw Lat	3	31.0400
Shwethwe Yin	3	32.7167
Sahel 108	3	33.8908
Sig.		.362

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 117.068.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Table A4: **Analysis of variance (DMRT) for root-tissue Fe concentrations (acid washed) of 5 cultivars (3 days after exposure to 1500 mg L^{-1} Fe(II) in nutrient solution at 4 week-old seedling stage).**

Post Hoc Tests

Homogeneous Subsets

Root Fe (washed with HCl)

Duncan[a,b]

Cultivars	N	Subset 1	Subset 2	Subset 3
Shwethwe Yin	3	4.4200		
IR 31785	3	6.1467	6.1467	
Shwewar Tun	3		7.2767	
ITA 320	3		7.2933	
yebaw Lat	3			9.2267
Sig.		.065	.218	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 1.038.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Curriculum Vitae

Name	Tam Aung
Date of birth	27 December 1970
Place of birth	Mindat, Myanmar
Nationality	Myanmar (Chin)
Education	M.Sc.
	Institute of Crop Science and Resource Conservation
	Department of Plant Nutrition
	Thesis title: **Physiological mechanisms of iron toxicity tolerance in lowland rice.**
	B.Agri.Sc
	Yezin Agricultural University
	Myanmar
Occupation	Assistant supervisor
	MCSE, Ministry of Agriculture and Irrigation
	Myanmar
Trainings	Desert Agriculture and Drip Irrigation
	Israel, 1997-1998.