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Developing a Standardized Procedure to Screen Lowland Rice (*Oryza sativa*) Seedlings for Tolerance to Iron Toxicity.

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Abstract

Iron toxicity during the seedling stage widely affects the yield of lowland rice in West Africa. 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 a standardized screening methodology. Thus, cultivars that reportedly showed Fe(II) tolerance in Asia, failed to produce reliable results in Africa and *vice versa*. The aim of the present research was to develop a simple, cheap and rapid method for screening rice cultivars for Fe(II) tolerance and for studying tolerance mechanisms under standardized conditions.

Four experiments were conducted in a phytotron of the University of Bonn, Germany in 2003 with the following objectives: (1) establish a standardized growth and nutritional conditions for rice seedling growth in agar culture, (2) determine the rate and the timing of Fe(II) addition that allows for a rapid and reproducible visual differentiation (leaf bronzing score) of tolerant and sensitive rice cultivars; (3) determine the effect of relative air humidity-rH (vapour pressure deficit) on Fe uptake (acropetal transport) and bronzing symptom expression in leaves; and (4) validate the set-up using a range of cultivars with known responses to Fe(II).

Growing rice seedlings in 150 ml pots containing a 1% agar and 150 ml of culture solution, both of half strength of the standard rice-growing Yoshida medium, allowed to grow healthy seedlings for up to four weeks. Three ml of paraffin oil largely avoided the oxidation of Fe(II) in the medium. Addition of 0-3000 mg Fe(II) l^{-1} differentially affected the time of occurrence and the severity of expression of leaf bronzing symptoms in tolerant (*Suakoko8*) and sensitive (*IR31785-58.1-2-3-3*) test cultivars. A clear and reliable visual cultivar's differentiation was possible already three days after exposure of the seedlings to a concentration of 2000 mg Fe(II) l^{1}. Slight variations in the vapour pressure deficit (60-65% rH) had no significant effect on seedling growth or symptom score but tended to increase leaf Fe content in the sensitive check cultivar. Leaf bronzing scores and tissue Fe analysis of 2 and 4 week-old seedling exposed for three days to 2000 mg Fe(II) l^{-1} allowed for the classification of the 14 test cultivars into (1) Fe-sensitive plants (leaf bronzing, tissue Fe > solution Fe); (2) tolerant Fe-excluders (low leaf bronzing, tissue Fe < solution Fe) and (3) tolerant includers (low leaf bronzing, tissue Fe > solution Fe). It may be concluded that the proposed culture set-up allows within 3 days of Fe(II) addition to 2 and 4 week-old rice seedlings to reliably differentiate tolerant from susceptible cultivars. A validation using a wider range of cultivars and breeding lines and further studies on the effect of the vapour pressure deficit are required.

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List of Abbreviations

IRRI:	International Rice Research Institute
RH:	Relative humility
SARI:	Savanna Agricultural Research Institute
VPD:	Vapour pressure deficit
WARDA:	West African Rice Development Association
WAS:	Weeks after sowing
wks.	Weeks

1 INTRODUCTION

1.1 Background

Rice has become a commodity of strategic importance across much of Africa. Driven by changing food preferences in both urban and rural areas and compounded by high population growth rates, rice consumption in Sub-Saharan Africa has increased by 5.6% per annum between 1961 and 1992 which was more than double the rate of population growth. Projections by the FAO suggest rice consumption in West Africa to remain high, and continue to increase at 4.5% through the year 2000 and beyond (WARDA, 2000).

West Africa's rice production, however, has not been able to meet the growing demand due to a number of production constraints. The increasing gap between regional supply and demand is been filled by rising import at a growth rate of 8.4% per annum since 1997. Rice imports amounts to up to 4 million tons in the year 2000, raising spending of the scarce foreign exchange to more than $ 1 billion (WARDA, 1997). The desired increase in domestic rice production can be achieved by increasing the production area beyond the currently 5 million hectares and or to increase productivity on existing rice lands. Area expansion is possible since only about 20% of the available inland valley swamps are currently in use for agriculture (Windmeijer *et al*., 1998). Much of this available area however, is characterised by a number of abiotic constraints of which uncontrolled flooding and the occurrence of iron toxicity are the most prominent. Maschner (1995) stated that Fe toxicity is the second most important yield limiting abiotic stress in lowland rice production. Productivity gains on existing rice land are seemed to be tied primarily to progress in the areas of weed control, nitrogen supply and management of iron toxicity. WARDA (1998) projected that advances in the control of iron toxicity alone is likely to result in regional production gains in the in the order of 50,000 tons of grain per year (WARDA, 1999).

Flooded soils are characterised by periodic changes of aerobic and anaerobic conditions. Oxygen diffuses faster in air than in water or in water saturated soils (Amstrong, 1979). Hence it is rapidly depleted by the respiration of micro organisms and plant roots. In flooded soils, after the depletion of oxygen, NO_3; Mn^{4+}, Fe^{3+}, SO_4^{2-} act as electron acceptors for facultative anaerobic micro-organisms and are subsequently reduced. The reduction of O_2 and NO_3 takes place within a couple of hours and is followed by reduction of Mn^{4+} and Fe^{3+} which can occur within a few

days after flooding (Ponnamperuma, 1972). Therefore, shortly after the 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 characterised by light textured soils with high extractable acidity (Benckiser *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 landscapes of humid and sub-humid West Africa. It also occurs when large amounts of ferrous iron are mobilised *in-situ* in soil solution or when interflow brings ferrous ions from adjacent slopes (Ponnamperuma 1972, Sahrawat, 1999).

Iron toxicity is a nutrient disorder which is brought about by the uptake of Fe(II) in amounts that disrupts or over express a number of metabolic processes, resulting in damage of the rice plant. It is characterised by rusty leaf spots (bronzing), stained leaf edges and a dark brown and rigid poorly developed root system. It has been reported to widely occur in several Asian countries, including China, India, Indonesia, Thailand, Malaysia, and the Philippines. In West Africa, it is widespread throughout the humid forests and Savanna zones in about 30 to 40 % of all cultivated lowlands (WARDA, 1997) and there, rice yields are reportedly reduced by 12-100% depending on the severity of toxicity and the tolerance of the rice cultivars (Benckiser *et al*., 1982, Sahrawat *et al*.,1995, Audebert *et al*., 2000,).

First reported by Pounamperuma *et al*., (1955), iron toxicity in rice has been subject to various types of investigation. These include disciplines such as microbiology, plant nutrition, soil chemistry, water management and plant breeding. Breeding approaches to address Fe toxicity are generally favoured as they are low cost to farmers. The absence of a mechanistic understanding of the factors resulting in the expression of Fe toxicity symptoms in rice has resulted in a ``black-box screening´´ in most rice breeding programs of Asia and Africa and has lead to variable results with inconsistencies (M. Becker, personal communication). Thus cultivars or breeding lines that have been described as tolerant in Asia have succumbed to iron toxicity in Africa or when planted under different climatic conditions.

Some investigations indicate mechanisms by which tolerant rice varieties may be able to grow quite healthily under conditions of high Fe without displaying symptoms of toxicity. Thus Green and Etherigton (1966) reported the oxidizing power of rice

roots to be the dominate mechanism for tolerance for rice to plants conditions of excess of Fe. Other mechanisms reported include the iron-exclusion by the roots, which exclude Fe at the root surface which may prevent Fe (II) from entering the roots (Tadona, 1975) or the iron-retaining power in root tissues, reducing the Fe(II) translocation from the roots (compartmentation). Another possible mechanism is the retention of iron in above-ground plant parts such as Fe^2 retention in the stem tissue (Audebert and Sahrawat, 2000) and in the apoplasm of the leaf (Kosegarten *et al*.,1999).

For Fe toxicity to occur, Fe(II) must pass the oxidation barrier of the rhizosphere before it can be taken up by the root. Rice roots diffuse molecular oxygen into the root medium though the 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 passed the barrier of the caspirian strip (Marschner,1993). There are indications that an apoplasmic pH of more than 6.5 prevents influx of Fe^2 into the symplasm (Kosegarten *et al*., 1999) which may be one mechanism explaining a high tissue tolerance describe the high levels of Iron detected in the leaf tissue.
The understanding of the mechanisms of uptake and tissue tolerance of Fe(II) is necessary to improve the targeting of interventions and to develop physiology – based breeding tools.

1.2 Hypothesis and Objectives

The determination of physiological mechanism of tolerance and the development of improved plant breeding and selection strategies, requires a standardized, and repeatable screening set-up and procedure. The objectives of the study were therefore to the following:
a) Develop standardized growth and nutritional conditions for rice seedling growth in agar culture.
b) Determine the amount of Fe(II) required to visually differentiate between standard checks varieties for Fe(II) tolerance and sensitivity.
c) Determine the effect of relative air humidity (VPD) on Fe uptake and the severity of symptom expression in leaves.
d) Validate the standard set-up developed with a range of cultivars and breeding lines, reported to be sensitive/tolerant to excess Fe(II).

2 LITERATURE REVIEW

Iron toxicity is a nutritional disorder in plants caused by excess uptake of Fe by plants, resulting in brown spots starting from the tip or whole leaves coloured reddish-brown (leaf bronzing). It also results in root damage and may lead to high yield loses.

2.1 Characteristics of iron toxic soil

A first requirement for Fe toxicity to occur is the presence of iron in the soil which may be formed from various base rocks. Iron toxicity can occur where the parent rock material is rich in iron (Abifarin, 1990, Zhao 1992)). Thus, Alfisols derived from gneiss have relatively higher amounts of total iron than those derived from granite (Rajkumar *et al*., 1997). Wang *et al*. (1994) found that iron accumulation was high in granitic soils, particularly in upland soils. The relation between iron (II) and iron (III), however depends on other environmental conditions.

Soil texture is an other important factor in iron toxic soils since it exerts an influence on the iron dynamics in the soil. The net transfer of iron depends on the bulk density and gaseous porosity of soils (Kirk and Solivas,1990). Fe content of plants decreases significantly in fine textured soils, indicating a positive influence of the soil clay content towards reducing iron concentrations in the plant (Das *et al*., 1998). Clay was found to control the content and distribution of residual iron in both Alfisols and Vertisols (Rajkumar *et al*., 1998). Clay content therefore seems to be important for Fe dynamics in the soil because of it's influence on CEC. Thus, iron toxicity can be found in soils where the predominant clay mineral is kaolinite with their relatively low cation exchange capacity whereas absent in areas dominated by smectites or kaolinites associated with illites.

2.2 Interaction of Fe with other nutrients

In the process of the microbial reduction of Fe(II) oxides and hydroxides and other nutrients such as phosphorous, zinc, silicon and molybdenum (Ponnamperuma, 1972, 1977 a/b). Apart from these nutrients, toxic elements such as aluminium may also be released and cause additional hazards to the rice plant. Large amounts of salts can further enhance the accumulation of reduced Fe in the soil solution since salts are likely to compete for binding sites in the soil and thereby increase Fe (II) in the soil solution (Ponnamperuma, 1977b).

In most cases, iron will interact with other nutrients in the soil through antagonistic or synergistic interaction. One of the antagonist is manganese. Application of manganese can suppress iron desorption and subsequently total iron uptake from the soils. Similarly, high amounts of iron suppress the uptake of manganese. On the other hand, iron oxides are known to have a strong zinc binding capacity. The reduction of iron oxides and the resulting increase in Fe availability to plants is generally associated with an increased availability of Zn. Silicon as a beneficial (not essential) element in the soil can reduce concentration and uptake of iron in the plant (Verma and Minhas, 1989). In general, the uptake by plant of cationic nutrients is reduced with increasing iron concentration Fe(II) in the growth medium. The inhibition plant nutrient uptake by iron can be put in the in the order; P > K > N > Mg > Ca and for micronutrients- Mn > Zn > Cu. Consequently, with increasing iron concentrations in lowlands, phosphorous, potassium, and zinc deficiencies are likely to be the first to appear (Fageria, 1988). Wang *et al* (1994) conclude that Fe(III) deposition on plant roots and in rhizoshpere might block the uptake of other nutrients. Similarly, Jugsujinda and Patrick (1993) indicate that root growth was limited when root surfaces were coated with iron and manganese oxides by reducing the root's capacity to absorb nutrients from the soils. The inhibitory effect of Fe(II) on the uptake of PO_4^{2-} is not yet well understood.

2.3 Fe uptake and distribution

Rice plants have the tendency of taking up more iron than most other plants. The natural habitat of the wild form of rice is similar to the cultivated paddy field. As a rule, Fe(II) is the species taken up by the plant. Since Fe(II) can easily be taken up by the plant and is available in large quantities in waterlogged conditions where wetland rice is grown, the uptake mechanism of Fe(III) is probably of less importance. Nevertheless, plants have developed two different pathways for Fe uptake and assimilation. Dicots and non-grass monocots obtain iron as Fe(II) after reduction by a trans-plasma-membrane electron transport system (strategy I) (c). Under iron deficiency, enhancement of plasma membrane iron reducing activity is accompanied by an increase of H^+ secretion and release of phenolic compounds, capable of reducing/chelating iron (Bienfait, 1985). Grasses use phytosiderophores that are able to form complexes with Fe(III) (Römheld, 1987). These complexes are resorbed by the plant (Bienfait, 1985). Siderophore excretion by the rice plant is usually low as it is usually not required in the anaerobic environments where rice is grown (Takagi,

1976). The strategies mentioned above are important mainly for mobilizing Fe(III) under defieciency conditions. However, excess amounts of Fe(II) is the main problem in iron toxic soils.

2.4 Fe in the plant

Iron content in the leaves of the rice plant as much as 100-200 mg l^{-1} are considered to be normal. Above an iron concentration of 300 mg l^{-1} in the leaves the rice plant may already experiences iron toxic stress (Tanaka and Yoshida, 1972). In green leaves, about 80 % of the iron is accumulated in the chloroplast (Bienfait, 1985, Marschner, 1993). Iron is stored in the plant cells in the stroma of plastids as phytoferritin. This is a hollow shell which can store up to 5000 atoms of iron as Fe(III), often in well-defined crystalline form (Seckbach, 1982). The localization of phytoferritin is not confined to the chloroplasts but can also be found in the xylem and phloem (Smith, 1984).Ferrous iron Fe(II) is the physiologically active form of iron and the fraction which undergoes reversible Fe(III)/Fe(II) oxidoreduction (Machold ***et al***, 1968). Physiologically active iron present within plant tissues may catalyse the generation of active oxygen species. The presence of large amounts of Fe(II) greatly enhance formation of reactive oxygen species such as superoxide, hydroxyl-radical, and H_2O_2 resulting in oxidative stress which can eventually damage plant cells. It can be concluded that excess amounts of Fe(II) in the plant tissue causes elevated production of toxic oxygen radicals.

Radicals within biological tissues cause a multitude of damages. Membrane lipids (Thompson and Legge, 1987), membrane charge proteins and nucleic acids (Elstner, 1982) are irreversibly damaged by oxygen radicals. Free radical formation will eventually lead to stimulation of chlorophyll oxidation and subsequently to a decrease of chlorophyll content upon accumulation of high concentrations of iron (Monteiro ***et al***., 1988). Thus, to prevent oxidation damage iron has either to be tightly bound or incorporated into structures (e.g. heme-, nonheme proteins) which allow controlled oxidation/reduction reactions; Fe(II) - e^- <=> Fe(III) + e^- including those in antioxidant

2.5 Symptoms of Fe toxicity

In case of iron toxicity the activity of polyphenol oxidases is increased and oxidised polyphenols are the direct cause of the discolouring of rice leaves commonly referred to as "bronzing" - the symptomatic expression of iron toxicity in rice. Symptoms may

vary, depending on the variety and associated imbalances. Bronzing of the leaves starting at the tips is characteristic, oranging or yellowing of the leaves may occur; stunting may also occur, with scanty root production and spikelet sterility (Virmani, 1977).

The visual symptoms of iron toxicity first appear as many brownish spots on the tips of older leaves, which in time develop towards the leave base. Eventually the leave (except the midrib) turns brown and dries. Thus, as younger leaves become affected many older affected leaves completely die. Simultaneously, the roots turn dark brown and become damaged (Tanaka *et al*., 1966). Older leaves suffer from iron toxicity more severely because they accumulate more iron and because they have higher transpiration rates (Yamanouchi and Yoshida, 1981). Shoot dry matter shows higher sensitvity to excess iron than root dry weight (Fageria *et al*., 1984). Flowering is delayed and yields are generally low whereas highly susceptible varieties do not flower at all (Ayotade, 1979).

During the growth cycle of the rice plant toxicity symptoms can be found to occur at booting early tillering, maximum tillering and heading stage (Abu *et al*., 1989). Although symptoms may occur at any growth stage, symptoms commonly develop at early tillering and heading stage. This corresponds to a maximum concentration of the micronutrients at tillering stage, which thereafter declines with the crop age. Variation of symptom expression which is often related to soil heterogeneity and represents a big problem in the field selection of rice for tolerance to excess iron (Gunawardena *et al*., 1982).There is a correlation between the iron toxicity symptoms mentioned above and yield. It can be concluded that the symptoms of iron toxicity are directly or indirectly responsible for yield reduction in rice (Audebert and Sahrawat, 2000). This correlation does not necessarily have to be strong and can vary from year to year (Sahrawat and Diatta,.1995). However, yield reduction (of up to 30%) as a result of excess iron can also occur without the appearance of any foliar symptoms (Abifarin, 1988).

2.6 Mechanisms for Fe tolerance

Evidently rice plants have developed physiological avoidance/tolerance mechanisms to survive in these adverse iron toxic soil conditions to cope with large amounts of iron in the soil. These mechanisms are important in the selection of resistant 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 tissue (Marschner, 1993). So far 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 root tissue (Tadano, 1976)
4.) Leaf tissue tolerance to excess amount of iron (Yamanouchi and Yoshida, 1981

2.6.1 Oxidation power of the root

Fe(II) must pass the oxidation barrier in the rhizosphere before it can be absorbed by the root. Rice roots diffuse molecular oxygen into the root medium through aerenchyma in the leaves, stems nodes and roots which make the rhizosphere more oxidative than the bulk of the growing media. In primary roots the aerenchyma forms when its cortex cells degenerate upon flooding of the soil. In secondary roots the aerenchyma forms through big intercellular lumina (Ando, 1983). Aerenchyma in the basal zones may occupy 20 to 50 % of the total root volume of well-drained and flooded soils, respectively (Armstrong, 1979). There is also some evidence that genotypical differences in tolerance to flooding are positively correlated with differences in the rate of ethylene formation in root in response to flooding (Blake and Reid, 1981). Due to the bigger diameter of the primary roots, the aerenchyma of the secondary roots is usually bigger in volume than the aerenchyma of the primary roots. As a result of oxidization of the rice rhizosphere the soil near the roots shows that a large quantity of the mobile Fe(II) is oxidised in that region, resulting in the accumulation of immobile $Fe(OH)_3$ deposits.

The iron plaque on rice roots is thought to reduce the toxicity of ferrous iron for the plant (Tanaka *et al.*, 1966). There is some evidence that apart from rhizosphere oxidation through the aerenchyma there is also some enzymatic iron oxidation taking place at the rhizosphere. However, this only contributes up to 10 % to the total iron oxidation in the rhizosphere (Ando, 1983). There are reports that apart from reducing excess uptake of iron, iron plaque can impede nutrient uptake, especially phosphate uptake, possibly leading to nutrient deficiency (Howeler, 1973). Other reports suggest that iron plaque can act as a nutrient reservoir (Zhang *et al.*, 1998). The iron plaque on rice roots can in some cases become a hazard for the plant. This would be the case if the rice plant finds itself unable to maintain the oxidative environment in the rhizosphere because of some physiological malfunction. Fe-reducing bacteria are then forced to use the Fe-oxides of the iron plaque as their O_2 source thereby increasing the amount of Fe(II) in the proximity of the rice root (Prade, 1987).

The oxidation power of rice plants is generally high in early development stages

Given the possible timing of iron toxicity occurrence and the fact that the oxidation power of rice plants is generally high in early growth stages (Tanaka, 1966, Tanado, 1976) a vigorous early development of effective aerenchyma formation should be a breeding objective. Assuming that the oxidation mechanism is efficient in preventing iron toxic stress dense rice transplanting of rice with high root oxidation power may avoid the accumulation of excess iron.

2.6.2 Exclusion of Fe in the rhizosphere

Bleeding sap analysis indicated that rice roots have a high degree of iron excluding ability (Yamanouchi and Yoshida, 1981). The iron excluding power of a healthy rice plant can be as high as 87 %, meaning that 87 % of the iron that reaches the root surface by mass flow is excluded. The iron excluding power can be decreased by respiratory inhibitors such as potassium cyanide (KCN). This may indicate that iron-excluding power is an active process of exclusion rather than a passive process of resisting the entry of iron (Tadano, 1975). The critical iron concentration in the growth media in which the iron-excluding mechanism primarily operates is between 10 and 50 ppm. Above these concentrations iron exclusion is impaired (Tadano, 1976). The nutritional status (Ca and K) of the plant exerts a strong influence on the plants' ability to exclude iron (Ottow *et al.*, 1981). Other deficiencies such as Mg, P and Mn deficiencies are known to weaken the rice roots' power to exclude iron (Yoshida,

1981). Iron excluding power of rice plants in early growth stages is extremely low (Tadano and Yoshida, 1978).

2.6.3 Retention of Fe in root tissues

Iron may be immobilized in the plant and deposited at specific sites ("dumping sites") within the plant, possibly in the root cortex or any other tissue between the root and the leaf. Under iron toxic stress highest iron content are often found in the roots. There is also a substantial amount of iron immobilized in the stem (Tanaka *et a.l*, 1966). One can observe that the iron-tolerant rice cultivars transport less iron from roots to leaves, indicating the presence of a physiological avoidance mechanism (Audebert and Sahrawat, 2000). Part of the iron entering the roots is translocated to the shoots whereas the rest is retained in the roots. However, it is not yet clear to what extent sequestering of iron in vacuoles by certain organic acids contribute to the inhibition of iron transport to the shoot (Marschner, 1993). The iron retaining power of the roots is also associated with the metabolic activity of the roots and is in turn impaired by a very large amount of iron (Tadano, 1976). Deficiencies of K, Ca, Mg, Mn, or Si decrease the rice roots' ability to retain iron (Tadano and Yoshida, 1978). The ability to retain iron in the roots decreases with growth stage (Tadano, 1976).

2.6.4 Leaf tissue tolerance

Leaf tissue tolerance may also contribute to genotypic differences in tolerance to iron toxicity. This is especially true when the rice plant is injured during transplanting or exposed to toxic substances in the soil (Yamauchi and Peng, 1995). Trials begun in 1969 identified 479 of 6140 rice cultivars as being relatively tolerant to excess iron in the soil and this is thought to be based on tolerance rather than resistance to penetration of iron into the plant (Lantin and Neue, 1989). The fact that the majority of the tolerant varieties contain high concentrations of iron which can vary from 500 to 5000 mg kg^{-1} (Lantin and Neue, 1989) also suggests that varietal tolerance to iron toxicity is a degree of tolerance for excess iron rather than a mechanism of resisting the entry of iron into roots (Jayawardena *et al*., 1977). It has been suggested that when uptake of iron by plants is relatively slow the cell walls and associated polysaccharides are able to exclude Fe(II) from the symplast (Yamauchi and Peng, 1995). Deficiencies of nitrogen, phosphorus, potassium, magnesium and calcium decrease leaf tissue tolerance (Yamanouchi and Yoshida, 1981). As with the other mechanisms tolerance of rice to high iron concentration is also related to the

development stage of the rice plant. Tolerance is lower at early growth stage than at later growth stages (Tadano and Yoshida, 1978).

2.6.5 The role of other nutrients

Excessive Fe(II) reduces N, P, K, and Mg uptake and therefore iron toxicity is often accompanied by deficiency symptoms of N, P and K. Other nutrients such as zinc simply help the plant to tolerate high levels of iron. The rice plant reacts to high levels of Fe(II) in the plant by increasing superoxide dismutase (SOD) activity. Zinc is an essential element for the SOD enzyme. Consequently the symptoms observed in case of zinc deficiency resemble those of iron toxicity (Ottow *et al*., 1981).

Potassium seems to play an important role in regulating iron in the rice plant. Prade (1987) demonstrated that especially potassium nutrition was important in reducing excess iron contents in the plant. To avoid confusion a distinction should be made between Oranging disease and iron toxicity ("bronzing"). Oranging disease is caused by relatively high concentrations of iron in solution. The coating of existing roots by iron reduces their nutrient absorption capacity and leads to nutrient deficiencies in the plant. Thus one significant trait of oranging disease is the deficiency of nutrients. In one case oranging disease was also associated with ammonium toxicity (Liao *et al*., 1994). Bronzing, on the other hand, is the result of direct iron toxicity resulting only from high levels of iron in the soil solution (Howeler, 1973) and nutrient deficiency in the plant is not necessarily a prerequisite for bronzing. This implies that there is an interaction between iron toxicity and nutrient availability which is poorly understood at present.

Iron toxicity can further be divided into two subclasses of toxicity. Iron toxicity in connection with nutrient deficiency induced by iron or aluminium which can be amended through fertilizer treatment is also called pseudo toxicity. However the role of these nutrients is quite complex because many nutrients can also influence the rice plant's ability to tolerate iron toxicity through root functions. The other type of iron toxicity (true toxicity) is caused without any apparent nutrient deficiency and appears while the toxic level of water-soluble iron in plants is at least 300 mg kg^{-1} in the leaf blade at tillering (Lantin and Neue, 1989). However, it is possible that in the case of true toxicity a nutrient imbalance may be caused by high concentrations of iron in the medium. It is evident that a high concentration of iron in the soil may cause nutrient

imbalance in the plant. It is not clear however whether the nutrient imbalance observed in the plant tissue is the cause or the result of excess iron uptake.

2.7 Varietal tolerance to Fe toxicity

During recent years the problem of iron toxicity has become even more severe due to the introduction of modern high-input rice varieties susceptible to excess iron (Razafinjara, 1999). The realization of these difficulties led to a shift in the approach towards adapting the plant to the soil. Current procedures use conventional breeding methods in transferring tolerance from known donors. Repeatable screening methods are not yet available for isolating genotypes with tolerance to excess iron. Genetic studies are required but may only be feasible when additional knowledge of mechanisms is available (Neue *et al*., 1998).

Experimental results show that rice cultivars differ largely in their tolerance to excessive amounts of iron (Fageria *et al*., 1984) and that genetic tolerance to excess iron can significantly improve rice production in iron-toxic soils with some cultivars producing more than 5 t / ha on such sites (Sahrawat and Diatta, 1996).

Rice genotypes absorbing potassium strongly had higher root oxidation power. In a similar way the involvement of manganese in oxidation of iron might be one of the reasons for different tolerance among rice cultivars to iron toxicity (Kulkarni *et al*., 1991). Microbial activity in the rhizosphere is higher in susceptible than in tolerant rice varieties. This indicates that tolerant rice genotypes have a better control over the release of organic compounds into the rhizosphere. Thus, they are able to limit the continuous reduction of ferric compounds in the plaque of rice.

Somewhat contradictory to the above Sahrawat (1999) found, that both iron-tolerant and susceptible cultivars had a high iron content, well above the critical limit (300 mg Fe/kg plant dry wt) and that observations made in several wetland iron-toxic swamp soils in West Africa suggest that 'real' iron toxicity is a single nutrient (iron) toxicity caused by excessive amounts of iron rather than a multiple nutrient deficiency stress. Clear varietal differences exist in leaf tissue tolerance to excessive amounts of iron. Therefore some authors report that varietal tolerance to these excessive amounts of iron is not a degree of resisting the entry of iron into roots (exclusion, oxidation etc.) but rather a mechanism of tolerating excess iron in the plant (Yamanouchi and Yoshida, 1981, Jayawardena *et al*., 1977). Possibly tolerant rice genotypes resort to

a combination of both avoidance and tolerance mechanisms. This was demonstrated by Audebert and Sahrawat (2000) who found that a higher net photosynthetic rate in the tolerant cultivars was partly due to avoidance (less iron accumulation in leaves) and tolerance (superior photosynthetic potential in the presence of absorbed iron in the leaves). Furthermore data indicated that these mechanisms could be further enhanced through the application of P, K and Zn (Audebert and Sahrawat, 2000).

Markedly higher Fe(II) induced enzyme activities can be observed in plants tolerant to excess iron. The change in enzyme activity in some cultivars will probably cause higher leaf chlorophyll content (Baruah and Nath, 1996) and consequently, as in one case observed, a higher net photosynthetic rate in tolerant cultivars (Audebert and Sahrawat, 2000).

2.8 The role of the development stage of rice

Both the external concentration of Fe(II) and the susceptibility of the plant to excess iron vary with time. The importance of the mechanisms for overall iron toxicity resistance depends on the rice plants' development stage. The oxidation-power is high in early growth stages. Iron retaining power is high in early growth stages and decreases with age. The iron-excluding power is low at the early stages of growth, increases with growth till the flowering stage, and then decreases gradually (Tadano, 1976). A combination of these changes with age makes the rice plant more susceptible to excess iron in the early and later stages, and more resistant in the middle stages of development (Tadano, 1976). Prade (1987) observed two peeks of iron toxicity depending on the development stage of the rice plant. The first symptoms occur soon after transplanting (primary toxicity), the second peek occurs at the flowering stage (secondary toxicity). After flooding of the field the concentration of soluble iron is often at a maximum. In addition young transplanted plants are often still poorly adapted to flooded conditions and are often damaged when being transplanted. This and an apparent sensitivity of freshly transplanted rice seedlings may explain primary toxicity. The tillering stage seems to be a phase of recovery from iron toxic stress. Secondary iron toxicity occurs during the heading stage of the rice plant and may be described to the excessive Fe(II) uptake. This is caused by an increased root permeability (potassium deficiency) and enhanced microbial iron reduction in the rhizosphere (intensive exudation) during the physiological active phase between heading and flowering (Prade 1987).

3 MATERIAL & METHODS

3.1 Growing conditions

Pot experiments were conducted under climate-controlled conditions in a growth chamber of the Institute of Plant Nutrition at the University of Bonn, Germany, between January and May 2003 in view of developing a standard screening tool for iron toxicity in lowland rice. The climatic conditions in the phytotron were adjusted to a constant temperature of 26°C and an average relative humidity of 60%. Following a 14-hour dark phase, a light intensity of 18 µE (micro Einstein) for a duration of 10 hours was obtained using sodium vapour lamps.

3.2 Plant material

Seeds of lowland rice cultivars (*Oryza sativa* L. and *O. glaberrima* Steud.), varying in their reported degree of sensitivity towards reduced iron, were obtained from the INGER-Africa germplasm collection (WARDA, Bouake, Côte d'Ivoire) and from the Savanah Agriculture Research Institute (SARI, Nyankpala, Ghana). The cultivars originated from breeding programs at the International Rice Research Institute - IRRI, Philippines (*IR31785-58.1-2-3-3, IR12979-24-1* and *IR4630-22-2*), the International Institute for Tropical Agriculture –IITA, Nigeria (*ITA320, ITA306, TOX4004-8-1-2-3*, Sikamo, MR123), the West Africa Rice Development Association – WARDA, Côte d'Ivoire (*Suakoko8, WITA7, CG14*) and from WARDA-Sahel, Senegal (*Sahel108*, *I Kong Pao*). These cultivars are listed with regards to their sensitivity towards excess amounts of reduced iron (Table 1).

Table 1. Rice cultivars used in the experiment classified according to their sensitivity towards reduced iron (Fe toxicity).

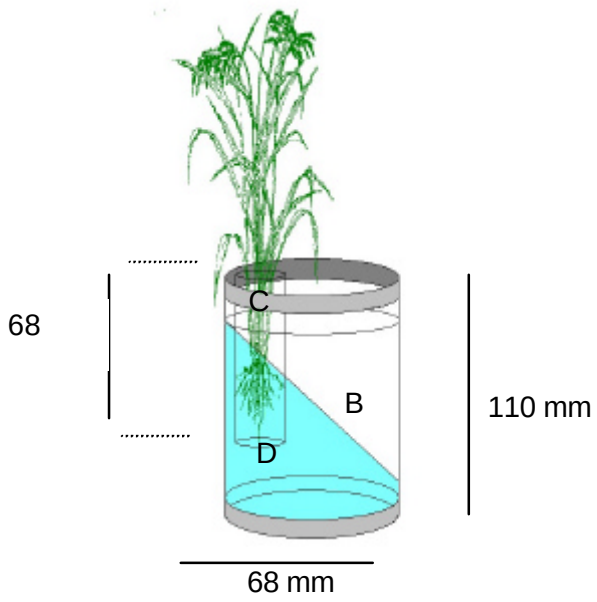
Fe tolerant	Moderately tolerant	Fe-sensitive
Sahel108[4]	*ITA320*[3]	*IR31785-58.1-2-3-3*[4]
Suakoko8[1]	*ITA306*[3]	*MR123*[4]
Tox4004-8-1-2-3[3]	*CG14*[2]	*Sikamo*[3]
WITA7[3]	*I Kong Pao*[4]	*IR12979-24-1*[4]
CK4[2]		*IR4630-22-2*[4]

1-WARDA 1997
2-WARDA 2001/2002
3-SARI
4-Not available

3.3 Growth media and plant culture

Rice plants were cultivated in Yoshida medium, a culture solution preparation developed specifically for rice (Yoshida, 1978). It contained 40mg l^{-1} (NH_4NO_3), 10 mg l^{1} P ($NaH_2 PO_4 . 2H_2O$), 40 mg l^{1} K (K_2SO_4), 40 mg l^{1} Ca ($CaCl_2$), 40 mg l^{-1} Mg ($MgSO_4 . 7H_2O$), 0.5 mg l^{1} Mn ($MnCl_2 . 4H_2O$), 0.05 mg l^{1} Mo ($(NH_4)_6 . MO_7O_{24} . 4H_2O$), 0.2 mg l^{1} B (H_3BO_3), 0.01 mg l^{1} 0.01 mg l^{1} Zn ($ZnSO_4 . 7H_2O$), 0.01 mg l^{1} Cu ($CuSO_4$ $5H_2O$), 2 mg l^{1} Fe ($FeCl_3 . 6H_2O$ (in monohydrate Citric acid)). and buffer adjusted to a pH of 5. Transparent polysterol pots with 68 mm diameter and a pot height of 110 mm were obtained from Greiner bio-one, Bonn Germany. The pots were filled with 150 ml of Yoshida growth medium autoclaved with 1% Agrar (10 g agar l^{-1} solution) at 121° and 1.2 mb pressure (Tuttnauer Systec Autoclave, ELV 3850). The medium was slanted and let to cool at a 45° angle. The slant agar pots were wrapped in aluminium foil and stored in a cold room at 4°C until further use.

Rice seeds were soaked over-night in water and incubated and pre-germinated on filter paper in petri dishes for 60 hours in the growth chamber. Before transferring the 3-day-old rice seedlings into the slant agar pots, a PVC tube of 15 mm diameter and 68 mm length was inserted to a depth of 25 mm into the agar slant in such a way that rice seedling growing within the tube were protected from direct contact with the liquid medium. After the transfer of the rice seedling, the pots were filled with 150 ml ½strength Yoshida solution, covered with aluminium foil to exclude light in the root zone and placed for 2-4 weeks of growth in the phytotron (Figure 1).



A. 1% agar slant prepared from ½ strength Yoshida medium

B. ½strength Yoshida solution

C. 3 mm paraffin oil layer to minimize oxygen diffusion into the medium

D. PVC tube to prevent contact of above-ground plant parts with paraffin oil

Figure 1: Schematic presentation of the experimental set-up for rice seedling growth in the phytotron.

3.4 Methods of analysis

The effect and differential response of various lowland rice cultivars to added Fe(II) was determined both visually (leaf score), gravimetrically (dry matter) and chemically (iron content in leaf tissues).

3.4.1 Leaf scoring

Table 2 Score of leaf area damaged by uptake of excess Fe(II).

Percentage leaf area affected	Score
0	1
1-9	2
10-19	3
20-29	4
30-39	5
40-49	6
50-59	7
60-69	8
70-79	9
80-89	10
90-99	11

A subjective visual assessment of Fe toxicity symptoms on fully expanded leaves (bronzing symptoms) followed the standards leaf scoring for leaf blast (*Pyricularia oryzae*) lesions prepared by the International Network for the Genetic Evaluation of Rice – INGER (IRRI, 1996), with the percentage leaf area affected by iron-induced leaf spots is being translated into a 1-10 score.

3.4.2 Dry weight and Fe content in plant tissues

The above-ground biomass of 2 and 4 week-old rice seedlings was determined after oven-drying for 48 hours at 70°C. Four µl of saturated ammonium nitrate solution (approximately 650 g NH_4NO_3 l^{-1} H_2O) was added to each sample (0.1 and 0.6 g) and heated at 180° C for 7 hours (pressure digestion). The samples were cooled and transferred into 100mL volumetric flasks and filled to volume with distilled water. After shaking, the content of the flasks was filtered into collecting bottles. The Fe content in the samples was determined by Atomic Absorption Spectrometry using a .Perkin-ELMER Atomic Absorption Spectrometer 1100B, Überlingen, Germany.

3.5 Treatment application

A series of four pot experiments was conducted in order to develop and test a simple, cheap and easy-to-handle standard set-up for individual seedlings of lowland rice cultivars for their tolerance to reduced iron. The experiments comprised the evaluation of suitable growth media, determining the critical iron concentration, the study of the effect of relative air humidity and the validation of the findings using a range of commonly recommended lowland rice cultivars.

3.5.1 Growing medium for rice seedlings (Experiment 1)

The aim of experiment 1 was the identification of the optimal strength of the standard Yoshida medium so as to avoid the occurrence of leaf spots or discolorations that may interfere with the scoring of leaf bronzing symptoms in 2 and 4 week-old rice seedlings. Semi-solid 1% agar slants were prepared from either full and from half strength Yoshida culture solution (see chapter 3.3) and watered with either demineralised water, half strength or full strength Yoshida solution (six treatment combinations). The rice cultivar Sahel-108 was grown for periods of up to 28 days in the six culture media combinations in three replicates. Seedlings were monitored for plant height and the occurrence of leaf spots or leaf discolorations. The dry biomass of the seedlings was determined at 28 days after transplanting. The differential behaviour of rice roots in different growth media (proportion of roots growing out of the agar into the nutrient solution) was also recorded.

3.5.2 Iron {Fe(II)} addition to the growth medium (Experiment 2)

This experiment aimed to determine the rate of Fe(II) required to visually differentiate between sensitive and tolerant rice cultivars. Iron concentration were varied in such a way that Fe toxicity symptoms can be induced in the seedlings of the sensitive

cultivars while no symptoms occur in the tolerant check. Three cultivars were used for this experiment. They included the sensitive check cultivar *IR31785-58.1-2-3-3*, the moderately tolerant cultivar *Suakoko8* and the relatively tolerant Sahel-108. At seedling growth stages of 14 and 28 days, Fe(II) (applied as $FeSO_4$) was added to the culture solution so as to be present in concentrations of 0, 1000, 2000, 3000 mg Fe(II) l^{-1}, respectively. After iron addition, the nutrient solution was covered with 3 ml of liquid paraffin creating a 3 mm diffusion barrier for oxygen in view of maintaining the redox potential sufficiently low to prevent the oxidation to Fe(II) into Fe(III). The presence of the paraffin layer necessitated the use of a PVC tube in the agar (see Figure 1, chapter 3.2) to avoid oil damage on the plants. Seedling leaves were assessed visually for the expression and the severity of Fe(II) toxicity symptoms (bronzing) at daily intervals for up to five days after Fe addition. Thereafter, the seedlings were harvested for dry biomass and the determination tissue Fe content.

3.5.3 Effects of vapour pressure deficit (Experiment 3)

Changes in the relative air humidity are likely to modify the transpiration rate of rice and thus the acropetal transport of iron in the xylem vessels. The effect that such changes in the vapour pressure deficit may elicit on the occurrence and the severity of toxicity symptom expression in rice leaves was studied in Experiment 3. At plant ages of 14 and 28 days, the moderately sensitive and the tolerant test cultivars *Suakoko8* and *Sahel108* received the Fe concentration as in Experiment 2 (1000, 2000, 3000 mg l^{-1}. At the times of Fe addition, the seedlings were subjected to two levels of relative air humidity (i.e. average of, 60 and 65%). The lower vapour pressure deficit was achieved by placing the pots in a water bath and covering them with a transparent plastic frame (Figure 2). The humidity in the enclosure was monitored using a Tinytag Plus Moisture Meter (Gemini Data Loggers, Part No : TGP - 1500 West Sussex, UK). Leaf scoring was done at 1, 2 and 3 days after treatment application. At plant ages of 17 and 31 days (14 or 28 days of unstressed seedling growth plus three days of Fe and VPD treatment applications), dry biomass and tissue Fe content were determined.

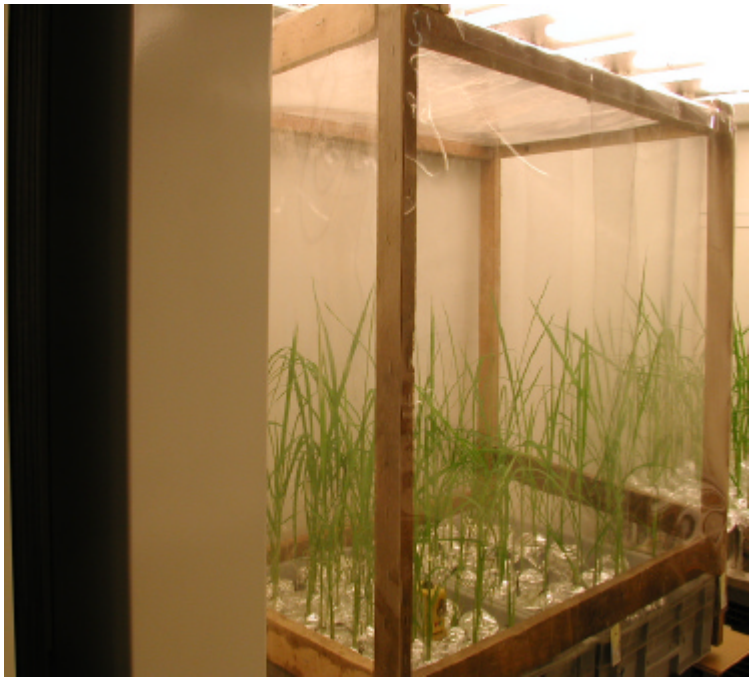


Figure 2.: Experimental set-up in the phytotron to modify the vapour pressure deficit (average rel. humidity 60% outside, 65% inside the box)

3.5.4 Validation of the experimental set up (Experiment 4)

In this last experiment, the validity of the previous findings and its suitability for reliably differentiating Fe-tolerant and Fe-sensitive rice cultivars at seedling stages was tested. Twelve rice cultivars of various origin and with known field behaviour under Fe toxicity conditions were compared in the standard set-up (Experiment 1) both in the absence and in the presence of the "optimal" Fe concentration (Experiment 2) and at a relative air humidity of 60%. Leaf scoring, dry biomass and tissue Fe content were determined three days after treatment application (17 and 31 day-old seedlings).

3.6 Data analysis

Data analysis was carried out with All experimental data (leaf symptoms scores, plant height, dry biomass and tissue Fe concentration) were subjected to analysis of variance and mean separation using SPSS Statistical Software for Windows (Version 10.0).

4 RESULTS

4.1 Optimising the nutrient medium for rice seedling growth in the phytotron

The recommended standard growth medium for the hydroponic culture of rice (Yoshida) can result during the early growth stages in retarded seedling growth and the appearance of leaf spots that may interfere with iron toxicity scoring. A first experiment was conducted to optimise the strength of the Yoshida medium for the cultivation set-up developed in the course of the present research. The effect of a range of combinations of culture media strengths in both agar and in the nutrient solution on plant height, dry matter chlorophyll content, and root development is presented in Table 3.

The plant height of four week-old seedlings ranged from 42.7 to 49.5 cm. Tallest plants were obtained with treatments T2, T5 and T6, where plant height was significantly more than with T1 and T4, which received less nutrients in either the agar or the solution. Seedling dry weights ranged from 0.36 to 0.87 g. The highest shoot dry weight was observed in treatment T6 but it was not significantly different from treatment T2 (0.65 g). The leaf chlorophyll content as a proxy for the N nutritional status, was estimated by reflectance measurements. SPAD values ranged from 35.6 to 38.3 with maximal values obtained again in T6. However, there were no significant differences in the SPAD values between treatments.

The scores for the number of root growing out of agar and into the culture solution ranged from none to high. In treatments T5 (with higher nutrient concentrations in the solution than in the agar) and T2 (same concentration in both agar and solution) a larger share of roots grew into the culture solution than in the other treatments and was therefore potentially more exposed to Fe additions to the solution medium. In summary, the treatment with half strength medium in both agar and culture solution for 4 weeks (T2) appeared the most promising to be used in the follow-up experiments

Table 3: Performance parameters of 4 week-old seedlings of the rice cultivars in different nutrient media (Mean values ± standard errors).

Treatment	Plant height (cm)	Above-ground dry weight (mg)	SPAD values	Proportion of roots growing into the culture solution
T1	42.70 ±.1.42	360 ±0.23	37.5 ± 0.7	medium
T2	47.5 ± 0.6	650 ± 0.41	35.6 ± 1.04	high
T3	46.7 ± 2.4	750 ± 0.11	38.1 ± 1.9	low
T4	44.2 ± 0.7	400 ± 0.21	36.1 ± 0.8	medium
T5	48.3 ± 1.7	450. ± 0.73	36.4 ± 0.7	high
T6	49.5 ± 1.8	870 ± 0.11	38.3 ± 0.8	medium

T 1 – Half strength Yoshida nutrient solution in agar with distilled water as culture solution for first two weeks and half strength of the nutrient solution for third and fourth weeks.
T 2-. Half strength Yoshida nutrient solution in agar with half strength in culture solution over the 4 weeks.
T 3. - Half strength Yoshida nutrient solution in agar with half strength in the culture solution for the first and second weeks and then full strength of the nutrient solution for the third and fourth weeks.
T 4 – Full strength Yoshida nutrient solution in agar with distilled water as culture solution for first two weeks and half strength of the nutrient solution for third and fourth weeks.
T5 – Full strength Yoshida nutrient solution in agar with half strength in culture solution over the 4 weeks.
T 6 – Full strength Yoshida nutrient solution in agar with half strength in the culture solution for first and second weeks and then full strength of the nutrient solution for third and fourth weeks.

4.2 Optimising the Fe(II) concentration to visually differentiate rice cultivars.

With increasing rates of added Fe(II), rice cultivars differentially responded in terms of symptoms expression and Fe uptake. *Suakoko8* (tolerant) and *IR31785-58.1-2-3-3* (sensitive) were used to determine the concentration of Fe(II) that allows for an easy visual differentiation (symptoms expression) between tolerant and sensitive cultivars. They were exposed to different Fe (II) concentration at two seedling growth stages (two and four weeks) over a four-day period. Scores for leaf bronzing, shoot dry matter, Fe concentration and Fe content in the shoot were recorded.

4.2.1 Toxicity symptom expression

The exposure of *Suakoko8* and *IR31785-58.1-2-3-3* to different Fe(II) concentrations produced a distinct trend of increasing symptoms expressions over the period of exposure in both cultivars and at both seedling growth stages (two and four week-old) Day three after addition of 2000 mg l^1 Fe(II)produced the largest differences in symptom expression in both cultivars (Figures 3) and in both two and four weeks old seedlings. Symptom scores ranged from 1 to 7.8 and 1 to 8 in the two week-old

seedlings of *Suakoko-8* and *IR31785-58.1-2-3-3* respectively. In the four week-old seedlings, scores ranged from 1 to 9 and 1 to 9.5 (*Suakoko8* and *IR31785-58.1-2-3-3* respectively). The largest difference in toxicity(leaf bronzing) score between cultivars was 6 in two week-old seedling and 3.3 in four week-old seedlings. Two and four week-old seedlings of *IR31785-58.1-2-3-3* exhibited earlier and much more severe symptoms of leaf rolling when exposed to 2000 and 3000 mg l^{1} Fe(II) than those of *Suakoko8* .

Roots of both cultivars showed brown coatings with the colour intensity increasing with increasing Fe(II) concentration of the culture solution. Root damage occurred from the third and fourth day after treatment in seedlings exposed to 3000 mg l^{-1} Fe(II) and this was observed earlier in the two week-old seedlings than in the four week-old seedlings of both cultivars.

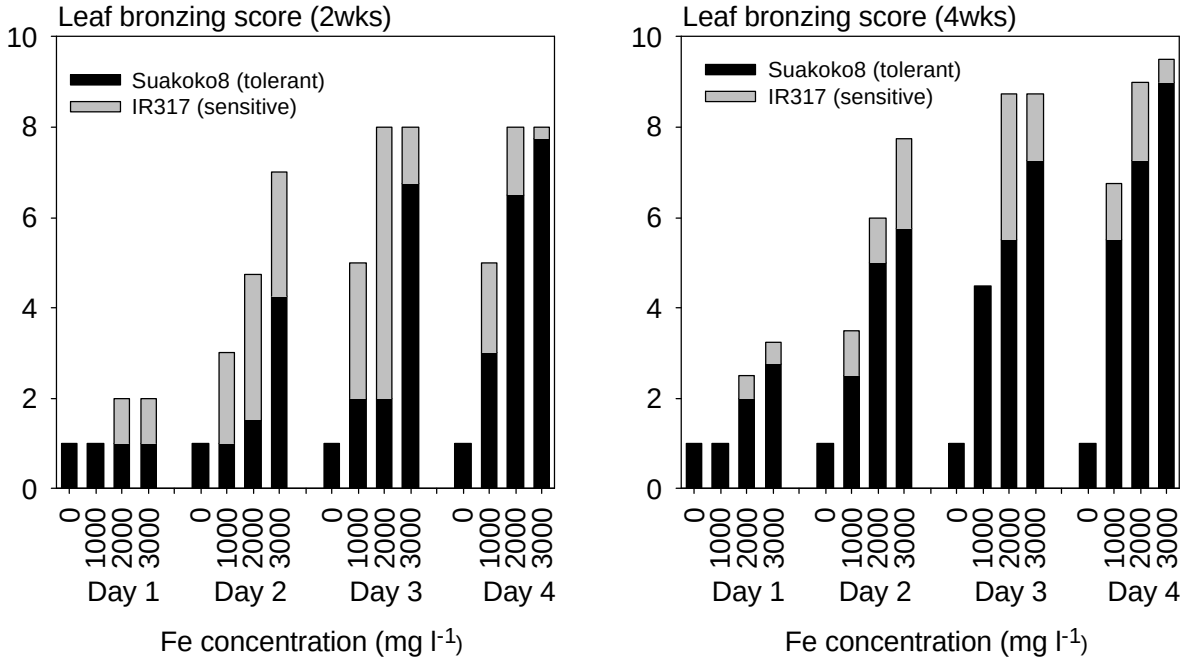


Figure 3. Differential response (leaf bronzing score) of rice seedlings (*Suakoko8* and *IR 31785-58.1-2-3-3*) to increasing Fe(II) concentrations in the culture solution over a 4-day period.

4.2.2 Effect of Fe(II) stress on shoot dry weight and Fe content

The shoot dry weight of two week-old seedlings exposed for four days to different Fe(II) concentrations ranged from 0.09 to 0.11 g in *Suakoko8* and from 60 to 90 mg in *IR31785-58.1-2-3-3* (Figure 4). The lowest dry weight was recorded in seedlings exposed for four days to 3000 mg l1 of Fe(II) in the culture solution while control

plants showed the highest dry matter accumulation. However, these differences in shoot dry weight accumulation were not significant either among different Fe(II) concentration or among cultivars (p>0.05).

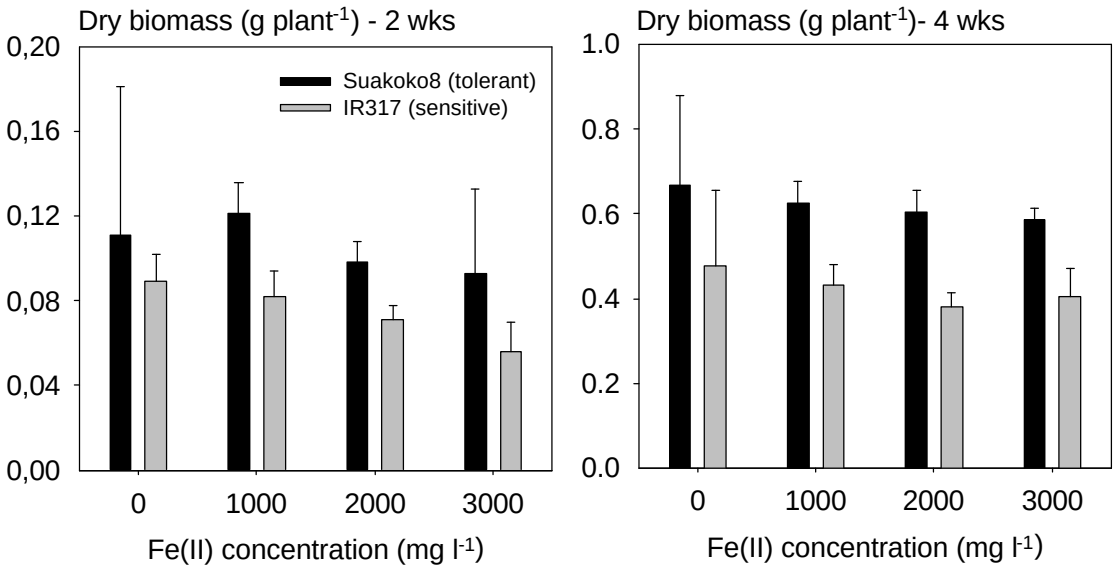


Figure 4. Effect of 4-day exposure to increasing Fe(II) concentration in the nutrient solution on above-ground dry biomass accumulation of 2 and 4 week-old seedlings.

In the four week-old seedlings, shoot dry weight accumulation followed a similar trend and the observed differences were again not significant (Figure 4). The Fe concentration in shoots biomass of both cultivars and at both growth stages (two and four weeks) showed a linear, increase with increasing Fe(II) concentration in culture solution (Figure 5). The Fe uptake was in all instances more in the sensitive check than in the moderately Fe tolerant *Suakoko8*. In *IR 31785-58.1-2-3-3*, shoot Fe concentration ranged from 211 to 7703 mg l^{-1} in two week-old seedlings whilst in *Suakoko8*, it ranged from 180 to 3955 mg kg^{-1}. In the four week-old seedlings, shoot biomass Fe concentration ranged from 170 to 4893 mg kg^{-1} in *Suakoko8* and from 170 to 8343 mg l^{1} in *IR31785-58.1-2-3-3.* The Fe content per seedling followed a similar trend as that of the Fe concentration (Figure 5 and Figure 6). Values ranged from 82 to 3045 µg in *Suakoko8* and from 71 to 2527 µg in *IR31785-58.1-2-3-3.*

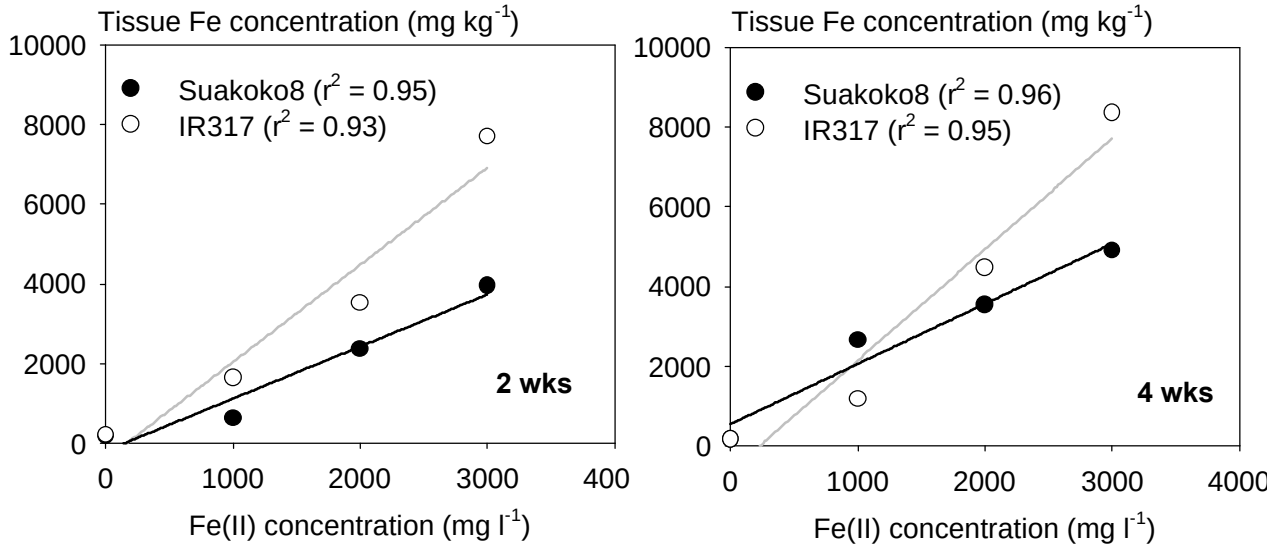


Figure 5. **Relation between the shoot Fe concentration of 2 and 4 week-old seedlings (Suakoko8 and IR31785-58.1-2-3-3) and the Fe(II) concentration of in the culture solutions.**

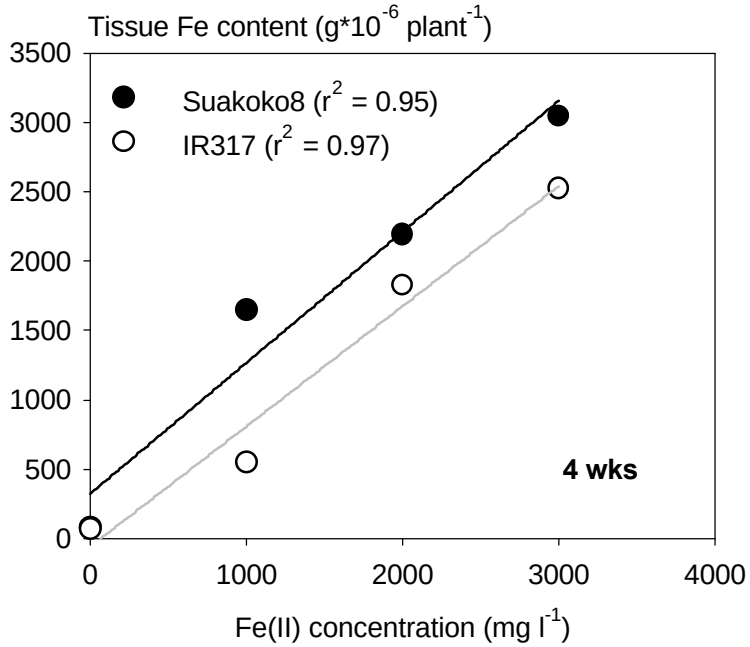


Figure 6. **Relation between the shoot Fe content in the 4 week-old seedlings (*Suakoko8* and *IR31785-58.1-2-3-3*) and the Fe(II)) concentration of in the culture solutions.**

4.3 Effect of vapour pressure deficit (VPD) on iron toxicity.

The climatic signal (vapour pressure deficit – VPD) is likely to affect the acropetal transport of ions in the xylem and may thus influence the expression of Fe toxicity

symptoms in rice. The time of occurrence and the severity of toxicity symptoms were evaluated in the cultivars *Suakoko8* and *Sahel108* at different Fe concentration and at two levels of VPD (average air humidity of 60 and 65%). Besides the Fe toxicity expression (leaf bronzing score), shoot dry matter and Fe content in 2 and 4 weeks old seedlings were determined.

The increase in VPD (reduction in air humidity from 60 to 65%) for a four day duration after Fe(II) addition had no effect on the dry biomass accumulation of rice seedlings except in 4 weeks seedlings of *Sahel108* (Appendix, Figure 1). It did not enhance the expression of leaf bronzing symptoms and the uptake of Iron.

4.3.1 Toxicity symptom expressions

The reportedly Fe-tolerant cultivar *Suakoko8* showed Fe toxicity symptoms starting two days after Fe addition. These symptoms increased within four days of exposure to a Fe concentration of 2000 mg l^1 to a score of 7.2 (low VPD) and 8.5 (high VPD) in 4 week-old seedlings. In case of the moderately tolerant cultivar,*Sahel108*, the same Fe(II) concentration (2000 mg l^1) produced symptoms scores 8.3 (low VPD) and 9 (high VPD). Similar trends were observed at higher concentrations of Fe(II). Irrespective of the Fe concentration and the duration of exposure, increasing the VPD had no significant effect in symptoms expression neither 2 nor 4 week-old seedlings of either *Suakoko8* or *Sahel108* (Figure 7).

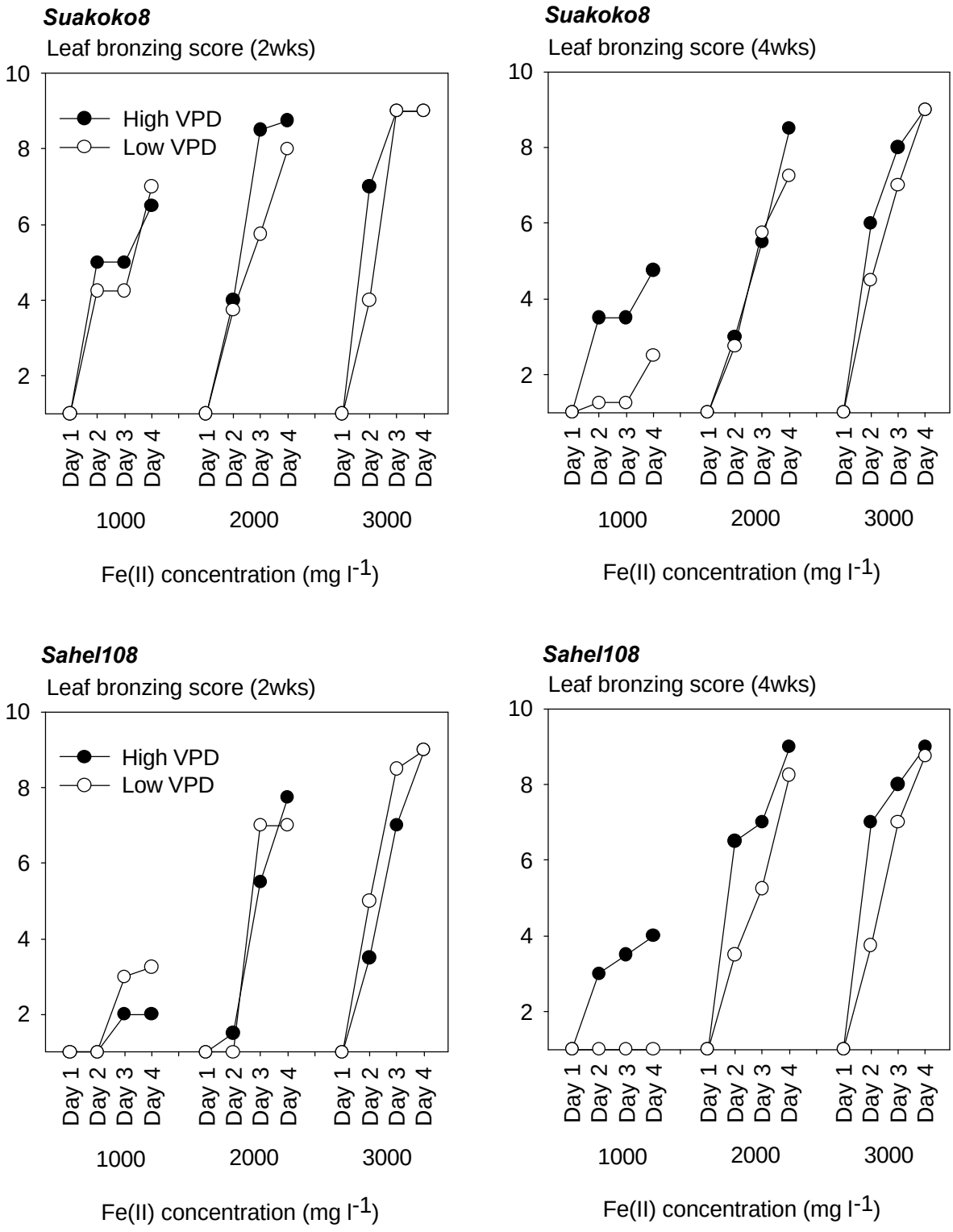


Figure 7. **Differential response of leaf bronzing symptoms in 2 and 4 week-old rice seedlings during a 4-day exposure to increasing Fe(II) concentrations at two levels of relative air humidity (high VPD = 60% rH, low VPD = 65% rH).**

4.3.2 Effect of VPD on shoot dry weight and Fe content

In the 2 week-old seedlings of *Suakoko8*, shoot dry weight ranged from 45 to 98 mg (low VPD) and from 44 to 68 mg (high VPD). In the four week-old seedlings, shoot dry weight ranged from 340 to 360 (low VPD) and 330 to 400 (high VPD). Highest mean dry shoot weight were produced from the control and lowest with 3000 mg l^{-1} of Fe(II) at both seedling growth stages (Appendix 1 Figure 1). In *Sahel108* (Figure 8) mean shoot dry weight ranged from 57 to 74 mg (low VPD) and 64 to 81 mg (high VPD) in the two week-old seedlings. In the four week-old seedlings, shoot dry weight ranged from 32 to 38 mg (at low VPD) and 23 to 35 mg (at high VPD). Generally, shoot dry weight were higher in the controls than with Fe(II) treatment. Differences in shoot dry weight with Fe(II) treatments were significant only in the two week-old seedlings of *Sahel108* (Table 4).

Data on shoot Fe concentrations are presented in Figures 8 and 9. In *Suakoko8*, Fe concentration in shoots of two week-old seedlings ranged from 179 to 10716 mg l^{1} (low VPD) and 277 to 8898 mg l^{1} (high VPD), while in the four week-old seedlings it ranged from 143 to 7744 mg l^{-1} (low VPD) and 168 to 5069 (high VPD). In the *Sahel108* cultivar, Fe concentration of shoot biomass recorded ranged from 173 to 4753 mg l^{-1} (low VPD) and 219 to 4206 mg l^{-1} (high VPD) in the two week-old seedlings. In the four week-old seedlings it ranged from 218 to 3254 mg l^{-1} (low VPD) and 208 to 4812 mg l^{1} (high VPD). The lowest Fe concentration were recorded in control seedlings and highest in seedlings exposed to 3000 mg l^{-1} of Fe in culture solution, with shoot Fe concentration and increasing with Fe(II) concentration in culture solution irrespective of cultivar growth stage or VPD. Differences in shoot Fe concentration irrespective of the cultivar, growth stage or VPD were not significantly ($p>0.05$).

Shoot biomass Fe content per plant (four week-old seedlings) in *Suakoko8* ranged from 54 to 2153 µg under low VPD and from 81 to 1591 µg under high VPD. In the *Sahel108*, it ranged from 65 to 1973 µg at high VPD and from 99 to 1216 µg at low VPD.

These differences were however, not significant at ($p> 0.05$). There was a similar trend of increasing Fe content per plant as observed for the Fe concentration.

Table 4. Effect of vapour pressure deficit (VPD) on above-ground dry matter accumulation and Fe toxicity symptom score in rice seedlings exposed to different Fe concentration in the culture solution.

2 week-old seedling					
	Shoot dry matter (mg plant^{-1})			Symptom score	
Fe(II) concentration (mgl^{-1})	Low VPD (65% rH)	High VPD (60% rH)	*Prob*[a] *(0.05)*	Low VPD (65% rH)	High VPD (60% rH)
Suakoko8					
0	9.80	6.8	*0.34*	1.00	1.00
1000	5.3	6.1	*0.08*	7.00	6.50
2000	5.3	4.9	*0.54*	8.00	8.75
3000	4.5	4.2	*1.00*	9.00	9.00
Sahel108					
0	5.8	7.5	*0.04*	1.00	1.00
1000	7.4	8.1	*0.66*	3.25	2.00
2000	5.7	7.5	*0.09*	7.00	7.75
3000	6.2	6.4	*0.73*	8.50	9.00

4 week-old seedling					
Shoot dry matter (mg plant^{-1})				Symptom score	
Fe(II) concentration (mg l^{-1})	Low VPD (65% rH)	High VPD (60% rH)	*Prob*[a] (*0.05)*	Low VPD (65% rH)	High VPD (60% rH)
Suakoko8					
0	35.4	33.8	*0.86*	1.00	1.00
1000	33.7	40.2	*0.25*	2.50	4.75
2000	35.7	37.2	*0.75*	7.25	8.50
3000	36.2	33.2	*0.67*	9.00	9.00
Sahel108					
0	31.8	23.0	*0.21*	1.00	1.00
1000	37.7	31.2	*0.39*	1.00	4.00
2000	37.3	27.6	*0.17*	8.25	9.00
3000	38.3	35	*0.37*	8.750	9.00

Prob[a] level of significance for differences between shoot weights at the two VPDs

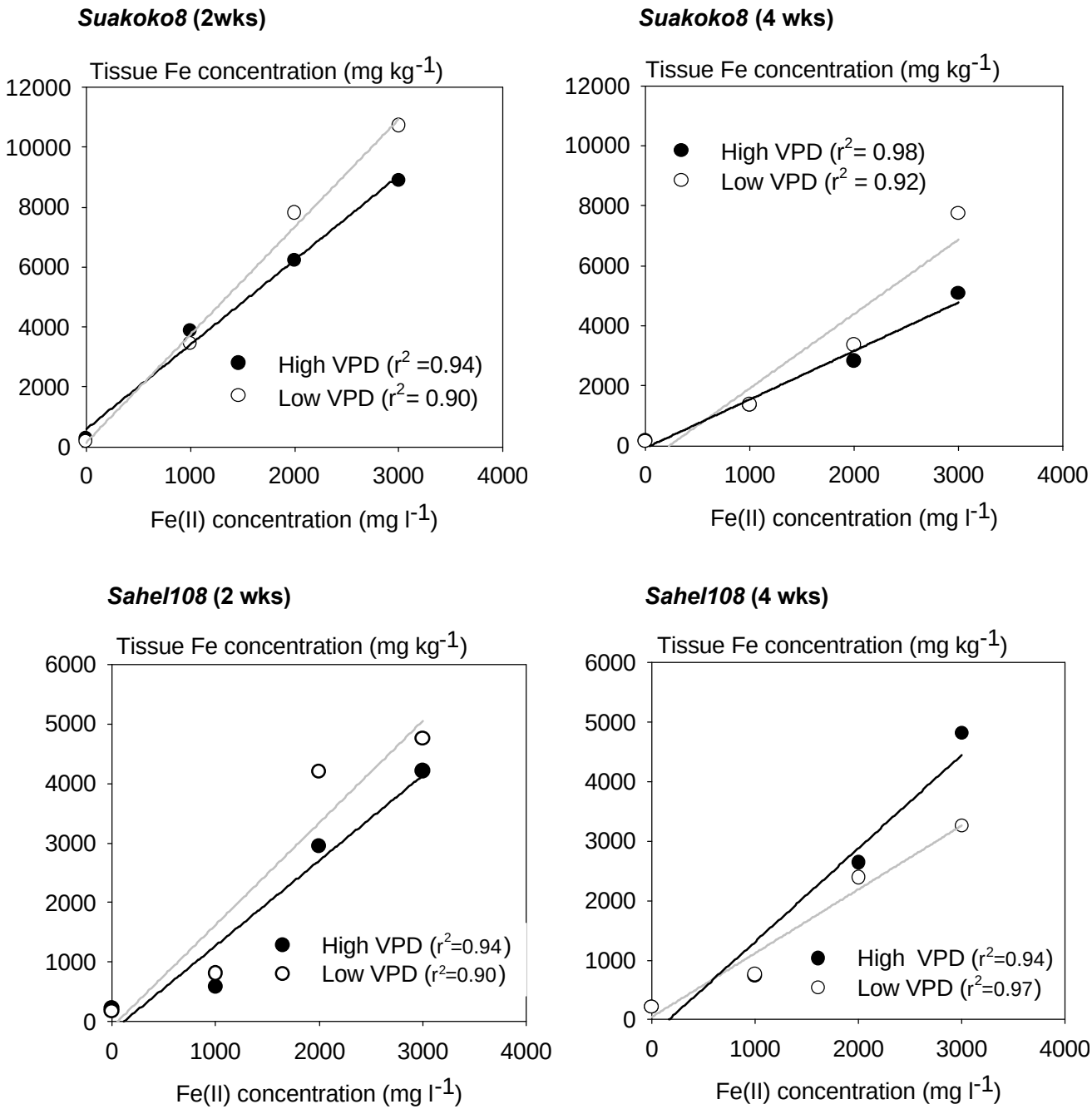


Figure 8. Relationship between the Fe concentration in 2 and 4 week-old rice seedlings and the Fe(II) concentration in culture solution at two levels of relative air humidity (high VPD = 60% rH; low VPD = 65% rH).

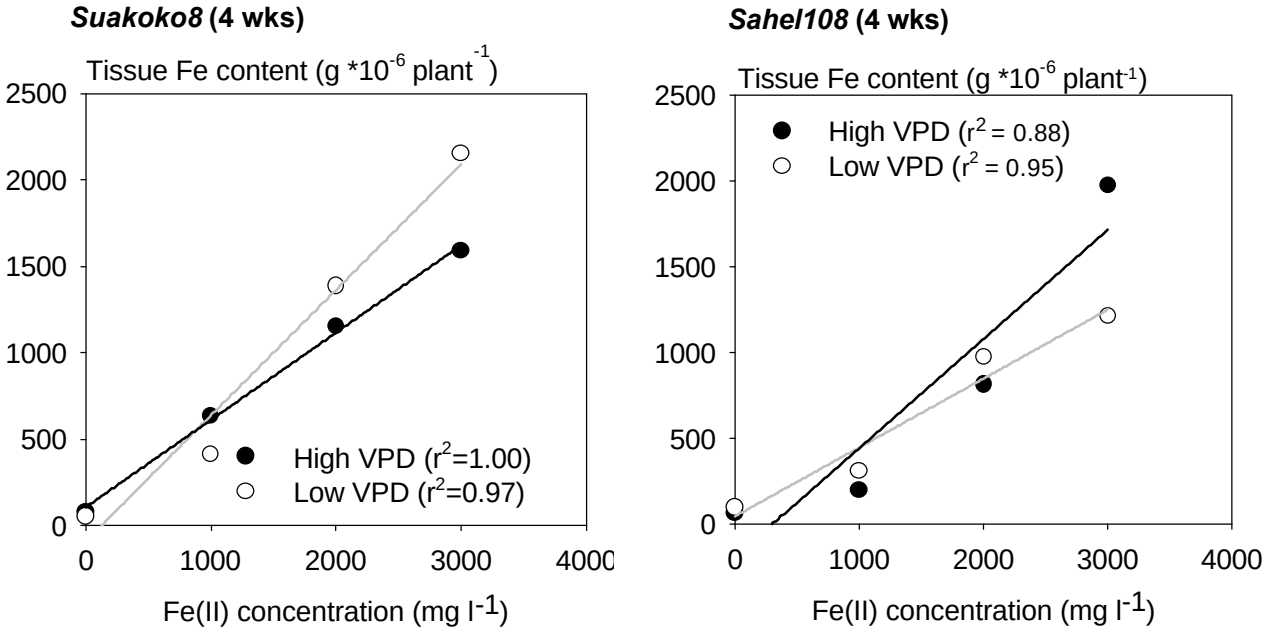


Figure 9. Relationships between the shoot Fe content of 4 week-old rice seedlings (Suakoko8 and Sahel108) and Fe(II) concentration in the culture solutions at two levels of relative air humidity (high VPD = 60% rH; low VPD = 65% rH).

4.4 Validation of the screening set-up

Results from the previous experiments indicate that a growth media with half strength Yoshida nutrient solution in both the agar and culture solution to be most appropriate for the present study. Also, Fe(II) concentration of 2000 mg l^{-1} with three days of exposure to

Fe(II) addition provided the best differentiation between tolerant and sensitive cultivars. Slight modification in vapour pressure deficit had no significant effect on the studied plant parameters. Based on these findings, a range of cultivars was compared at two seedling stages (two and four weeks) after three days of exposure to an added Fe(II) concentration of 2000 mg l^{-1}.

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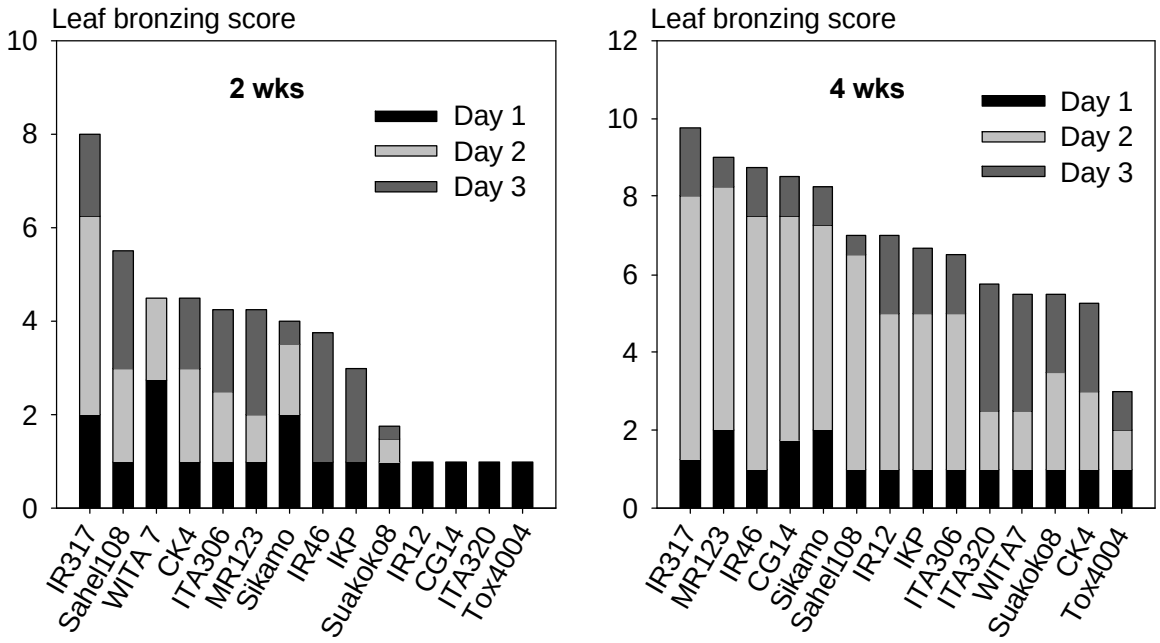

Figure 10. Differential response of rice cultivars (seedlings) to a 3-day exposure to 2000 Fe(II) mg l^{-1} in the culture solution.

4.4.1 Toxicity symptom expression of screened cultivars

In the two week-old seedlings bronzing symptom scores ranged from 1 to 8, while in four week-old plants, it ranged from 3 to 9.8 (Figure 10). The lowest scores were recorded in *CG14, ITA320, Tox4004-8-1-2-3* and *IR12979-24-1* in the two week-old seedlings while four week-old seedlings of *Tox4004-8-1-2-3* showed least bronzing symptoms. *IR31785-58.1-1-2-3-3* appeared to be most sensitive to Fe addition at both seedling growth stages. *Suakoko8* had a score of 1.8 at two weeks and 5.5 at four weeks. The other cultivars (*WITA7, ITA306, Sahel108, CK4, MR123, Sikamo, IR 4630-22-2* and *I Kong Pao*) took an intermediate position with toxicity scores ranging fro 3 to 9. Bronzing symptom expression was generally lower in the two weeks old seedlings than in the four weeks old seedlings. Leaves of the two week-old seedlings were observed to roll and die earlier than in the four week-old seedlings.

Generally, the IR cultivars recorded higher scores Fe-sensitive than the ITA Fe-Tolerant cultivars. Bronzing symptoms were observed from the first day after treatment application in leaves of two week-old *IR31785-58.1-2-3-3, WITA7* and *Sikamo* and starting day two in the four week-old seedling with 50% of the plants showing leaf rolling. On the third day after Fe addition, leaf rolling also occurred in *CG14, IR12979-24-1* and *MR123*.

4.4.2 Shoot dry Matter

In the two weeks old seedlings, shoot dry weight was lower in all cultivars exposed to 2000 mg l-1 of Fe(II) compared to their respective controls with the exception of I Kong Pao (Table 5). These differences in shoot dry weight were however significant (p>0.05) in *IR4630-22-2, IR31785-58.1-2-3-3, Sikamo, MR123 Tox4004-8-1-2-3* and *ITA306*.

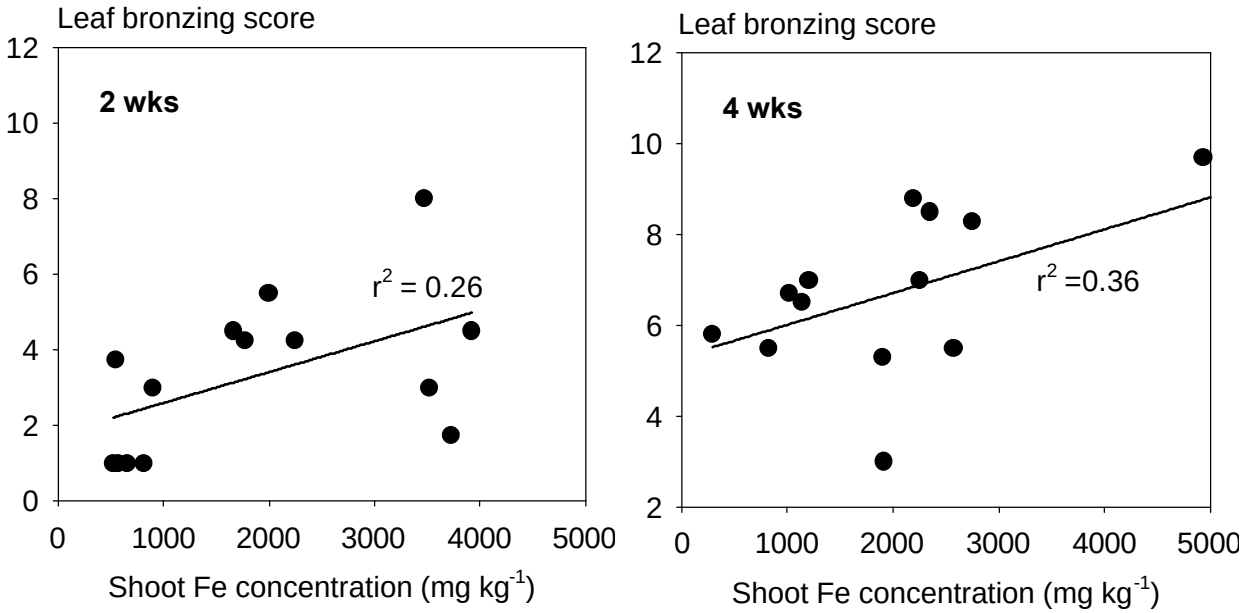

Figure 11. Relationship between tissue Fe concentration and symptom expression (leaf bronzing) in 2 and 4 week-old seedlings of different cultivars (phytotron, 2000 mg Fe(II) l^{-1} in nutrient solution).

Table 5. Effect of Fe(II) addition on above-ground dry matter accumulation of different rice cultivars at 2 and 4 weeks after seeding (3 days of exposure to 0 and 2000 mg l^{-1} of Fe(II)).

Cultivar	Seedling dry weight (mg)					
	2 WAS			4 WAS		
	0 mg l^{-1} Fe	2000 mg l^{-1} Fe	*Prob*[a]	0 mg l^{-1} Fe	2000 mgl^{-1} Fe	*Prob*[a]
Sahel108	9.6	9.0	*ns*	54.1	49.9	*ns*
Suakoko8	11.1	9.9	*ns*	46.3	43.2	*ns*
CK4	8.7	8.3	*ns*	50.6	41.1	*ns*
IR12979-24-1	8.6	7.0	*ns*	45.3	39.7	*ns*
ITA306	9.9	7.3	*	40.7	39.0	*ns*
Sikamo	9.5	6.2	*	39.2	36.9	*ns*
CG14	9.2	8.3	*ns*	39.4	36.4	*ns*
IR31785-58.1-2-3-3	7.6	5.8	*	37.6	35.9	*ns*
WITA7	7.2	6.1	*ns*	35.1	32.9	*ns*
IR4630-22-2	8.8	6.8	*	35.1	31.6	*ns*
MR123	8.6	6.0	*	32.5	31.3	*ns*
I kong Pao	6.9	7.7	*ns*	35.1	29.0	*ns*
ITA320	5.8	5.4	*ns*	29.2	28.9	*ns*
Tox4004-8-1-2-3	8.1	5.7	*	35.1	28.7	*ns*

[a] level of significance for differences between shoot weights at 0 mg Fel^{-1} and 2000 mg Fe l^{-1}.
ns and * indicates not significant and significant at p=0.05 levels, respectively.

4.4.3 Shoot Fe concentration and content

Shoot Fe concentrations in the two-week-old seedlings ranged from 522 to 3920 mg l^{-1} and from 292 to 6102 mg l^{-1} in the four week-old seedlings. The lowest Fe concentration was recorded in *CG14 (Oryza glaberrima)*and the highest in *WITA7. CG14* recorded a bronzing symptom score of 1 and *WITA7* had a score of 4.5. The highest symptom score was recorded in *IR31785-58.1-1-2-3-3.* There was no linear correlation between shoot Fe concentration and bronzing symptoms score (Figure 11).

We conclude from figure 13 that all cultivars that show a tissue concentration of less than 2000mg kg^{-1} (the Fe strength in the nutrient medium) have the ability to discriminate and may thus be considered “excluders”. In some cultivars this ability to reduce (exclude) iron is associated with less toxicity symptoms and thus a certain degree of tolerance. Examples of such tolerant excluders are *Tox4004*-8-*1-2-3* and

ITA320 and to a lesser extent *CK4* and *WITA7.* All cultivars that accumulate iron in concentrations beyond the Fe strength of the medium are not able to discriminate against iron or may be considered "includers". Most includers associate increased Fe uptake with increased toxicity symptoms. A typical example is *IR31785-58.1-2-3-3,* which may be used as a sensitive check in future studies. The only notable exception is *Suakoko8*, which may be classified as a tolerant includer.

In the four week-old seedlings, *ITA320* recorded the least shoot Fe content per plant and *MR123* recorded the highest. *ITA320* had a bronzing symptoms score of 5.7 and *MR123* recorded 9. *Tox4004*-8-*1-2-3* with the least score (3) recorded Fe content of 1919 µg. *Suakoko8* with a score of 5.5 recorded shoot Fe content of 2574 µg per plant. *IR31785-58.1-2-3-3* with the highest score of 9.7 recorded Fe content of 4937 µg per plant.

There was no linear correlation between bronzing symptoms scores and shoot Fe content of the cultivars screened (Figure 12).

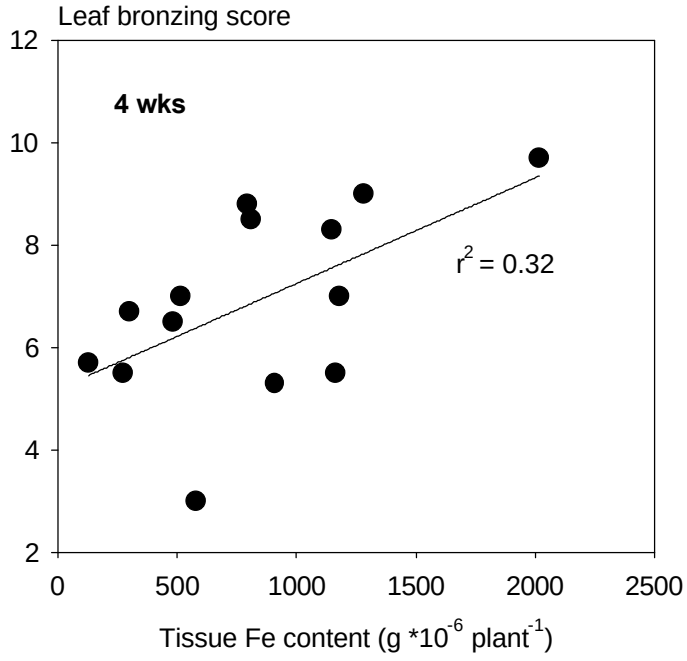

Figure 12. Relationship between shoot Fe content per plant and symptom expression (leaf bronzing) in 4 week-old seedlings (phytotron, 2000 mg Fe(II) l^{-1} in nutrient solution.

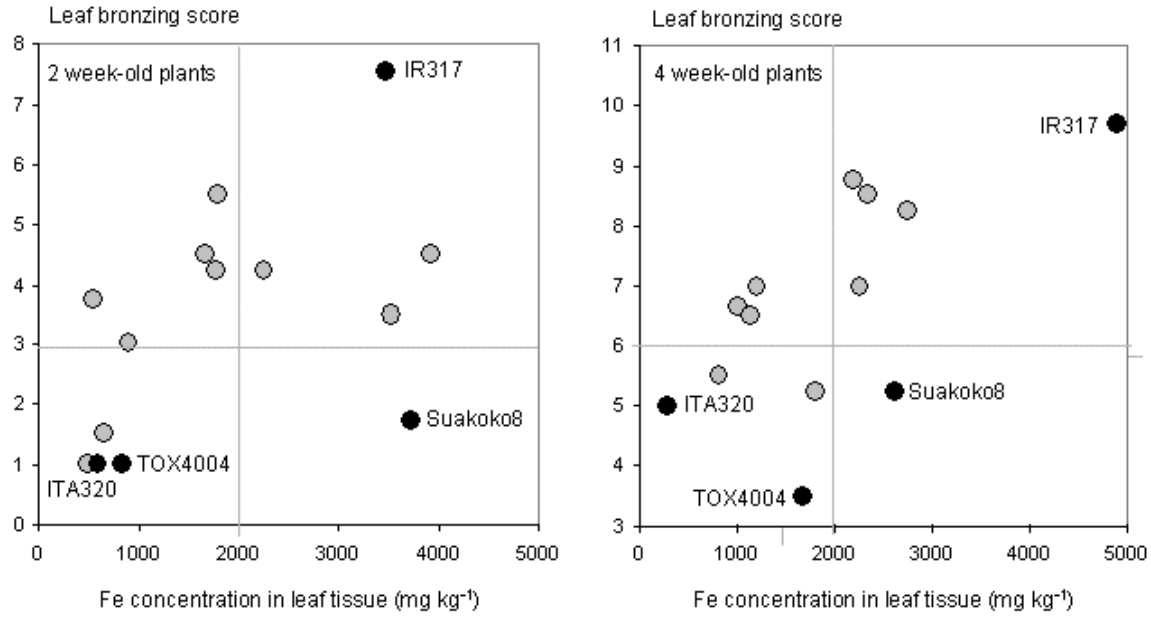

Figure 13. Relationships between tissue iron concentration and the expression of iron toxicity symptoms (leaf bronzing score) in 2 and 4 week-old rice seedlings.

5 DISCUSSION

5.1 Culture set up and medium strength.

Fe toxicity can be damaging at the early growth stages or at grain filling (when arenchyma starts to degrade and can no longer oxidize the rhizosphere). However, it is the seedling toxicity that is most damaging and can result in 100% yield loss. That is why screening tools should primarily address the seedling stage of rice. The tool needs to be small (to handle a large number of accession), cheap, and easy to handle and producing repeatable results in a very short period of time, allow for in-depth studies on plants for physiological mechanism involved in tolerance to reduced Fe.

Small pots about 330ml in size were used per plant with 150 ml of culture solution and 150ml of semi solid agar. These pots can be re-used after each experiment. The agar allows roots to be visible hence suitable for physiological studies such as the O_2 gradient at the rhizosphere, and the bulk medium. The set-up is comparatively low cost. To screen 100 plants costs in all, about 60.00 euro in material (100 pots costs about 33.00 euro, 300ml of paraffin at about 10.00 euro, 100g of agar at 14 euro and chemicals for nutrient solution at about 4 euro). The paraffin used blocked the culture solution-oxygen interface, hence reduced oxidation of Fe(II) to Fe(III). The silicon tube around the stem of the rice seedlings also prevented the paraffin from interacting with rice stems. A set-up of with 100 pots requiring a space of $1.2m^2$ space in the phytotron The materials used in the set-up are readily available and easy to handle. Visual scoring of leaf bronzing symptom may replace expensive chemical analyses. The set-up provides a rapid turnover time of experiments since symptom are already observed within 4 days after Fe(II) addition to two and four week-old seedlings (18-32 days in total).

Optimal nutrient strength in the medium was required to avoid the development of brown spots on seedlings that may occur as a result of high ammonium N in culture medium. Spots developed on leaves of rice seedlings are likely to interfere with leaf bronzing symptoms and were therefore not desired. The selected treatment (T2) containing half strength nutrient solution in both agar and culture solution resulted in healthy rice seedlings and recorded the least SPAD (a proxy of N nutritional status) value but above the critical value of 35 reported by Peng *et al.,* 1996. Besides, T2 resulted in one of the treatments with highest proportion of seedling roots growing out

of agar into the culture solution, which was desired to achieve interaction with culture solution.

Above all, the procedure resulted in the occurrence of Fe toxicity symptoms, comparable to other screening methods used. For example, Tox and ITA series (cultivars originating from the breeding programs of IITA) have been reported in other screening trials by WARDA (1997) to be tolerant as was observed in this study.

Suakoko8, a reportedly Fe(II) tolerant cultivar and *IR31785-58.1-2-3-3*, a sensitive cultivar were used as references in determining the rate and timing of Fe(II) addition at which visual differentiation between cultivars was possible. This was to enable the screening of a number of cultivars to determine their tolerance to Fe(II) with reference to the test cultivars. www.warda.org.warda1/main/publications quoted a breeder at WARDA stating the long time involved in screening rice cultivars for Fe(II) tolerance as an impediment. In this set-up, symptom expressions were recorded over a four-day period in contrast to 8 days reportedly required after Fe(II) addition for leaf bronzing symptom to occur (Bode *et al.* 1995) and in field trials. Breeders would therefore welcome the set-up developed.

Fe(II) concentration of 2000 mg l^{-1} over a duration of three days allowed for the differentiation between cultivars. Previous studies reported Fe(II) concentration of 300 mg l^{-1} with constant renewal of solution to be enough to produce toxicity symptoms. To make the developed procedure less cumbersome, changing of culture medium was not required, Fe(II) is added once and therefore the need for Fe(II) concentration to be high enough to induce symptoms expression in the shortest possible time.

5.2 Vapour pressure deficit

Effect of relative air humidity (vapour pressure deficit) on Fe uptake (acropetal transport), plant growth and bronzing symptom expression in leaves were investigated with the aim of providing standards for the set-up developed. Differences in biomass of rice seedlings due to varied relative humidity were not expected as plants were exposed to the different levels of relative humidities (60 and 65%) only for a period of 3 days. More so, the difference in relative air humidity (5%) was also low. Temperature and moisture (water) are important determinants of relative air humidity. For the relative air humidity in the cage to be significantly different from that

outside it, it's temperature should be higher than that of the surroundings. However, the temperature within the cage was similar to that outside it. This may explain the slight difference in relative air humidity even in the presence of water in the enclosure. An increase in transpiration is likely to increase the acropetal transport of all xylem-mobile elements (Marschner, 1993). This could explain why cultivars bred for Fe toxicity in humid Asia (Indonesia, The Philippines) succumbed to Fe toxicity in sub-humid West Africa, particularly during the dry season (Sahrawat et al. 1995). Sahrawat (1993) also indicated that iron uptake is highly affected by transpiration when rice plants are exposed to high concentrations of Fe(II). This may explain the relatively higher leaf bronzing symptom expression at all Fe(II) concentrations at the 60% relative air humidity than at the 65% in the 4 week-old seedlings of *Sahel108*. This implies that cultivars that are not able to resist or control the uptake of reduced Fe at the rhizosphere and lack tissue tolerance for large amounts of Fe(II) are likely to be affected by differences in relative air humidity. Thus, *IR31785-58.1-2-3-3* the sensitive check with high Fe concentration and leaf bronzing score may not be recommended for drier environments that are noted for have high levels Fe toxicity occurrence. The relative response to change in relative air humidity may be a criterion to be used as a screening tool to differentiate between cultivars that resist the uptake of large amounts of reduced Fe ('excluders') and those that do not control the uptake of large amounts of reduced Fe ('includers') without performing chemical analysis.

5.3 Cultivars differentiation

Differentiation of cultivars according to their sensitivity or tolerance to iron toxicity was achieved with the developed set-up. *Suokoko8* and *WITA7* among others produced results comparable to field trials aimed at screening rice cultivars for tolerance to Fe toxicity (WARDA, 1997). In the initial screening trials of the new African Rice (NERICA: interspecific cross of *Oryza sativa* and *Oryza glaberrima*) the O. glaberima cultivar, *CG14* was reported to be relatively Fe tolerant as observed in the current study. This cultivar, a traditional variety is however prone to lodging when fertilizer is applied. *ITA* and *Tox* cultivars were both found to be have the potential of reducing uptake of high amounts of reduced Fe into their shoots probably by oxidizing Fe(II) at the rhizosphere to Fe(III) or retaining the Fe in the root tissues (tolerant excluders). Their ability to control uptake of reduced Fe is evident in their low tissue Fe content observed after three days of exposure to 2000 mg Fe(II) l^{1}.

This may explain their fairly stable performance across environments in Africa and Asia.

A plot of the tissue Fe content of *Suakoko8* has identified it as a tolerant cultivar ("includer"). Thus, *ITA* and *Tox* obviously avoid the uptake of iron while *Suakoko8* accumulates Fe but apparently is able to partition it in it's tissue or immobilize excess Fe in apoplasm (Kosegarten *et al*., 1999). The mechanism of tissue tolerance is important and needs to be introgressed into cultivars that are exposed to grain-filling toxicity (i.e. Mekong Delta) and to enable them cope with late season toxicity.

CK4 and *WITA7* were both bred for Fe toxicity tolerance and are widely used in West Africa region. Their performances in regional yield trials are fairly constant which may be related to a combination of Fe tolerance mechanisms. On one hand, the present research could classify these cultivars as moderately efficient in discriminating against reduced Fe(II) ("excluders"). On the other hand, Auderbert *et al*. (1999) could show their ability to partition Fe (immobilization in the stem), providing a certain degree of tissue tolerance. *ITA 320* obviously avoids the uptake of iron while *Suakoko8* accumulates iron but has an apparently high tissue tolerance. Mechanisms used in these two cultivars could be investigated in further studies. This would allow for the development of a germplasm that shows a constant and stable iron-toxicity tolerance under a range of environmental conditions. Such a germplasm could reduce rice yield loses attributed to iron toxicity which have been reported to range from 50 to 100% in West Africa (www.warda.org.warda1/main/publications).

IR31785-58.1-2-3-3 was classified as a sensitive cultivar lacking the ability to discriminate against reduced Fe hence taking up large amounts into its' tissue. It also cannot cope with Fe, resulting in high susceptibility. Investigation into the tolerance mechanisms in *Suakoko8, ITA 320,* and *Tox 4004-8-1-2-3* and the identification and isolation of the genes responsible for this trait could be used to improve Fe susceptible cultivars with desirable qualities to enable their cultivation in a wider rage of environment.

6 Conclusions and Research needs

The developed set-up is relatively cheap, requiring 60 Euro to screen 100 plants and about 1.2m^2 of space in the phytotron. With the turnover time of 18 and 32 days, large numbers of rice accessions can be reliably screened for their response to Fe toxicity. No medium change is required and one-time addition of 2000 mg Fe(II) l^1 is enough to visually differentiate tolerant from susceptible cultivars within 3 days. A slight change in relative air humidity (5% rH) did not affect plant growth and leaf bronzing symptom but tended to increase iron content in susceptible cultivars. In conjunction with chemical analysis, the developed set-up allows for a classification of the cultivars by Fe tolerance mechanisms (e.g. Fe “excluder” vs. Fe “includer”).

Research needs

For further breeding and screening strategies, larger number of cultivars should be used to validate the set-up. Secondly, a wider range of air humidity levels should be compared to determine the influence on Fe uptake or symptom expression.

The suitability of the set-up should be evaluated to study physiological mechanisms of Fe tolerance such as cultival differences in the oxidation power of the roots (Redox indicators could be incorporated in the agar medium) or O_2 gradients between rhizosphere and the agar medium. In addition, the Fe partitioning (immobilization of Fe in specific tissues) may be studied both in long distance transport (root-stem-leaf) as well as in short distance transport (immobilization of Fe in apoplasm i.e. apoplast-symplast).

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Appendices

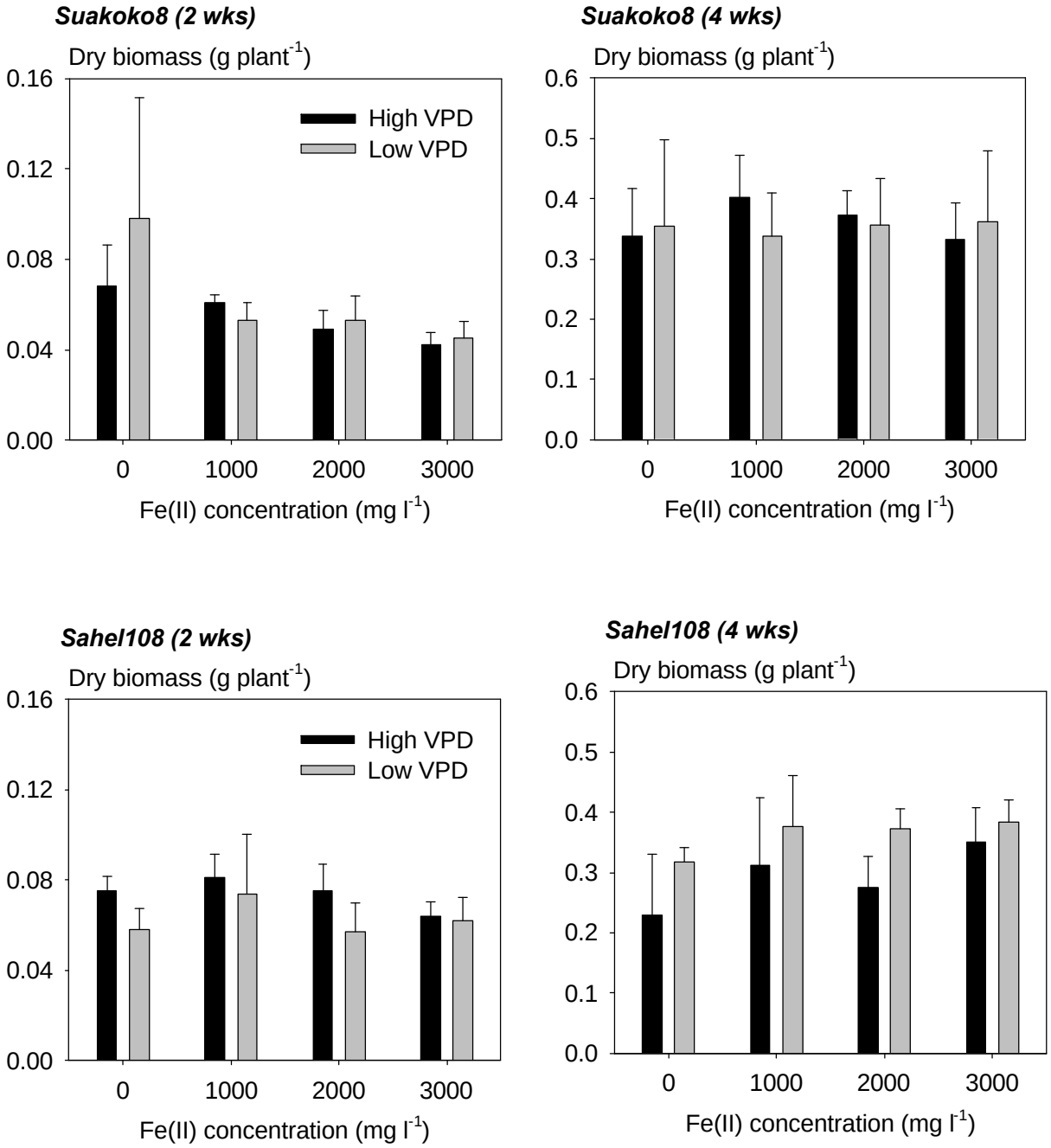

Figure A1: Effect of relative air humidity (low VPD =65% rH; high VPD =60% rH) on above ground dry matter accumulation of 2 and 4.week-old rice seedlings, exposed for a duration of four days to increasing concentrations of Fe(II) in the nutrient solution.

Table A1 Effect of vapour pressure deficit (VPD) on above ground dry weight of *Suakoko8* and *Sahel108* exposed for 4 days to different Fe (II) concentrations.

Plant age	Suakoko8			Sahel108		
Shoot dry weight (mg.plant^{-1})	Low VPD (65% rH)	High VPD (60% rH)	*Prob*[a] *(0.05)*	Low VPD (65% rH)	HighVPD (65% rH)	*Prob*[a] *(0.05)*
2 WAS	6.2	5.5	*0.457*	6.4	7.4	*0.066*
4 WAS	36.1	35.3	*0.713*	29.2	36.7	*0.016*

Prob[a] level of significance for differences between shoot weights between the two VPDs.

Table A2 Effect of vapour pressure deficit (VPD) on shoot Fe concentration of rice seedlings exposed to increasing concentration of added Fe(II).

	Low VPD				High VPD			
Fe concentration in culture solution (mg l^{-1})	0	1000	2000	3000	0	1000	2000	3000
Fe concentration (mg kg^{-1})								
2 week-old seedling								
Suakoko8	179	3454	7802	10716	277	3877	6529	8898
Sahel108	173	807	4610	4753	219	589	2947	4206
4 week-old seedling								
Suakoko8	143	1365	3357	7744	168	1344	2824	5069
Sahel108	218	759	2386	3254	208	728	2638	4812

Table A3 Differential response (toxicity symptom expression) of two and four week-old seedlings of different rice cultivars to Fe(II) addition (3 days of exposure to 2000 mg l^{-1} Fe (II))

Cultivars	Symptom score	
	2 week-old seedlings	4 week-old seedlings
IR31785-58.1-2-3-3	8.0	9.8
MR123	4.3	9.0
IR4630-22-2	3.8	8.8
CG14	1.0	8.5
Sikamo	3.0	8.3
Sahel108	5.5	7.0
IR12979-24-1	1.0	7.0
I Kong Pao	3.0	6.7
ITA306	4.3	6.5
ITA320	1.0	5.8
WITA7	4.5	5.5
Suakoko8	1.8	5.5
CK4	4.5	5.3
Tox4004-8-1-2-3	1.0	3.0

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