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Characteristics of Iron Tolerant Rice Lines Developed at IRRI under Field Conditions

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Abstract

In the 2003 wet season, IR64 (check variety) and four lines of rice (*Oryza sativa* L.) developed at IRRI were cultivated in a field with iron (Fe) toxicity at Iloilo City, Philippines, and also under normal soil conditions at IRRI farm. Two of the lines used in the experiments were the near isogenic lines (NILs) of IR64, selected as Fe-tolerant lines in solution culture in the greenhouse. The other two lines were elite breeding lines that were selected as Fe-tolerant lines in the field trial at Iloilo. The tolerances of NILs were not expressed at the Iloilo field site because the yield reductions due to Fe-toxicity were similar to or larger than those for IR64. The yield reduction of elite breeding lines was smaller than that of IR64, indicating that the tolerance of elite breeding lines was expressed at the Iloilo field. At Iloilo, the shoot dry weights of IR64 and its NILs hardly increased after 73 days of cultivation. On the contrary, those of elite breeding lines kept on increasing. While the root dry weight of IR64 and its NILs decreased abruptly after 50 days of growth, those of elite breeding lines remained constant or decreased gradually. This finding suggests that one of the factors that suppressed the growth of IR64 and its NILs during the late stage was early root senescence. Since the tolerance of elite breeding lines was associated with the maintenance of root activity during the late stage of growth, the long maturity period of elite breeding lines presumably helped to alleviate the toxicity.

Discipline: Soil, fertilizers and plant nutrition
Additional key words: Fe-tolerance, Fe-toxicity, paddy soil, root

Introduction

Iron (Fe) toxicity is one of the most serious problems in the tropics and sub-tropics. Under irrigated or rainfed conditions, rice growth is suppressed when a large amount of Fe is mobilized in soil solution or when interflow brings Fe ions from upper slopes[23]. The toxicity occurs in acid Ultisols and Oxisols, and acid sulfate soils[29]. Typical symptoms of Fe-toxicity are generally manifested as tiny brown spots starting from the tips and spreading towards the bases of the lower leaves[10]. The toxicity reduces rice yields by 12–49%, and this ratio depends on the Fe tolerance of the genotype, intensity of Fe toxicity stress, and soil fertility status[28,29]. High concentration of Fe in soil solution can also decrease the absorption of other nutrients such as phosphorus (P) and potassium (K)[13,36].

Evidently, rice plants have developed physiological avoidance and/or tolerance mechanisms to survive under Fe-toxic condition. These mechanisms are important in

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the selection of rice genotypes. However, problems in selection of rice genotypes for tolerance to Fe-toxicity still relate to the inadequacy of knowledge on physiological mechanisms of tolerance[5,14]. It has been reported that an Fe-tolerant variety absorbed less Fe or transported less from roots to leaves, indicating the presence of physiological avoidance mechanisms[4,30]. The following mechanisms have been found to be relevant in rice plant in coping with excess Fe concentrations[5].

(1) Oxidation of Fe at the root surface (Fe-oxidizing power)[1]
(2) Exclusion of Fe at the root surface (Fe-excluding power)[30,31]
(3) Retention of Fe in the root tissue (Fe-retaining power)[30,31]
(4) Leaf tissue tolerance to excess amounts of Fe[7]

The physiological status of the rice plant growing under submerged conditions modifies its ability to tolerate high concentrations of Fe[29]. The Fe-oxidizing power is associated with tolerance for other toxic metals because Fe plaque on the root could act as a barrier to the other metals[20]. The Fe-excluding power of the root was markedly decreased by respiratory inhibitors such as potassium cyanide (KCN) and sodium azide (NaN_3)[30]. Therefore, the Fe-excluding power was associated with metabolic activity. A high concentration of sodium chloride (NaCl) did not affect the Fe-excluding power[31]. On the contrary, it decreased the Fe-oxidizing power indicating those two powers are different mechanisms[31].

It was reported that three major types of adaptation strategies can be differentiated in relation to “includer” and “excluder” as well as “avoidance” and “tolerance” mechanisms[6]. Namely, plants employing strategy I (exclusion/avoidance) excluded Fe^{2+} in soil solution at the root level to avoid Fe^{2+} damage to the shoot tissue (rhizospheric oxidation and root Fe selectivity). With strategy II (inclusion/avoidance), although Fe^{2+} was taken up into the rice root, tissue damage was avoided by either compartmentation (immobilization of active Fe in old leaves that consist of photosynthetically less active leaf sheath tissue) or exclusion from the symplast (immobilization in the leaf apoplast). With strategy III (inclusion/tolerance), plants tolerate elevated levels of Fe^{2+} within leaf cells, presumably by using enzymatic alleviation of toxicity in the symplast. Whereas Fe-exclusion by oxidation in the rhizosphere and the detoxification of leaf cells were well established as Fe-tolerance mechanisms of rice, the other mechanisms are not yet well understood and are not considered in rice breeding or screening for iron tolerance.

Although some knowledge of the mechanisms underlying those strategies existed, Fe-tolerant lines were not screened based on actual mechanisms such as avoidance or tolerance but on leaf symptoms and yields[14,22]. Furthermore, the mechanisms were hardly investigated under field conditions. Those facts were partly because the physiological mechanisms proposed were difficult to investigate and were not suited for mass-screening methods[3]. The International Rice Research Institute (IRRI) has developed many lines and varieties with Fe toxicity tolerance. However, the characteristics of this tolerance are not well understood under field conditions. The present study was aimed at confirming the Fe-tolerance of the breeding lines under a field condition, and investigating the growth characteristics of the lines relating to the Fe-tolerance.

Materials and methods

In the 2003 wet season, experiments in a field with Fe-toxicity were conducted at San Dionisio, Iloilo City, Panay Island, Philippines. As a reference for rice growth under normal conditions, the same experiments were conducted at IRRI field, Los Baños, Philippines. Some characteristics of the soils were analyzed. Free iron oxide was extracted using the mixture of sodium hydrosulfite ($Na_2S_2O_4$) and ethylenediaminetetraacetic acid (EDTA) with the procedure described by Asami and Kumada[2]. The ratio of soil to deionized water for the analysis of pH was 1 to 2.5[33]. Total nitrogen (N) was analyzed using the Kyeldahl method[8]. Organic carbon (C) was determined by using the method of Walkley and Black[24,34]. This method quantifies the amount of oxidizable soil carbon as determined by reaction with potassium bichromate ($K_2Cr_2O_7$) and sulfuric acid (H_2SO_4). After the digestion of soil using the mixture of hydrogen fluoride (HF), perchloric acid ($HClO_4$) and H_2SO_4, total Fe was determined by atomic absorption spectrophotometry[21].

Two near isogenic lines (NILs), FTB-7 and FTB-11, with an Indica-type variety IR64 genetic background, were developed by backcross breeding, with an iron-tolerant Indian variety Bg90-2 that was crossed five times with IR64 as a donor and the recurrent parent, respectively. In each generation, these hybrid progenies were selected with a solution culture for Fe-tolerance in a greenhouse. The cultivation periods were less than 30 days. The other two lines (Fe0013 and Fe0014) were selected as Fe-tolerant lines in Iloilo field trials. The lines were described as elite breeding lines in this study. Plots were laid out in a randomized complete block design with four replications. Pre-germinated seeds were sown in seedling trays to produce uniform seedlings. Fourteen (IRRI)- or 21 (Iloilo)-day-old seedlings were transplanted at a spacing of 20 ×

20 cm. The seedlings were transplanted on 21 August 2003 at Iloilo, and on 24 July 2003 at IRRI. Basal fertilizer was applied as 30 kg N ha^{-1}, 20 kg P ha^{-1} and 20 kg K ha^{-1}, incorporated at puddling. Nitrogen was topdressed at 20 kg ha^{-1} each at midtillering and panicle initiation. At Iloilo, rain water was collected in some pools in which the water was introduced to the fields as flooded water. At IRRI, ground water was used for the flooded water. To keep the reductive status of soil both at IRRI and Iloilo, the fields were flooded throughout the cultivation.

During the experiments at Iloilo, plant and soil solutions were sampled at 28, 51, 73, 91, 102, and 105 days after planting. Data from each plot was the mean of four samples. To collect the soil solutions, a porous cup, 10 cm long, was set in the soil vertically around 2 cm below the soil surface. The cup was connected to a flexible plastic tube, and the soil solution obtained was introduced into a 10 mL evacuated test tube[25]. The amount of Fe in soil solution was determined by the colorimetric method using *o*-phenanthroline[21]. The fresh samples of rice plants were washed by water with careful attention to remove soil. After shoots and roots of collected plant samples were separated, those were dried at 70 ºC for 3 days, and weighed. For the analysis, a part of the dried shoot and root were ground. A part of the plant samples were digested with the method of dry ashing for the chemical analysis[9]. The dry ashing was conducted in a muffle furnace at temperature of 550 ºC for 8 h. After this sample was cooled, the ash was dissolved in dilute nitric acid (HNO_3). The amount of P and K were analyzed with the Infra Analyzer 405 (Braun+Lube, Norderstedt, Germany)[16]. The amount of Fe was determined with atomic absorption spectrophotometry[15].

Results and discussion

The soil pH of Iloilo was lower than that of IRRI (Table 1). Although the total amount of soil Fe in Iloilo was smaller than that in IRRI, free iron oxide of the former was greater than that of the latter. Both total N and organic C in Iloilo were smaller than those in IRRI. The yields of IR64 and all lines at Iloilo were lower than those at IRRI (Table 2).

The reported levels of Fe in culture solution that caused toxicity varied from as low as 10 mg Fe L^{-1} up to 500 mg Fe L^{-1} or higher[32]. The concentration of Fe in soil solution that developed symptoms of Fe-toxicity varied with the pH of soil solution[29]. The Fe-toxicity can occur under low-pH conditions because Fe in soil solution hardly forms precipitations as various oxides, hydroxides and carbonate at low pH. Generally, soils with pH below 5.0 were prone to Fe toxicity[11]. The critical limit was about 100 mg L^{-1} at pH 3.7 and 300 mg L^{-1} or higher at pH 5.0[24,27]. In the current experiment at Iloilo, the concentrations of Fe in soil solution ranged from 40 to 140 mg L^{-1} throughout the growth period (Fig. 1). The Fe concentrations in affected plants are usually high, and the critical concentrations depend on plant age and general nutritional status. The critical level for toxicity in leaf was higher than 0.3–0.5 g / kg[11,28]. In the current study, the Fe concentrations of leaf were 0.5–2.0 g kg^{-1} throughout the growth (Fig. 2). Those high concentrations of iron oxide under low soil pH and Fe in leaf support the expression of Fe-toxicity at Iloilo.

Table 1. Chemical properties of soils used in this study

Site	pH (H_2O)	Total N (g kg^{-1})	Organic C (g kg^{-1})	Free iron oxide (as Fe g kg^{-1})	Total Fe (g kg^{-1})
IRRI	6.6	2.10	21.0	16.5	60.1
Iloilo	4.2	1.30	12.3	24.0	27.0

Table 2. Grain yields (t ha^{-1}) and growth period (d) at IRRI and Iloilo

Lines	IRRI			Iloilo			B/A (%)
	Yield (A) ± SD[a)]	Growth period for		Yield (B) ± SD[a)]	Growth period for		
		flowering	maturity		flowering	maturity	
IR64	4.3 ± 0.5	67	88	2.2 ± 0.1	63	89	51
FTB7	5.0 ± 0.8	69	89	2.4 ± 0.2	65	93	48
FTB11	4.8 ± 0.5	68	91	1.4 ± 0.3	66	93	29
Fe0013	3.8 ± 0.3	84	102	3.2 ± 0.2	78	106	84
Fe0014	5.4 ± 0.2	85	105	3.8 ± 0.5	80	108	70

a): Standard deviation (n = 4).

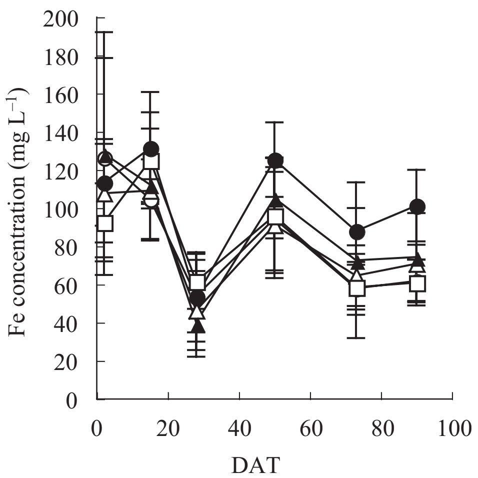

Fig. 1. Change in Fe concentrations in soil solution during growth of rice plant at Iloilo
Vertical bars indicate standard deviation (n = 4).
○:IR64, △:FTB7, □: FTB11, ●:Fe0013, ▲:Fe0014.

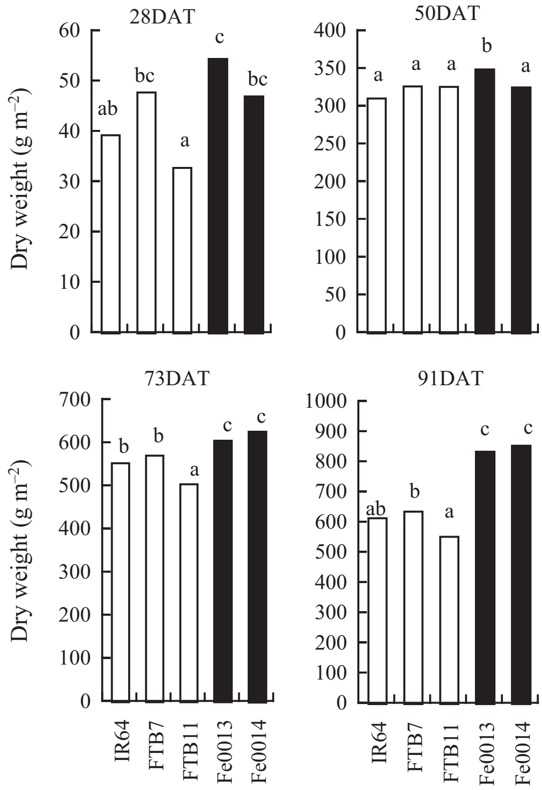

Fig. 3. Shoot dry weight of IR64 and four lines at Iloilo
Symbols with different letters denote a significant difference at the 5% level by Duncan's protected LSD (n = 4). DAT: Days after transplanting.

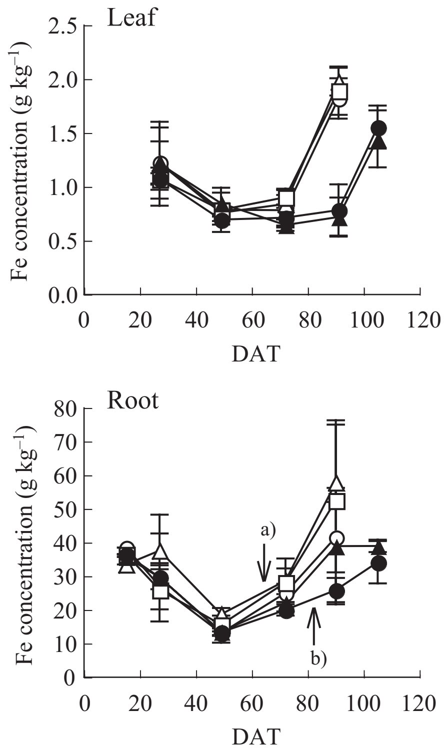

Fig. 2. Change in Fe concentration of roots and leaf during growth of rice plant at Iloilo
Vertical bars indicate the standard deviation (n = 4).
a): flowering stage of IR64, FTB7 and FTB11;
b): flowering stage of Fe0013 and Fe0014.
○:IR64, △:FTB7, □: FTB11, ●:Fe0013, ▲:Fe0014.

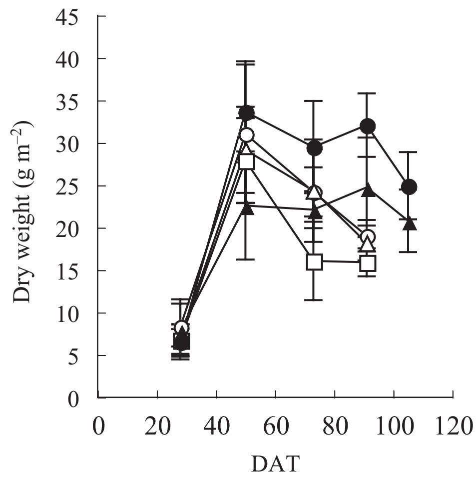

Fig. 4. Root dry weight of IR64 and four lines at Iloilo
Vertical bars indicate standard deviation (n = 4).
○:IR64, △:FTB7, □: FTB11, ●:Fe0013, ▲:Fe0014.

Genetic characteristics were reported to be able to significantly improve rice production in Fe toxic soils[12,27]. The periods of elite breeding lines for maturity and flowering were more than 10 days longer than those of IR64 and its NILs irrespective of the fields (Table 2). This finding indicates that the longer maturity period of elite breeding lines than those of IR64 and its NILs was not caused by the Fe-toxicity but by genetic factors.

On the basis of the report of Ladha et al.[19] and Yanagihara (unpublished data), the yields of IR64 and elite breeding lines were similar to those in other experiments conducted at IRRI (Table 2). To analyze the Fe-toxicity at Iloilo, the yields of the lines at Iloilo (B) was divided by those at IRRI (A). Based on the quotient (B/A), the tolerances of NILs were not expressed at the Iloilo field site because the yield reductions due to Fe-toxicity were similar to or bigger than those for IR64. The yield reduction of elite breeding lines were smaller than that of IR64, indicating that the tolerance of elite breeding lines was expressed at the Iloilo field. At Iloilo, whereas the shoot dry weights of IR64 and its NILs hardly increased after 73 days of cultivation, those of elite breeding lines kept on increasing (Fig. 3). Therefore, the yield reduction of IR64 and its NILs (Table 2) was attributed to the poor growth due to Fe-toxicity during the late stage. Although the root dry weight in the plots of IR64 and its NILs decreased abruptly after the 50 days of growth, those of elite breeding lines were kept constant or decreased gradually (Fig. 4). This finding suggests that the decrease in the amount of root of IR64 and its NILs was caused by earlier root senescence than those of elite breeding lines, susceptibility of root to Fe-toxicity and/or proper growth duration.

According to the report by Kpongor[18], two peaks of sensitivity to Fe-toxicity were present during the growth. The first symptoms occurred soon after transplanting because the roots of plants were damaged by the transplanting. Secondly, Fe-toxicity occurred during the heading stage because of the increase in root permeability and enhancement of microbial Fe-reduction in the rhizosphere. The current Fe-toxicity was categorized into the second toxicity. In the late stage of growth at Iloilo, the shoot dry weights of elite breeding lines were greater than those of IR64 and its NILs (Fig. 3). Especially after flowering stage, the Fe concentrations in the roots of IR64 and NILs increased rapidly (Fig. 2). During this period, the root of IR64 and its NILs might have not operated Fe-avoiding mechanisms such as Fe-oxidizing and Fe-excluding powers because of root senescence (Fig. 4). On the contrary, the root of elite breeding lines absorbed Fe gradually (Fig. 2). As a result, the final Fe-concentration in the leaf of IR64 and its NILs were higher than those of elite breeding lines (Fig. 2). The longer period for maturity in the elite breeding lines than that in IR64 and its NILs could be one of the factors that were responsible for the tolerance because it could keep the root activity for a longer time, and alleviate the Fe-toxicity.

Under the solution culture during less than 30 days, NILs might have shown a high survival rate to the Fe-toxicity because of vigorous growth during the early stage of cultivation. However, the Fe-tolerances of NILs were not expressed under the field condition (Table 2). Therefore, the growth operating factor such as root senescence might have attributed to the Fe-toxicity during the late stage (Fig.4). Further studies are needed to clarify the tolerance for Fe-toxicity under solution culture and field experiments.

After the flowering stage, the Fe concentration in the roots of either of the elite lines increased uniformly or remained constant, whereas those of IR64 and its NILs increased rapidly (Fig. 2). The Fe concentrations in leaf of IR64 and all the lines increased after flowering stage, and the final Fe concentration of IR64 and its NILs were higher than those of elite breeding lines. The findings suggest that the absorption of Fe in root was responsible for the changes in Fe concentration of leaf especially during the late stage of cultivation.

There are many reports relating Fe-toxicity with deficiencies for other nutrient elements such as P and K[4,12,13,17,26,35]. However, Sahrawat[27] showed the presence of Fe toxicity without any apparent deficiency of other nutrients. For both Fe-tolerant and susceptible cultivars in his experiment, there were no differences in the P and K concentrations in shoot except for that of Fe. The critical level for P-deficiency on leaf is 1.0 g kg^{-1} at panicle initiation stage, and 1.8 g kg^{-1} at flowering stage[10]. In the current study, the leaf P concentrations of IR64 and all lines were over the critical level throughout the growth (data not shown). There were no significant differences between the K concentration in IR64 and its NILs, and that in elite breeding lines throughout the growth (data not shown). Those findings suggest that neither the deficiencies in P nor that in K was associated with the growth inhibition under the current conditions.

Conclusion

Selection of Fe-tolerant lines in nutrient solution experiments did not produce lines with tolerance to Fe-toxicity under field conditions. However, selection in field trials produced improved tolerance. The long maturity period of tolerant lines helped to alleviate the toxicity, since the tolerance of improved lines was associated with the maintenance of root growth until the late stage of growth.

References

1. Ando, T. (1983) Nature of oxidizing power of rice roots. *Plant Soil*, **72**, 57–71.
2. Asami, T. & Kumada, K. (1959) A new method for determining free iron in paddy soils. *Soil Plant Food*, **5**, 141–146.

3. 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.
4. Audebert, A. & Sahrawat, K. L. (2000) Mechanisms for toxicity tolerance in lowland rice. *J. Plant Nutr.*, **23**, 1877–1885.
5. Aung, T. (2006) Physiological mechanisms of iron toxicity tolerance in lowland rice. Thesis of Master of Science, Rheinische Friedrich Wilhelms Universität zu Bonn.
6. Becker, M. & Asch, F. (2005) Iron toxicity – conditions and management concepts. *J. Plant Nutr. Soil Sci.*, **168**, 558–573.
7. Bienfait, H. F. (1989) Prevention of stress in iron metabolism of plants. *Acta Bot. Neerl.*, **38**, 105–129.
8. Bremner, J. M. (1996) Nitrogen-total. *In* Methods of soil analysis, Part 3- Chemical methods, eds. Sparks, D. L. et al., Soil Science Society of America, American Society of Agronomy, Madison, Wis., 1085–1121.
9. Campbell, C. R. & Plank, C. O. (1988) Preparation of plant tissue for laboratory analysis. *In* Handbook of reference methods for plant analysis, ed. Karla, Y. P., CRC Press, New York, 37–61.
10. Dobermann, A. & Fairhurst, T. (2000) Phosphorus deficiency. *In* Rice: Nutrient disorders and nutrient management, eds. Dobermann A. & Fairhurst, T., International Rice Research Institute, Manila, 60–71.
11. Dobermann, A. & Fairhurst, T. (2000) Iron toxicity. *In* Rice: Nutrient disorders and nutrient management, eds. Dobermann A. & Fairhurst, T., International Rice Research Institute, Manila, 121–125.
12. Fageria, N. K. & Rabelo, N. A. (1987) Tolerance of rice cultivars to iron toxicity. *J. Plant Nutr.*, **10**, 653–661.
13. Genon, J. G. et al. (1994) Iron toxicity and other chemical soil constraints to rice in highland swamp of Burundi. *Plant Soil*, **166**, 109–115.
14. Gunawardena, I., Virmani, S. & Sumo, F. J. (1982) Breeding rice for tolerance to iron toxicity. *Oryza*, **19**, 5–12.
15. Hanlon, E. A. (1988) Elemental determination by atomic absorption spectrophotometry. *In* Handbook of reference methods for plant analysis, ed. Karla, Y. P., CRC Press, New York, 157–164.
16. Jimenez, R. R. & Ladha, J. K. (1995) Assessing the potential of the near infrared reflectance (NIR) method for the reliable analysis of nitrogen in rice tissues. *In* Leaping ahead with near infrared spectroscopy, eds. Batten, G. D. et al., The NIR Spectroscopy Group, North Melbourne, Victoria, Australia, 208–213.
17. 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**, 273–243.
18. Kpongor, D. S. (2003) Developing a standardized procedure to screen lowland rice (*Oryza sativa*.) seedlings for tolerance of iron toxicity. Thesis of Master of Science, Rheinische Friedrich Wilhelms Universität zu Bonn. pp.53.
19. Ladha, J. K. et al. (2000) Long-term effects of urea and green manure on rice yields and nitrogen balance. *Soil Sci. Soc. Am. J.*, **64**, 1993–2001.
20. Liu, W. J. et al. (2005) Do iron plaque and genotypes affect arsenate uptake and translocation by rice seedlings (*Oryza sativa* L.) grown in solution culture? *J. Exp. Bot.*, **275**, 57–66.
21. Loeppert, R. L. & Inskeep, W. P. (1996) Iron. *In* Methods of soil analysis, Part 3- Chemical methods, eds. Sparks, D. L. et al., Soil Science Society of America, American Society of Agronomy, Madison, Wis., 659–661.
22. Mahadevappa, M. et al. (1991) Field screening of rice for growth, yield and nutrient uptake in acidic soil. *Dev. Plant Soil Sci.* **45**, 959–965.
23. Moormann, F. R. & van Breemen, N. (1978) Soil and land properties that affect the growth of rice. *In* Rice: soil, water, land, eds. Moormann, F. R. & van Breemen, N., International Rice Research Institute, Manila, 107–142.
24. Nelson, D. W. & Sommers, L. E. (1996) Total carbon, organic carbon, and organic matter. *In* Methods of soil analysis, Part 3- Chemical methods, eds. Sparks, D. L. et al., Soil Science Society of America, American Society of Agronomy, Madison, Wis., 961–1010.
25. Nozoe, T. et al. (1999) Effects of the addition of Fe-containing slag fertilizers on the changes in Eh in paddy soil. *Soil Sci. Plant Nutr.*, **45**, 729–735.
26. Olaleye, A. O. et al. (2001) Effect of toxic iron concentrations on the growth of lowland rice. *J. Plant Nutr.*, **24**, 441–457.
27. Sahrawat, K. L. et al. (1996) The role of tolerant genotypes and plant nutrients in the management of iron toxicity in lowland rice. *J. Agric. Sci. Cambridge*, **126**, 143–149.
28. Sahrawat, K. L. (2000) Elemental composition of rice plant as affected by iron toxicity under field conditions. *Commun. Soil Sci. Plant Anal.*, **31**, 2819–2827.
29. Sahrawat, K. L. (2004) Iron toxicity in wetland rice and the role of other nutrients. *J. Plant Nutr.*, **27**, 1471–1504.
30. Tadano, T. (1975) Devices of rice roots to tolerate high iron concentration in growth media. *JARQ*, **9**, 34–39.
31. Tadano, T. (1976) Studies on the methods to prevent iron toxicity in lowland rice. *Hokudai Nougakubu Houbun Kiyou (Mem. Fac. Agric. Hokkaido Univ.)*, **10**, 22–68 [In Japanese with English summary].
32. Tanaka, A., Loe, R. & Navasero, S. A. (1966) Some mechanisms involved in the development of iron toxicity symptoms in the rice plant. *Soil Sci. Plant Nutr.*, **12**, 158–164.
33. Thomas, G. W. (1996) Soil pH and soil acidity. *In* Methods of soil analysis, Part 3- Chemical methods, eds. Sparks, D. L. et al., Soil Science Society of America, American Society of Agronomy, Madison, Wis., 475–490.
34. Walkley, A. & Black, I. A. (1934) An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Sci*., **37**, 29–38.
35. Yamauchi, M. (1989) Rice bronzing in Nigeria caused by nutrient imbalances and its control by potassium sulfate application. *Plant Soil*, **117**, 275–286.
36. Yoshida, S. (1981) Iron. *In* Fundamentals of rice crop science, International Rice Research Institute, Manila, Philippines, 156–160.