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Endurance Test of Three Paddy Genotypes to Different Iron Toxicity Level

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Abstract: Iron toxicity in rice plants may occur both in vegetative and generative phase. Each genotype shows different response to iron toxicity both in morphology and physiology. The objective of this study was to evaluate morphological and physiological responses as well as the level of tolerance of paddy genotypes to iron toxicity. This study was conducted at the greenhouse, Bogor Agricultural University from October-December 2012. The design was two factors completely randomized design with three replications. The results showed that there were significant differences among each genotype of the ethylene content, aerenchyma size, plaque content, Fe content in root, leaf bronzing and Fe content in the shoot. Based on several parameters, Indragiri was very tolerant genotype to iron toxicity as indicated by the highest ethylene content (116.71 nL g^{-1} wet weight h^{-1}), the highest Fe content in roots (21271 ppm), the largest size of Aerenchyma (80230.11 nm), the highest plaque content (1864.12) and the lowest value of bronzing score (6). Base on the observations, it can be concluded that Indragiri genotype is very tolerant to iron toxicity, whereas IR64 genotype is very sensitive to iron toxicity.

Key words: Paddy genotype, ethylene, tolerance, iron toxicity

INTRODUCTION

Iron (Fe^{2+}) is one of the essential micro nutrients. This element is needed by plants in small amounts ranging from 300-500 ppm (Sahrawat, 2000), around 500-2000 ppm (Nozoe *et al*., 2008) and in the soil solution between 1000-2000 ppm (Asch *et al*., 2005). Plants with iron excessive absorption lead to impaired metabolic processes, e.g., arising brown spots on the leaves (Bronzing), interference on the growth of roots and shoot (little, short and blunt) (Peng and Yamauchi, 1993). Tolerance level of paddy to Fe is quite extensive, so to determine the level of tolerance to the toxicity, it needs some testing from the roots to the leaves. The results will indicate the level of tolerance to iron toxicity (either sensitive or tolerant).

According to Marschner (1995), there are two types of mechanisms of rice tolerance to Fe toxicity. The first is excluder type in which plants accumulate excessive Fe^{2+} in the roots, the ions Fe^{2+} inhibited to enter into the root zone. Before entering into the root tissue, iron must be pass through oxidative barriers in the rhizosphere area. Paddy plant has aerenchyma as air diffusion paths into the roots so that the rhizosphere becomes more oxidative. The results of ions Fe^{2+} oxidation in rhizosphere is iron plaques (Fe_2O_3) and will be accumulated in root surface so that the soluble ions Fe^{2+} is reduced. The second is includer type inwhich plant roots absorb the ions Fe^{2+} and hold it in the leaves. The tolerance mechanism of this type is excessive, Fe^{2+} ions are absorbed by the roots and then neutralized by SOD enzyme (Super Oxide Dismutase) to produces H_2O_2. Furthermore, the resulting H_2O_2 with the help of peroxides enzyme and/or catalase will produce H_2O and oxygen triplet that is not toxic to the plants.

The purpose of this study was to evaluate morphological and physiologisal responses as well as the level of tolerance of rice genotypes to iron toxicity.

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MATERIALS AND METHODS

The experiment was conducted in a controlled environment in greenhouse, at Bogor Agricultural University, from October-December 2012. The experiment was designed two factors completely randomized design. The first factor was 4 levels of iron concentration namely, 0 (control), 500, 1000 and 1500 ppm, respectively. The second factor was the paddy genotypes consisting of three genotypes, namely, IRH108, IR64 and Indragiri. The media was half concentration of (Yoshida *et al*., 1976) nutrient solution with the source of Fe was $FeSO_4.7H_2O$ (Audebert and Sahrawat, 2000). Planting containers (tub) 27×14×7 cm, each filled with nutrient solution as much as 9 liters.

The implementation phases were as follow. First, paddy seeds soaked with deionized water for 2-3 days. Second, the soaked seeds germinated on straw paper for 5 days, the seedlings remain in wet conditions. Third, the seedlings were transferred to the nutrient solution for 2 weeks. The 2 weeks old plants were treated with Fe stress. The concentration of Fe ($FeSO_4.7H_2O$) were 0, 500, 1000 and 1500 ppm. Planting tubs containing nutrient solution with Fe ($FeSO_4.7H_2O$) set at pH = 4.

When one of the genotype has already reached the maximum stress (death), observations of the morphological and physiological were carried out. The observed parameters was including plant biomass, Fe content in roots and leaves, Fe distribution in root and root aerenchyma. Observation of Fe distribution in root tissue can be done using dye solution of Perl's Prusian Blue. The root section was part of the root tip (0-5 mm) from tip of the main root. The observation was conducted using Olympus BX51 microscope with magnification of 4×10. Root aerenchyma observations were made on the roots 0-10 mm from the base of the main root. Aerenchyma measurements performed using Olympus B×51 microscope with magnification of 10×10. Scoring the leaf bronzing was done based on the directives issued by IRRI and INGER (1996).

RESULTS AND DISCUSSION

Ethylene content: Results of analysis of variance showed that each genotype (IRH108, IR64 and Indragiri) has significant differences in the content of ethylene in root. At a zero (0) concentration (control) and 500 ppm, ethylene content in the roots was not significantly different among three genotypes. At concentration of 1000 and 1500 ppm, there was no marked difference on ethylene content in roots between IRH108 and Indragiri genotypes but both genotypes were significantly different to IR64. Interaction between genotypes and Fe concentrations also significantly affected the content of ethylene. The higher the Fe concentration in the nutrient solution, the higher ethylene production in the roots. The increase in ethylene production allegedly due to stagnant roots conditions (anaerobic). Anaerobic conditions can stimulate the formation of ACC. The ACC is derived from S-adenosyl methionine. The precursor ACC with sufficient oxygen can stimulate the formation of ethylene (Yang, 1980). Furthermore, ethylene hormone formed in the roots will induce the development of aerenchyma at the root (Kawase, 1981).

In addition to anaerobic conditions, ethylene production can also occur if the plants were exposed with iron toxicity. Ions of Fe^{2+} will be accumulated in plant root tissues, followed by ethylene biosynthesis in roots, root growth dropped dramatically and can reduce the yield (Yamauchi and Peng, 1995; Becker and Asch, 2005; Dorlodot *et al*., 2005). Increased ethylene production in this experiment presumably due to root waterlogged condition (anaerobic) and high Fe^{2+} concentration in the nutrient solution. Different ethylene content in the roots can be seen in Fig. 1.

Roots aerenchyma: Plants were flooded in a short time can experience hypoxia (O_2 deficiency). Plants that are able to adapt to the waterlogged condition characterized by the development of aerenchyma and increased soluble sugars (Sairam *et al*., 2008). Aerenchyma is a cavity among cells that serves as air diffusion channel (O_2)

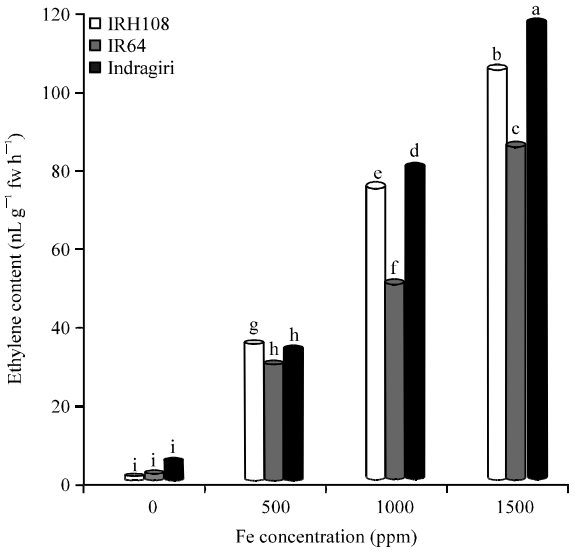

Fig. 1: Effect of different Fe concentrations on ethylene in root tissue in each gentype (IRH108, IR64 and Indragiri genotypes)

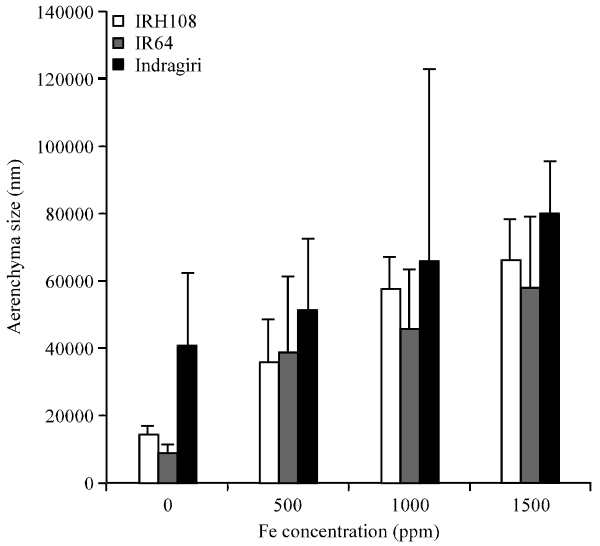

Fig. 2: Aerenchyma size in root tissue at different concentration of Fe in IRH108, IR64 and Indragiri genotypes

(Mulyani, 2006). Aminocyclopropanacarboxilyc acid (ACC), which is formed from S-adenosyl methionine can be converted into ethylene in root and able to stimulate the formation of aerenchym (Kawase, 1981). This study showed that the production of ethylene has positive relationship with aerenchyma development. The higher ethylene production in the root, the greater aerenchym development in roots. The highest aerenchyma development found in Indragiri genotype (80230.11 nm) at Fe concentration of 1.500 ppm. Aerenchyma formation in the roots can reach 20-50% of the total volume of rice root in flooded condition (Armstrong, 1979). The results showed that the size of aerenchyma increased with increasing concentrations of Fe ions in solution. In this study, observations of the size of aerenchyma performed under a microscope Olympus B×15 with magnification 10×10. The difference of the size of aerenchyma in the roots of each genotype at level 0 ppm (control) and 1500 ppm can be seen in Fig. 2. Differences in the size of the root aerenchyma in cross section with a concentration of 0, 500, 1000 and 1500 ppm Fe on IRH108, IR64 and Indragiri genotypes can be seen in Fig. 2 and 3.

Plaque content: The occurrence of air diffusion from the leaves, stems to the root and the root zone to becomes more oxidative is facilitated by the air cavity called aerenchyma. Air cavity that known as aerenchyma is able to facilitate the process of O_2 diffusion from leaves, stem to the roots that make the rhizosphere area more oxidative. More oxidative rooting environment can prevent ions Fe^{2+} enter to root tissue by means of oxidizing ions Fe^{2+} to Fe^{3+} at the root surface and known as iron plaque (Asch *et al*., 2005). Oxidation of iron at the root surface is one of avoidance strategies to an excess of Fe^{2+} (Ando *et al*., 1983). In these experiments, it is known that the size of aerenchyma positively associated with plaque formation. The larger aerenchyma development in root surface, the larger plaque formation. This can be evidenced in Indragiri genotype that has the largest size of aerenchyma (80230.11 nm) and the highest content of plaque (1864.12 ppm).

Iron plaque (Fe_2O_3) which is attached on the root surface can hinder Fe^{2+} ions enter into the root tissue (Asch *et al*., 2005). The root ability to oxidize Fe^{2+} to Fe^{3+} at the root surface is one of the avoidance mechanisms (Marschner, 1995). Iron tolerance range in the plant tissue is quite extensive which are 300-500 ppm (Sahrawat, 2000) and 500-2000 ppm (Nozoe *et al*., 2008). Plants that are tolerant to iron toxicity has ability to oxidize Fe^{2+} in the roots. But the formation of plaques cannot be used to measure the level of plant tolerance to iron toxicity. Therefore, it is required observation of the Fe content in the roots and shoot. The differences of root plaque content of each genotype can be seen in Fig. 4.

Distribution of Fe in root tissues: Distribution of Fe in root tissue was observed using Perl's Prusian Blue dye (Pearse, 1982). Roots were containing Fe ions will blue in color. The results showed that at Fe concentration of 500 ppm, Fe ions detected in epidermal tissue for Indragiri and IRH108 while for IR64 Fe^{2+} ions detected in epidermal and cortex tissue. Fe^{2+} ions can enter into the epidermal and cortex tissue by simplast and apoplast. High concentration of Fe ions in solution can make Fe^{2+} ions enter to the root tissue after passing through the barrier of oxidative rhizosphere area. At a concentration of 1000 ppm, Fe^{2+} ions have been detected in epidermis and cortex tissues on IRH108 and Indragiri. For IR64 genotype, the distribution of Fe has been detected in epidermis, cortex and vascular (xylem) tissues. At a concentration of 1500 ppm, distribution of Fe^{2+} ions have been detected in all root tissues (epidermis, cortex and xylem) at the apoplast and simplast.

Fe^{2+} ions were detected in the cortex tissue can entered into the xylem tissue by means simplast path and a part passes through apoplast (Yeo *et al*., 1987; Yamanouchi and Yoshida, 1981). The results showed that the higher concentrations of Fe application produce more root layers tissue were detected containing Fe^{2+} ions. Fe^{2+} ions that have entered into the vascular tissue (xylem) will then be transported to leaf via transpiration stream (Tanaka *et al*., 1966). Observations on Fe^{2+} distribution in

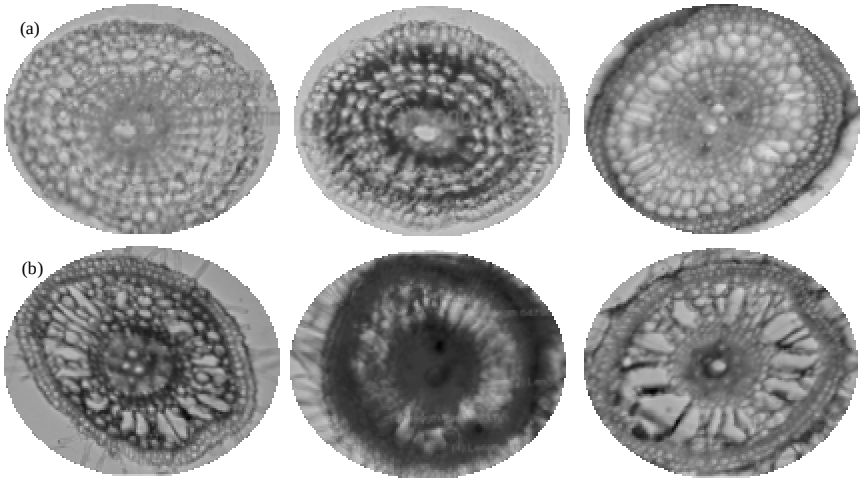

Fig. 3(a-b): Roots cross section showing aerenchyma size at differennt Fe concentrations in each genotype IRH108, IR64 and Indragiri (left to right), (a) Control (0 ppm) and (b) 500 ppm

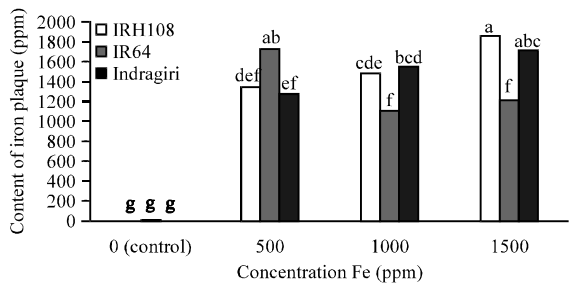

Fig. 4: Content of iron plaque in root at different concentration Fe in IRH108, IR64 and Indragiri genotypes

Table 1: Content of Fe in root and shoot with different concentration of Fe in IRH108, IR64 and Indragiri genotypes

Concetration Fe (ppm)	Genotypes	Content of ion Fe in Root tissue	Shoot tissue
0 (control)	IRH108	2189±190^{f}	255±32^{j}
	IR64	3208±100^{f}	231±32^{j}
	Indragiri	2737±63^{f}	236±32^{j}
500	IRH108	64332±7668^{b}	1951±72^{i}
	IR64	88475±3612^{a}	2320±72^{g}
	Indragiri	65944±0^{b}	2093±72^{h}
1000	IRH108	20467±543^{e}	4030±72^{e}
	IR64	17962±1038^{ed}	4196±72^{d}
	Indragiri	21271±733^{e}	3746±72^{f}
1500	IRH108	13991±1690de	6228±72^{c}
	IR64	15410±2530de	7394±72^{a}
	Indragiri	11534±1466^{f}	6303±72^{b}

the roots was done under Olympus BX51 microscope with a magnification of 4×10. Differences in the distribution of individual genotypes with different concentrations of Fe in solution can be seen in Fig. 5.

Fe content in root and shoot tissues: Fe ions which have entered into the vascular tissue (xylem) partially accumulated in root tissue and the other part will be transported to the shoot tissue. Tolerant plants to iron toxicity will store more Fe^{2+} in root tissue. Results of analysis of variance showed that each genotype (IRH108, IR64 and Indragiri) has significant difference on Fe^{2+} content in roots and shoot tissues. Changing treatment from no Fe (control) to 500 ppm Fe application showed an increase in Fe content in root tissue. However, application with 1000 and 1500 ppm will decrease Fe ion in roots. This applies to all genotypes tested. An increase in Fe^{2+} content from control to 500 ppm makes Fe^{2+} ions detected in epidermis and a part is cortex. Thus, absorbed Fe^{2+} ions by the roots will be accumulated in root tissues. While at concentration of 1000 and 1500 ppm, Fe^{2+} detected in epidermal, cortex and xylem tissue. Fe^{2+} ions were detected in the xylem tissue will then be transported to shoot tissue via transpiration tissue simultaneously with the process of transpiration (Tanaka *et al*., 1966). Fe^{2+} ions which have entered the vascular tissue can be transported along with the process of transpiration.

The increase in Fe^{2+} content in shoot along with the increasing of Fe^{2+}concentration in the nutrient solution may also be caused by reduction process that occurs continuously causing root tissue becomes damaged and the influx of Fe^{2+} cannot be avoided (Makarim *et al*., 1989)

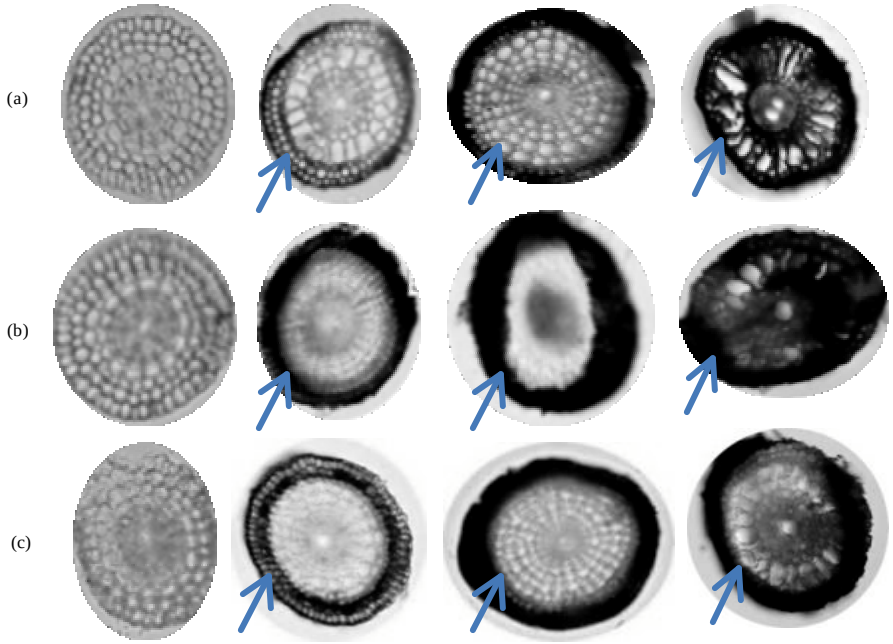

Fig. 5(a-c): Roots cross section showing Fe distribution at different Fe concentrations (control, 500, 1000 and 1500 ppm, form left to right) in each genotype, (a) IRH108, (b) IR64 and (c) Indragiri. The roots tissue containing Fe^{2+} ions appear blue and black color and the arrows show that the root tissue contained detectable Fe^{2+}

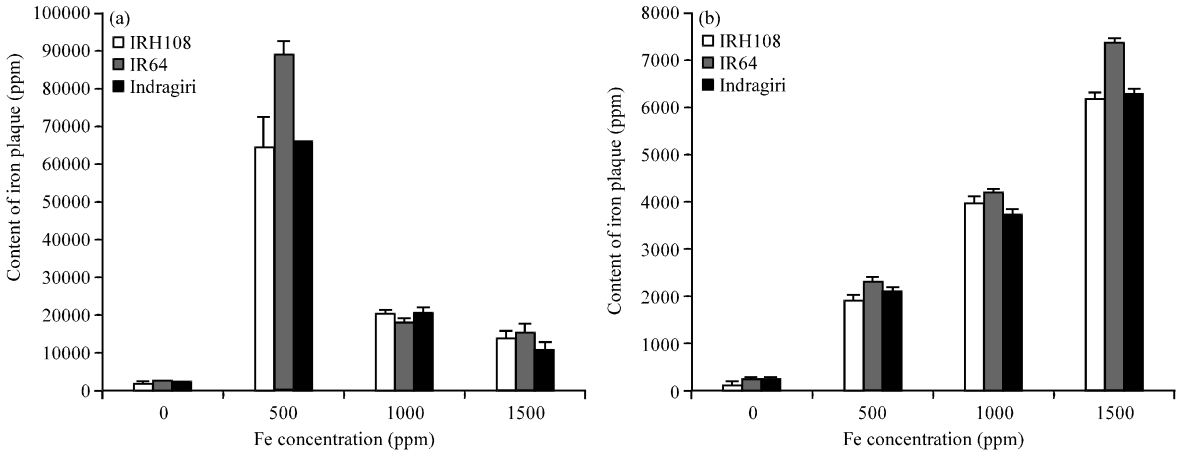

Fig. 6(a-b): Content of Fe in (a) Root and (b) Shoot tissue at different concentration of Fe in IRH108, IR64 and Indragiri genotypes

and Fe^{2+} ions in the xylem tissue can be easily transported to shoot tissues along with the process of transpiration (Tanaka *et al*., 1966). Differences of Fe^{2+} content in the roots and shoot of each genotype with differences in Fe^{2+} concentration in solution can be seen in Table 1 and Fig. 6.

Bronzing score in leaf: Plants have a strategy to tolerate Fe^{2+} ions that have entered into the leaves tissue. The presence of Fe^{2+} ions in the leaves tissue will help to develop several active oxygen e.g., superoxide, hydroxide radicals and H_2O_2 (Marschner, 1995). Then the Fe^{2+} ions will be neutralized by the enzyme of Super Oxide

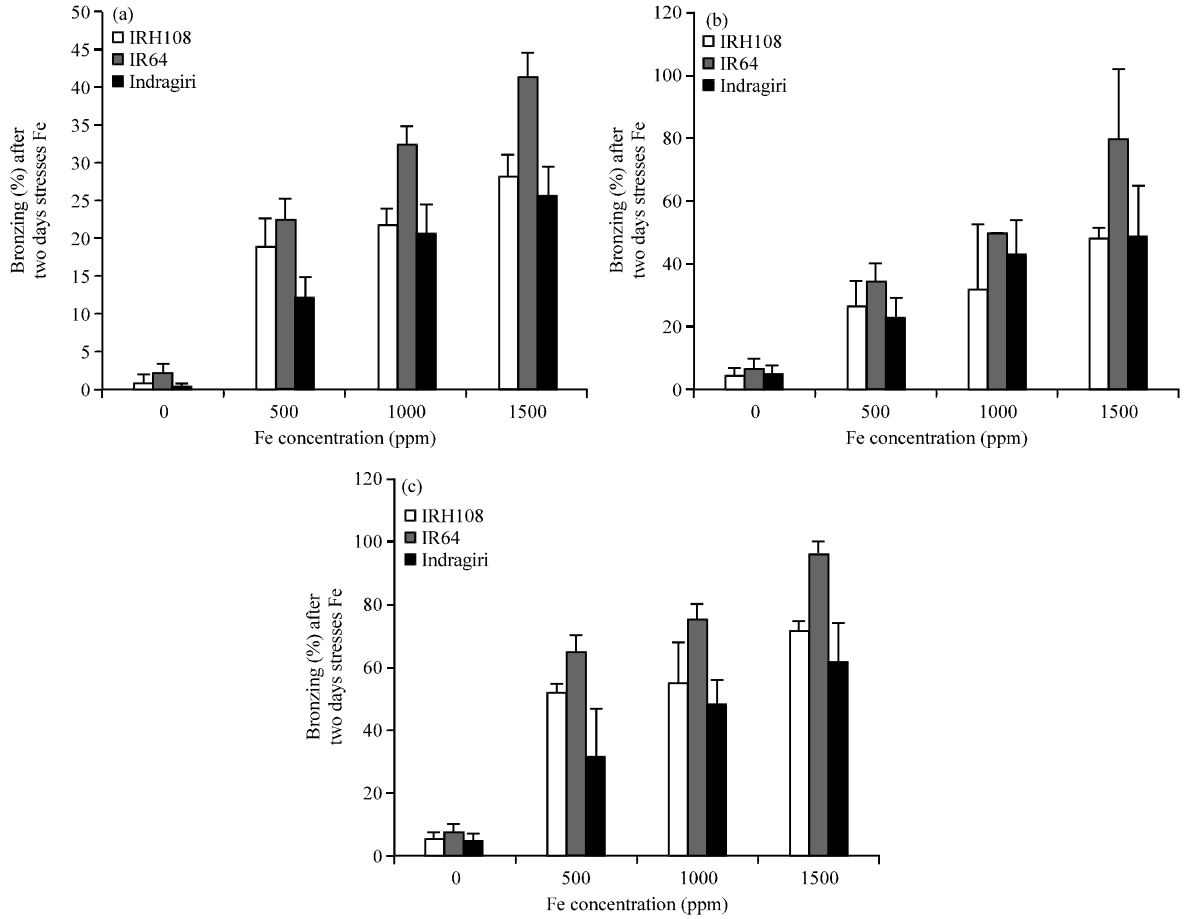

Fig. 7(a-c): Percentage of leaf bronzing on each genotype, (a) After two days of treatment of Fe application, (b) After four days of treatment of Fe application and (c) After six days treatment Fe application

Dismutase (SOD) and produces H_2O_2. Peroxides enzyme catalase produce H_2O and triplet oxygen that not toxic to plants. This strategy is includer type mechanism in plants (Marschner, 1995). In addition, the plant can also tolerate Fe^{2+} ions in the leaves by means of enzymatic detoxification in simplast (Becker and Asch, 2005). Sensitive plants generally do not have a detoxification strategy, so that the leaves will experience bronzing when high Fe^{2+} ions present in leaf tissue. This can be seen in IR64 genotype, that is after experiencing Fe^{2+} stress at 1500 ppm for 6 days these plants experiencing maximum stress (death) and showed bronding score 10. Score for IRH108 is 8 and Indragiri was 6. Iron toxicity in rice is more influential on the top (stem and leaf) (Lubis and Noor, 2012).

Plants with includer tolerance mechanisms type absorb high Fe^{2+} ions and stored on the leaf tissue but experiencing slightly bronzing. Not all of Fe^{2+} ions that absorbed by the leaves is toxic and the Fe^{2+} ions can be detoxified in the simplast (Becker and Asch, 2005). Plants that are sensitive to iron toxicity has no selectivity towards Fe^{2+} ions. The absorption of Fe^{2+} ions occur continuously and do not have barrier between the different organs. Genotype of differences in leaf bronzing score at different measurement time can be seen in Fig. 7.

CONCLUSION

Based on several parameters such as the differences observed thylene content, aerenchyma size, Fe content in root tissue, Fe content in shoot tissue and the percentage of leaf bronzing, Indragiri was considered as highly tolerant genotyp to iron toxicity. This genotype is not only had excluder tolerance (avoidance) but also had includer tolerance mechanism.

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