Relationship of Ethylene Production and Aerenchyme Formation on Oxidation Ability and Root Surfaced-Iron (Fe^{2+}) Accumulation under Different Iron Concentrations and Rice Genotypes

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Abstract

Iron toxicity in rice plant can occur during the vegetative and generative phase. Different rice genotypes show different responses to iron toxicity both morphologically and physiologically. The aim of this study was to evaluate the relationship of root ethylene production and aerenchyme formation on the ability of rice root to oxidize and accumulate iron (Fe^{2+}) on root surface under different iron concentration and rice genotypes. This research was conducted at the greenhouse, Bogor Agricultural University, Indonesia from October to December 2012. experiment that consisted of two factorial was completely randomize designed with three replications. The first factor was four level Fe concentrations, i.e.: 0, 500, 1000, and 1500 ppm and the second factor was IRH108, IR64 and Indragiri rice genotypes. The results showed that there was significant differences on the ethylene production, aerenchyme size, plaque content, and Fe^{2+} distribution in root tissue among each genotype. It was concluded that Indragiri was a tolerant genotype to iron toxicity as indicated by the highest ethylene production (116.71 nl.g^{-1}fresh $weight^{-1}$.h^{-1}), the highest root Fe^{2+} content (21,271 ppm), the largest size of aerenchyme (80,230.11nm), and the highest plaque content (1,864.12).

Keywords: Ethylene, Aerenchyme, Rice Genotype, Concentration, Iron Toxicity

Introduction

Iron (Fe^{2+}) is one of the essential micronutrients, which is required by plants in a small amount ranging from 30-150 ppm. While critical deficiency of iron content ranging from 50 to150 mg Fe kg^{-1}dry weight of leaves (Marschner, 1995). If plants absorb excessive iron, the root growth will be retarded and the root become few, short with blunt tip (Yamauchi and Peng,1993). The range of rice tolerance Fe toxicity is quite extensive. The critical limit of Fe content in plant tissue is between 300-500 ppm (Sahrawat, 2000). However, other research reported that the critical limit of Fe content in plant tissue is between 500-2000 ppm (Becker and Asch, 2005; Nozoe *et al* 2008). Plant could tolerate to 1000-2000 ppm of Fe^{2+} in the soil solution (Asch *et al*. 2005). Koesrini and William (2001) reported that Margasari varietiy that absorbs 400 ppm of Fe^{2+} could survive; however, plants will die when absorb about 600 ppm of Fe^{2+} .

Depending on the nature of tolerance or sensitivity to iron toxicity, different rice genotype respond differently to iron toxicity. According to Marschner (1995) there are two types of mechanisms of plant tolerance to iron toxicity. The first is exclusion mechanism, which is plant accumulate excess Fe^{2+} in the roots, and the Fe^{2+} ions were inhibited to enter the root tissue. Before entering into the root tissue, iron must be through oxidative barriers that exist in the rhizosphere area. Aerenchyme of the rice root diffuse air into the root surface making the rhizosphere becomes more oxidative. The results of the oxidation of Fe^{2+} ions in the rhizosphere will form iron plaque (Fe_2O_3) that decrease soluble Fe^{2+} ions. The second is inclusion mechanism, which is plant roots absorb the Fe^{2+} ions and hold it in the leaves. The absorbed Fe^{2+} ions were then neutralized by the SOD (Super Oxide Dismutase) enzyme produces H_2O_2. Furthermore, the resulting H_2O_2 with the help of peroxides and/or catalase enzymes will produce H_2O and oxygen triplet that is not toxic to the plants.

The plant root is part of the plant that contact with the soil, therefore it becomes the target of the iron toxicity (Zhang *et al*. 2012). Plants that tolerant to iron toxicity has the ability to oxidize Fe^{2+} to Fe^{3+} which is known as iron plaque (Asch *et al*. 2005). Oxidizing ability corelate to the production of ethylene in the roots and aerenchyme formation in the roots. Research on the relationship between ethylene production and aerenchyme formation to oxidation ability has not been done, therefore the research on root oxidation ability associated with the ethylene production and aerenchyme size needs to be done.

The objective of this study was to evaluate the relationship of root ethylene production and aerenchyme formation on the ability of rice root to oxidize and accumulate iron (Fe^{2+}) on root surface under different iron concentration and rice genotypes.

Materials and Methods

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

The implementation phases are as follow. First, rice 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 seed which has been moved to 5-day-old the culture media (bath) that is already filled with a mixture of nutrient solution and Yoshida half concentration as much as 9 liters of distilled water. Let stand for 2 weeks in the growing media. After 2 weeks in nutrient solution was replaced with a new nutrient solution with the same composition. Each concentration Fe ($FeSO_4$ $7H_2O$) were 0, 500, 1000 and 1500 ppm and set pH=4 . When one of the genotype has already reached the maximum stress (death), observations of the morphology and physiology will be carried out. Observations on the content of iron plaque on the roots, the distribution of Fe^{2+} in the roots, Fe^{2+} content in root, root a erenchyma size and content of ethylene in the roots. Observation of the distribution of Fe^{2+} in root tissue was conducted using Perl's Prusian Blue dye solution (Pears, 1982). Taken root section is part of the root tip (0-5 mm) from the tip of the main root. At first the root tips were washed with water flowing slowly, and then soaked for 10 minutes in a solution of HCl and potassium ferrosianida (1:1).

After that soak into the match dye eosin 0.5% for 10 min, then dehydrated with 95 % alcohol. Observations were made under a microscope Olympus BX51 with 4x10 magnification. The root portion were taken for observation aerenchyme is at the base of the main roots (0-10 mm) from the base of the root. Aerenchyme measurements performed using Olympus BX51 microscope with a magnification of 10x10. Measurement of ethylene content is done by cutting the roots from the base of the root, then washed, after which the roots were weighed fresh weight, then insert into the bottle tightly closed. Subsequently incubated for 24 hours, after it sucked up by using the tools injection (sheryng) Ethylene content was then measured using Chromatograph. Measurement of the content of the plaque was done by soaking the roots for $\pm$30 minutes to a solution of 2% HCl. After the Fe^{2+} content was measured by means of Spectrofotometer (AAS).

Results and Discussion

Root Ethylene Production

Ethylene that formed in the roots induced the formation of root aerenchymes (Kawase, 1981). In this experiment, root ethylene production was affected by interaction between genotypes and Fe concentrations (Figure 1, Table 1). At zero ppm Fe, there were no differences on ethylene production among genotypes. However, when Fe concentration increased above 500 until 1500 ppm, rice var. Indragiri and IRH108 produced ethylene significantly higher than that of rice var. IR64. It was indicated from this experiment and other researchs that when the plants are experiencing to iron toxicity, the plants synthesized more ethylene in the roots resulting the inhibition on root growth (Yamauchi and Peng 1995; Becker and Ash 2005; Dorlodot *et al*. 2005). Ethylene production can also occur due to the flooding condition, which stimulate the formation of amynocyclopropane carboxylic acid (ACC). Under sufficient oxygen, the ACC will be changed to ethylene. (Yang, 1980). Once ethylene produce in the root, then it could stimulate root aerenchyme formation (Kawase, 1981).
The results of the research that has been conducted on some rice genotypes showed that ethylene production was highest in genotype Indragiri (116.71 $nl.g^{-1}FreshWeight^{-1}.h^{-1}$) at a concentration of 1500 ppm Fe and the lowest was 29.53 in genotype IR64 ($nl.g^{-1}FreshWeight^{-1}.h^{-1}$) at a concentration of 500 ppm Fe

Root Aerenchyme

The observation that has been made known that each genotype was not significantly different to the size of aerenchyme. but the concentration of Fe gives a real difference to the size of aerenchyme in roots. The results showed that the higher the concentration of Fe in solution led to the greater size of the root aerenchyme. In addition, ethylene production also encourages the development of aerenchyme in roots. The higher ethylene production also increased aerenchyme development.
Root aerenchyme formation was affected by genotypes and Fe concentrations separately. The higher Fe concentration, the larger size of root aerenchyme formed. Rice var. Indragiri formed largest aerenchyme size (80230.11 µm) among three genotypes followed by IRH108 and IR64, respectively (Figure 2). The results obtained form this experiment indicated that there was positive correlation between root ethylene production and root aerenchyme size. The higher ethylene produced from the root, the larger size of root aerenchyme formed. Armstrong (1997) reported that aerenchyme formation on the roots can reach 20-50 % of the total volume of the rice roots. One of the aerenchyme functions is as a cavity for air diffusion from the rice leaves and stems to the roots to supply O_2 to the roots.

Iron Plaque

Oxidation of Fe^{2+} to Fe^{3+} by the roots is one of the avoidance mechanisms in plant when exposed to the excess of Fe^{2+} through the formation of iron plaque on root surface to reduce the risk of iron toxicity (Ando, 1983; Marschner, 1995; Asch. e*t al* 2005). In this study it was known that iron plaque formation were affected by interaction between genotypes and Fe concentration (Figure 3, Table 2). Iron plaque formation was positively correlated with the concentration of Fe in the nutrient solution. The higher Fe concentration, the higher iron plaque formed. There was no difference on iron plaque formation among genotypes when they grown on 0 ppm Fe.

However, at 500 ppm Fe rice var. IR64 formed iron plaque significantly higher than that of var. Indragiri and IRH108. Interestingly, when the Fe concentrations increased to 1000 and 1500 ppm, rice var. Indragiri and IRH108 formed iron plaque higher than that of IR64, while both genotypes no difference in plaque formation.
An Fe-tolerant rice has the ability to form plaques higher than the sensitive one. The plaque formation was influenced by the size of the root aerenchyme, which was influenced by ethylene production in the root. The higher production of ethylene in the root, the larger size of aerenchyme formed. The larger size of root aerenchyme, more oxidative area in the rhizosphere that can formed iron plaque on root surface.

The results of this research showed that rice var. Indragiri and IRH108 has strategy to tolerate to iron toxicity by producing ethylene (116.71 and 105.23 $nl.g^{-1}FreshWeight^{-1}.h^{-1}$), forming aerenchyme (80230.11 and 66668 µm) and iron plaque on the root surface surface (1864.1 and 1717.14 ppm) at 1500 ppm of Fe.

Distribution of Fe in Root Tissue

Observations on the distribution of Fe in the root tissues were calculated using Perl's Prusian Blue dye (Pearse, 1982). The roots containing Fe^{2+} ions appeared in blue color. Root cross section analysis showed that there was no detected Fe on root tissue when it was treated with 0 ppm Fe. When the root was treated with 500 ppm Fe, the iron was detected in root epidermal tissue. The increasing Fe concentration to 1000 ppm did not affect Fe distribution on Indragiri and IRH108 root tissue, but significantly affected the distribution in IR64 root tissue, which could penetrate until root cortex. Iron was detected in all root tissue (epidermis, cortex and vascular tissue) including apoplast and symplast when those three rice genotypes stressed with1500 ppm Fe. The higher the concentration of Fe in the solution caused more Fe absorbed by roots through the barrier in the area of oxidative rhizosphere. Fe ions are absorbed by the roots and then transported to the leaves via the transpiration stream (Tanaka *et al*. 1966) after passing the ribbon Casparry (casparyan strip) through symplast or apoplast (Yeo *et al* 1987; Yamanouchi and Yoshida 1981).

Fe Content in Root Tissues

Fe ions that entered into root cell partially accumulated in root tissue and the other part will be transported to the shoot tissue. Fe-tolerant plants will stores more Fe^{2+} in root tissue beside transported part of the Fe ion to the shoot tissue. Results of analysis of variance showed that each genotype (IRH108, IR64 and Indragiri) has significant difference on Fe^{2+} content in roots tissues. Changing treatment from zero (control) to 500 ppm Fe showed an increase in Fe content^{2+} in root tissue. However, treatment with 1000 and 1500 ppm decreased Fe^{2+} ion in roots. This phenomenon occurred in 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 Fe concentration of 1000 and 1500 ppm, Fe^{2+} detected in epidermal, cortex and xylem tissue. Fe^{2+} ions that were detected in the xylem tissue will then be transported to shoot tissue via transpiration strean simultaneously with the process of respiration (Tanaka *et al*. 1966).

The process of Fe ion influx into plant tissue starting from the increasing permeability of plant root cells to Fe^{2+} ions with increasing Fe reduction process in the root zone and cause the absorption of the ferrous ion increased rapidly. Reduction of Fe^{3+} that occurs in the root zone can cause damage to Fe oxidation, so that the uncontrolled influx of Fe^{2+} entry into rice roots (Makarim et al. 1989). In this research note that the genotype of plants tolerant to iron toxicity save more Fe in root tissue and a few are transported to tissues canopy. This can be seen in genotype Indragiri more Fe ions accumulate in the roots while genotype IR64 more Fe ions accumulate on the shoot.

The increase in Fe^{2+} content in leaves along with the increasing of Fe^{2+} concentration in the nutrient solution may also be caused by: 1) reduction process that occurs continuously causing root tissue becomes damaged and the influx of Fe^{2+} cannot be avoided (Makarim *et al.* 1989), 2) Fe^{2+} ions in the xylem tissue can be easily transported to shoot tissues along with the process of respiration (Tanaka *et al.* 1966). Differences of Fe^{2+} content in the roots of each genotype with differences in Fe^{2+} concentration in solution can be seen in figure 5.

Conclusion

Rice genotypes that tolerant to iron toxicity has ability to oxidize Fe^{2+} on the root surface. The higher ethylene production, the larger root aerenchyme size which ultimately the larger capacity to oxidize Fe on root surface and the more tolerant the genotype. Based on those criteria, it was conclude that rice var. Indragiri was a tolerant rice to iron toxicity followed by IRH108.

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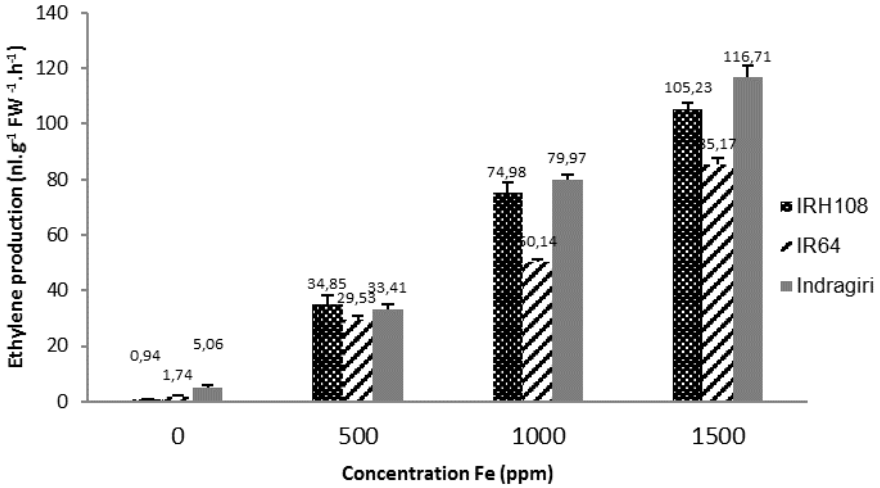


Figure1. Content of ethylene and iron plaque in root in with different concentration Fe in IRH108, IR64 and Indragiri genotypes

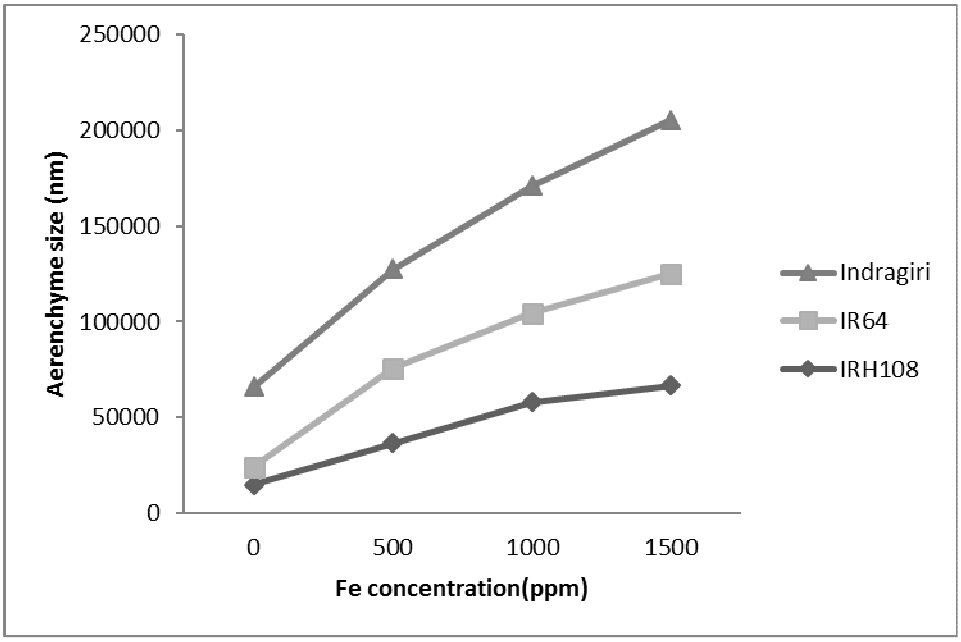


Figure 2. Differences in the size of the root aerenchyme in cross section with a concentration of 0 ppm, 500 ppm, 1000 ppm and1500 ppm Fe on IRH108, IR64 and Indragiri genotypes

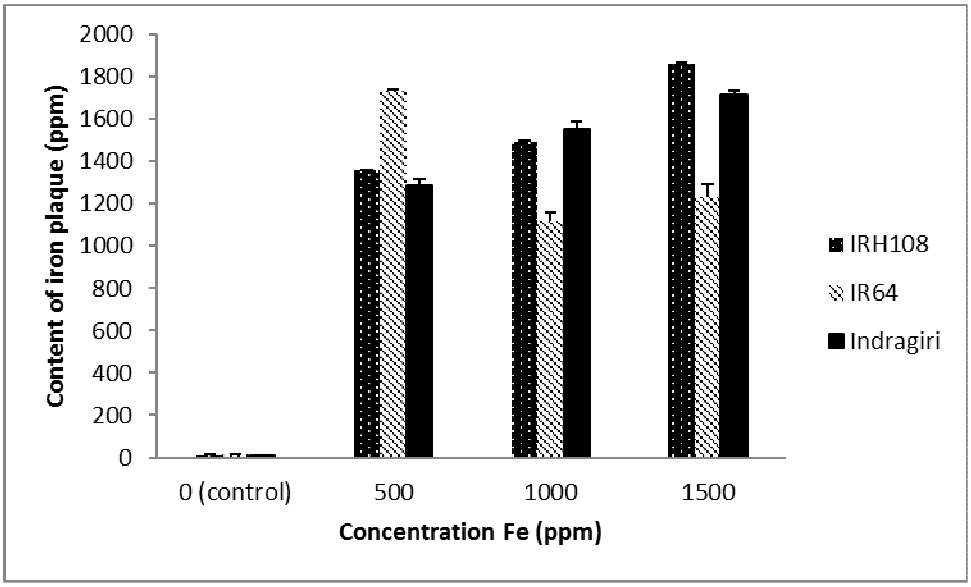
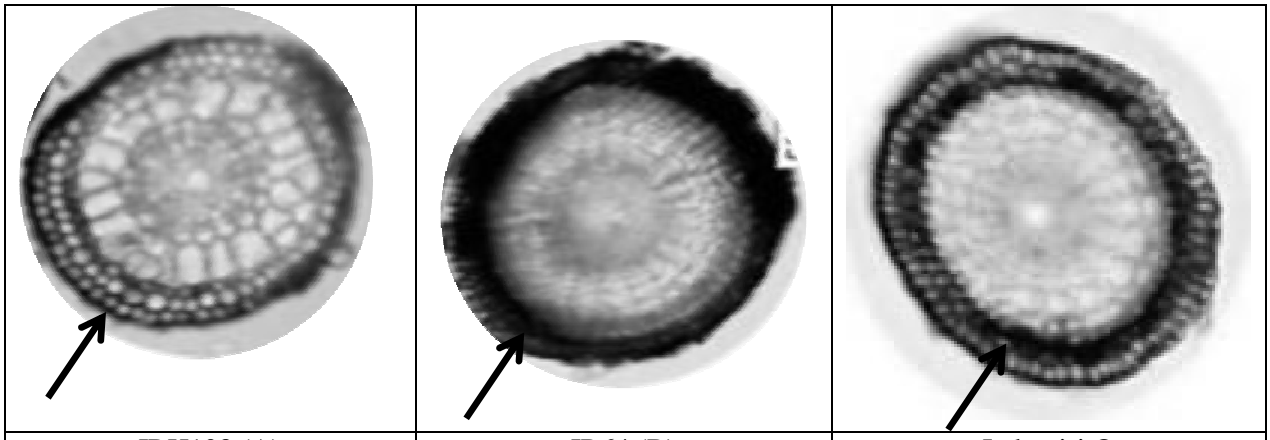


Figure 3. The effect of Fe concentrations on iron plaque content in root of IRH108, IR64 and Indragiri genotype



IRH108 (A)	IR64 (B)	Indragiri ©

Figure 4. Roots cross section to observe Fe^{2+} distribution in Fe concentrations 500 ppm in each genotype (IRH108=A, , IR64 =B and Indragiri=C). The roots tissue were detected containingFe^{2+}ions appear blue and black color. and the arrows shown in the picture is to show that the root tissue contained detectable Fe^{2+}

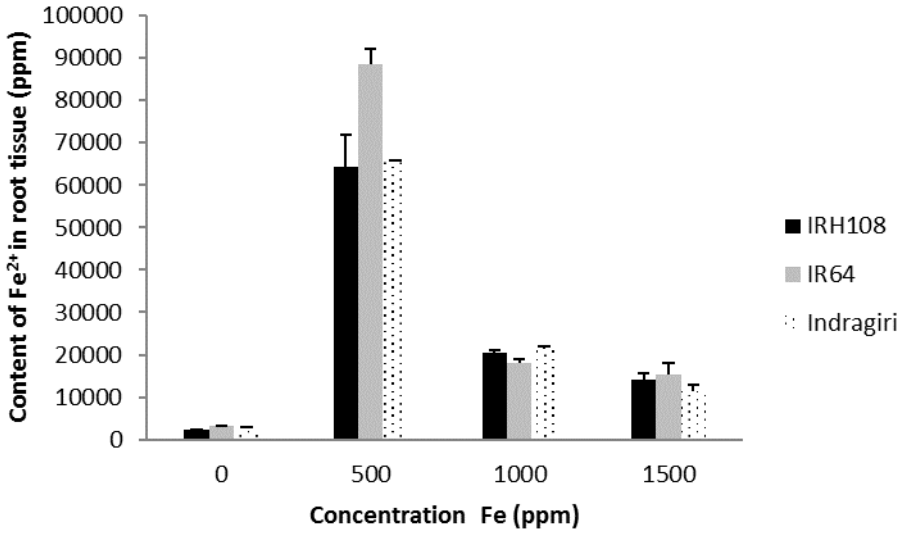


Figure 5. Fe content in root tissue of each genotype (IRH108, IR64andIndragiri) with different concentrations of Fe in the nutrient solution

Table 1. The Ethylene production in root tissue of each genotype (IRH108, IR64 and Indragiri) in difference Fe concentrations on the nutrient solution

Concentration Fe (ppm)	Genotypes	Ethylene production
0 (control)	IRH108	0.94 i
	IR64	1.74 i
	Indragiri	5.06 i
500	IRH108	34.85 g
	IR64	29.53 h
	Indragiri	33.41gh
1000	IRH108	74.98 e
	IR64	50.14 f
	Indragiri	79.97 d
1500	IRH108	105.23 b
	IR64	85.17 c
	Indragiri	116.71 a

Note: Values followed by the same letter in the same column are not significantly different according to the Duncan's Multiple Range Test at *P<5%*

Table 2. The content of iron plaque in root surface and the content of Fe in root tissue of each genotype (IRH108, IR64 and Indragiri) in difference Fe concentrations on the nutrient solution

Concentration Fe (ppm)	Genotypes	Content of iron plaque (ppm)	Content of ion Fe in root tissue
0 (control)	IRH108	11.9 g	2189 f
	IR64	14.07 g	3208 f
	Indragiri	10.07 g	2737 f
500	IRH108	1356.15 def	64332 b
	IR64	1736.53 ab	88475 a
	Indragiri	1289.12 ef	65944 b
1000	IRH108	1489.43 cde	20467 c
	IR64	1118.75 f	17962 cd
	Indragiri	1551.35 bcd	21271 c
1500	IRH108	1864.12 a	13991 de
	IR64	1227.91 f	15410 de
	Indragiri	1717.14 abc	11534 f

Note: Values followed by the same letter in the same column are not significantly different according to the Duncan's Multiple Range Test at $P<5\%$