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The response of East Kalimantan, Indonesia local rice cultivars against iron stress

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Abstract. *Nurhasanah, lestari HS, Sunaryo W. 2019. The response of East Kalimantan, Indonesia local rice cultivars against iron stress. Biodiversitas 20: 273-282.* Iron (Fe) toxicity is one of the most problematic metal elements in acidic soil. Besides being as an essential micronutrient, an excessive iron can cause mineral and nutrients absorption disorder which leads to disruption of plant metabolism and cell development. Reduction of plant growth and yield will be the further consequences of the excessive soil iron content. This study aimed to evaluate the response of East Kalimantan local rice cultivars and to screen rice genotypes tolerant to iron stress. Twenty-five rice genotypes were used in this study, consisted of twenty-three local rice cultivars of East Kalimantan and two control of iron sensitive (IR64) and tolerant (Mekongga) varieties. Uniform sprouts (3 days old) having 1-1.5 cm root length were used for iron stress experiment. The seedlings were grown in nutrient solution using hydroponic system in an aerobic condition. The seedlings were treated for one week in iron stress condition by adding an extra iron source of 100 and 200 ppm $FeSO_4.7H_2O$ (pH 4.0). The seedlings grown in the nutrient solution without an extra iron treatment at normal acidity growth condition (pH 5.8) were used as the control. The growth responses were observed from root, shoot, and biomass of the plants. The tolerance index of the plant growth characters was calculated to classify the rice genotypes into tolerant, moderate, and sensitive to iron stress. The results showed that 100 and 200 ppm of FeSO4.7H2O treatments inhibited the root and shoot growth and also reduced the plant biomass. The plant growth reduction was in parallel with the increase of iron concentration. There was a significant differential response of East Kalimantan local rice genotypes to iron stress treatment. Some genotypes showed an extreme reduction of plant growth, whereas several genotypes had an increased growth under stressed situation. In the contrary, the sensitive genotype IR 64 was consistently sensitive based on the tolerance index of the root, shoot, and plant biomass characters. Among all growth parameters, the most selective character for iron toxicity screening was maximal root length character. This character caused the most severe symptoms for most of the genotypes. Two local rice genotypes, Bentian and Bogor Hitam, were consistently tolerant based on the maximal root growth, total root growth, shoot length and plant biomass.

Keywords: East Kalimantan, local rice, iron stress, iron-tolerant, tolerance index

INTRODUCTION

Soil acidity is a plant growth limiting factor causing significant yield reduction in the crop plants, including rice. Acid soil spreads widely, from lowlands to highlands. Acid soil is generally found in wet climates (high rainfall> 2,000 mm per year) and can be formed from various types of soil materials. Acidic land is characterized by low pH, low cation exchange capacity (CEC), low nutrient content, low organic matter content, and excessive metal content (Sahrawat, 2004; Chérif et al. 2009).

One of the problematic metal elements in the acidic soil is iron (Fe) toxicity. Actually, iron is an essential micronutrient playing a critical role in metabolic processes (Briat et al. 1995; Kim and Guerinot, 2007; Lan et al., 2011). Iron is also a cofactor for several important enzymes (Morrissey and Guerinot, 2009). However, excessive iron can be toxic to plants (Anjum et al. 2015; Tripathi et al. 2015). Iron toxicity will disrupt absorption of essential mineral nutrients in plants, which then further disrupt plant metabolism processes and cell development (Connolly and Guerinot, 2002; Rout and Sahoo, 2015). In severe condition, iron toxicity can cause abnormalities of growth, reduce yields, even crop failure and plant death (Audebert and Sahrawat, 2000; Curie and Briat 2003; Audebert and Fofana, 2009).

Several factors in the soil that can lead to iron poisoning include mineralogy (kaolinite), clay content, amount of soil exchangeable Fe, soil acidity, poor and unbalanced nutrient content, and lack of root oxidation due to the accumulation respiratory-inhibiting materials (Sahrawat and Diatta, 1995; Dobermann and Fairhurst 2000). The critical limit of Fe concentration in the soil causing iron poisoning in rice is around 100 ppm at pH 3.7 and 300 ppm or higher at pH 5.0 (Sahrawat et al. 2000). According to Asch et al. (2005), toxic Fe content in the solution varies widely from 10-500 ppm Fe.

The use of iron-sensitive genotypes will severe the Fe poisoning symptoms in the plants, including rice. The negative effects of Fe poisoning can be reduced by environmental manipulation through water regulation (Yang et al., 2004), balanced fertilization (Ramirez et al., 2002), lime application (Jena et al. 2008), and soil amelioration (Nozoe et al. 2010). However, these treatments are difficult to be implemented as they are high cost, time-consuming and laborious. Therefore, the use of iron tolerant varieties is highly recommended for improving rice productivity in the affected areas.

East Kalimantan has been known as one of rice biodiversity spots in Indonesia having hundreds of local rice genotypes (Nurhasanah et al. 2016; Nurhasanah et al. 2017). Their genetic potency as genes sources for several important rice diseases have been previously reported (Nurhasanah et al. 2018); however, their response to abiotic stress, especially the iron toxicity tolerance, was limitedly known. In addition, several studies proposed that iron toxicity tolerance study might reflect potential impacts in alleviating the stresses of other metal ions in plants (Emamverdian, 2015; Tripathi et al. 2018). Therefore, this study was carried out to evaluate the response of East Kalimantan local upland rice cultivars as an early screening for iron stress tolerance in the seedling stage using a hydroponic system.

MATERIALS AND METHODS

Plant materials

Twenty-three local rice cultivars originated from Kutai Kartenegara District, East Kalimantan were screened for their tolerance to iron toxicity. The local rice cultivars consisted of 17 non-glutinous and six glutinous cultivars. An iron sensitive rice variety IR64 (Nugraha et al. 2016; Mackill and Khush 2018); and tolerant variety Mekongga (Suprihatno et al., 2009; Nurhasanah 2017) were also included in this study.

Seeds germination

The selected rice seeds were sterilized using 10% (v/v) NaOCl for 15 min, and rinsed with distilled water. The seeds were soaked for 24 h in deionized water at room temperature under dark condition. The rice seeds sinking in the water were then germinated on moisture germination paper for three days in room temperature (28-31 ºC) under dark condition.

Iron stress treatment

A uniform sprouts having 1-1.5 cm root length were further used for iron stress experiment. The selected sprouts were grown in nutrient solution (Yoshida, 1976) using raft foam of 5 mm in height to prevent the seedlings from drowning in the hydroponic growth medium. The nutrient solution containing macronutrients ((NH_4NO_3, $NaH_2PO_4.2H_2O$, K_2SO_4, $CaCl_2$, $MgSO_4.7H_2O$) and micronutrients (($(NH_4)_6.Mo_7O_{24}.4H_2O$, MnSO4, H_3BO_3, $ZnSO_4.7H_2O$, $CuSO_4.5H_2O$, $FeSO_4.7H_2O$) in deionized water was placed in plastic culture containers. The solution was maintained at pH 5.8.

The seedlings were then treated in iron stress condition for one week. Iron stress condition was applied by adding an extra iron source compound of 100 and 200 ppm $FeSO_4$.7H2O in the nutrient solutions at pH 4.0. In the control treatment, the seedlings were grown in the nutrient solution without addition of extra iron (the nutrient solution contains iron according to the normal iron requirement concentration for rice seedlings in Yoshida solution) at normal acidity growth condition at pH 5.8. The pH of all experiments was constantly maintained daily. The nutrient solution in all experiments was not refreshed with the new nutrient until the last experiment. All experiment units were placed in a growth chamber with 16 h photoperiod in room temperature (28-31 ºC). The experiment was repeated six times. The experiment was conducted in aerated condition.

Plant growth observation

Several root and shoot growth characters were observed to evaluate the response of the rice seedling against iron stress treatment. The shoot length (SL) was measured from the base of the shoot to the highest leaf tip. The maximal root length (MRL) was measured as the longest root growth (from the tip of root to the root base). The total root growth (TRG) was the sum of all seminal root length. Root number was counted for all seminal roots in the rice seedlings. Plant fresh and dry biomass were also observed.

Data analysis

Observed data of root, shoot and plant biomass were used to calculate tolerance index (TI) (Rout et al. 2014). The TI was calculated by dividing the growth in stress condition with the growth in normal condition. The TI value then was used to classify the tolerant level of the tested rice genotypes. Pearson correlation was used to analyze the correlation between MRL and TRG parameters.

The percentage of root and shoot growth reduction was calculated using the formula (Richard et al. 2015):

Growth reduction % = ((Root or shoot growth in stress condition - Root or shoot growth in normal condition) / Root or shoot growth in normal condition) X 100%

RESULTS AND DISCUSSION

Root growth

In a normal growth condition, the root growth of the rice genotypes in this study differed since they represented the different genetic diversity. Several genotypes had short root length either the maximal length or total root growth as observed in Bogor Hitam, Buyung and Ketalun Tawar, and others had longer root length as in Jala Mengo, Ketan Lekatan and ketan Linjuang cultivars (Table 1). In iron stress condition, the root growth of most of genotypes was inhibited in an addition of 100 and 200 ppm of $FeSO_4$.7H2O. The maximal root length (MRL) and the total root growth (TRG) were decreased compared to the control growth condition (Table 1). It means that iron concentration of 100 ppm and 200 ppm of FeSO4.7H2O at pH 4.00 has been able to inhibit the growth of all rice genotype. According to Sahrawat et al (2000) Fe concentration at 100 ppm could already be a toxic concentration to the rice plants. Several toxic iron concentrations were reported earlier showing that higher iron concentration increased the toxic effect to the plants (Asch et al 2005). Mild to severe iron toxicity symptom was observed in the presence of around 50 to 300 ppm of iron in rice seedling (Noor et al 2012). In addition, the concentration of Fe in the soil which causes toxicity varies depending on the acidity of the soil solution (Sahrawat 2004).

The root growth reduction varied among the rice genotypes at different extra iron concentrations. Generally, the percentage of root growth reduction was higher when the iron stress concentration increased from 100 to 200 ppm either based on the MRL (Figure 1) or TRG (Figure 2) parameters. The highest root growth reduction in the MRL was -31.33 % in the 100 ppm and -38.52 % in the 200 ppm of $FeSO_4.7H2O$, observed in the control of sensitive genotype IR64. The same result was also noticed in the TRG parameter, in which the root growth of genotype IR 64 decreased up to -38.30 and -39.38% in the 100 and 200 ppm $FeSO_4.7H2O$, respectively. Root growth significantly interferes in the presence of metal ions. Therefore to quantify the inhibitory effect of iron or other metal ions, root growth was widely used to evaluate the response of the plants to that abiotic stress condition (Rout et al. 2014). Plants with iron poisoning have a small, rough, short and blunt root (Sahrawat 2004). However, several genotypes showed an opposite phenomenon, in which the root growth was increased in the toxic iron condition. These genotypes might have tolerant character against iron stress.

Table 1. Mean value of Maximal Root Length and Total Root Growth of the rice genotypes at a different $FeSO_4.7H2O$ concentration

Id	Geno-type	Rice type	Maximal root length (cm)			Total root growth (cm)		
			0 ppm	100 ppm	200 ppm	0 ppm	100 ppm	200 ppm
V1	IR 64	Non-glutinous	9.00	6.18	5.53	12.97	8.00	7.86
V2	Mekongga	Non-glutinous	8.85	6.55	6.27	13.63	13.60	13.40
V3	Jala Mengo	Non-glutinous	14.30	11.08	10.82	25.08	18.33	18.38
V4	Mayas	Non-glutinous	9.68	8.52	7.55	10.85	12.62	13.25
V5	Kawit	Non-glutinous	8.72	7.15	6.95	13.20	11.42	9.42
V6	Awang	Non-glutinous	9.60	9.54	10.57	16.55	13.98	12.07
V7	Bentian	Non-glutinous	9.88	9.13	9.00	13.67	17.02	14.43
V8	Ritam	Non-glutinous	10.95	8.18	8.15	12.88	10.77	11.28
V9	Bogor Hitam	Non-glutinous	6.47	8.03	8.60	8.22	11.22	10.20
V10	Bogor Putih	Non-glutinous	8.75	10.08	7.38	10.83	11.97	11.92
V11	Sungkai	Non-glutinous	8.80	7.30	6.95	10.30	9.67	14.62
V12	Tumiyang	Non-glutinous	7.78	5.62	5.22	18.30	13.68	13.60
V13	Melak	Non-glutinous	10.37	9.27	8.02	12.28	11.45	10.90
V14	Bogor	Non-glutinous	9.37	9.02	7.57	16.73	12.92	11.45
V15	Mayas Kuning	Non-glutinous	10.35	8.25	7.03	14.67	14.83	11.98
V16	Mayas Putih	Non-glutinous	11.62	10.65	8.43	14.98	18.70	12.37
V17	Buyung	Non-glutinous	7.97	9.08	8.83	9.78	11.73	10.40
V18	Serai Gunung	Non-glutinous	9.82	7.30	6.27	11.53	9.70	11.07
V19	Ketalun Tawar	Non-glutinous	7.67	9.57	7.65	7.67	10.35	10.82
V20	Ketan Putih	Glutinous	11.88	9.67	9.58	18.42	20.12	18.05
V21	Ketan Huan	Glutinous	7.75	6.72	5.70	10.83	10.37	12.57
V22	Ketan Lekatan	Glutinous	13.18	11.65	11.40	15.38	13.87	13.17
V23	Ketan Putek Iting	Glutinous	12.13	8.97	10.78	18.38	14.05	12.22
V24	Ketan Mayang	Glutinous	10.15	8.47	8.45	13.65	11.62	11.40
V25	Ketan Linjuang	Glutinous	13.90	11.98	11.93	20.18	17.87	16.95

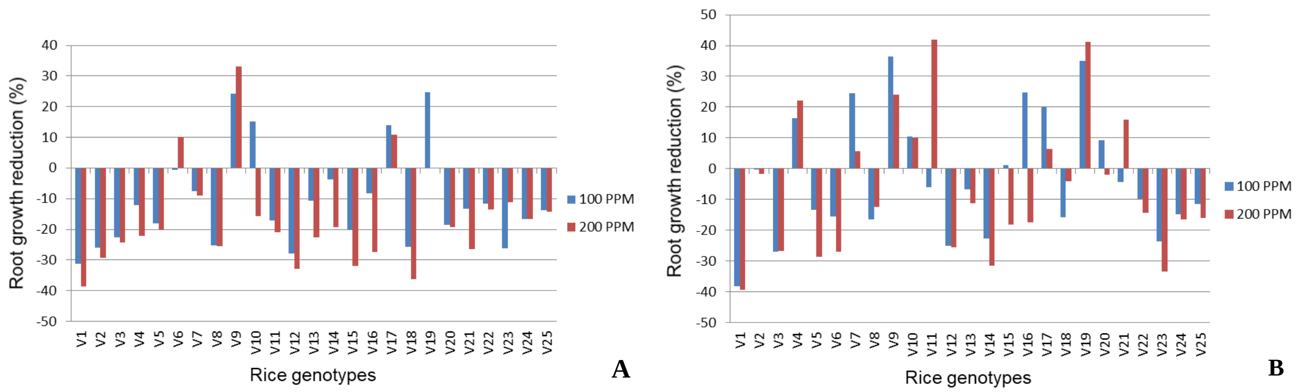

Figure 1. Percentage of the root growth reduction or accretion of the rice genotypes at different iron stress concentration. A. Maximal Root Length; B. Total Root Growth. V1 (IR 64), V2 (Mekongga), V3 (Jala Mengo), V4 (Mayas), V5 (Kawit), V6 (Awang), V7 (Bentian), V8 (Ritam), V9 (Bogor Hitam), V10 (Bogor Putih), V11 (Sungkai), V12 (Tumiyang), V13 (Melak), V14 (Bogor), V15 (Mayas Kuning), V16 (Mayas Putih), V17 (Buyung), V18 (Serai Gunung), V19 (Ketalun Tawar), V20 (Ketan Putih), V21 (Ketan Huan), V22 (Ketan Lekatan), V23 (Ketan Putek Iting), V24 (Ketan Mayang), V25 (Ketan Linjuang)

Root Tolerance Index (RTI)

Root tolerance index (RTI) is one of the most important markers to screen genotypes and varieties for metal tolerance (Wu et al. 1997; Famoso et al. 2010), including iron (Rout et al. 2014). Tolerance index is considered very useful to characterize tolerant genotypes by comparing the growth data of different treatments and the control. It shows the level of growth reduction or accretion in the stressed condition compared to control/normal growth, meaning that the lower the tolerance index value, the more severe the plant growth as a result of the plant stress response, vice versa.

The RTI of the MRL ranged from 0.69 to 1.25 in 100 ppm $FeSO_4.7H2O$, and between 0.61 and 1.33 in the presence of 200 ppm of $FeSO_4.7H2O$ (Table 2). The tolerance level was grouped based on the RTI value, in which the genotypes with RTI value lower than 0.7 were categorized as sensitive, larger than 0.9 as tolerant, and in between 0.7-0.9 are moderately tolerant (Wu et al. 1997). All of the local rice genotypes were grouped as moderately tolerant or tolerant at iron stress concentration of 100 ppm $FeSO_4.7H2O$, while only two local rice genotypes "Mayas Kuning" and "Serai Gunung" were sensitive at 200 ppm of $FeSO_4.7H2O$. Among eight genotypes tolerant to 100 ppm $FeSO_4.7H2O$, only five local rice genotypes were constantly tolerant in the 200 ppm of the iron source. The iron-sensitive control genotype IR64 remained susceptible to iron in both of iron-stressed levels. On the other hand, Mekongga variety which was described as abiotic tolerant genotype, such as aluminum (Sari et al. 2013) and iron (Nurhasanah, 2017), was grouped as moderately tolerant to iron stress based on the maximal root length character.

A larger range of RTI value was observed when the tolerance index was applied for the TRG character (Table 3). There was a different tolerance level of several genotypes using RTI value from different character of MRL or TRG. For example, the moderately tolerant level shifted to tolerant as observed in Mekongga variety. In another situation, "Awang" cultivar which was tolerant based on the RTI value of MRL became moderate using the TRG parameter. Even, a drastically alteration from sensitive to tolerant category occurred in "Serai Gunung" cultivar. The different tolerance level of the genotypes, when screened using the MRL or TRG characters, might be due to the different response of the root growth in dealing with iron stress condition. Both characters might correlate with the plant's tolerance mechanisms against iron stress. However, an inconsistent correlation between MRL and TRG was obtained in different stress concentration. A quite high coefficient of correlation (r= 0.72) was obtained when the plants were subjected iron stress in 100 ppm $FeSO_4.7H2O$. On the contrary, the correlation was low (r= 0.33) in the higher iron concentration of 200 ppm of $FeSO_4.7H2O$. A low correlation between the longest and the total root growth (r2=0.172) was also observed in the previous study for aluminum tolerance phenotyping in cereals (Famoso et al. 2010).

More genotypes were classified as tolerant using the RTI value of TRG parameter compared to MRL, either in 100 ppm or 200 ppm of $FeSO_4.7H2O$ (Table 4). This seems to be related to the number of seminal roots, since the TRG is measured by the sum of lengths of all seminal roots in the root system of rice seedlings. Almost all of the rice genotypes had more root number in the iron stress treatment as compared to the control (Figure 2). Iron stress might induce the seminal root growth as a defense mechanism in dealing with the stress. Therefore, all of the tolerant genotypes according to the TRG parameter produced considerably higher number of seminal roots than the sensitive genotypes under iron stressed condition (Figure 2). The sensitive genotypes "Bogor" and "Ketan Putek Iting" based on the RTI of TRG produced lower number of seminal roots in iron stressed condition as compared to that in the normal growth condition (Figure 2), thus had an extremely low total root growth (Table 1), and greatly total root growth reduction (Figure 1B). In contrast to those two genotypes, "Serai Gunung" which was sensitive based on the MRL was grouped as tolerant based on the TRG parameter (Table 2, Table 3). This genotype produced more root number in the iron stress treatment with an average root number 1.67, 2.00 and 3.00 in 0 ppm, 100 ppm and 200 ppm $FeSO_4.7H2O$, respectively. Therefore the total root growth was higher (Table 1, Figure 1B), even though the plant had short maximal root length (Table 1, Figure 1A). The control of sensitive genotypes "IR64" remained sensitive based on the TRG value, since this genotype has not tolerance mechanisms against iron stress condition, the plant failed to form or extend the seminal roots under stress conditions.

Table 2. The iron tolerance level of the rice genotypes at different iron stress concentration based on the RTI value of the MRL

Id	Genotype	100 ppm $FeSO_4.7H2O$		200 ppm $FeSO_4.7H2O$	
		RTI	Category	RTI	Category
V1	IR 64	0.69	Sensitive	0.61	Sensitive
V2	Mekongga	0.74	Moderate	0.71	Moderate
V3	Jala Mengo	0.78	Moderate	0.76	Moderate
V4	Mayas	0.88	Moderate	0.78	Moderate
V5	Kawit	0.82	Moderate	0.80	Moderate
V6	Awang	0.99	Tolerant	1.10	Tolerant
V7	Bentian	0.92	Tolerant	0.91	Tolerant
V8	Ritam	0.75	Moderate	0.74	Moderate
V9	Bogor Hitam	1.24	Tolerant	1.33	Tolerant
V10	Bogor Putih	1.15	Tolerant	0.84	Moderate
V11	Sungkai	0.83	Moderate	0.79	Moderate
V12	Tumiyang	0.72	Moderate	0.71	Moderate
V13	Melak	0.89	Moderate	0.77	Moderate
V14	Bogor	0.96	Tolerant	0.81	Moderate
V15	Mayas Kuning	0.80	Moderate	0.68	Sensitive
V16	Mayas Putih	0.92	Tolerant	0.73	Moderate
V17	Buyung	1.14	Tolerant	1.11	Tolerant
V18	Serai Gunung	0.74	Moderate	0.64	Sensitive
V19	Ketalun Tawar	1.25	Tolerant	1.00	Tolerant
V20	Ketan Putih	0.81	Moderate	0.81	Moderate
V21	Ketan Huan	0.87	Moderate	0.74	Moderate
V22	Ketan Lekatan	0.88	Moderate	0.86	Moderate
V23	Ketan Putek Iting	0.74	Moderate	0.89	Moderate
V24	Ketan Mayang	0.83	Moderate	0.83	Moderate
V25	Ketan Linjuang	0.86	Moderate	0.86	Moderate

Table 3. Iron tolerance level of the rice genotypes at different iron stress concentration based on the RTI value of the TRG

Id	Genotype	100 ppm $FeSO_4.7H2O$		200 ppm $FeSO_4.7H2O$	
		RTI	Category	RTI	Category
V1	IR 64	0.62	Sensitive	0.61	Sensitive
V2	Mekongga	1.00	Tolerant	0.98	Tolerant
V3	Jala Mengo	0.73	Moderate	0.73	Moderate
V4	Mayas	1.16	Tolerant	1.22	Tolerant
V5	Kawit	0.86	Moderate	0.71	Moderate
V6	Awang	0.84	Moderate	0.73	Moderate
V7	Bentian	1.25	Tolerant	1.06	Tolerant
V8	Ritam	0.84	Moderate	0.88	Moderate
V9	Bogor Hitam	1.37	Tolerant	1.24	Tolerant
V10	Bogor Putih	1.10	Tolerant	1.10	Tolerant
V11	Sungkai	0.94	Tolerant	1.42	Tolerant
V12	Tumiyang	0.75	Moderate	0.74	Moderate
V13	Melak	0.93	Tolerant	0.89	Moderate
V14	Bogor	0.77	Moderate	0.68	Sensitive
V15	Mayas Kuning	1.01	Tolerant	0.82	Moderate
V16	Mayas Putih	1.25	Tolerant	0.83	Moderate
V17	Buyung	1.20	Tolerant	1.06	Tolerant
V18	Serai Gunung	0.94	Tolerant	1.02	Tolerant
V19	Ketalun Tawar	1.35	Tolerant	1.41	Tolerant
V20	Ketan Putih	1.09	Tolerant	0.98	Tolerant
V21	Ketan Huan	0.96	Tolerant	1.16	Tolerant
V22	Ketan Lekatan	0.90	Tolerant	0.82	Moderate
V23	Ketan Putek Iting	0.76	Moderate	0.66	Sensitive
V24	Ketan Mayang	0.85	Moderate	0.84	Moderate
V25	Ketan Linjuang	0.89	Moderate	0.84	Moderate

Table 4. The tolerant genotypes based on the total root growth and maximal root length at different iron concentration.

Id.	Total Root Growth (TRG)		Maximal root length (MRL)	
	100 ppm	200 ppm	100 ppm	200 ppm
V2	Mekongga	Mekongga	-	-
V4	Mayas	Mayas	-	-
V6	-	-	Awang	Awang
V7	Bentian	Bentian	Bentian	Bentian
V9	Bogor Hitam	Bogor Hitam	Bogor Hitam	Bogor Hitam
V10	Bogor Putih	Bogor Putih	Bogor Putih	-
V11	Sungkai	Sungkai	-	-
V13	Melak	-	-	-
V14	-	-	Bogor	-
V15	Mayas Kuning	-	-	-
V16	Mayas Putih	-	Mayas Putih	-
V17	Buyung	-	Buyung	Buyung
V18	Serai Gunung	-	-	-
V19	Ketalun Tawar	-	-	-
V20	Ketan Putih	Ketan Putih	-	-
V21	Ketan Huan	Ketan Huan	-	-
V22	Ketan Lekatan	-	-	-

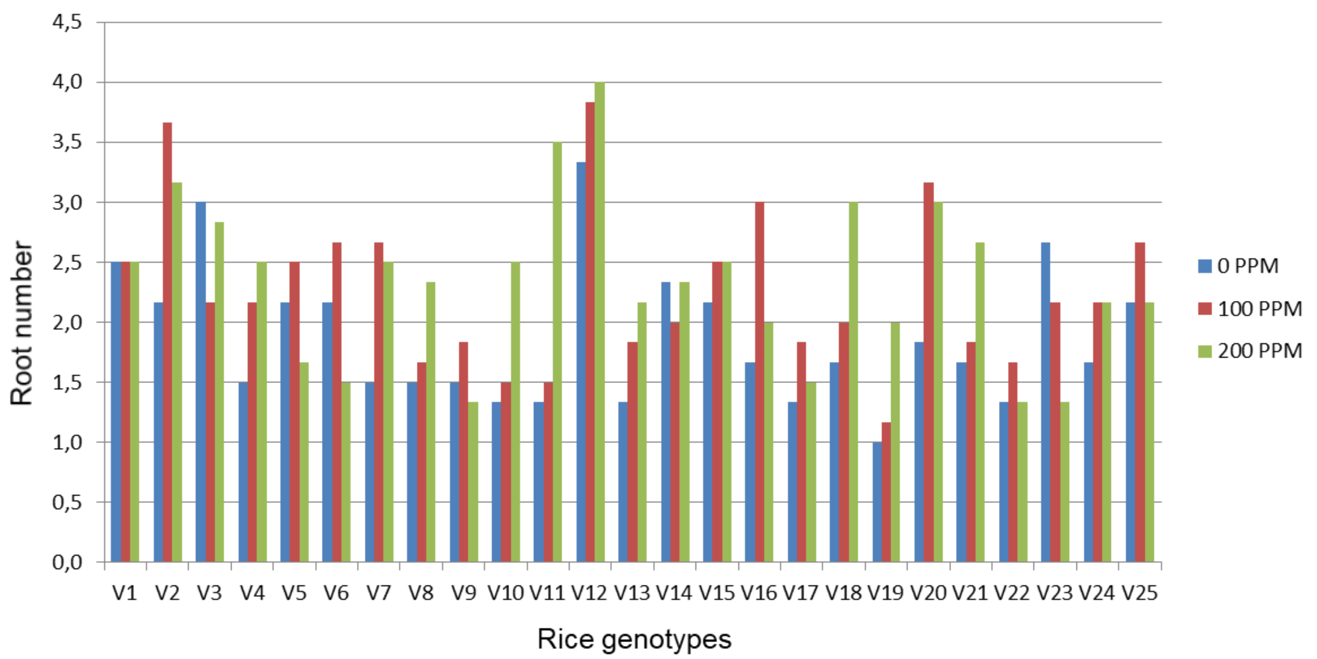

Figure 2. Root number of the rice genotypes at different iron stress concentration. V1 (IR 64), V2 (Mekongga), V3 (Jala Mengo), V4 (Mayas), V5 (Kawit), V6 (Awang), V7 (Bentian), V8 (Ritam), V9 (Bogor Hitam), V10 (Bogor Putih), V11 (Sungkai), V12 (Tumiyang), V13 (Melak), V14 (Bogor), V15 (Mayas Kuning), V16 (Mayas Putih), V17 (Buyung), V18 (Serai Gunung), V19 (Ketalun Tawar), V20 (Ketan Putih), V21 (Ketan Huan), V22 (Ketan Lekatan), V23 (Ketan Putek Iting), V24 (Ketan Mayang), V25 (Ketan Linjuang).

Shoot growth analysis

The shoot growth of the rice genotypes varied, showing the genetic diversity of the rice genotypes. In normal growth condition, the shoot length of Ketan Huan cultivar was observed as the shortest and Ritam cultivar was the highest among all rice genotypes (Table 5). In iron stress condition, the shoot length of the local rice genotypes was reduced for most of genotypes. However, in particular genotypes the shoot length was increased in the stress situation as observed in Ketan Huan, Sungkai, and Bentian cultivars (Table 5). The shoot length reduction percentage was in line with the iron stress concentration. The higher iron stress concentration, the higher the shoot length reduction (Figure 3). The highest shoot length reduction was -24.83% and -36.65% in 100 ppm and 200 ppm of $FeSO_4$.7H2O, respectively. Nevertheless, the reduction percentage was considered lower than that of the root growth (Figure 1). The shoot growth seems to be less affected by iron poisoning condition compared to the root growth.

Several studies reported the association between iron toxicity and plant height reduction (Asch et al. 2005; Majerus et al. 2007, Fageria et al. 2008). The stunted shoot growth is resulted from the mineral and nutrient deficiency (Rout et al 2014) due to the ‘iron plaque’ coated the root surface (Sahrawat 2004; Chen et al. 2006; Elec et al. 2013) disrupting their absorption by the plant. This condition will cause multiple nutritional disorders interfering plant metabolism processes (Rout and Sahoo, 2015) and further affected plant growth (Dorlodot et al. 2005; Majerus et al. 2007), or death of the plant (Chérif et al., 2009). In this case, the shoot growth disruption can be stated as the secondary effect from the iron toxicity inhibiting the root growth. Therefore, the shoot growth disorders were not as severe as the root growth disturbance due to iron poisoning.

If we calculate the tolerance index (TI) based on the shoot growth, in this study we used shoot length, the tolerance index will range from 0.75 to 1.24 and from 0.63 to 1.33 in 100 ppm and 200 ppm $FeSO_4$.7H2O, respectively (Table 6); in which none of the genotypes was sensitive except for IR64 variety at the 200 ppm of $FeSO_4$.7H2O. Based on this, twenty from twenty-three of the local rice genotypes were grouped as tolerant in 100 ppm, and fifteen of them remain tolerant in the higher iron toxicity concentration. It means that the iron concentration of 100 ppm of $FeSO_4$.7H2O was not high enough for selecting the sensitive genotype based on the shoot length; a higher iron poisoning concentration is needed. Other morphological symptoms of iron toxicity on the leaf such as the presence of purplish-brown spots followed by leaves drying or leaf bronzing (Yamauchi dan Peng 1993; Becker and Asch 2005) were not present in this study. A higher iron concentration or longer stress duration might be needed for observing such condition in rice seedlings of the genotypes used in this study. The leaf-bronzing symptom on affected plant depends on the intensity and the duration of the Fe stress and genotype-specific tolerance mechanisms (Becker and Asch 2005). In other studies, growth reduction might present without significant leaf bronzing (Onaga et al. 2012; Sikirou et al 2016). There is also no clear information between Fe concentrations in the plant with the leaf symptoms (Elec et al. 2013).

Plant biomass analysis

Iron stress condition influences biomass accumulation on the plants (Sahrawat 2004; Quinnet et al., 2012). However, according to Sikirou et al. (2015), shoot length and plant biomass were included as secondary traits for abiotic stress tolerance indirect selection. In this study, iron stress treatment also resulted in the reduction of fresh and dry biomass of the plants (Table 7) for most of the rice genotypes. The level of biomass reduction varied depending on the rice genotypes (Figure 4). Several genotypes showed a reduction of biomass especially in 200 ppm of $FeSO_4$.7H2O as observed in the sensitive control IR64, in which the plant biomass remarkably reduced up to more than 50%. Whereas other genotypes had a higher biomass accumulation in the iron stress condition, as observed in Bogor, Sungkai, Tumiyang, and Melak cultivars. Those genotypes were grouped as tolerant and moderately tolerant genotype based on the root tolerance analysis. The fresh weight tended to be more interfered by the iron toxic condition compared to the dry weight (Figure 4).

Table 5. Shoot length of the rice genotypes at a different FeSO4.7H2O concentration

Id	Genotype	Rice type	Shoot Length (cm)		
			0 ppm	100 ppm	200 ppm
V1	IR 64	Non-glutinous	13.05	11.40	8.27
V2	Mekongga	Non-glutinous	16.17	14.57	14.30
V3	Jala Mengo	Non-glutinous	15.68	15.85	15.02
V4	Mayas	Non-glutinous	13.17	15.80	12.47
V5	Kawit	Non-glutinous	16.02	14.72	14.67
V6	Awang	Non-glutinous	16.67	16.12	15.83
V7	Bentian	Non-glutinous	17.17	17.40	20.63
V8	Ritam	Non-glutinous	19.95	15.70	15.62
V9	Bogor Hitam	Non-glutinous	17.92	17.67	16.20
V10	Bogor Putih	Non-glutinous	18.23	17.93	17.67
V11	Sungkai	Non-glutinous	11.05	12.37	11.22
V12	Tumiyang	Non-glutinous	16.15	18.08	15.88
V13	Melak	Non-glutinous	17.97	18.02	17.97
V14	Bogor	Non-glutinous	13.22	12.93	12.57
V15	Mayas Kuning	Non-glutinous	14.00	12.92	12.70
V16	Mayas Putih	Non-glutinous	16.82	15.82	13.65
V17	Buyung	Non-glutinous	14.97	11.25	12.72
V18	Serai Gunung	Non-glutinous	13.87	14.93	13.50
V19	Ketalun Tawar	Non-glutinous	12.83	11.77	13.05
V20	Ketan Putih	Glutinous	16.65	14.22	14.88
V21	Ketan Huan	Glutinous	9.87	9.95	13.12
V22	Ketan Lekatan	Glutinous	16.95	21.05	18.07
V23	Ketan Putek Iting	Glutinous	15.88	15.95	12.82
V24	Pulut Mayang	Glutinous	13.12	13.52	11.78
V25	Pulut Linjuang	Glutinous	13.47	12.70	11.58

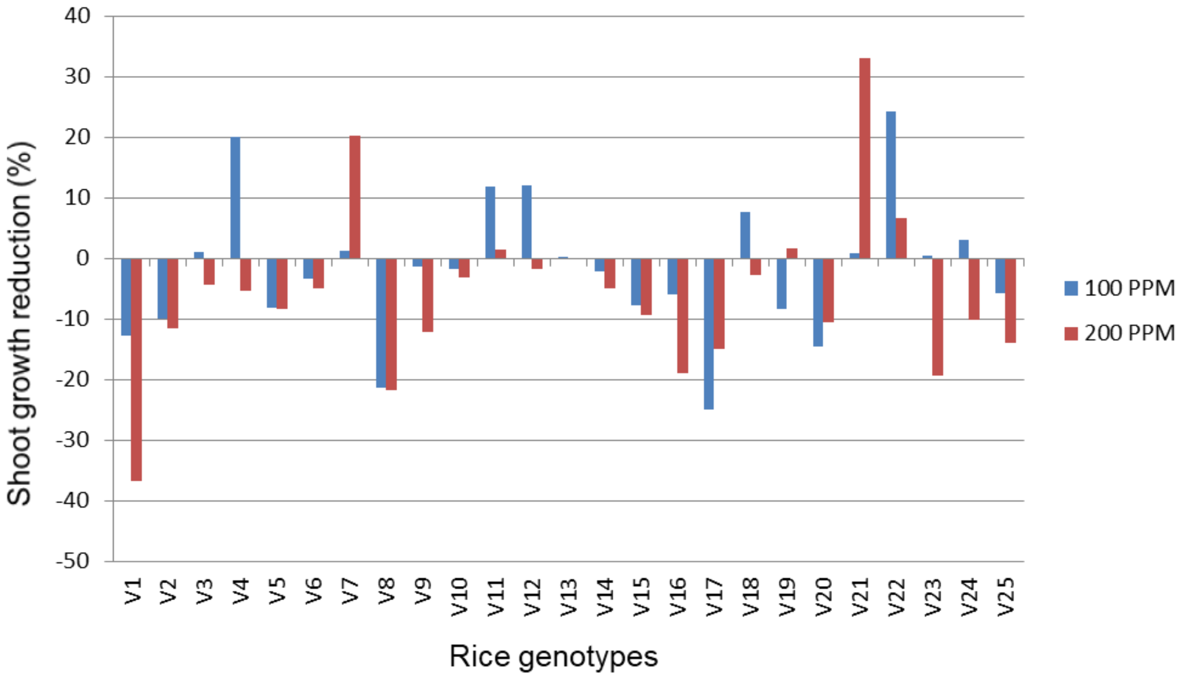

Figure 3. Percentage of the shoot growth reduction or accretion of the rice genotypes at different iron stress concentration. V1 (IR 64), V2 (Mekongga), V3 (Jala Mengo), V4 (Mayas), V5 (Kawit), V6 (Awang), V7 (Bentian), V8 (Ritam), V9 (Bogor Hitam), V10 (Bogor Putih), V11 (Sungkai), V12 (Tumiyang), V13 (Melak), V14 (Bogor), V15 (Mayas Kuning), V16 (Mayas Putih), V17 (Buyung), V18 (Serai Gunung), V19 (Ketalun Tawar), V20 (Ketan Putih), V21 (Ketan Huan), V22 (Ketan Lekatan), V23 (Ketan Putek Iting), V24 (Ketan Mayang), V25 (Ketan Linjuang).

Table 6. The tolerance level of the rice genotypes at different iron stress concentration based on the shoot length (SL)

Id	Genotype	100 ppm $FeSO_4.7H2O$		200 ppm $FeSO_4.7H2O$	
		TI	Category	TI	Category
V1	IR 64	0.87	Moderate	0.63	Sensitive
V2	Mekongga	0.90	Tolerant	0.88	Moderate
V3	Jala Mengo	1.01	Tolerant	0.96	Tolerant
V4	Mayas	1.20	Tolerant	0.95	Tolerant
V5	Kawit	0.92	Tolerant	0.92	Tolerant
V6	Awang	0.97	Tolerant	0.95	Tolerant
V7	Bentian	1.01	Tolerant	1.20	Tolerant
V8	Ritam	0.79	Moderate	0.78	Moderate
V9	Bogor Hitam	0.99	Tolerant	0.90	Tolerant
V10	Bogor Putih	0.98	Tolerant	0.97	Tolerant
V11	Sungkai	1.12	Tolerant	1.02	Tolerant
V12	Tumiyang	1.12	Tolerant	0.98	Tolerant
V13	Melak	1.00	Tolerant	1.00	Tolerant
V14	Bogor	0.98	Tolerant	0.95	Tolerant
V15	Mayas Kuning	0.92	Tolerant	0.91	Tolerant
V16	Mayas Putih	0.94	Tolerant	0.81	Moderate
V17	Buyung	0.75	Moderate	0.85	Moderate
V18	Serai Gunung	1.08	Tolerant	0.97	Tolerant
V19	Ketalun Tawar	0.92	Tolerant	1.02	Tolerant
V20	Ketan Putih	0.85	Moderate	0.89	Moderate
V21	Ketan Huan	1.01	Tolerant	1.33	Tolerant
V22	Ketan Lekatan	1.24	Tolerant	1.07	Tolerant
V23	Ketan Putek Iting	1.00	Tolerant	0.81	Moderate
V24	Pulut Mayang	1.03	Tolerant	0.90	Tolerant
V25	Pulut Linjuang	0.94	Tolerant	0.86	Moderate

The tolerance index (TI) of fresh biomass showed the inconsistent result, in which two local rice genotypes, Ketan Putih and Ketan Huan which were sensitive in 100 ppm became tolerant in the higher iron concentration of 200 ppm (Table 8). On the other hand, in dry biomass character, almost all of the rice genotypes were tolerant to the iron stress treatment (Table 9). It was suspected that these characters were less sensitive to screen the tolerance level of the plants against iron stress, as observed in the shoot length character. The reason might be because the shoot growth and biomass accumulation are the secondary effects resulted from the root growth disorder (Sahrawat et al 2004; Dorlodot et al. 2005). Therefore a higher or longer term of iron poisoning treatment should be done to use these characters as selection criteria in evaluating plant response against the stress (Mehraban et al. 2008; Rout et al 2014).

In conclusion, iron concentration of 100 and 200 ppm of $FeSO_4.7H2O$ inhibited the growth of root, shoot, and biomass of rice seedling. The effect was increasing in line with the increase of iron concentration. The response of each East Kalimantan local rice genotypes varied in facing iron stress condition, showing a different level of tolerance. Shoot length and plant biomass were less disturbed by the iron toxicity treatment of 100 and 200 ppm of $FeSO_4.7H2O$ for a week. Among growth parameters, MRL and TRG can be used for early screening of the tolerant genotypes in the seedling stage. The tolerance index values of the TRG selected more genotypes which were tolerant compared to the MRL. The iron stress condition might induce the seminal root growth as a defense mechanism in tolerant genotypes thus increasing the total root number and total root growth. However, the most selective character for iron toxicity screening causing more severe symptoms for most of the genotypes was MRL character, compared to the total root growth. Most all of the tolerant genotypes based on the tolerance index of the MRL were also tolerant based on the TRG. Two local rice genotypes, Bentian and Bogor Hitam, were consistently tolerant based on the maximal root growth, total root growth, shoot length, and plant biomass.

Table 7. Mean value of fresh and dry biomass of the rice genotypes at different iron stress concentration

Id	Genotype	Rice type	Fresh biomass (g)			Dry biomass (g)		
			0 ppm	100 ppm	200 ppm	0 ppm	100 ppm	200 ppm
V1	IR 64	Non-glutinous	0.0523	0.0352	0.0234	0.0097	0.0085	0.0047
V2	Mekongga	Non-glutinous	0.0762	0.0678	0.0703	0.0121	0.0114	0.0111
V3	Jala Mengo	Non-glutinous	0.0786	0.0773	0.0795	0.0133	0.0144	0.0143
V4	Mayas	Non-glutinous	0.0364	0.0371	0.0379	0.0066	0.0086	0.0093
V5	Kawit	Non-glutinous	0.0521	0.0488	0.0458	0.0083	0.0081	0.0076
V6	Awang	Non-glutinous	0.0719	0.0765	0.0787	0.0114	0.0110	0.0124
V7	Bentian	Non-glutinous	0.0598	0.0663	0.0790	0.0103	0.0123	0.0124
V8	Ritam	Non-glutinous	0.0620	0.0483	0.0540	0.0096	0.0097	0.0087
V9	Bogor Hitam	Non-glutinous	0.0536	0.0692	0.0528	0.0083	0.0120	0.0093
V10	Bogor Putih	Non-glutinous	0.0532	0.0722	0.0633	0.0098	0.0119	0.0115
V11	Sungkai	Non-glutinous	0.0448	0.0543	0.0510	0.0074	0.0096	0.0090
V12	Tumiyang	Non-glutinous	0.0767	0.1062	0.0819	0.0106	0.0160	0.0145
V13	Melak	Non-glutinous	0.0601	0.0619	0.0705	0.0101	0.0109	0.0133
V14	Bogor	Non-glutinous	0.0603	0.0538	0.0500	0.0091	0.0098	0.0096
V15	Mayas Kuning	Non-glutinous	0.0425	0.0451	0.0410	0.0080	0.0096	0.0090
V16	Mayas Putih	Non-glutinous	0.0572	0.0663	0.0505	0.0084	0.0113	0.0092
V17	Buyung	Non-glutinous	0.0527	0.0480	0.0523	0.0077	0.0073	0.0092
V18	Serai Gunung	Non-glutinous	0.0625	0.0626	0.0488	0.0090	0.0092	0.0082
V19	Ketalun Tawar	Non-glutinous	0.0405	0.0426	0.0526	0.0070	0.0071	0.0091
V20	Ketan Putih	Glutinous	0.0822	0.0565	0.0799	0.0109	0.0101	0.0117
V21	Ketan Huan	Glutinous	0.0404	0.0280	0.0455	0.0056	0.0043	0.0089
V22	Ketan Lekatan	Glutinous	0.0615	0.0895	0.0905	0.0145	0.0148	0.0145
V23	Ketan Putek Iting	Glutinous	0.0637	0.0700	0.0590	0.0101	0.0109	0.0098
V24	Ketan Mayang	Glutinous	0.0677	0.0710	0.0933	0.0110	0.0118	0.0121
V25	Ketan Linjuang	Glutinous	0.0935	0.0847	0.0604	0.0146	0.0140	0.0122

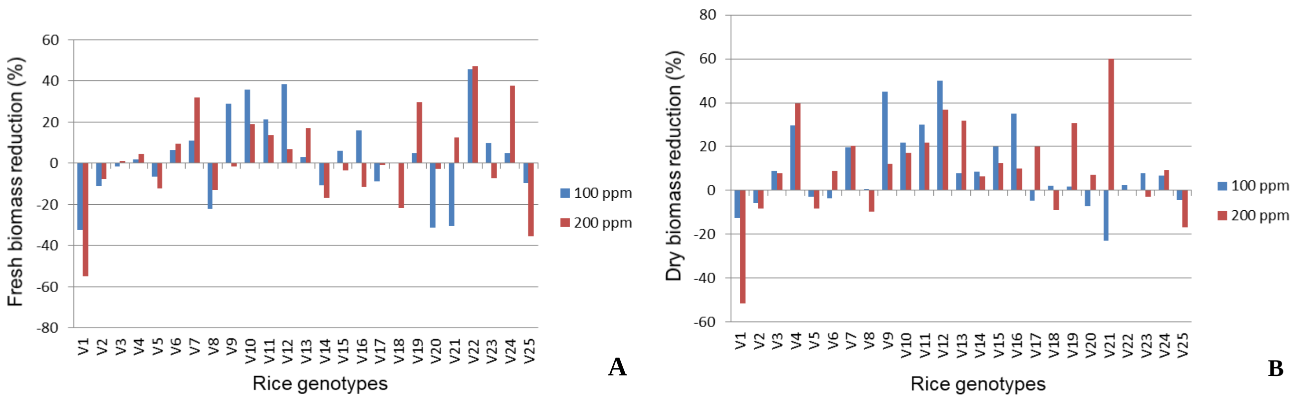

Figure 4. Percentage of the biomass growth reduction or accretion of the rice genotypes at different iron stress concentration. A. Fresh biomass; B. Dry biomass. V1 (IR 64), V2 (Mekongga), V3 (Jala Mengo), V4 (Mayas), V5 (Kawit), V6 (Awang), V7 (Bentian), V8 (Ritam), V9 (Bogor Hitam), V10 (Bogor Putih), V11 (Sungkai), V12 (Tumiyang), V13 (Melak), V14 (Bogor), V15 (Mayas Kuning), V16 (Mayas Putih), V17 (Buyung), V18 (Serai Gunung), V19 (Ketalun Tawar), V20 (Ketan Putih), V21 (Ketan Huan), V22 (Ketan Lekatan), V23 (Ketan Putek Iting), V24 (Ketan Mayang), V25 (Ketan Linjuang).

Table 8. The tolerance level of the rice genotypes at different iron stress concentration based on the total fresh biomass character

Id	Genotype	100 ppm $FeSO_4.7H2O$		200 ppm $FeSO_4.7H2O$	
		TI	Category	TI	Category
V1	IR 64	0.6730	Sensitive	0.4481	Sensitive
V2	Mekongga	0.8893	Moderate	0.9222	Tolerant
V3	Jala Mengo	0.9843	Tolerant	1.0115	Tolerant
V4	Mayas	1.0192	Tolerant	1.0431	Tolerant
V5	Kawit	0.9361	Tolerant	0.8779	Moderate
V6	Awang	1.0640	Tolerant	1.0950	Tolerant
V7	Bentian	1.1093	Tolerant	1.3211	Tolerant
V8	Ritam	0.7785	Moderate	0.8704	Moderate
V9	Bogor Hitam	1.2909	Tolerant	0.9838	Tolerant
V10	Bogor Putih	1.3578	Tolerant	1.1898	Tolerant
V11	Sungkai	1.2113	Tolerant	1.1384	Tolerant
V12	Tumiyang	1.3844	Tolerant	1.0673	Tolerant
V13	Melak	1.0288	Tolerant	1.1724	Tolerant
V14	Bogor	0.8927	Moderate	0.8294	Moderate
V15	Mayas Kuning	1.0612	Tolerant	0.9647	Tolerant
V16	Mayas Putih	1.1592	Tolerant	0.8828	Moderate
V17	Buyung	0.9108	Tolerant	0.9924	Tolerant
V18	Serai Gunung	1.0016	Tolerant	0.7802	Tolerant
V19	Ketalun Tawar	1.0502	Tolerant	1.2985	Tolerant
V20	Ketan Putih	0.6873	Sensitive	0.9724	Tolerant
V21	Ketan Huan	0.6922	Sensitive	1.1262	Tolerant
V22	Ketan Lekatan	1.4553	Tolerant	1.4721	Tolerant
V23	Ketan Putek Iting	1.0995	Tolerant	0.9267	Tolerant
V24	Pulut Mayang	1.0492	Tolerant	1.3781	Tolerant
V25	Pulut Linjuang	0.9055	Tolerant	0.6460	Sensitive

Table 9. The tolerance level of the rice genotypes at different iron stress concentration based on the total dry biomass character

Id	Genotype	100 ppm $FeSO_4.7H2O$		200 ppm $FeSO_4.7H2O$	
		TI	Category	TI	Category
V1	IR 64	0.8733	Moderate	0.4863	Sensitive
V2	Mekongga	0.9423	Tolerant	0.9176	Tolerant
V3	Jala Mengo	1.0879	Tolerant	1.0779	Tolerant
V4	Mayas	1.2965	Tolerant	1.3970	Tolerant
V5	Kawit	0.9720	Tolerant	0.9160	Tolerant
V6	Awang	0.9650	Tolerant	1.0875	Tolerant
V7	Bentian	1.1948	Tolerant	1.2045	Tolerant
V8	Ritam	1.0069	Tolerant	0.9028	Tolerant
V9	Bogor Hitam	1.4516	Tolerant	1.1210	Tolerant
V10	Bogor Putih	1.2177	Tolerant	1.1701	Tolerant
V11	Sungkai	1.2986	Tolerant	1.2172	Tolerant
V12	Tumiyang	1.5016	Tolerant	1.3668	Tolerant
V13	Melak	1.0792	Tolerant	1.3168	Tolerant
V14	Bogor	1.0846	Tolerant	1.0625	Tolerant
V15	Mayas Kuning	1.2008	Tolerant	1.1255	Tolerant
V16	Mayas Putih	1.3506	Tolerant	1.0996	Tolerant
V17	Buyung	0.9524	Tolerant	1.1991	Tolerant
V18	Serai Gunung	1.0221	Tolerant	0.9114	Tolerant
V19	Ketalun Tawar	1.0191	Tolerant	1.3062	Tolerant
V20	Ketan Putih	0.9268	Tolerant	1.0701	Tolerant
V21	Ketan Huan	0.7725	Moderate	1.5988	Tolerant
V22	Ketan Lekatan	1.0230	Tolerant	1.0000	Tolerant
V23	Ketan Putek Iting	1.0792	Tolerant	0.9703	Tolerant
V24	Pulut Mayang	1.0665	Tolerant	1.0937	Tolerant
V25	Pulut Linjuang	0.9567	Tolerant	0.8314	Moderate

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