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SCREENING METHOD FOR IRON TOLERANT RICE SUITED FOR TIDAL SWAMP AREA

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ABSTRACT

Previous research conducted in the greenhouse at the Cikabayan University Farm, Bogor Agricultural University, Indonesia from July to September 2010, using cultivar IR 64 and Margasari found that Fe concentration in solution that causing low Fe toxicity symptoms was ≤ 52 ppm Fe, moderate toxicity was 143 ppm Fe, and severe toxicity ≥ 325 ppm Fe. This research aims at: 1) To observe the effects of two levels of Fe concentrations on growth of rice; 2) to find out rice genotypes that tolerant or semi tolerant to iron toxicity; 3) to verify toxicity levels in the green house and in the field. The green house experiment was conducted at the Cikabayan University Farm, Bogor Agricultural University from July to September 2010 using Yoshida's nutrient solution with Fe added according to the treatment levels. This research used factorial design with two factors, which are: Fe concentrations (143 and 325 ppm Fe) and 20 paddy genotypes. The field experiment was done in two tidal swamp areas, in Belandean and Danda Jaya, Barito Kuala District, South Kalimantan Province, Indonesia, consisting of four genotypes chosen in the green house experiment and IR64 as a control. Results showed that increasing in Fe concentration from 143 to 325 ppm and period of Fe stress increase Fe toxic symptoms in rice. Four paddy genotypes that had been selected in the green house (Inpara-1, Inpara-2, Inpara-4, dan TOX4136 line) showed lower Fe toxic symptom after planted in the tidal swamp area. Inpara-1 and Inpara-4 genotypes indicated as tolerant genotypes to iron toxicity and perform higher yield than the other genotypes in both of field experimental locations in the tidal swamp area. Fe concentration at 325 ppm in Yoshida's solution can be used for screening of rice tolerant genotypes to iron toxicity.

Key words: iron toxicity, rice geonotypes, green house, field

INTRODUCTION

Iron toxicity is one limiting factor of rice production in the paddy field; it reportedly occurs in Asian countries such as China, India, Indonesia, Thailand, Malaysia, and Philipines (Asch *et al*. 2005). Specific visual symptoms related to iron toxicity, are primarily associated with accumulation of oxidation products of polyphenols which is called bronzing or yellowing in rice (Yamauchi and

Yoshida, 1981). Iron toxicity in paddy results in poor growth, limited tillering and as a consequence, low yield or a failed harvest (Audebert and Sahrawat, 2000). Many researches show iron toxicity could decrease yield by around 12-100% (Sahrawat , 2004; Sahrawat, 2010).

Iron toxicity causes physiological stress in rice, and it is common in ultisol, oxisol, and sulfic acid tidal swamp land with high iron concentration (Sahrawat 2004). Tidal swamp area in Indonesia is 20.1 million ha, and about 6.7 million ha is sulfic acid soils (Alihamsyah, 2004), with high pyrites and soluble Fe content that potentially cause iron toxicity in paddy. Iron toxicity is not only caused by higher absorption of Fe^{+2} in paddy tissues, which is enhanced by Fe concentration in the soil, but also relates with other factors such as nutrient imbalance and reductive environment condition with poor drainage (Sahrawat , 2004). Iron toxicity also relates with sensitive rice cultivar such as IR64 that causes low productivity (Suhartini, 2004; Suhartini and Makarim, 2009). A sensitive cultivar (IR64) performed lower yield (58%) compared to a tolerant cultivar (Margasari) in tidal swamp land in South Kalimantan, in which soil Fe concentration was 719 ppm and pH 3.84(Noor *et al*. 2005). Using tolerant genotype against iron toxicity is the cheaper and easier way for farmers to solve iron toxicity problems. In order to get higher productivity, it is necessary to perform early selection for paddy genotypes that can tolerate high iron levels with potentially higher yield.

One of the problems in the evaluation for rice genotypes that tolerant to iron toxicity is if the number of genotypes to be evaluated is large, and the other problem is variation in Fe concentrations in the soils. As a consequence, results of selection for tolerant genotypes may not be consistent (Audebert and Sahrawat, 2000). In order to eliminate environmental variation in the field, selection for tolerant genotypes can be done under the controlled environment in the green house, using nutrient solution culture or hydroponics (Asch *et al*. 2005). An appropriate selection method with a short process period in the green house is important in order to get tolerant rice genotype for production in the field. A screening method in the green house having a high correlation with performance and productivity in the field is necessary in order to identify tolerant rice genotype in a fast, cheap and precise way. Previous researches showed that there are differences in rice selection methods using nutrient solution in the green house, primarily Fe concentrations in nutrient solution, solution pH, seedling age and period of Fe stress (Asch *et al*. 2005; Dorlodot *et al*. 2005; Aung, 2006; Kpongor, 2003). This research therefore sought to observe the effects of two levels of Fe concentrations on the growth of rice; to select rice genotypes that are tolerant or semi tolerant to iron toxicity and to verify the iron toxicity levels in the green house and in the field in order to develop a better method for screening.

MATERIALS AND METHODS

Experiments were done in a green house at the Cikabayan University Farm, Bogor Agricultural University from July to September 2010, and in two locations in a tidal swamp area in Barito Kuala, South Kalimantan from Fabruary to July 2011.

Soil analysis

Soil analysis was done in the Swamp Land Agricultural Research Agency in Banjarbaru, South Kalimantan. Soil pH was measured in a 1:1.5 (w/v) water solution using a pH meter. Clay minerals were identified by X-ray diffraction analysis (Rigaku RAD-2RS Diffractometer). The content of organic carbon (C) in soil was measured with a NC analyzer (Sumigraph NC analyzer NC-800-13 N, Sumika Chem. Anal. Service). Available P content was obtained by the Bray 1 method (Bray and Kurtz 1945), while the absorbance at 693 nm was determined using a UV-VIS spectrophotometer (UV-1200, Shimadzu Corporation, Japan). Cation exchange capacity (CEC) was obtained by extraction with 1 mol L^{-1} NH_4OAc pH 7.0 and the contents of exchangeable bases (calcium and magnesium) were determined by atomic absorption spectrophotometry (AA-640-12, Shimadzu Corporation, Japan) while those of exchangeable potassium and sodium were determined by flame emission spectrophotometry (AA-640-12, Shimadzu Corporation, Japan). Base saturation was defined as the ratio of total exchangeable bases to CEC, expressed as a percentage. Exchangeable Al was extracted with 1 mol L^{-1} KCl. Exchangeable Al was extracted with 1 mol L^{-1} KCl and measured with acid-base titration. Iron (Fe) was exracted with 1 mol L^{-1} NH_4 OAc (ammonium acetate) and then Fe in solution was determined by atomic absorption spectrophotometry. Pyrite was determined by oxidizing pyrite with hydrogen peroxide and soluble sulphate, which was equivalent with pyrite, was measured by turbidimetry.

Green house experiments

Rice genotypes tolerant to iron toxicitywere evaluated under two environmental stress conditions which caused moderate and severe iron toxicity symptoms. Previous research demonstrated that ≤ 52 ppm Fe concentration in solution caused low Fe toxicity symptoms with a score of ≤ 3 in IR64. Moderate toxicity with score = 5 was observed in 143 ppm Fe, and severe toxicity with score ≥ 9 was observed in ≥ 325 ppm Fe (Noor *et al*. 2012). In the experiment, a factorial in randomized block design was used with two factors, namely (1) Fe concentrations: 143 ppm, moderate Fe toxicity symptom and 325 ppm, severe Fe toxicity symptom) and (2) Genotypes: 20 genotypes that could be divided into three groups:

a) Genotypes that have been released as irrigated paddy rice (4 genotypes)
b) Genotypes that have been released as swamp or tidal swamp rice (4 genotypes)
c) Genotypes as promotion tidal swamp rice (8 genotypes) (Table 1).

Table 1. Rice genotypes used in this study

No.	Rice Genotype	Remarks
1	IR 64	Lowland /Rain Pad Rice
2	Ciherang	Lowland /Rain Pad Rice
3	Inpari-1	Lowland /Rain Pad Rice
4	Inpari-6	Lowland /Rain Pad Rice
5	Margasari	Swamp /Tidal Swamp Rice
6	Indragiri	Swamp /Tidal Swamp Rice
7	Dendang	Swamp /Tidal Swamp Rice
8	Inpara-1	Swamp /Tidal Swamp Rice
9	Inpara-2	Swamp /Tidal Swamp Rice
10	Inpara-3	Swamp /Tidal Swamp Rice
11	Inpara-4	Swamp/Flooding Tolerant Rice
12	Inpara-5	Swamp/Flooding Tolerant Rice
13	BP1031F-PN-25-2-4-KN-2	Tidal Swamp Promoted Line
14	B11586F-MR-11-2-2-2	Tidal Swamp Promoted Line
15	BP-1027F-PN-1-2-1-KN-MR-3-3	Tidal Swamp Promoted Line
16	B10891B-MR-3-KN-4-1-1-MR-1	Tidal Swamp Promoted Line
17	IR72049-B-R-22-3-1-1	Tidal Swamp Promoted Line
18	BP367E-MR-42-4-PN-3-KN-MR-4	Tidal Swamp Promoted Line
19	B10387F-MR-7-6-KN-3-KY-2	Tidal Swamp Promoted Line
20	TOX4136-5-1-1-KY-3	Tidal Swamp Promoted Line

Every experimental unit was replicated three times. Rice was transplanted into a plastic box with sand as a medium, where half concentration of Yoshida's nutrient solution at pH 5.0 was added. After 14 days, single seedlings were transferred into a PVC plastic pot (1200 ml capacity; 7.5 cm x 23 cm, diam. x length) with half concentration of Yoshida's nutrient solution (1000 ml) (Yoshida *et al*. 1976) at pH 4.5, and acclimated for 7 days. After acclimatisation, it was treated using $FeSO_4$ for two levels of Fe concentrations at pH 4.0 (Fig. 1). The top of the plastic pot was covered with a plastic liner to minimize oxygen loss and solution media evaporation. The solution with Fe was added everyday to replace the solution that was lost by absorption and evapotranspiration and nutrient culture was replaced once a week.

Fig. 1. Research activities in the green house: (a) Rice seedling in sand box, (b) Rice seedling at 14 days age transferred to a plastic pot (PVC) and acclimated for 7 days. (c) Rice that had been treated with Fe and grown for 4 weeks.

Observations conducted consisted of Fe toxicity levels on rice for a period of 1 to 4 weeks after transplanting, tiller numbers, root length, shoot weight and root weigt at 4 weeks after transplanting. The toxicity levels were indicated by leaf damage starting from the tip leaf that became brown and then black, and the scoring of iron toxicity symptoms referred to IRRI-INGER (1996), modification by Asch *et al*. (2005) and Aung (2006) (Table 2). Four genotypes were chosen for the field trial according to its tolerance to Fe toxicity (scoring) and plant growth.

Table 2. Fe toxicity symptom scores in rice

Fe Score	Fe Toxicity in Leaf (%)	Tolerancy Levels
1	0	Highly tolerant
2	1-9	Tolerant
3	10-29	Tolerant
5	30-49	Moderately tolerant
7	50-69	Sensitive
9	70-89	Very sensitive
10	90-100	Very sensitive

Sources : IRRI-INGER (1996), modification by Asch *et al*. (2005) and Aung (2006)

Verification of Iron Toxicity Symptoms in the Field

Verification of tolerant rice genotypes in the green house was done in field at two locations that potentially have different levels of iron toxicity stress. The field experiment was done in a B type of tidal swamp area in Belandean and Danda Jaya, Barito Kuala District, South Kalimantan Province. The B type of tidal swamp is characterized by temporary flooding by a big tide (sea or river) which occurs periodically. Rice genotypes used for the experiment were four genotypes chosen from the green house experiment. IR 64 was used as a sensitive control. Four genotypes TOX4136, Inpara-1, Inpara-2, and Inpara-4 which have been selected based on the concentration of 325 ppm Fe at 4 week stress with Fe toxicity symptom score 5.0 (moderate) and one sensitive variety IR 64 (score Fe 7.7) based on the results of the previous experiment (Noor *et al.*, 2012). The treatments were replicated three times and the 21 day olds seedlings were transplanted into (4 x 5) m^2 of plot at 20 x 20 cm^2 spacing with two seedlings per hole. Half of nitrogen and all of P and K fertilizer were applied at 7 days after transplanting, and the other half of N was given four weeks after first application. Observations on iron toxicity symptom were done at 4 and 8 weeks after transplanting.

Statistical analysis

Data analysis was done using analysis of variance, and the Least Significant Difference at 95% confidence level used for advance analysis.

RESULTS AND DISCUSSION

Green House Experiments

Iron toxicity symptom on rice in green house

Generally, iron toxicity symptoms in irrigated paddy (4 genotypes), swamp rice (8 genotypes), and promoted lines (8 genotypes) increased with increasing in iron toxicity, and period of observation and iron concentration in nutrient solution (Fig. 2).

In 143 ppm Fe condition, iron toxicity symptoms in the three genotype groups were almost similar, except in the week 4th iron toxicity symptom scores in paddy rice were higher than the other groups. Under 325 ppm Fe condition, paddy rice showed toxicity symptom scores higher than the other groups for all of observation periods. Average different iron toxicity symptom scores between 143 ppm Fe and 325 ppm Fe conditions at 1, 2, 3, and 4 weeks observation were 0.20, 1.88, 2.20 and 3.00 (Fig. 2).

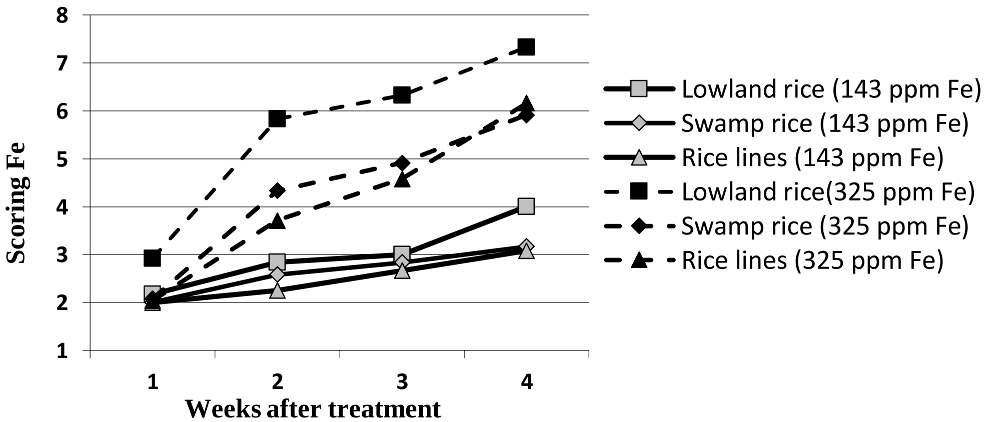

Fig. 2. Change of Fe toxicity average score of irrigated, swamp and promoted line rices in two levels of Fe concentrations for 4 weeks.

In the second week of observation, iron toxicity symptom scores significantly different among treatments, especially for 325 ppm Fe (2.3 – 6.3), Iron toxicity symptom in the second week at 143 and 325 ppm Fe treatments were different only for genotypes such as IR64 Ciherang, Inpari-1, Inpari-6, Indragiri, Dendang, and Inpara-1 (Table 3).

Table 3. Interaction between genotype and Fe concentrations for Fe toxicity symptoms at 2 and 4 weeks after treatment.

No.	Rice genotype	2 Weeks		4 Weeks	
		143 ppm Fe	325 ppm Fe	143 ppm Fe	325 ppm Fe
1	IR 64	2.7 a B	6.3 a A	5.0 a B	7.7 ab A
2	Ciherang	3.0 a B	5.7 ab A	4.3 ab B	6.3 bcd A
3	Inpari-1	2.7 a B	5.0 abc A	3.7 ab B	7.0 abc A
4	Inpari-6	3.0 a B	5.7 ab A	3.7 ab B	8.3 a A
5	Margasari	3.0 a B	5.0 abc A	3.0 b B	5.7 cde A
6	Indragiri	2.7 a A	4.3 bcd A	3.0 b B	6.3 bcd A
7	Dendang	2.7 a A	4.3 bcd A	3.0 b B	6.3 bcd A
8	Inpara-1	2.7 a A	4.3 bcd A	3.0 b B	5.0 def A
9	Inpara-2	3.0 a A	3.7 cde A	3.0 b B	5.0 def A
10	Inpara-3	2.7 a B	5.0 abc A	3.7 ab B	6.3 bcd A
11	Inpara-4	2.0 a A	3.0 de A	3.0 b B	5.0 def A
12	Inpara-5	2.0 a B	5.0 abc A	4.3 ab B	7.0 abc A
13	BP1031F-PN-25-2-4-KN-2	2.3 a A	3.7 cde A	3.0 b B	5.0 def A
14	B11586F-MR-11-2-2-2	2.0 a B	5.0 abc A	3.7 ab B	7.0 abc A
15	BP-1027F-PN-1-2-1-KN-MR-3-3	2.0 a A	3.0 de A	3.0 b B	7.0 abc A
16	B10891B-MR-3-KN-4-1-1-MR-1	2.0 a B	4.3 bcd A	3.7 ab B	7.0 abc A
17	IR72049-B-R-22-3-1-1	3.0 a B	4.3 bcd A	3.0 b B	5.7 cde A
18	BP367E-MR-42-4-PN-3-KN-MR-4	2.7 a A	4.3 bcd A	3.7 ab B	7.0 abc A
19	B10387F-MR-7-6-KN-3-KY-2	2.0 a A	2.7 de A	3.0 b B	5.7 cde A
20	TOX4136-5-1-1-KY-3	2.0 a A	2.3 e A	3.0 b B	5.0 def A

Values within columns having the same lowercase letters are not significantly different ($P<0.05$) using LSD test. (critical value of t test for 2 weeks = 1.9 and 4 weeks = 2.0)

Based on iron toxicity score observation at 4 weeks in the 325 ppm treatment, paddy field genotypes (IR64, Ciherang, Inpari-1, Inpari-6) showed high toxicity symptomp scores that were 6.3-8.3 (average 7.33). Iron toxicity symptom score in swamp paddy genotypes were between 5.0-7.0 (average 5.83) whereas iron toxicity in tidal paddy genotypes were between 5.0-7.0 (average 6.18) . Iron toxicity scores at 4 weeks for all paddy genotypes at 325 ppm treatment were higher than those of 143 ppm treatment (Table 3). Based on the score of iron toxicity symptoms at 4 weeks at 143 ppm treatment, 17 genotypes were classified as tolerant (3.0-3.7). There were 11 genotypes had the lowest score (3.0) from the 17 tolerant genotypes. Many selected genotypes were classified as tolerant, based on iron toxicity score at 143 ppm treatment, making it less effective as a basis for selection.

Based on the scores of iron toxicity symptoms at 4 weeks at 325 ppm Fe stress treatment, 11 genotypes performed moderately tolerant (score 5.0-5.7), with five genotypes with the lowest score (score of 5.0). Five genotypes considered to be moderately tolerant to iron toxicity were Inpara-1, Inpara-4, TOX4136-5-1-1-KY-3 line, Inpara-2, and BP1031F-PN-25-2-4 -KN-2 liner. The research results showed that Fe concentrations that causes Fe toxication in plants is very diverse. The Fe levels in solution which causes toxicity vary widely ranged between 10-500 ppm Fe (Bode *et al*., 1995; Asch *et al*., 2005; Fageria and Rabelo, 1987). The concentrations of Fe nutrient in solution of 250-500 ppm with pH 4.5-6.0 significantly boosted the levels of Fe in plant tissue and showed symptoms of Fe toxicity on sensitive plants (Majerus *et al*. 2007; Mehraban *et al*. 2008).

Verification of iron toxicity symptoms in the field

To verify the tolerance of varieties tested under greenhouse conditions, five varieties were tested in a field trial in the tidal swamp in Belandean and Danda Jaya, South Kalimantan Province.

Soil characteristics

Soil analysis showed that the soil was very acidic, with a pH of 3.8 and 4.1 in Belandean and Danda Jaya, respectively. Levels of toxic elements, such as exchangeable-Al (9.70 me/100g) and Fe concentration (631 ppm), in Belandean were higher than in Danda Jaya which had exchangeable-Al at 6.37 me/100 g and 425 ppm Fe. The depth of the layer of pyrites ($FeS_2 \geq$ 2%) in Belandean was more shallow ($\geq$ 40 cm) than in Danda Jaya ($\geq$ 54 cm), while pyrite content in Belandean was also higher (4.37%) than in Danda Jaya (2.48%).(Table 4). Belandean possesses higher level of stress than the Danda Jaya location based on the depth of the more superficial layers of pyrites and pyrites content, exchangeable-Al, higher Fe and the lower soil pH, higher soil Fe content and lower pH. Levels of pyrites was high and pyrites layer depth was more shallow at Belandean, and as a consequences, iron toxicity stress were more severe than in Danda Jaya (Table 4). The shallow pyrites layer in the soil has greater potential to cause iron toxicity in rice, because shallow layer is easy to be affected by oxidation. When the pyrite layer is oxydized, it would decrease soil pH, increase Al and Fe toxicity and lower nutrient content. Under reductive environment, excessive iron in the form of ferrous ions (Fe^{2+}) will appear in acid sulfate soils and may become toxic for rice (Dent, 1986).

The oxydation of pyrates produce ferric ions (Fe^{+3}) and H^+ that cause soil to become very acidic, based on the chemical reaction below (Dent, 1986) :

$$FeS_2 + 15/4\ O_2 + 7/2\ H_2O \rightarrow Fe(OH)_3 + 2\ SO_4^{2-} + 4\ H^+$$

Under flooded reductive conditions, ferric ions (Fe^{+3}) will reduce to ferrous ions (Fe^{+2}) which can be absorbed in larger amounts resulting in phytotoxicity. The reduction of ferricc to ferrous ions is commonly associated with iron reduction bacteria, and the reaction is as follows (Dent, 1986) :

$$Fe(OH)_3 + 3H^+ + e^- \Leftrightarrow Fe^{2+} + 3H_2O$$

Table 4. Soil characteristics of the field in tidal swamp area, KP. Blandean and Danda Jaya, Barito Kuala District, South Kalimantan

Soil Characteristic	KP. Blandean		Danda Jaya	
	Value	Criteria*	Value	Criteria*
pH (H_2O)	3.80	Very Acid	4.10	Very Acid
C. Organic (%)	5.01	High	4.16	High
N total (%)	0,25	Medium	0.27	Medium
P Bray I (ppm P_2O_5)	12.80	Medium	15.20	Medium
P total (mg/100g P_2O_5)	84.00	High	112	High
K total (mg/100 g K_2O)	8.00	Low	8.00	Low
Excngeable Base (me/100g) :				
Ca	1.50	Low	1.10	Low
Mg	1.37	Low	1.30	Low
K	0.09	Very low	0.09	Very Low
Na	0.62	Medium	0.62	Medium
KTK (me/100 g)	15.75	Medium	12.65	Medium
Al-dd (me/100 g)	9.70	High	6.37	High
Fe (ppm)	631	-	425	-
Texture (%):				
Clay	69	Silty Clay	63	Silty Clay
Silt	31		36	
Sand	0		1	

*Soepraptohardjo (1983)

Iron toxicity symptoms and rice yield

The level of iron toxicity symptoms in the field showed that more stress was found in Belandean than in Danda Jaya location, either in the 4th or 8th week of observation, especially for sensitive varieties such as IR 64. Higher toxicity in Belandean resulted in lower yield compared to that in Danda Jaya. Iron toxicity symptom scores of Inpara-1 and Inpara-4 varieties were lower and these varieties produced higher yield than the others in both locations. For both locations, IR 64 as a sensitive control variety showed higher iron toxicity symptom score and produced lower yield than the others (Table 5). Observations were done in the 4th or 8th week after transplanting because these refered to the results in the greenhouse experiments. Iron toxicity symptom scores at 8 weeks showed that green house tolerant varieties had lower level of symptom scores (3.0 – 4.3) than the control variety IR 64 (7.0) in Belandean and in Danda Jaya locations. Tolerant varieties also had lower level of symptom scores (2.0 – 3.7) than sensitive control variety IR 64 (5.7). Yield of Inpara-1, Inpara-2 and Inpara-4 performed were 3.85, 3.32 and 4.01 ton ha^{-1}, respectively in Belandean which were higher than that of IR 64 (2.1 ton ha^{-1}). In Danda Jaya location, Inpara-1, Inpara-2 and Inpara-4 performed yield of 4.51, 4.12 and 5.46 ton ha^{-1} respectively which were higher than yield of IR 64 as a sensitive control variety (2.33 ton ha^{-1}). The yield of TOX4136 line had no significant difference with the yield of IR 64 in both locations. The facts above showed that the results in the green house were confirmed by the results in the field.

Concentrations of Fe in the soil at 300-400 ppm cause iron toxicity in rice plant (Breemen and Moormann 1978). The critical border of Fe concentration (extraction by 1N NH_4OAC, pH 4.8) in the soil of tidal swamp land that can cause iron toxicity is 260 ppm Fe (Sulaiman *et al.* 1997). Initial soil analysis in the two locations were 425 ppm Fe with pH 4.1 (Danda Jaya) and 631 ppm with pH 3.8 (Belandean) were higher than the concentration that stated by Breemen and Moormann (1978) and

Sulaiman *et al.* (1997). Those conditions potentially cause toxicity for sensitive variety such as IR 64. Belandean, with lower pH and higher soil Fe concentration, has the potential to cause more severe iron toxicityon rice than Danda Jaya location.

Table 5. Fe toxicity symptom scores at 4 and 8 weeks after transplanting and rice yield in tidal swamp land in Blandean and Danda Jaya in the first planting season, 2011.

Rice Genotype	Belandean			Danda Jaya		
	Iron toxixity score (4 weeks)	**Iron toxixity score (8 weeks)**	**Rice Yield (t/ha)**	**Iron toxixity score (4 weeks)**	**Iron toxixity score (8 weeks)**	**Rice Yield (t/ha)**
TOX4136	3.0 c	4.3 b	2.46 b	3.0 b	3.7 b	3.33 ab
Inpara-1	3.0 c	3.0 b	3.85 a	2.0 b	2.0 b	4.51 ab
Inpara-2	4.3 b	4.3 b	3.32 a	3.3 b	3.7 b	4.12 ab
Inpara-4	3.0 c	3.0 b	4.01 a	2.7 b	2.0 b	5.46 a
IR 64	6.3 a	7.0 a	2.10 b	5.7 a	5.7 a	2.33 b
Average	3.92	4.32	3.15	3.34	3.42	3.95
Least significant difference of t-test	1.19	1.46	0.82	1.87	1.68	2.20

Values within columns having the same lowercase letters are not significantly different ($P<0.05$) using LSD test.

Trials conducted by other researchers in the tidal swamp land showed that the yield of rice was affected by the level of iron toxicity. Iron toxicity symptoms could appear at different growth stages and could influence vegetative as well as the reproductive growth. Iron poisoning at the vegetative stage can decrease plant height and dry matter production, and can affect tillering formation and number (Fageria, 1988). Ferrous ions that are absorbed by the plant causes leaf discoloration, decreases tiller number and significantly decreases yield. Decreasing rice yield by iron toxicity is also caused by disturbing in the metabolic processes in the plant that can change the physiology or agronomic characters of the rice plant. The scoring system for iron toxicity symptoms was demonstrated to correlate with rice yield, where increasing 1 point will reduce rice yield by 0.426 ton ha^{-1} (Audebert 2006). The condition without iron toxicity (score=1) gave a yield of 4,14 ton ha^{-1}, and increasing the iron toxicity score to 3, 5 and 7 decreased rice yield to 2.86, 2.01 and 1.16 ton ha^{-1}. Suhartini and Makarim (2009) also showed that the iron toxicity symptom score affected rice yield. Iron toxicity score < 3.5 supported rice yield > 4.3 ton ha^{-1} or was not so affected by iron toxicity. In contrast, by increasing iron toxity score > 4.5, the rice yield obtained was reduced to ≤ 2.01 ton ha^{-1}.

Iron toxicity symptom scores in green house and field

The longer the period of stress, the higher the iron toxicity levels in the green house as well as in the field, especially for sensitive or rather tolerant varieties in Belandean (Fig. 3). Iron toxicity scores of 5 genotypes at 4 weeks after transplanting (ATP) in the green house showed higher iron toxicity scores than in the two locations in the field at 4 or 8 weeks ATP. Inpara-1 and Inpara-4 showed lower toxicity symptom scores than the other genotypes in both locations in the field. Iron toxicity scores at 2 weeks ATP of TOX4136 genotype and Inpara-2 were lower than that in the field, while Inpara-1 and Inpara-4 genotypes in the green house had higher or similar iron toxicity scores than that in the field. IR 64 genotype showed iron toxicity symptom that was similar in the green house and in the field at 4 and 8 weeks ATP, and consistenly higher than the other genotypes.

Iron toxicity symptom scores of 4 genotypes that was chosen from green house showed lower scores (2.0-4.3) in the field experiment than that of the green house score (5.0) especially for Inpara-1 and Inpara-4 genotypes. These results indicate that selection of tolerant rice genotypes at Fe concentration of 325 ppm (Yoshidas'nutrient solution) in the green house for 4 weeks can be used as the criteria for selection, because it has verified that the symptom scores in the field were lower than those in the green house. Sensitive variety IR 64 constantly showed similar iron toxicity scores in both conditions, the green house (score 7.7) and in the field (score 7.0). The chosen genotypes from green house Inpara-1, Inpara-2 and Inpara-4 showed lower symptom scores and performed higher yield than that IR 64 (Table 4). Experimental results showed differences in iron toxicity level of the tested rice genotypes under green house conditions compared to field conditions. Inpara-1 and Inpara-4 in the green house was classified as rather tolerant varieties (Fe toxicity score = 5.0), and these changed to the tolerant genotypes class (score = 2.0-2.7) in the two field locations. TOX4136 line and Inpara-2 variety were classified as rather tolerant genotypes in the green house (5.0), and in Blandean (4.3), yet changed to the tolerant genotypes (3.7) when they were planted in Danda Jaya.

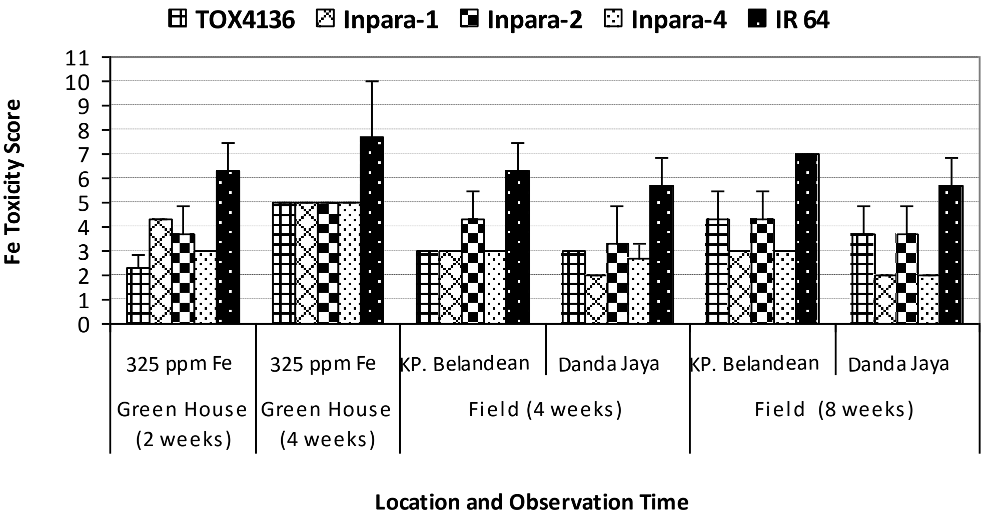

Fig. 3. Iron toxicity symptom of five rice genotypes in the green house (325 ppm Fe) and in the tidal swamp land (Blandean and Danda Jaya) in the first planting season, 2011.

The facts above indicate that Fe concentration of 325 ppm at pH 4.0 in Yoshida nutrient solution provide clearer Fe toxicity symptoms compared to soil in Blandean location (631 ppm Fe, pH 3.8) and soil of Danda Jaya location (425 ppm Fe, pH 4.1). These results indicate that Fe in solution in lower quantities can produce Fe toxicity faster than Fe in the soil. There is a variation of iron toxity in the soil according to the pH in the soil solution (Sahrawat, 2004). At pH below 5.0, the plant is susceptible to iron toxicity (Dobermann, and Fairhurst 2000). The critical limit of iron concentrations that causes iron toxicity are 100 ppm at pH 3.7 and 300 ppm or more at pH 5.0 (Sahrawat *et al.* 1996). The concentration of 250 ppm Fe or more in the Yoshidas' solution could be used to differentiate rice tolerance, because it showed clearly Fe toxicity symptom (*bronzing*), growth reduction and plant tolerance during the 4 weeks period of stress. Iron concentration at 250 ppm could be used to compare tolerance of various varieties in solution culture (Dorlodot *et al.* 2005). Iron concentration at 2000 ppm in Yoshidas' solution culture with 3 days period of stress could be used to differentiate sensitive and tolerant cultivars in nutrient + seaweed solution (Kpongor 2003). Clear iron toxicity symptoms were demonstrated after 8 days of Fe stress at a concentration of Fe > 300-500 ppm Fe (Bode *et al.* 1995).

CONCLUSIONS

A screening method in the green house for rice tolerant genotype that related with the level of Fe toxicity symptom and rice productivity in the field is needed in order to select tolerant genotypes in shorter time, cheaper cost, and better accuracy than a direct selection in the field. Based on the score of iron toxicity symptoms at 4 weeks after treatment, 17 genotypes were classified as tolerant using 143 ppm Fe, and 11 genotypes performed moderately tolerant using 325 ppm Fe. Inpara-1 and Inpara-4 indicated as the tolerant genotypes to Fe toxicity, and performed higher yields in the two field research locations. Rice genotypes that were selected in the green house showed lower Fe toxicity symptoms in the tidal land than those in the green house. Fe concentarion at 325 ppm under Yoshida' nutrient solution at pH 4.0 in the four weeks period of stress can be used for screening of rice tolerant genotypes to Fe toxicity.

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