Responses of Selected Indonesian Rice Varieties under Excess Iron Condition in Media Culture at Seedling Stage

Respon Varietas Indonesia Terpilih pada Kondisi Kelebihan Besi Fase Bibit di Media Kultur

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ABSTRAK

Keracunan besi dapat membatasi hasil padi pada tanah masam dan rawa di beberapa negara tropis. Penggunaan varietas toleran terhadap keracunan besi menjadi strategi alternatif dalam meningkatkan hasil padi pada daerah-daerah tersebut. Penelitian bertujuan untuk mempelajari variasi fenotipik dan mengkarakterisasi alur besi di dalam akar dan tajuk dari 24 genotipe padi yang ditanam pada kondisi tercekam Fe tinggi dengan konsentrasi 400 mg/l dan dalam kondisi normal. Hasil penelitian menunjukkan terdapat variasi akumulasi biomasa selama perlakuan cekaman Fe, yaitu genotipe toleran yang berbobot kering tajuk besar dan genotipe toleran dengan bobot kering tajuk kecil. relatif bobot kering berkorelasi sangat nyata dengan skor bronzing daun (LBS) pada kondisi cekaman besi. Berdasarkan kategorisasi ini beberapa genotipe dipilih untuk mempelajari keberadaan Fe di dalam akar dan tajuk menggunakan visualisasi pewarnaan 2,2 bypiridine untuk mengetahui keberadaan Fe di dalam akar dan tajuk. Hasil penelitian menunjukkan sejumlah genotipe dapat membentuk aerinkima di daerah akar dan tajuk akibat perlakuan cekaman besi. Kemampuan ini berhubungan dengan terbentuknya plak besi di akar dan kandungan besi di tajuk. Dengan demikian, genotipe padi dapat digolongkan menjadi tipe toleran includer (Inpara2) dan tipe toleran excluder (Mahsuri, Pokkali and Siam Saba). Informasi tentang strategi toleransi tanaman padi berguna untuk pemulia dalam merancang program pemuliaan tanaman padi toleran terhadap keracunan besi berbasis fisiologi.

Kata kunci: Tipe excluder, tipe includer, ferric, aerenkima, biomassa.

ABSTRACT

Iron toxicity could limit rice productivity on irrigated lowland acid and swampy soil. The use of iron toxicity tolerant rice is an alternative strategy to improve rice productivity in these areas. The research studied the phenotypic variation of twenty-four rice genotypes and characterized the fate of Fe^{2+} along its path between the roots and shoot of rice plant. Twenty-four rice genotypes from different agro-ecosystems were grown under agar nutrient solution conditions with 400 mg/l iron stress and under normal condition. Results showed variation in the biomass accumulation of rice seedling during stress of iron, differentiated as high accumulated biomass tolerant type and low accumulated biomass tolerant type. The relative biomass weight was highly correlated with the leaf bronzing scores (LBS) under excess iron. Based on these categorizations, six genotypes were chosen to observe the present of Fe in root and shoot using in vivo-staining 2,2 bypiridine. The results indicated that some genotypes were able to develop root and shoot aerenchym during iron stress. This was related to the development of root iron plaque and the iron content of the shoot of the rice seedling. In this present study, rice genotypes could be classified as the includer tolerant type (*Inpara 2*) and some others were the excluder tolerant type (*Mahsuri, Pokkali* and *Siam Saba*). This information on crop tolerance strategies is important for rice breeder to develop physiological-based breeding program of iron-toxicity tolerance in rice.

Keywords: Excluder-type, includer-type, ferrous iron, aerenchym, biomass.

INTRODUCTION

High concentration of ferrous iron (Fe^{2+}) negatively affects the growth of rice plants. Since rice is cultivated under flooding condition, most iron ion is transported by liquid mass flow from rhizosphere through the intercellular space of the root cortex to the Casparian strip, and uploaded into the xylem vessels (Stephan *et al.* 2002). Along this long-distance transport elevated Fe^{2+} concentrations catalyze the formation of reactive oxygen species (ROS) and may result in an irreversible damage to the leaf, so called leaf-bronzing (Becana *et al.* 1998). This leaf injury is followed by stunted plant growth, low tiller number, and poorly developed root system (Dobermann and Fairhurst 2000). The leaf-bronzing severity depends on the intensity and the duration of the Fe stress and genotype-specific tolerance mechanisms (Becker and Asch 2005). In acute cases, eventually it causes the death of leaves or whole plant and contributed up 12 to 100% yield loss (Audebert and Sahrawat 2000).

Some cultural practices can reduce the negative impact of iron excess, such as periodical drainage to oxidize the iron (Prasetyo *et al.* 2013); soil liming to increase soil pH (Fageria *et al.* 2008); and applying appropriate P, K and Zn fertilizers (Ramirez *et al.* 2002) or Si fertilizer (Dufey *et al.* 2014) to minimize the negative effect of excessive iron. However, farmers may find that these methods are often not practical and economical. They may prefer to plant iron toxicity tolerant variety. Although there had been number of iron toxicity tolerant varieties released, the breeding program for iron-toxicity still continue to meet farmer preferences and to improve characteristics the existing varieties (Ruskandar *et al*. 2011). The program may be done through physiological-based breeding, which based on the phenotypical-tolerance appearance and the physiological aspect of rice plant tolerance.

Screening for iron toxicity tolerance had been done among Indonesian rice germplasm pool (Suhartini 2004, Suhartini and Makarim 2009, Hanarida and Utami 2009, Utami and Hanarida 2014). Iron toxicity tolerant genotypes were mostly local varieties which are characterized as tall plant type, photoperiod sensitive and low yielded (Suhartini 2004). To adapt rice plants to Fe toxic conditions, four types of tolerance mechanisms had been proposed. Type I referred to exclusion of Fe^{2+} at the root level, thus avoiding Fe^{2+} from entering the shoots tissue via rhizospheric oxidation (Wu *et al.* 2014, Engel *et al.* 2012a) or through increasing pH by OH^- efflux (Suhartini and Makarim 2009). Type II referred to exclusion of Fe^{2+} and selectivity of root membrane via protein transporter regulation e.g. Iron regulated transporter (IRT) (Utami and Somantri 2014; Thomine and Vert 2013). Type III referred to inclusion and avoidance via internal compartmentation of Fe^{2+}, e.g. utilization iron storage protein, Ferritin (Briat *et al*. 2010; Majerus *et al.* 2007b) or preferential storage in old leaves or photosynthetically less active leaf sheath tissue (Audebert and Sahrawat 2000). Type IV referred to inclusion via increased thresholds to elevated Fe^{2+} within leaf cells, probably through enzymatic detoxification in the symplast (Majerus *et al.* 2007a, Majerus *et al.* 2009).

Among the tolerance-typed to iron toxicity, it can be simplified into two categories namely, exclusion tolerance-type and inclusion tolerance-type. This identification can be done simply by checking the presence of Fe^{2+} in the shoots and root of rice plant. However, up to now there is no clear information regarding the between leaf symptoms and Fe concentration in the plant (Elec *et al*. 2013, Engel *et al.* 2012a). It also had been demonstrated that iron plaque deposition on the root surface could explain different levels in iron toxicity tolerance (Dufey *et al*. 2009, Wu *et al*. 2014). For oxidizing iron, the root needs to transfer oxygen from atmospheric to root zone, which is facilitated by aerenchym (Harahap *et al.* 2014, Colmer 2003). Hence, the reason for these differences could be due to gas transport capacity among the cultivars and it is assumed due to anatomical differences of the rice aerenchym.

Little is known about the prevalence of different tolerance-type based on the presence of Fe^{2+} in the roots and in the shoot among the available rice germplasm pool. This knowledge is required to accelerate breeding efforts for iron-toxicity tolerance. Here, we study the identification of phenotypic variations among twenty-four rice genotypes based on tolerance, biomass production and iron concentration in the shoot. We also observe the fate of Fe^{2+} along its path between the roots and shoot, in order to know the tolerance-type of rice seedling to iron toxicity stress.

MATERIALS AND METHODS

Plant Materials and Media Culture

The experiment was conducted in greenhouse experimental station of Indonesian Center for Rice Research, Bogor from May to June 2014. Twenty-four rice genotypes of known tolerance degree of iron toxicity were used in this study. Germinated seeds were transferred to sheet-holed styrofoam, measuring 24 cm x 36 cm x 2 cm size that fitted to a 10-L plastic tray. Each sheet consisted of 100 holes with 2 cm x 3 cm spacing; each hole was used for growing one seedling. The plastic trays were filled with pre-culture solution using 1 L of 8×strength stock nutrient solution (Yoshida solution) added with 7 L of deionized water. After 14 days the pre-culture media solutions were replaced by new Yoshida solution with addition of 400 mg L-1 of Fe^{2+} supplied as FeSO4 and a 0.2 % agar, to minimize oxidation of ferrous iron (Nugraha *et al.* 2016). The initial pH was adjusted at 5.5 (±0.2). The nutrient solution of control was the same to that of the first experiment. We did not replace nutrient solution until 14 days for final leaf bronzing scored and samples were harvested for further analysis. The leaf bronzing score were determined by using scoring indexes 1 (no bronzing symptom on the leaf) to 7 (the whole leaves were bronzing) (Table 1). Ten samples were taken to observe the shoot and root length. The shoot length was measured from based of shoot to the highest tip of leaf, while the root length was measured from the

Table 1. Bronzing score classified into seven ranks according to inspection of leaf blades of rice (Shimizu *et al*. 2005)

Skor	Leaf order-				
	2	3	4	5	6
1	N	N	N	N	N
2	P	N	N	N	N
3	W	P	N	N	N
4	R	W	P	N	N
5	R	R	W	P	N
6	R	R	R	W	P
7	R	R	R	R	W

N: normal, T: bronzing on the leaf tip, P: partly bronzing, W: whole leaf bronzing, R: rolled or dead leaf.

longest root tip of root to the root base. Afterward the samples were oven dried at 70^o C for 3 days then weighed for root and shoot dry-weight. The relative value of shoot and root dry weight was determined by (dw under normal – dw under iron stress)/dw under normal.

Samples were harvested for measuring iron root plaque and shoot iron content. The fresh root of entire roots was incubated in 2 M HCl in 50 mL plastic flask for 60 minutes. The extract was filtered and transferred into new flask for analysis. Afterward the samples were oven dried at 70^o C for 3 days. The oven-dried shoot samples were ground and weighed 0.5 g into digestion tube. The samples were digested using 5 mL concentrate acid (HNO3:HClO4 =3:1). The following days, samples were heated on digestion block at 120^{o}C for 24 hours. After the tube had cooled, the digest was transferred to 25 mL flask with deionized water. Iron plaque and shoot concentration were measured by atomic absorption spectrophotometry.

In Vivo Staining of Rice Seedling After Exposed to Iron Toxicity

A total of six rice genotypes were chosen based on tolerance and biomass accumulation during plant growth under the same nutrient condition for screening under Fe-excess and normal condition. Four samples from each genotype were taken for observation. Segments of the roots and shoot from four individual plants were sliced using a razor blade with 4 0.1 mm thickness and placed on a glass carrier plate. The root segments of main root were taken from main root at 10 mm from the root apex. This cross section was viewed at 100-fold magnification using microscope Axiovert 70 Zeuss. The segment of the shoot was taken from 20 mm above the epicotyl and were viewed at a 40-fold magnification. Staining were done by dropping 0.1 ml 10 mM 2,2 bypiridine to the sample and allowed for 5 minutes to allow the iron in the sample diluted. This technique was a modification from the staining technique developed by Engel *et al.* (2012b), which needed an overnight iron rice seedling staining and used low concentration of bypiridine. The microscopic observations were documented using a high-resolution camera Canon A85. The visualization and measurement of aerenchym area used software Zeuss Zen 12 (Zeuss, Germany).

Statistical analyses were performed using variance (ANOVA) after verifying that the residuals met the criterion of normal distribution. Cate–Nelson analysis (Cate and Nelson 1976) was performed to divide the data into two groups: sensitive and tolerance genotypes. Those data where a change in the x variable is likely to correspond to a response in the variable and those data where a change in x is unlikely to correspond to a change in y. The Sum of Squares was presented by PROC ANOVA. This procedure was required to compute the potential critical-x value interactively to each the data set, and the procedure re-run each time, so that the critical-x value was maximizes. The critical-y value and the error would be determined manually afterwards.

RESULTS AND DISCUSSION

Relationship of the Bronzing Severity with Observed Characters

We did not find variation on leaf bronzing among genotypes after 5-d exposure with high iron concentration, resulting insignificant correlation between the observed characters and leaf bronzing (Table 2). However, after 10-d, LBS correlated with traits related to root and their relative comparison to normal condition, indicating that excess iron inhibited the development of root system in sensitive genotypes compared to those in tolerant genotypes. However, the observation of rice root system in media culture needs uprooting the plant and would be convicting the sample for analysis. Therefore, this method would not be applicable for selection criteria.

High susceptibility to iron toxicity resulted from treatment of 400 mg. L^{-1} was observed on IPB107-5-1-1 and IR64, with bronzing scores 5.3 and 5.2, respectively. Whereas *Siam Saba* (2.8), *Cilamaya Muncul* (2.9) and *Pokkali* (3.0) indicated the lowest bronzing scores among all tested genotypes (Fig 1). There was no significant correlation between LBS with shoot dry weight, however LBS was significantly correlated with the weight of its relative to normal conditions (r=0.625**). This was because tolerant genotypes could

Table 2. Simple correlation between leaf bronzing score (LBS) and each observed characters of rice seedlings exposure with 400 mg. L-1 of ferrous iron (N=24).

Characters	*LBS* at 5-d	*LBS* at 10-d
LBS at 10-d	0.476*	-
Root length	-0.314	-0.515*
Root dry weight	0.139	-0.419*
Shoot length	0.050	-0.412
Shoot weight	0.148	-0.362
Relative root length+	0.242	0.506*
Relative root dry weight+	-0.081	0.525**
Relative shoot length+	-0.059	0.010
Relative shoot dry weight+	0.380	0.625**
Shoot iron content	-	0.392

+ Relative value is determined by normal-iron stress, *LBS*, leaf bronzing score

belong to high or low accumulated shoot biomass (Fig 1). For example, the tolerance genotype *Pokkali* was the most vigorous seedling observed in this experiment (0.32 g per plant), while other tolerant genotypes like Siam Saba was only 0.15 g per plant. High biomass accumulation of *Pokkali* was also reported by other researchers (Engel *et al*. 2012a, Wu *et al*. 2014). In this present study, we found that the LBS also did not correlated with shoot iron content (r=0.392), indicating that some tolerant genotypes were able to store the iron in the shoot and the others tolerant excluded the iron from the leaves, left in the rhizosphere. Other researchers reported the total amount of Fe accumulated in the aboveground plant parts was not always related to the leaf-symptom scores (Engel *et al*. 2012b, Onaga *et al.*

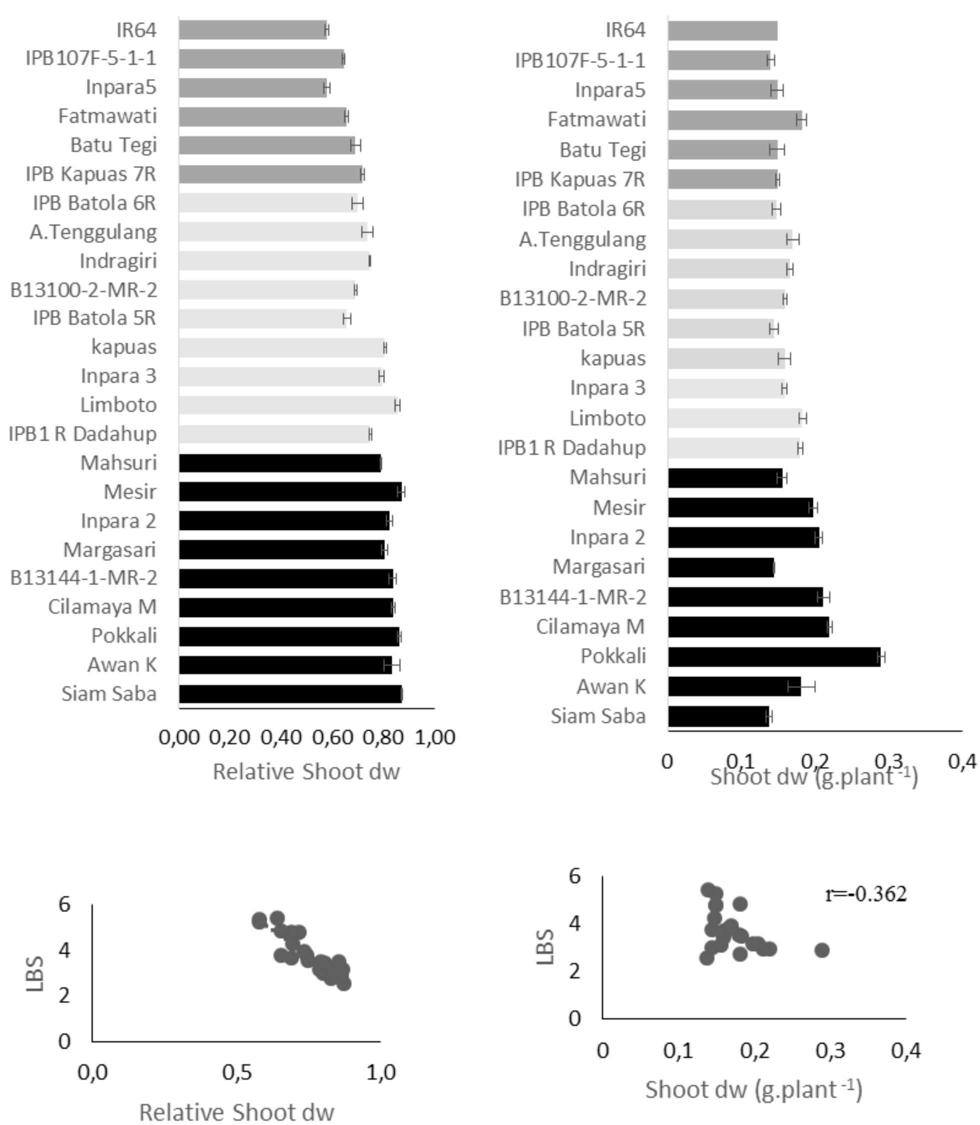

Fig 1. Shoot dry weight, relative shoot dry weight and relation with leaf bronzing scores of 24 rice genotypes under iron excess 400 mg. L^{-1} of $FeSO_4$. Error bar is standard error from three replications.

Table 3. LBS, Relative plant height, root length, shoot dry weight, and root dry weight of rice genotypes under 400 mg. L^{-1} of Fe^{2+} for 10 days.

Genotypes	LBS (score)	RL (cm)	RW (g)	SL (cm)	SW (g)	RRL	RRW	RSL	RSW
Siam Saba	2.53	12.7	0.04	35.7	0.14	**0.68**	**0.68**	**0.82**	**0.87**
Awan Kuning	2.73	14.8	0.06	40.8	0.18	**0.65**	**0.51**	**0.81**	**0.79**
Pokkali	2.87	19.5	0.11	53.3	0.29	**0.60**	**0.51**	**0.80**	**0.83**
Cilamaya M	2.90	16.8	0.07	36.8	0.22	**0.58**	**0.73**	**0.88**	**0.86**
B13144-1-MR-2	2.96	16.2	0.06	39.2	0.21	**0.56**	**0.77**	**0.86**	**0.84**
Margasari	2.97	10.8	0.04	36.3	0.14	**0.68**	**0.57**	**0.82**	**0.87**
Inpara 3	3.11	15.2	0.05	38.8	0.21	**0.51**	**0.47**	**0.83**	**0.79**
Mahsuri	3.11	14.5	0.06	40.2	0.20	**0.62**	**0.51**	**0.80**	**0.82**
Inpara 2	3.13	12.3	0.04	40.2	0.16	**0.66**	**0.61**	**0.78**	**0.84**
Mesir	3.16	16.3	0.04	38.0	0.18	**0.62**	**0.53**	**0.81**	**0.80**
Kapuas	3.40	10.2	0.05	37.3	0.18	**0.63**	**0.45**	**0.81**	**0.81**
Limboto	3.48	13.0	0.04	37.8	0.16	**0.59**	0.52	**0.86**	**0.85**
IPB Dadahp 1R	3.53	11.5	0.05	38.8	0.16	**0.54**	0.41	**0.79**	**0.75**
B13100-2-MR-2	3.67	13.7	0.04	34.5	0.14	0.51	0.42	0.77	**0.69**
IPB Batola 5R	3.73	13.0	0.04	37.8	0.16	0.49	0.4	0.79	0.66
Indragiri	3.77	11.5	0.05	34.8	0.17	0.53	0.52	0.79	0.75
A. Tenggulang	3.90	13.0	0.05	37.8	0.17	0.57	0.5	0.77	0.74
IPB Batola 6R	4.23	12.3	0.04	36.2	0.15	0.5	0.42	0.80	0.7
IPBKapuas 7R	4.73	10.0	0.04	32.8	0.15	0.43	0.4	0.76	0.72
Batu Tegi	4.77	11.8	0.06	40.2	0.15	0.48	0.58	0.77	0.69
Fatmawati	4.83	13.8	0.06	40.2	0.18	0.43	0.37	0.78	0.66
Inpara5	5.23	9.5	0.03	33.7	0.15	0.45	0.34	0.75	0.64
IR64	5.33	11.0	0.04	33.3	0.14	0.48	0.3	0.75	0.58
IPB107F-5-1-1	5.40	10.5	0.03	33.0	0.15	0.48	0.34	0.77	0.58
R^2	-	0.03	0.03	0.28	0.06	0.757	0.560	0.636	0.845
SS critical	-	507	1194	26.1	13.0	0.092	0.154	0.014	0.241
SS total	-	20902	39586	91.6	2056	0.122	0.258	0.022	0.285
ANOVA (Model)	ns	ns	**	ns	***	***	***	***	
CV (%)		14.5	12.3	23.2	15.5	10.9	14.4	9.4	13.6

LBS, leaf bronzing score, *RL,* root length, *RW*, root weight, *SL*, shoot length, *SW*, shoot weight, *RRL*, relative root length, *RRW,* relative root weight, Grey shaded values indicate tolerant genotypes after Cate-Nelson analysis using LBS as critical pivot point,
R^2, coefficient determination, *SS* partition, Sum of square critical partition between tolerant and sensitive groups, *SS* total, Sum Square of Total Anova model, *CV,* coefficient variation

2013). An iron dilution may have occurred in vigorously growing genotypes (i.e., *Pokkali*) and it may present an additional mechanism to cope with high Fe^{2+} in solution. The dilution of iron in the biomass may play an important role in the resistance to Fe toxicity. Therefore, this mechanism was further investigated in the next experiment of this study.

Determination Tolerance Level Based on Phenotypic Variation

Excess iron also inhibited growth and development of roots and shoot compared to normal condition, which was indicated by greatly reduced on the sensitive genotypes. For example, IR64 had only 75% and 48% of normal condition in shoot length and root length, respectively, comparing with tolerant genotype *Siam Saba* which had 82% and 68% (Table 3). All genotypes also showed reduction in root dry weight, but was the most pronounced in *IR64* by 30% of control condition. The less reduction of root dry weight was found in *B13144-1-MR-2* (77%).

Tolerance level could be determined based on visual appearance of leaf color changing from green to yellowish reddish or leaf bronzing, when rice plant was exposed to excess iron in the field or in the green house. This system certainly could cause personal error even from inherently skilled eyes in selection practices. An alternative method was proposed by determining the critical value of a visual score (LBS) with phenotypical performance. However, the correlation between LBS and direct phenotypical performance was weak (Table 2). We used the relative value to analyze the critical point of separation (Table 3). The separation follow the method developed by Cate and Nelson (1976) for phosphorus calibrations that would indicate an increase in crop yield or not.

The range of coefficient determination (R^2) was 0.560 to 0.845, the highest was relative shoot dry weight (0.845), which had critical point of LBS. 3.67. This could mean that genotypes with LBS score less than 3.67 could be categorized as tolerant genotypes, while the genotypes with LBS score above 3.67 could be categorized sensitive genotypes. The critical point of LBS based on observed phenotypical performance was quite narrow ranged from 3.40-3.67. Using this interactive method analysis was reported for determining critical value between growth and quality nitrogen exchange in turf grass (Kopp and Guillard 2002); adoption sustainable resource and quality water resource (Mangiafico *et al.* 2008); and critical value for survey respond (Hollingsworth *et al.* 2011).

Status of Fe^{2+} in the Shoot and Root

Based on the first experiment, we selected six genotypes representing different tolerance and biomass accumulation. *IR64* and *IPB107F-5-1-1* were each sensitive genotypes with low biomass accumulation, *Inpara2* and *Pokkali* were tolerant genotypes with high biomass accumulation, and *Siam Saba* and *Mahsuri* were each tolerant genotypes with low biomass accumulation. Differential expression of bipyridine-induced iron color formations in roots cross section are shown in Fig 2A. *IR64, Inpara2* had distinct red color in

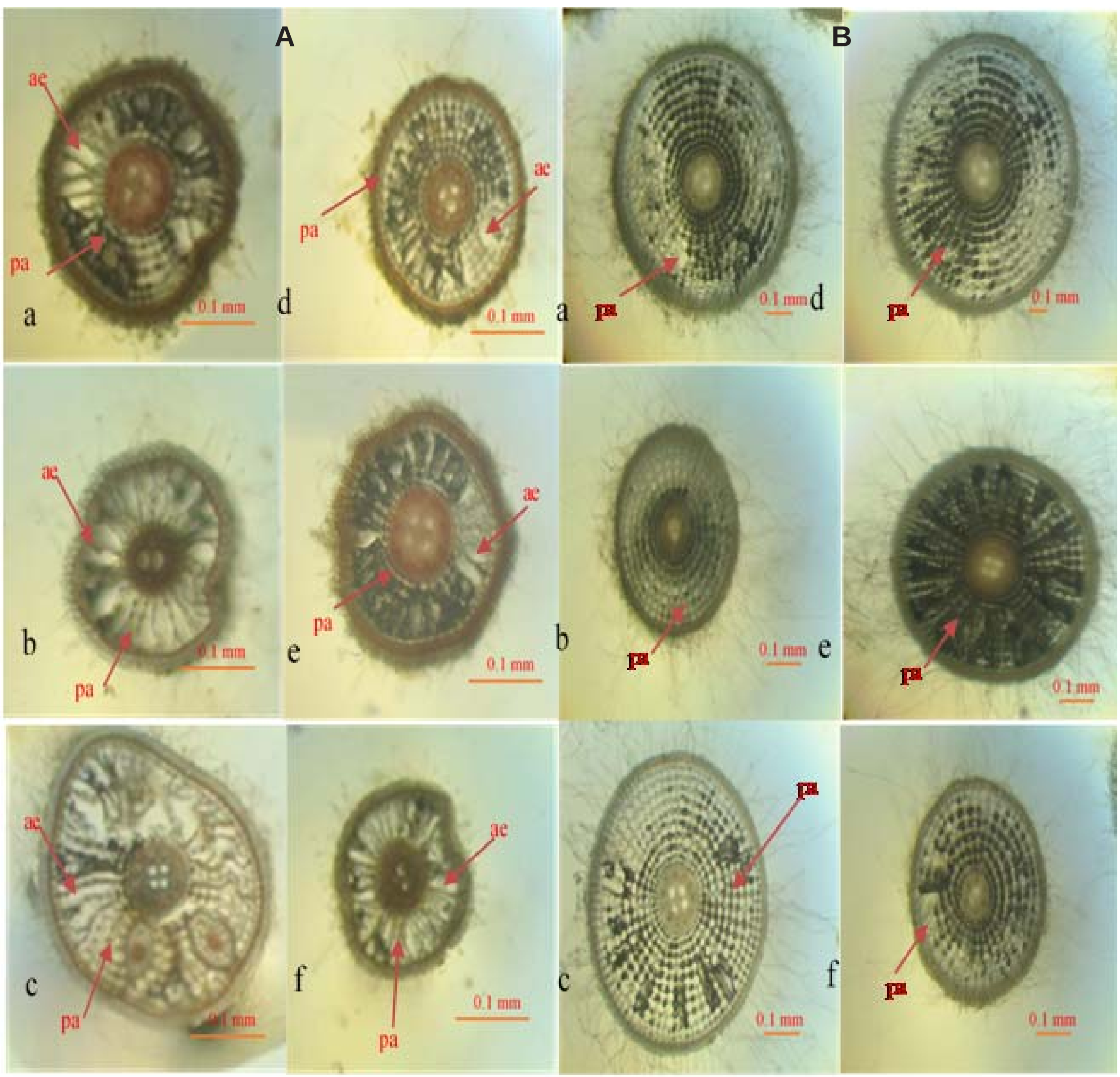

Fig. 2. Cross section of root at 10 mm of rice seedling after treated by 400 mg. L^{-1} of Fe^{2+} in Yoshida-Agar solution for 10 d. (A) and normal condition (B). The red color is stained by 2,2 bypiridine. *pa*, Parenchyma; *ae*, aerenchym; *lr*, lateral root, a, *IR64*; b, *Mahsuri*; c, *Pokkali*; d, *IPB107*; e, *Inpara2*; f, *Siam Saba*.

the center cylinder of the root while *Pokkali* was found only in the lateral root, indicating that iron had entered in to the root. The red color formation in shoot followed after iron had entered the root, where most of the iron was deposited in vascular bundle of *IR64* and *Inpara2* but it was not found in *Pokkali*. We found only parenchyma formation in 10 mm from the tips of main apical root under normal conditions (Fig 2B), but the present of space formation (aerenchym) was only found under iron stress. This result revealed that iron toxicity stress induced an aerenchym formation.

Fig 3 supports the result of iron staining where the less red color formation genotypes (*Mahsuri, Siam Saba,* and *Pokkali*) also had low shoot iron content. The ratio between total area of cross section of root with aerenchym formation (Fig. 4A) was also higher compared to that of the other genotypes. This pattern also was found in formation of aerenchym in the shoot (Fig. 4B). The wider aerenchym per root section facilitated the genotypes to oxidize the ferrous iron (Harahap *et al*. 2014) and we suspect that shoot aerenchym per cross section also has important role in transferring oxygen to the root. Higher iron plaque formation supported the oxidation ability of the genotypes. The plaque deposition is associated with substantial formation of aerenchym, leakage of oxygen into the root zone, and subsequently chemical oxidation of potentially toxic Fe^{2+} to precipitated Fe^{3+}. Iron plaque has also been proposed to act as a barrier or buffer to reduce the uptake of toxic elements (e.g. Al, Cd, As) into plant tissues (Liu *et al.*

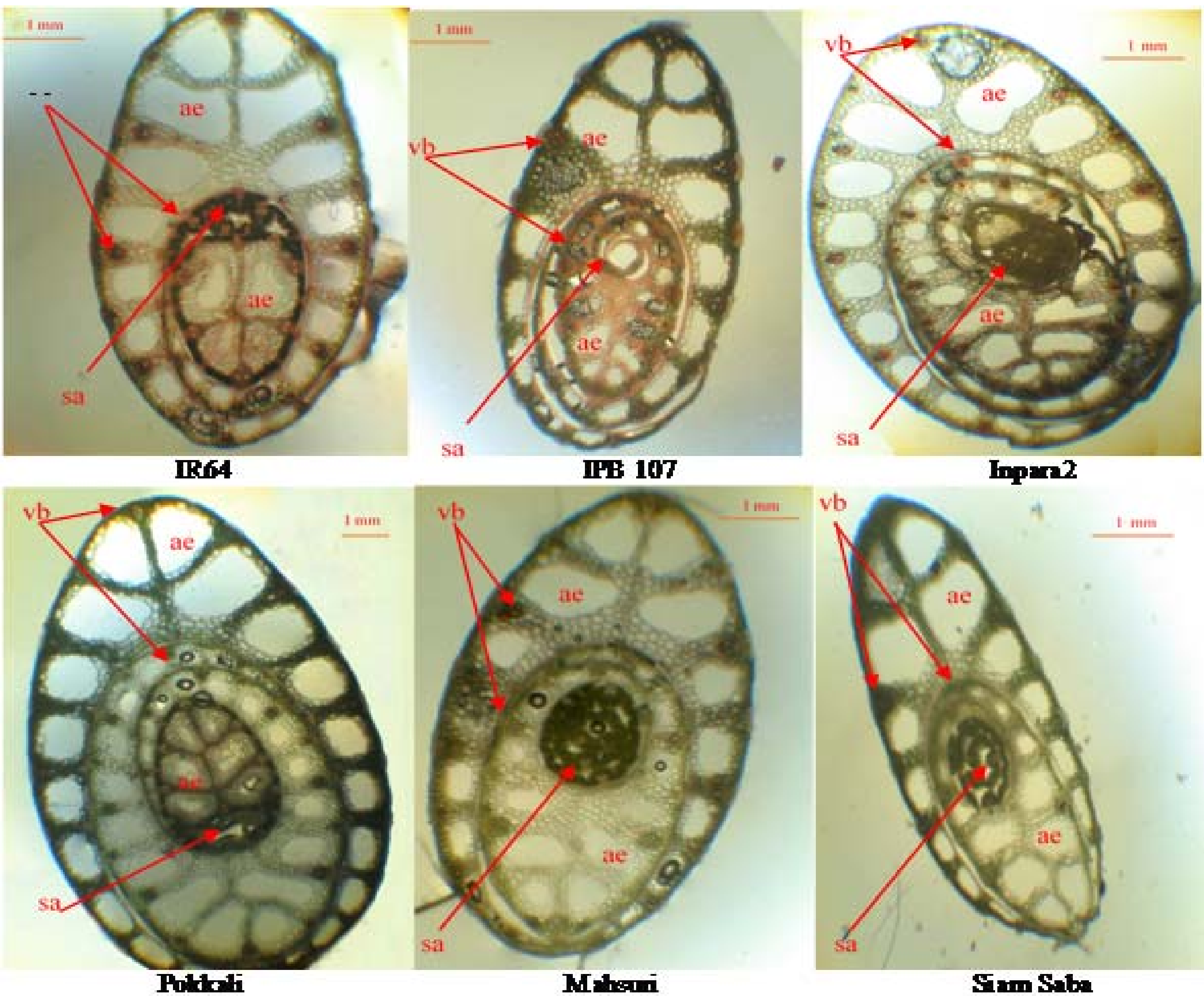

Fig. 3. Cross section of 20 mm above basal of shoot rice seedling after treated by 400 mg. L^{-1} of Fe^{2+} in Yoshida-Agar solution for 10 d. The red color is stained by 2,2 bypiridine. *vb*, vascular bundle, *sa*, shoot apical, *ae*, aerenchym.

2004, Liu *et al.* 2010, Chen *et al*. 2006). The amount of oxygen released was not quantified in the present study. However, the radial oxygen loss from rice roots could be determined using Eh microelectrodes or by methyl-blue staining of roots in agar (Kotula *et al*. 2009, Wu *et al.* 2014). The determination of this oxidation power of roots had been suggested as a screening tool for iron toxicity breeding of rice (Nozoe *et al*. 2008, Wu *et al.* 2014).

Based on these results we found that rice genotypes could be classified either as the includer tolerant-type such as *IR64, Inpara2* or the excluder tolerant-type such as *Mahsuri*, *Pokkali*, and *Siam Saba*. The research further confirmed the previously suggested tolerance mechanisms of rhizospheric exclusion or stress avoidance (Yoshida *et al*. 1976), Fe partitioning (Audebert and Sahrawat 2000) and leaf tissue tolerance (Thongbai and Goodman 2000, Engel *et al*. 2012b). The droplet-staining method developed in this research was simpler than that developed by Engel *et al.* (2012a), which needed to over-night sample staining. This method allows differentiating genotypes according to their tolerance type and accelerates the selection of candidate genotypes for future breeding of Fe-toxicity tolerance in rice.

Most of the genotypes reported in this study had different result with regard to that reported in the varietal description. For example, *IPB Kapuas 7R, IPB Batola 6R, A. Tenggulang, Indragiri, Kapuas and IPB Batola 5R*, each was reported as tolerant to Fe toxicity but reacted as moderately tolerant in this study. The different result could be due to the different characteristic of tolerance mechanisms or, due to the different methodology used in the determination of tolerance level. Most of the genotypes tested were improved cultivars which had been tested in many locations including at the iron toxic sites. Breeders might not be aware of the LBS as a selection criterion, as much as the grain yield. Therefore, it is possible to develop variety which has higher tolerance level similar to that *Siam Saba, Pokkali, Mahsuri,* and *Awan Kuning*, when the LBS was applied during course of selection.

The difference tolerance-type with regard to the Fe-upload in the shoot and root could be observed on the rice seedling. This study reveals that there is still a chance to develop more tolerant rice variety to iron toxicity by integrating these two distinct mechanisms through breeding program. Other implication of present study is that it is possible to develop high iron content in the grain. High iron concentration iron should be able to be taken up by root, transferred to the shoot via xylem loading, and stored in the grain (Masuda *et al*. 2012). High iron concentration in the shoot, however should not affect a negative effect on growth and development of rice plant. The two requisites are only possible when the genotypes has mechanism of includer tolerance-type, like that of *Inpara2*.

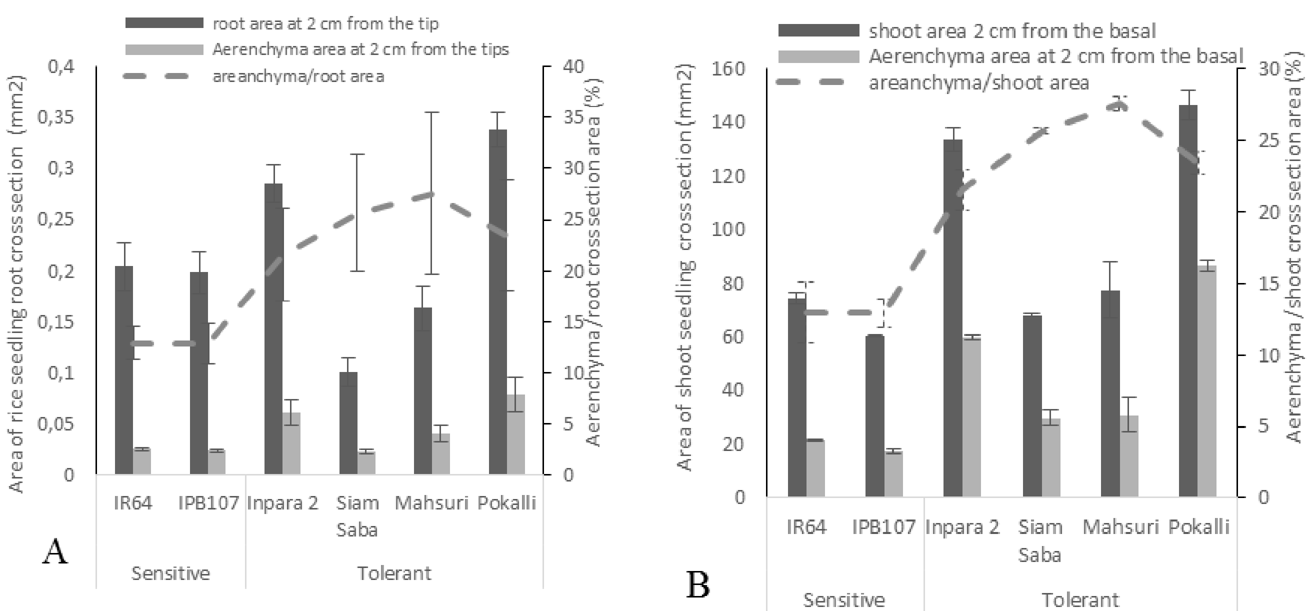

Fig 4. Area of root cross section, root aerenchym, and their ratio (A) and area of shoot cross section, shoot aerenchym and their ratio (B) of rice seedling under 400 mg. L^{-1} of ferrous iron. Error bar is standard error from three replications.

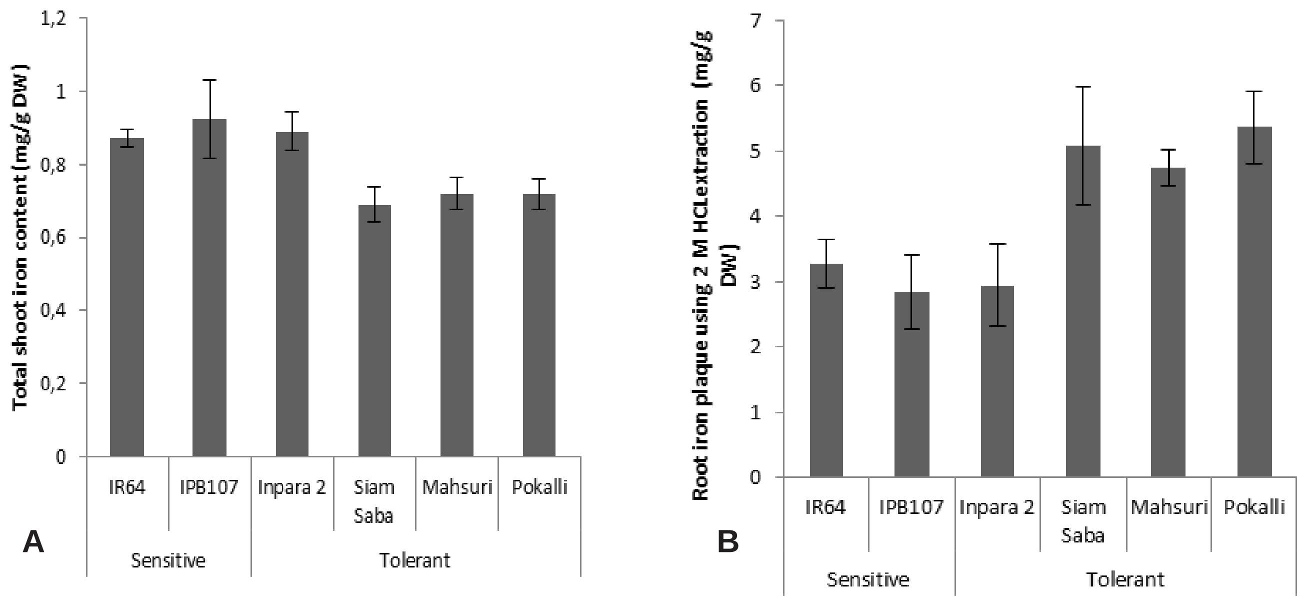

Fig 5. Root iron plaque (A) and total shoot iron content (B) of some genotypes of rice seedling under 400 mg. L^{-1} of ferrous iron. Error bar is standard error from three replications.

CONCLUSION

Variations on shoot dry weight, root dry weight, and shoot iron content were found among tested genotypes in respond to iron toxicity condition. The critical value for determining tolerant genotypes was 3.76 of LBS. Tolerant genotypes indicated either higher shoot dry weight (*Pokkali, Inpara2, Cilamaya Muncul*) or low shoot dry weight (*Siam Saba, Mahsuri, Awan Kuning*). Based on the status of Fe^{2+} in the root and shoot, we identified excluder-type tolerance (*Pokkali, Mahsuri and Siam Saba*) and an includer-type tolerance (*Inpara2*).

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