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Selection of local swamp rice cultivars from Kalimantan (Indonesia) tolerant to iron stress during vegetative stage

VINA NOVIANTI[1], DIDIK INDRADEWA[2], MARYANI[1], DIAH RACHMAWATI[1,♥]
[1]Department of Tropical Biology, Faculty of Biology, Universitas Gadjah Mada. Jl. Teknika Selatan, Sekip Utara, Sleman 55281, Yogyakarta, Indonesia. Tel./fax.: +62 274-580839, ♥email: drachmawati@ugm.ac.id; vina.novianti@mail.ugm.ac.id
[2]Department of Agronomy, Faculty of Agriculture, Universitas Gadjah Mada. Jl. Flora, Bulaksumur, Sleman 55281 Yogyakarta, Indonesia

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Abstract. *Novianti V, Indradewa D, Maryani, Rachmawati D. 2020. Selection of local swamp rice cultivars from Kalimantan (Indonesia) tolerant to iron stress during vegetative stage. Biodiversitas 21: 5595-5606.* Kalimantan with the large swamp area is a potential region in Indonesia to develop local rice cultivars with high tolerance to iron toxicity in tidal lowlands. This research was conducted to select iron toxicity-tolerant in local Kalimantan-rice cultivars, namely: Amas (AMS), Pandan Ungu (PDU), Kambang (KMB), Suatek Merah (SM), Siam Unus Mayang (SUM), Siam Unus Kuning (SUK), and Siam 11 Panjang (S11P). As control, two rice cultivars: Ciherang (iron toxicity-susceptible) and Inpara 5 (iron toxicity-tolerant) were also used. The seeds were grown hydroponically for 35 DAP (days after planting) in Yoshida nutrient solution containing 0 ppm (control), 100 ppm (low), and 400 ppm (high) FeS_2 as pyrite treatments. Morphological analysis (plant height, leaf length and width, and leaves number) showed that PDU and KMB cultivars have better characters for tolerating iron toxicity. The highest level of chlorophyll and carotenoid contents during iron toxicity treatment was observed in KMB cultivar. AMS cultivar had the highest proline level under high iron stress. Antioxidant enzyme analysis showed that AMS, PDU, and KMB cultivars had a high percentage increase in the activity of superoxide dismutase (SOD), hydrogen peroxide (H_2O_2), and ascorbate peroxidase (APX) enzymes when exposed to high pyrite conditions. Based on morphophysiological characteristics, biochemical component, and enzymatic antioxidant activity, it can be concluded that AMS, PDU, and KMB cultivars are potential candidates as iron toxicity-tolerant rice.

Keywords: Iron toxicity, Kalimantan, local rice, pyrite, tidal lowlands

INTRODUCTION

Indonesia is one of the countries whose population consumes rice as a staple food. The rice demand continues to increase every year in line with the rate of population growth. To compensate for this problem, efforts are needed to increase rice production in order to maintain sustainable food for self-sufficiency. One approach that can be done to increase rice productivity is by utilizing suboptimal land as additional rice production areas.

Tidal lowland is a suboptimal land that can be utilized for rice production. Tidal lowlands are widespread in Indonesia and very prospective as a substitute for fertile lands that are limited in Java and Bali. According to Sopandie (2013), Indonesian tidal lowland area is estimated to be around 20.11 million ha spread across the east coast of Sumatra (Lampung, South Sumatra, Jambi, Riau), the southern coast of South Kalimantan, and the southern coast of Irian Jaya. Tidal lowland is characterized by an irrigation system that relies on tides, low soil pH, pyrite (FeS_2) layer, nutrient deficiency, deep inundation, accumulation of Fe^{2+}, and Al^{3+} and low organic matter content (Anwar et al. 2001). The characteristics of tidal lowlands pose obstacles to the growth and productivity of rice, mainly due to the low soil pH and presence of pyrite. In aerobic conditions, pyrite will react with oxygen to form Fe^{3+} and SO_4^{2-} causes decreasing soil pH and be toxic to plants (Sopandie 2013). Asch et al. (2005) reported that iron toxicity is one limiting factor of rice production in the paddy field occurring in Asian countries such as China, India, Indonesia, Thailand, Malaysia, and Philippines.

The mechanism of plant adaptation to tidal lowlands is focused on the adaptation of plants to Fe, Al, and SO_4. Iron toxicity causes oxidative stress and superoxide radicals as the product of biological oxidation (Marschner 2012). The toxicity of the relatively unreactive superoxide radical and H_2O_2 will increase when it turns into a highly reactive hydroxyl radical, which causes severe damage to membranes, proteins, and DNA (Frei et al. 2016). Increased oxygen free radicals will trigger the formation of the enzyme superoxide dismutase (SOD) which encourages the formation of excess H_2O_2. H_2O_2 must also be detoxified with the enzyme ascorbate peroxidase (APX), peroxidase (POD), or catalase (CAT) (Nugraha et al. 2016).

Iron toxicity increases the activity of polyphenol oxidase and the oxidation of these polyphenols causes "bronzing" in leaves (Nugraha et al. 2016). Besides leaves bronzing, iron toxicity also causes stunted roots in plants and delayed harvesting age, while plant biomass and crop production decreased (Sahrawat 2004). Iron toxicity at the vegetative stage causes a decrease in plant height, dry weight, chlorophyll content, and the number of productive tillers (Fageria et al. 2008). At the further growth stage, iron toxicity causes a decrease in the number of panicles, an increase in the number of empty grains, delays in flowering, and maturation, thereby reduces productivity and yield (Audebert and Sahrawat 2000).

In general, there are three types of tolerance mechanisms to iron toxicity in rice, namely avoidance exclusion, avoidance inclusion, and tolerant inclusion (Wu et al. 2014). In addition to the three previous mechanisms, there is type-IV, membrane selectivity through the iron-regulating transporter protein (Nugraha and Rumanti 2017). Higher uptake of Fe by plants is known to reduce protein synthesis in leaves. According to Saikia and Baruah (2012), ferritin is considered important because it consists of a multimeric ball protein called phytoferritin, which is able to store up to 4500 Fe atoms in the cavity, in a non-toxic form. Ferritin functions as a cellular Fe buffer. Tolerant cultivars can accumulate more phytoferritin that forms complexes with Fe^{2+} reducing damage due to Fe toxicity (Saikia and Baruah 2012).

Crop tolerance to iron toxicity has become extremely important in agricultural development, especially in the areas of humid tropics. One approach that can be used to improve crop yields in tidal lowlands is to create rice cultivars that are tolerant against high levels of iron.

By observing morphological, physiological, and biochemical changes during iron stress associated with the tolerance of local Kalimantan swamp rice, the iron toxicity tolerant rice cultivar can be determined. From this study, information on the tolerance of these rice plants against iron stress conditions can be obtained to select potential iron toxicity tolerant rice cultivars.

MATERIALS AND METHODS

Plant material

Seven local swamp rice (*Oryza sativa* L.) cultivars collected from three different locations in Kalimantan Island, Indonesia, and two control rice cultivars of *O. sativa* 'Ciherang' (Iron susceptible cultivar) and *O. sativa* 'Inpara 5' (Iron tolerant cultivar) were used in this study (Table 1, Figure 1).

Pyrite toxicity experiment

The experiment was conducted at Plant Physiology Laboratory, Faculty of Biology, Universitas Gadjah Mada, Indonesia with mean temperature 27ºC (RH 82%) in the morning, and 31ºC (RH 62.5%) in the afternoon. This experiment was carried out in a completely randomized design (CRD) with two factors. The first factor was iron in the form of Fe-EDTA with three levels which were 0 ppm (control), 100 ppm (low iron), and 400 ppm (high iron), and the second factor was rice cultivar with nine rice cultivars (7 Kalimantan local rice and 2 control rice cultivars). The pyrite treatment was carried out by dissolving pyrite powder (FeS_2) using EDTA 0.1 M solution as a solvent in Yoshida's nutrient solutions. This treatment was chosen because it can represent 3 levels of iron in the field: 0 ppm (control), 50-250 ppm (low), and above 300 ppm (high) (Tanaka and Yoshida 1972; Lubis et al. 2016). The pyrite treatment was applied during the vegetative period from 14 to 35 DAP by hydroponic.

Table 1. Agronomic characteristics of Kalimantan (Indonesia) local rice and control of iron tolerant and sensitive rice cultivars

Abv	Cultivars	Origin	Seed description	Pericarp color	Hull color	Days to maturity (days)
AMS	Amas	Tana Tidung	The shape of the lancet, the ribs are not very clear	Pale white	Bright yellow	130-135
PDU	Pandan Ungu	Tana Tidung	Oval shape, slightly lanceolate, the centerline is clearly visible	Milky white	Bright yellow	107-113
KMB	Kambang	Tana Tidung	Long-form, lanceolate, less firm lines, the tip of the seeds have sharp-like structures	Pale white	Bright yellow	122-125
SM	Suatek Merah	Ketapang	Oval shape, seed diameter is firm, there are 4 rib lines on the seed	Pale white	Reddish light brown	165
SUM	Siam Unus Mayang	Barito Kuala	Very long and slim shape, the tip of the seeds have sharp-like structures, there are 4 rib lines on the seed	Ivory	Golden yellow	120-140
SUK	Siam Unus Kuning	Barito Kuala	Very long and slim shape, the tip of the seeds have sharp-like structures, less firm lines	Ivory	Golden yellow	120-140
S11P	Siam 11 Panjang	Barito Kuala	Very long and slim shape, lanceolate, the tip of the seeds have sharp-like structures, less firm lines	Ivory	Pale yellow	120-150
INP5	Inpara 5	Introduction from IRRI	Long-form, lanceolate, the centerline is clearly visible	Pale white	Bright yellow	115
CHR	Ciherang	IR18349-53-1-3-1-3/^{3}*IR19661-131-3-1-3//^{4}*IR64	Long-form, lanceolate, the ribs are not very clear	Pale white	Bright yellow	116-125

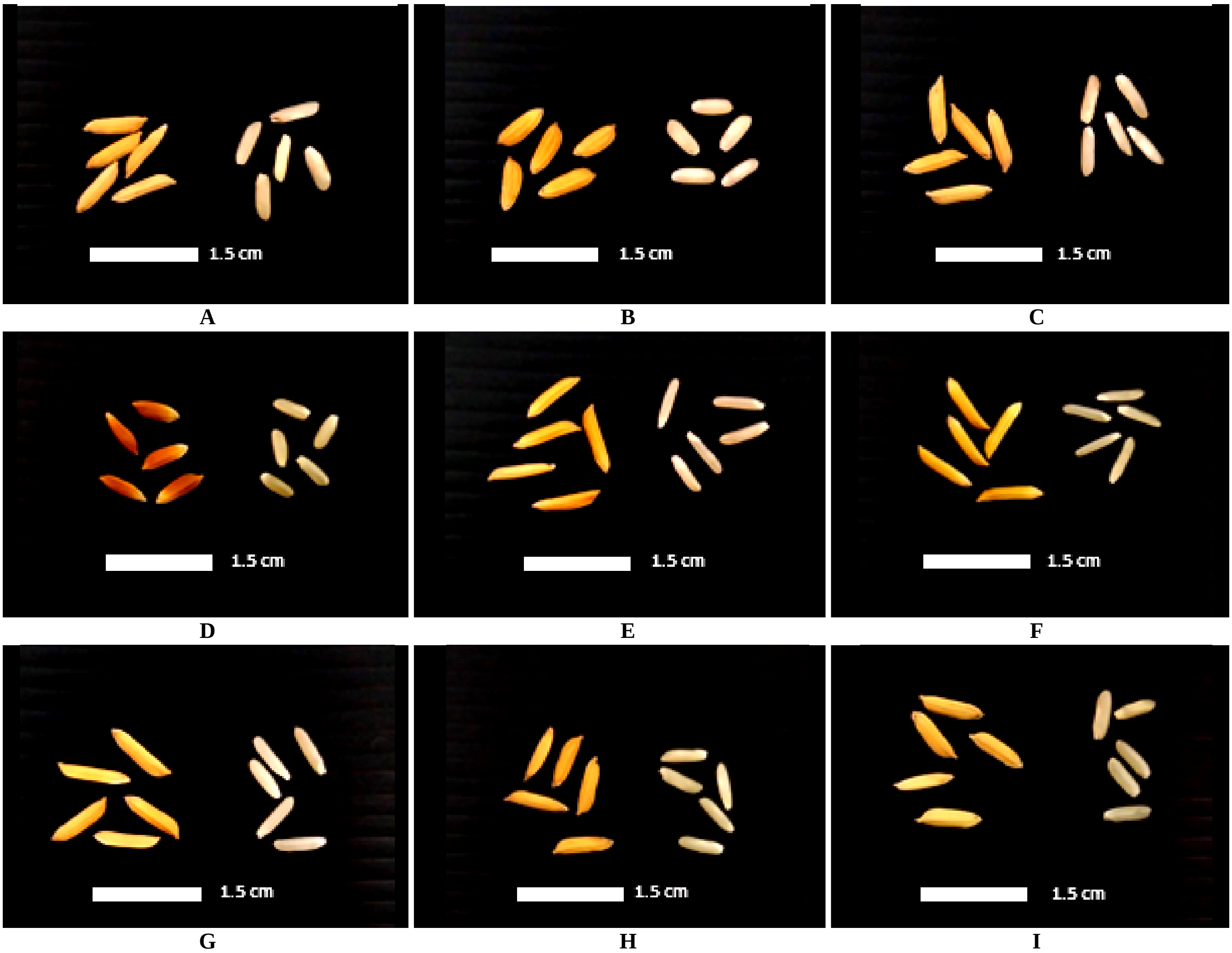


Figure 1. Kalimantan local rice seeds: A. Amas, B. Pandan Ungu, C. Kambang, D. Suatek Merah, E. Siam Unus Mayang, F. Siam Unus Kuning, G. Siam 11 Panjang, and two control rice seeds: H. Inpara 5, and I. Ciherang

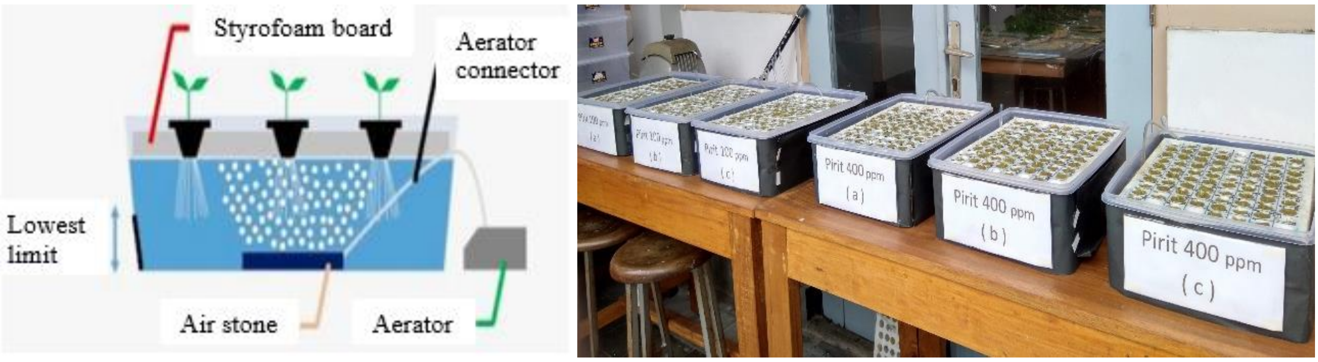
The rice seeds were sterilized using 10% sodium hypochlorite for 15 minutes, washed with distilled water and soaked for 24 hours. Germination was carried out for 3 days at room temperature. Three days old uniform seedlings were then hydroponically grown and acclimatized for 14 DAP (Days After Planting) in Yoshida medium as designed in Figure 2. To maintain nutrient needs, the Yoshida nutrient solution was refreshed once a week.

Each styrofoam board was in 33 × 26 × 2 cm^3 size and consisted of 80 holes. Each hole was 8 mm in diameter with a distance of 3 cm to each other. The styrofoam board was placed on top of plastic container containing 8 L of Yoshida nutrient solution. Each hole was used to grow one seedling. Each tray was used to randomly grow 9 cultivars and each iron level treatment had three trays.

Observation of morphological characters

Growth characters including plant height, leaf length and width, and leaf number were measured every 7 days, starting from the 14 DAP up to 34 DAP. Plant height was measured as the distance between the longest leaf tip and the root base above the rockwool surface. The leaf length and width were measured on the second leaf. Leaf number was determined by counting the number of total leaves.

Root morphology and leaf bronzing score (LBS) were carried out at 35 DAP. Plants were taken from the container, then the length of the roots was measured using a ruler. Measurements were made from the base to the longest root tip (base ± 2-3 cm above the planting surface) (Yoshida, 1981). Leaf bronzing scores (LBS) were measured on the three youngest fully expanded leaves of the main tiller. Leaf bronzing score indicates the severity of Fe toxicity ranging from 0 (healthy leaf without symptom) to 10 (dead leaf) (IRRI 2002). The score is rated on a scale of 0 - 10 where 0 means normal growth and 10 indicates that almost all plants die or are dying (Table 2).



A B

Figure 2. A. Floating raft hydroponic system design, and B. An installation of floating raft hydroponic systems

Table 2. Leaf bronzing scores (LBS) category and tolerance level (IRRI 2002)

Percentage of leaf area affected (%)	Bronzing score	Tolerance level
0	0	Very tolerant
1-9	1	Very tolerant
10-19	2	Tolerant
20-29	3	Tolerant
30-39	4	Moderate
40-49	5	Moderate
50-59	6	Susceptible
60-69	7	Susceptible
70-79	8	Very susceptible
80-89	9	Very susceptible
90-99	10	Very susceptible

Determination of chlorophyll and carotenoid levels

Physiological characters were measured on chlorophyll and carotenoid content according to the Harborne (1984) method with several modifications. A leaf sample of 0.3 g was ground with a mortar and homogenized with 3 mL of 80% cold acetone solution. Chlorophyll levels were determined through spectrophotometer readings (GENESYS 10 UV Scanning, Thermo Scientific) at multiwavelength of 470, 645, and 664 nm and expressed in $mg.g^{-1}$ FW (fresh weight).

Determination of proline content

Proline content was determined using method from Bates et al. (1973). Leaf samples of 0.25 g were ground and homogenized with 5 mL of 3% sulfosalicylic acid solution. In a ratio of 1: 1: 1 the sample was mixed with the ninhydrin reagent (containing ninhydrin, acetic acid, and phosphoric acid) and glacial acetic acid and then heated at a temperature of 95ºC for 60 minutes in a water bath (Memmer GmbH + Co.KG.WNB-7). The solution was cooled to 25ºC and reacted with toluene to form two layers. Proline levels were determined by comparing the absorbance of the solution at a wavelength of 520 nm and compared with the standard proline curve.

Determination of antioxidant activity

The procedure began with the extraction of enzymes from the leaves of rice plants, following the method described by Sunkar (2010). A sample of fresh leaves (200 mg) was ground with liquid nitrogen and homogenized in 2 mL potassium phosphate buffer 0.2 M (pH 7.8) containing 0.1 mM EDTA and 1.0% PVP. The sample was then centrifuged at 15,000 rpm for 20 min at 4℃. The homogenate was centrifuged at 8000 rpm at 4℃ for 15 min.

SOD activity was assayed using a protocol described in Kim et al (2017). Crude enzyme extract (8 µL) was added to 1 mL of Tris-HCl buffer (pH 8.2) containing 1 mM EDTA, 10 µL of pyrogallol 2mM, and 1 mL distilled water. The prepared assay mixture was immediately analyzed at 325 nm against a blank (which did not contain tissue homogenate or pyrogallol) at 3 min intervals in a spectrophotometer. The oxidation data for pyrogallol were taken each minute for 3 min and used to define 100% auto-oxidation. The measured data were expressed as units per milligram of protein (1 unit was the amount of enzyme utilized to inhibit 50% of pyrogallol auto-oxidation per minute).

APX activity was measured using the method described in Sunkar (2010). Crude enzyme extract (10 µL) was added to the reaction mixture, consisting of 1.3 mL 0.05 mM potassium phosphate buffer pH 7.8 containing 0.1 mM EDTA, 0.8 mL of 0.5mM ascorbate acid, 0.9 mL of 3% hydrogen peroxide, and the decrease in absorbance was measured after 1 min incubation from 1 to 3 min at 290 nm using a spectro-photometer. The collected data were used to define the reaction rate for H_2O_2 independent of ascorbate oxidation.

Determination of hydrogen peroxide (H_2O_2) content

The method used to extract and quantify H_2O_2 has been described by Bouazizi (2008). Fresh leaves sample (0.5 g) were ground with liquid nitrogen and homogenized in 3 mL of 5% TCA in cold conditions. The sample was then centrifuged at 10,000 rpm for 8 min at 4℃. The homogenate was used for H_2O_2 content determination.

The reaction mixture consisted of 0.8 mL homogenate, 0.2 mL of 5% TCA, 0.2 mL of 10 mM ferrous ammonium sulfate, and 0.2 mL of 2.5 M KCN was incubated for 60 min at room temperature. The mixture was vortexed until the color was changed to orange-peach, then directly pipet the mixture to a cuvette for measuring H_2O_2 contents using a spectrophotometer at 390 nm. The H_2O_2 content was determined by comparing the absorbance of the solution at a wavelength of 390 nm and compared with the standard H_2O_2 curve.

Data analysis

The plant growth characters (plant height, leaf length, and width, leaves number), leaf bronzing score, plant photosynthesis pigments, and other biochemical characteristics were analyzed using One-Way ANOVA and continued with Duncan's Multiple Range Test at 95% confidence level using IBM-SPSS Ver. 23.0 (US).

RESULTS AND DISCUSSION

Utilization of tidal lowlands for food crop cultivations, especially rice, faces several problems including low soil fertility, acidic soil reactions due to aerobic conditions, the pyrite layers, and high levels of Fe and organic acids (Arsyad et al. 2014). The obstacles to rice growth and productivity in that area are mainly due to the pyrite layer (Sopandie 2013). Under aerobic conditions, pyrite will react with oxygen to form Fe^{3+} and SO_4^{2-} which are toxic to plants. Iron levels that can be absorbed and do not interfere with the plant growth range between 50-200 ppm; the toxic level is above 300 ppm (Tanaka and Yoshida 1972). Lubis et al. (2016) showed that the increase of iron concentration from 143 to 325 ppm and duration of exposure to iron stress enhanced iron toxicity symptoms in rice. The process of identification through morphological growth characters, plant photosynthesis pigments, biochemical changes in proline levels, and enzymatic antioxidant responses, can be determinant characters of the tolerant genotype against iron poisoning conditions (Onyango et al. 2019).

Morphological growth characters

Plant growth of rice can be observed through changes in plant height, leaf length and width, and increase in the leaf number. The ability to grow to a certain height, leaf length, leaf width, and leaf numbers depends on genetics and environment interaction.

After 21 days of iron treatment, the growth characters including plant height, leaf length, and leaf width of each cultivar decreased significantly (P <0.05) in line with the increased iron level applied (Table 3). Increasing levels of pyrite from 0 to 100 ppm caused a decrease in plant height, leaf length, and width in all cultivars. But there was an increase in leaf number in Inpara 5, Pandan Ungu, and Kambang cultivars. Increasing levels of pyrite from higher than 100 to 400 ppm no longer reduced this variable for all cultivars tested. Based on the reduction percentage of morphological characters (Table 4), 'Pandan Ungu' cultivar has the lowest reduction of plant height (23.88%), leaf length (16.88%), and leaf width (30%), followed by 'Amas' with the reduction of plant height (31.60%), leaf length (28.46%), and leaf width (36%), and also 'Kambang' with the reduction of plant height (40.04%), leaf length (35.95%), and leaf width (46.97%). These three cultivars had lower reduction levels when compared to control tolerant plant (Inpara 5) in high level of iron stress conditions (pyrite 400 ppm). But in leaf number, 'Siam Unus Mayang' cultivar has the lowest reduction (2.58%). Meanwhile, there was an increase in leaf number in 'Inpara 5', 'Ciherang', 'Pandan Ungu', 'Kambang', dan 'Siam 11 panjang' cultivars. The increase of leaf number in 'Pandan Ungu' and 'Kambang' cultivars showed almost as same value as Inpara 5.

Based on these data it can be said that Pandan Ungu and Kambang showed tolerance to high pyrite levels. Thus, 'Pandan Ungu', 'Amas', and 'Kambang' cultivars are potentially tolerant to iron toxicity. These results obtained are in line with research by Fageria et al. (2008) that iron toxicity at the vegetative stage caused a decrease in plant height, dry weight, chlorophyll content, and the number of productive tillers. At the later stage of growth, iron toxicity caused a decrease in the number of panicles, an increase in the number of empty grains, delays in flowering and maturation, thereby reduced productivity and yields (Audebert and Sahrawat 2000). Besides, the development of shoots and tillers in the early vegetative stage is an important process that determines the number of productive tillers which not only contribute to the seed yield of a rice crop but also the ability to recover from inappropriate growth conditions (Singh et al. 2017; Lakunthod et al. 2018).

Table 3. Morphological characters of Kalimantan local swamp rice cultivars after 3 weeks treated with iron

Abv	Plant height (cm)			Leaf length (cm)			Leaf width (cm)			Leaf number		
	Pyrite 0 ppm	Pyrite 100 ppm	Pyrite 400 ppm	Pyrite 0 ppm	Pyrite 100 ppm	Pyrite 400 ppm	Pyrite 0 ppm	Pyrite 100 ppm	Pyrite 400 ppm	Pyrite 0 ppm	Pyrite 100 ppm	Pyrite 400 ppm
INP5	31.46^{efg}	21.99^{bc}	18.66^{ab}	21.14^{def}	16.78^{bc}	14.16^{ab}	0.67^{g}	0.38^{e}	0.36^{de}	$2.56^{c\text{-}f}$	3.45^{g}	3.44^{g}
CHR	32.96^{g}	17.86^{ab}	17.89^{ab}	23.11^{ef}	13.48^{ab}	13.51^{ab}	0.66^{g}	$0.32^{b\text{-}e}$	0.34^{cde}	$2.42^{c\text{-}f}$	$2.14^{a\text{-}e}$	2.98^{fg}
AMS	26.17^{cde}	19.68^{ab}	17.90^{ab}	19.01^{cd}	15.03^{abc}	13.60^{ab}	0.50^{f}	$0.32^{b\text{-}e}$	$0.32^{b\text{-}e}$	$2.39^{c\text{-}f}$	$2.18^{b\text{-}f}$	$2.55^{c\text{-}f}$
PDU	27.39^{def}	21.50^{abc}	20.85^{abc}	19.31^{cde}	16.67^{bc}	16.05^{bc}	0.50^{f}	0.35^{de}	0.35^{de}	$2.09^{a\text{-}d}$	$2.83^{d\text{-}g}$	$2.79^{d\text{-}g}$
KMB	30.02^{efg}	17.89^{ab}	18.00^{ab}	21.14^{def}	13.69^{ab}	13.54^{ab}	0.66^{g}	$0.29^{a\text{-}e}$	0.35^{de}	$2.17^{a\text{-}f}$	$2.57^{c\text{-}f}$	2.94^{efg}
SM	39.32^{h}	22.40^{bcd}	20.72^{abc}	28.48^{g}	17.46^{bcd}	15.93^{abc}	0.78^{h}	$0.32^{b\text{-}e}$	$0.32^{b\text{-}e}$	$2.43^{c\text{-}f}$	$2.07^{a\text{-}d}$	$2.24^{b\text{-}f}$
SUM	32.31^{fg}	20.90^{abc}	19.72^{ab}	23.47^{f}	15.34^{abc}	16.18^{bc}	$0.32^{b\text{-}e}$	0.22^{ab}	0.19^{a}	1.94^{abc}	1.50^{ab}	1.89^{abc}
SUK	34.56^{gh}	17.06^{ab}	15.82^{a}	25.18^{fg}	13.35^{ab}	11.65^{a}	0.37^{de}	0.20^{a}	0.23^{abc}	$2.08^{a\text{-}d}$	1.34^{a}	$2.14^{a\text{-}e}$
S11P	33.95^{g}	20.05^{ab}	20.25^{ab}	24.35^{f}	14.46^{ab}	15.25^{abc}	0.39^{e}	0.23^{abc}	$0.26^{a\text{-}d}$	$2.06^{a\text{-}d}$	$2.03^{a\text{-}d}$	$2.39^{c\text{-}f}$

Note: The mean value followed by the same letters in the row and column of each character indicates no significant difference at the 95% confidence level based on Duncan's multiple range test. pyrite 0 ppm: Control; pyrite 100 ppm: Low stress; and pyrite 400 ppm: High stress. INP5: Inpara 5, CHR: Ciherang, AMS: Amas, PDU: Pandan Ungu, KMB: Kambang, SM: Suatek Merah, SUM: Siam Unus Mayang, SUK: Siam Unus Kuning, S11P: Siam 11 Panjang

Table 4. Reduction percentage of morphological characters of rice cultivars after 3 weeks of iron treatment (pyrite 400 ppm).

Cultivars	Reduction Percentage (%)			
	Plant height	Leaf length	Leaf width	Leaf number
Inpara 5	40.69	33.02	46.27	-34.38
Ciherang	45.72	41.54	48.48	-23.14
Amas	31.60	28.46	36.00	-6.69
Pandan Ungu	23.88	16.88	30.00	-33.49
Kambang	40.04	35.95	46.97	-35.48
Suatek Merah	47.30	44.07	58.97	7.82
Siam Unus Mayang	38.97	31.06	40.63	2.58
Siam Unus Kuning	54.22	53.73	37.84	-2.88
Siam 11 Panjang	40.35	37.37	33.33	-16.02

Note: The reduction percentage indicating the delta value between control and iron treatment (pyrite 400 ppm). The more reduction percentage value is, the more decreasing of each parameter in each cultivar

Root morphology

The ability of plants to optimize the absorption of water and nutrients from the planting medium is influenced by the root character (Li et al. 2016). The morphology of the root system is influenced by many factors including the amount of fertilizer, temperature, planting media, and also environmental stress factors including pyrite (Jian et al. 2010).

Generally, the longer of root, the greater the ability to absorb water from the growing media. In normal conditions, variation among the root system of each cultivar represented genetic diversity (Nurhasanah 2019). In 0 ppm pyrite condition, most of the cultivars tested had root lengths that were not different from Inpara 5 cultivar, except for the local cultivars Kambang and Siam Unus Mayang which had shorter roots. When the soil medium contains 100 ppm pyrite (low iron stress), all cultivars have shorter roots than Inpara 5. In 400 ppm pyrite (high iron stress), local cultivars Amas, Pandan Ungu, Kambang, and Suatek Merah have roots that are no different from Inpara 5. Meanwhile, local cultivars Siam Unus Mayang, Siam Unus Kuning, and Siam 11 Panjang had shorter roots than Inpara 5, and were no different from the roots of the Ciherang (Figure 3 and Table 5).

Table 5. The root length (cm) of Kalimantan local swamp rice cultivars after 3 weeks treated with iron.

Cultivars	Pyrite Treatment		
	0 ppm	100 ppm	400 ppm
Inpara 5	16.10 ij	18.43 j	13.67 ghi
Ciherang	13.30 f-i	13.10 e-i	10.23 b-f
Amas	18.43 j	13.57 ghi	13.50 ghi
Pandan Ungu	16.03 ij	13.17 e-i	13.27 f-i
Kambang	10.10 b-e	12.40 d-h	11.17 c-g
Suatek Merah	16.10 ij	15.33 hi	13.43 ghi
Siam Unus Mayang	12.67 d-h	9.90 a-d	9.33 abc
Siam Unus Kuning	13.17 e-i	13.17 e-i	7.13 a
Siam 11 Panjang	15.53 hij	15.40 hi	7.93 ab

The mean value followed by the same letters in the row and column indicates no significant difference at the 95% confidence level based on Duncan's multiple range test

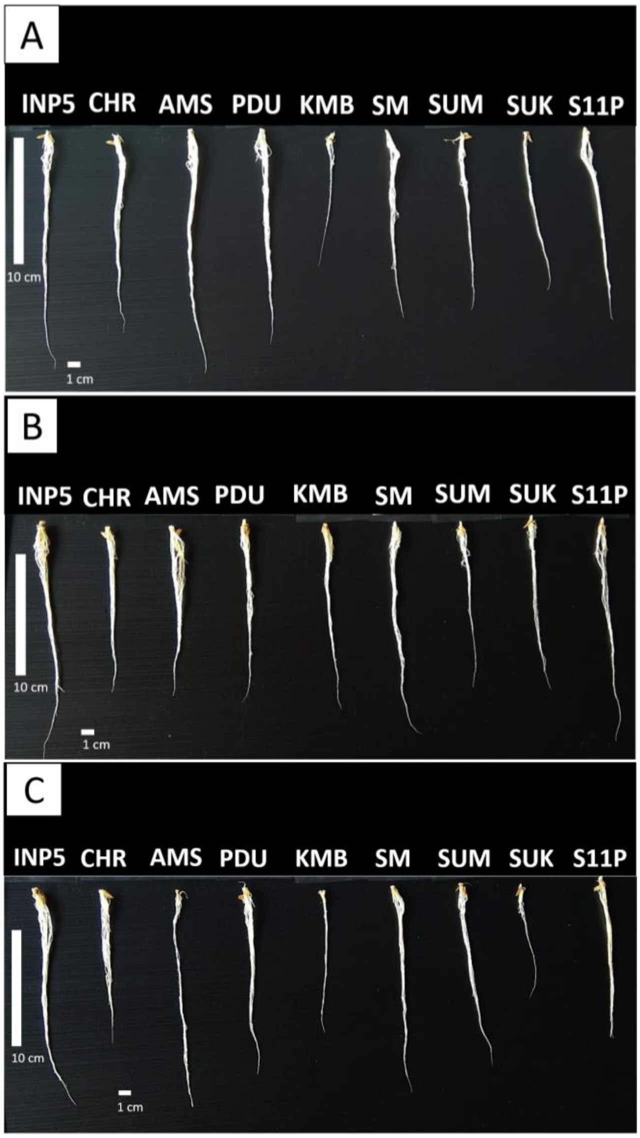


Figure 3. Morphology of rice roots after 3 weeks iron treatment. Control (A), pyrite 100 ppm (B), pyrite 400 ppm (C). Note: INP5: Inpara 5, CHR: Ciherang, AMS: Amas, PDU: Pandan Ungu, KMB: Kambang, SM: Suatek Merah, SUM: Siam Unus Mayang, SUK: Siam Unus Kuning, S11P: Siam 11 Panjang. Bars = 1 cm

Almost all cultivars decreased their root length when the iron level increased from 0 ppm to 400 ppm, while cultivar KMB did not. The longest root under high iron stress (pyrite 400 ppm) was observed in 'Amas' as compared to other local cultivars (Figure 3 and Table 5). When compared to iron susceptible cultivar (Ciherang) in high iron stress (pyrite 400 ppm) 'Amas', 'Pandan Ungu', 'Kambang', and 'Suatek Merah' cultivars have longer roots (Table 5). Based on the reduction percentage of root length (Table 7), when compared to other cultivars in high level of iron stress conditions (pyrite 400 ppm) 'Suatek Merah' cultivar has the lowest reduction of root length (16.58%), followed by 'Pandan Ungu' with the reduction of root length (17.22%). This result showed almost as same value as Inpara 5 (control tolerant plant). Meanwhile, there was an increase in root length in 'Kambang' cultivar.

These results are in line with research conducted by Alia et al. (2015) which showed that high concentrations of Fe and Al significantly inhibited root growth, and shrunk at the root and root surface, root surface tissue appeared to tear and form a bulge/torn. The condition of iron toxicity can also reduce the development of roots and reduce the biomass of rice seedlings (Zhang et al. 2015). This research showed that 4 of 7 local Kalimantan swamp rice cultivars ('Amas', 'Pandan Ungu', 'Kambang', and 'Suatek Merah') have longer roots in high iron stress (pyrite 400 ppm) and for 'Amas', 'Pandan Ungu', and 'Suatek Merah' cultivars were almost as same length as iron tolerant cultivar (Inpara 5). It is indicated that some local Kalimantan swamp rice cultivars have an ability to adapt and grow well in iron toxicity conditions.

Leaf Bronzing Score (LBS)

Audebert and Fofana (2009) reported that for each increase in leaves bronzing score reduced yields by 500 $kg.ha^{-1}$. Other studies also reported the negative correlation between bronzing scores and crop yields under iron stress conditions (Onaga et al. 2013). The relationship between leaf bronzing score and yields depend on rice genotypes and experimental conditions.

Fe toxicity causes plants to "bronze" on the leaves. Due to the presence of Fe toxicity, the activity of polyphenol oxidase increased and the oxidation of these polyphenols caused leaf bronzing (Nugraha et al. 2016). The high level of iron applied (pyrite 400 ppm) increased leaf bronzing score (LBS) in rice leaves. Leaf bronzing was significantly increased ($p < 0.05$) in all rice cultivars both in low iron stress levels (pyrite 100 ppm) and high iron stress levels (pyrite 400 ppm) compared to controls (pyrite 0 ppm) (Table 6). In a high iron stress (pyrite 400 ppm) condition, the lowest LBS (most tolerant to iron stress) was shown by two local cultivars namely 'Pandan Ungu' and 'Kambang', while the highest LBS (most sensitive to iron stress) was observed in 'Siam Unus Mayang' cultivar. In leaf bronzing score, increasing levels of iron stress from 0 ppm pyrite to 100 ppm and 400 ppm increased leaf bronzing score in all rice cultivars (Table 6). But in this condition, the lower score of leaf bronzing on high iron stress levels could be related to the higher tolerance level of the plant to iron toxicity. The response of tolerant plants in iron toxicity tends to be demonstrated through lower LBS scores as a form of enzymatic antioxidant defense against oxidative reactions and the emergence of ROS due to iron stress in cells (Sikirou et al. 2015; Xie et al. 2019).

Table 6. Leaf Bronzing Score (LBS) in rice cultivars after 3 weeks iron treatment

Cultivars	**Pyrite Treatment**		
	0 ppm	**100 ppm**	**400 ppm**
Inpara 5	1.25^{a}	$3.16^{c\text{-}h}$	$3.45^{d\text{-}i}$
Ciherang	$2.17^{a\text{-}d}$	$3.67^{d\text{-}i}$	$4.22^{f\text{-}j}$
Amas	1.25^{a}	$3.76^{e\text{-}j}$	$3.43^{d\text{-}h}$
Pandan Ungu	1.54^{ab}	$2.96^{b\text{-}f}$	$2.63^{a\text{-}e}$
Kambang	1.13^{a}	$3.17^{c\text{-}h}$	$3.05^{c\text{-}g}$
Suatek Merah	1.75^{abc}	4.68^{hij}	$4.51^{g\text{-}j}$
Siam Unus Mayang	1.83^{abc}	4.67^{hij}	5.19^{j}
Siam Unus Kuning	1.83^{abc}	$4.29^{f\text{-}j}$	4.97^{ij}
Siam 11 Panjang	$2.21^{a\text{-}d}$	5.20^{j}	$4.28^{f\text{-}j}$

The mean value followed by the same letters in the row and column indicates no significant difference at the 95% confidence level based on Duncan's multiple range test

Table 7. Reduction percentage of root length of rice cultivars after 3 weeks of iron treatment (pyrite 400 ppm)

Cultivars	**Reduction percentage (%) of root length**
Inpara 5	15.09
Ciherang	23.08
Amas	26.75
Pandan Ungu	17.22
Kambang	-10.59
Suatek Merah	16.58
Siam Unus Mayang	26.36
Siam Unus Kuning	45.86
Siam 11 Panjang	48.94

Note: The reduction percentage indicating the delta value between control and iron treatment (pyrite 400 ppm). The more reduction percentage value is, the more decreasing of each parameter in each cultivar

Figure 4. Leaf Bronzing Score (LBS) scale in rice (Wu et al. 2014). Note: The score is rated on a scale of 0 - 10 where 0 means normal growth and 10 indicates that almost all plants die or are dying.

Symptoms of leaf bronzing (LBS) is a secondary key character assessed visually in rice (IRRI 2002). This key character can be rapidly and easily identified (Figure 4). Secondary key characters must have sufficient genetic variability, easier and cheaper to measure than grain yields, must have high heritability and positive genetic correlation with yields (Lafitte et al. 2003). It is possible to develop iron toxicity-tolerant varieties by combining high yields with low LBS scores (Audebert et al. 2006; Gridley et al. 2006). Audebert and Sahrawat (2000) and Dramé et al. (2010) showed a strong negative correlation of 0.98 and 0.50 respectively, between LBS or leaf Fe content and grain yield for respective trials conducted in Korhogo (a Fe toxicity hotspot in Ivory Coast) and in three countries (southern Benin, Nigeria, and Burkina Faso).

Plant photosynthesis pigments

The photosynthetic performance of a plant can be observed from changes in photosynthetic pigment chlorophyll and carotenoids. This character is related to the plant growth character under iron stress conditions (Nenova 2008).

Iron stress treatment significantly ($P < 0.05$) affected total chlorophyll content in all rice cultivars. Plants experienced a decrease in chlorophyll levels in line with an increase in iron stress levels (Table 8). The decrease of total chlorophyll content varied in different rice cultivars. Most of Kalimantan local rice cultivars, experienced a significant decrease of chlorophyll content in the high iron stress treatment, except 'Pandan Ungu' and 'Kambang' cultivars. These two local rice cultivars had higher total chlorophyll content in 400 ppm pyrite treatment. These results assumed that those two cultivars had a high tolerance in iron toxicity conditions so that allowing the photosynthetic system to sustain photosynthesis under normal conditions. Meanwhile the cultivar "Siam 11 Panjang" showed the lowest chlorophyll content, this cultivar susceptible to iron stress. Photosynthetic pigments tend to decrease with increasing iron stress (Onyango et al. 2019).

The same trend of decreasing carotenoid levels was observed in the local Kalimantan rice cultivars exposed to iron stress (Table 8). The carotenoid reduction level varied in different cultivar. A higher level of carotenoids content was observed in Kambang and Pandan Ungu compared to other cultivars, especially in the low concentration of iron stress treatment (pyrite 100 ppm). Meanwhile, cultivar 'Siam 11 Panjang' had the lowest carotenoid levels, indicating that this cultivar was the most susceptible to iron toxicity.

Under stress conditions, plants will form self-defense and reduce the rate of metabolism to survive. For this reason, carotenoid as one of the plant photosynthetic pigments will be decreased. Carotenoids are potential scavengers for ROS that protect pigments and unsaturated lipid fatty acids from oxidative damage during iron toxicity (Strzalka et al., 2003). Previous studies showed that carotenoids also protect plant tissue by modulating photosynthetic compartments involving the xanthophyll cycle (Zhai et al. 2016).

Changes in proline levels

In the process of defense against iron stress conditions (mainly through osmosis adjustment), plants accumulate osmoprotectants in tissues. The osmoprotectant produced belongs to the sugar and amino acid group, including the highest proline level. Proline acts as an osmoregulator so that the higher the level, the greater the tolerance to iron poisoning stress.

Rice cultivars that accumulate higher proline have a better morphological performance with higher survival compared to plants with lower proline content. Both iron treatments, 100 and 400 ppm, increased proline levels in all cultivars compared to control (pyrite 0 ppm) (Table 9).

Table 9. Proline levels in rice after 2 weeks in iron treatment.

Cultivars	Proline (ug/g FW)		
	Pyrite 0 ppm	**Pyrite 100 ppm**	**Pyrite 400 ppm**
Inpara 5	0.17^{a}	0.52^{bcd}	0.62^{b-f}
Ciherang	0.15^{a}	0.68^{c-g}	0.82^{fg}
Amas	0.18^{a}	0.83^{g}	1.28^{h}
Pandan Ungu	0.14^{a}	0.75^{efg}	0.44^{b}
Kambang	0.18^{a}	0.56^{b-e}	0.65^{c-g}
Suatek Merah	0.17^{a}	0.56^{b-e}	0.55^{bcd}
Siam Unus Mayang	0.10^{a}	0.42^{b}	0.57^{b-e}
Siam Unus Kuning	0.17^{a}	0.49^{bc}	0.71^{d-g}
Siam 11 Panjang	0.14^{a}	0.55^{bcd}	0.76^{efg}

The mean value followed by the same letters in the row and column indicates no significant difference at the 95% confidence level based on Duncan's multiple range test.

Table 8. Changes in chlorophyll and carotenoid pigments in rice cultivars after 2 weeks of iron treatment

Cultivars	Chlorophyll (mg/g FW)			Carotenoid (mg/g FW)		
	Pyrite 0 ppm	**Pyrite 100 ppm**	**Pyrite 400 ppm**	**Pyrite 0 ppm**	**Pyrite 100 ppm**	**Pyrite 400 ppm**
Inpara 5	3.07^{e}	1.71^{a-e}	1.81^{a-e}	0.19^{g}	0.09^{a-f}	0.09^{a-f}
Ciherang	2.80^{de}	2.10^{b-e}	1.34^{a-e}	0.17^{fg}	0.10^{a-g}	0.07^{a-e}
Amas	2.48^{cde}	1.12^{a-d}	1.32^{a-e}	0.16^{efg}	0.06^{a-d}	0.08^{a-f}
Pandan Ungu	1.78^{a-e}	2.13^{b-e}	1.42^{a-e}	0.11^{b-g}	0.12^{b-g}	0.09^{a-f}
Kambang	1.65^{a-e}	2.63^{de}	2.10^{b-e}	0.10^{a-g}	0.13^{c-g}	0.10^{a-g}
Suatek Merah	1.46^{a-e}	0.69^{ab}	0.72^{abc}	0.09^{a-f}	0.04^{abc}	0.05^{a-d}
Siam Unus Mayang	2.26^{b-e}	1.06^{a-d}	1.19^{a-d}	0.14^{d-g}	0.06^{a-d}	0.07^{a-e}
Siam Unus Kuning	1.64^{a-e}	1.28^{a-d}	0.70^{ab}	0.10^{a-g}	0.06^{a-e}	0.05^{a-d}
Siam 11 Panjang	0.85^{abc}	0.21^{a}	0.51^{ab}	0.05^{a-d}	0.01^{a}	0.03^{ab}

Note: The mean value followed by the same letters in the row and column indicates no significant difference at the 95% confidence level based on Duncan's multiple range test.

Proline works through the osmoregulation mechanism by regulating the osmotic potential in the cell through the transfer of dissolved compounds to gain osmotic potential balance between internal and external of the cell (Slama et al. 2015). Plants with higher levels of osmotic enhancement are assumed to be plants that are more tolerant of iron poisoning. Based on the data obtained, it can be seen that the cultivar with the highest proline level under 400 ppm of pyrite is the local rice cultivar 'Amas'. This result indicates that Amas is a potential cultivar to be tolerant of high iron treatment. According to Hayat et al. (2012), proline is an osmoprotectant of the amino acid group that functions to maintain cells from osmotic stress and oxidative stress as well as metal chelators, sub-cellular structure stabilizers (membranes and proteins), regulators of $NADP^+$ / NADPH ratios, and acts as a protein hydrotrope. Hayat et al. (2012) also stated that proline is responsible for scavenging ROS and other free radicals. An exogenous proline application at an excessive level (40-50 mM) provides a low growth effect in rice plants with abiotic stress. The accumulation of proline in cells can regulate the balance of osmotic differences between the cell surroundings and the cytosolic environment (Slama et al. 2015; Singh et al. 2017). Osmoprotectants are universal in regulating osmotic adjustment in cells, preventing the effects of damage by ROS, and preventing damage to membranes and metabolic enzymes (Ashraf and Foolad 2007).

Based on the reduction percentage of chlorophyll and carotenoid (Table 10), 'Pandan Ungu' cultivar has the lowest reduction of chlorophyll (20.22%) and carotenoid (18.18%). But in ‘Kambang’ cultivar, there was an increase of chlorophyll and no reduction of carotenoid. These two cultivars had lower reduction levels when compared to control tolerant plant (Inpara 5) in high level of iron stress conditions (pyrite 400 ppm). In proline, increasing levels of iron stress from 0 ppm pyrite to 100 ppm and 400 ppm increased proline levels in all rice cultivars. However, in Pandan Ungu, Kambang, and Suatek Merah cultivars, the increase of proline levels was almost the same value as Inpara 5 (control tolerant plant).

Table 10. Reduction percentage of chlorophyll, carotenoid, and proline of rice cultivars after 2 weeks of iron treatment (pyrite 400 ppm).

Cultivars	Reduction Percentage (%)		
	Chlorophyll	**Carotenoid**	**Proline**
Inpara 5	41.04	52.63	-264.71
Ciherang	52.14	58.82	-446.67
Amas	46.77	50.00	-611.11
Pandan Ungu	20.22	18.18	-214.29
Kambang	-27.27	0.00	-261.11
Suatek Merah	50.68	44.44	-223.53
Siam Unus Mayang	47.35	50.00	-470.00
Siam Unus Kuning	57.32	50.00	-317.65
Siam 11 Panjang	40.00	40.00	-442.86

Note: The reduction percentage indicating the delta value between control and iron treatment (pyrite 400 ppm). The more reduction percentage value is, the more decreasing of each parameter in each cultivar

Enzymatic antioxidant activity

The mechanism of plant adaptation to tidal lowland ecosystems is focused on the adaptation of plants to Fe, Al, the acidity of pH and SO_4. Fe toxicity causes oxidative stress, toxic reduced O_2 which is a by-product of biological oxidation. The toxicity of the relatively unreactive superoxide radical and H_2O_2 will increase when it turns into a highly reactive hydroxyl radical, which causes severe damage to membranes, proteins, and DNA (Frei et al. 2016).

Increased oxygen free radicals trigger the formation of the enzyme superoxide dismutase (SOD) which encourages the formation of excessive H_2O_2, which must also be detoxified with the enzyme ascorbate peroxidase (APX), peroxidase (POD) or catalase (CAT) (Nugraha et al. 2016).

Table 11. Changes in SOD, H_2O_2, and APX in rice cultivars after 1 week of iron treatment.

Cultivars	SOD (U/L)			H_2O_2 (ppm)			APX (U/L)		
	Pyrite 0 ppm	**Pyrite 100 ppm**	**Pyrite 400 ppm**	**Pyrite 0 ppm**	**Pyrite 100 ppm**	**Pyrite 400 ppm**	**Pyrite 0 ppm**	**Pyrite 100 ppm**	**Pyrite 400 ppm**
Inpara 5	0.33^{a-e}	0.60^{e-h}	0.65^{fgh}	7.63^{b-g}	10.74^{ij}	6.90^{bcd}	0.58^{b-e}	0.51^{bcd}	0.36^{a-d}
Ciherang	0.43^{b-f}	0.38^{b-f}	0.74^{gh}	7.05^{b-e}	8.16^{b-h}	6.44^{bc}	0.51^{bcd}	0.57^{b-e}	0.28^{abc}
Amas	0.25^{a-d}	0.27^{a-d}	0.42^{b-f}	7.68^{b-g}	9.57^{f-i}	8.73^{c-i}	0.73^{de}	0.38^{a-d}	0.30^{abc}
Pandan Ungu	0.20^{abc}	0.39^{b-f}	0.44^{b-f}	7.86^{b-h}	12.13^{j}	9.85^{ghi}	0.94^{e}	0.37^{a-d}	0.08^{a}
Kambang	0.25^{a-d}	0.53^{d-h}	0.48^{d-g}	6.77^{bcd}	9.32^{e-i}	8.84^{d-i}	0.38^{a-d}	0.30^{abc}	0.33^{a-d}
Suatek Merah	0.40^{b-f}	0.27^{a-d}	0.18^{ab}	6.28^{b}	10.17^{hij}	7.40^{b-f}	0.62^{cde}	0.24^{abc}	0.29^{abc}
Siam Unus Mayang	0.18^{ab}	0.57^{e-h}	0.50^{d-g}	7.75^{b-g}	7.25^{b-f}	8.99^{d-i}	0.52^{bcd}	0.25^{abc}	0.35^{a-d}
Siam Unus Kuning	0.32^{a-e}	0.44^{b-f}	0.46^{c-f}	4.30^{a}	7.07^{b-e}	8.49^{b-i}	0.61^{cde}	0.45^{a-d}	0.28^{abc}
Siam 11 Panjang	0.09^{a}	0.25^{a-d}	0.79^{h}	9.83^{ghi}	9.42^{e-i}	9.51^{f-i}	0.40^{a-d}	0.46^{a-d}	0.17^{ab}

Note: The mean value followed by the same letters in the row and column indicates no significant difference at the 95% confidence level based on Duncan’s multiple range test.

Table 12. Reduction percentage of SOD, H_2O_2, and APX of rice cultivars after 1 week of iron treatment (pyrite 400 ppm)

Cultivars	Reduction percentage (%)		
	SOD	H_2O_2	APX
Inpara 5	-96.97	9.57	37.93
Ciherang	-72.09	8.65	45.10
Amas	-68.00	-13.67	58.90
Pandan Ungu	-120.00	-25.32	91.49
Kambang	-92.00	-30.58	13.16
Suatek Merah	55.00	-17.83	53.23
Siam Unus Mayang	-177.78	-16.00	32.69
Siam Unus Kuning	-43.75	-97.44	54.10
Siam 11 Panjang	-777.78	3.26	57.50

Note: The reduction percentage indicating the delta value between control and iron treatment (pyrite 400 ppm). The more reduction percentage value is, the more decreasing of each parameter in each cultivar

From Table 11 and 12, it can be seen that iron treatment promote SOD level to all cultivars treated, but only Ciherang and Siam 11 Panjang that give significant increases in high iron stress levels (pyrite 400 ppm) compared to control (pyrite 0 ppm) after 1-week iron treatment. Ciherang as a control susceptible cultivar has a highly increased SOD enzyme activity in high iron stress levels (pyrite 400 ppm). From local Kalimantan cultivar, Siam 11 Panjang also has a highly increased SOD enzyme activity compared to other local cultivars. This indicated that Siam 11 Panjang cultivar is the more susceptible rice cultivars to iron toxicity. Inpara-5 as a control tolerant cultivar showed the increasing SOD enzyme activity but not too drastic, same as Amas, Pandan Ungu, Kambang, Siam Unus Mayang, and Siam Unus Kuning cultivars. Mittler (2002) reported that SOD activity also increases at the beginning of the stress period and decreases in line with the increase in the stress period. The results in Table 11 show that SOD enzyme activity affected to the level of H_2O_2 produced. This is because H_2O_2 is a product of SOD enzyme activity in plants.

It is shown that Pandan Ungu cultivar has the highest H_2O_2 levels in low (pyrite 100 ppm) and high iron stress levels (pyrite 400 ppm) (Table 11 and 12). The same pattern is shown by Inpara 5 as a control tolerant cultivars. SOD activity is correlated with H_2O_2 production. Plants with a high tolerance to iron toxicity showed higher levels of H_2O_2. SOD converts singlet oxygen ($O2^{-*}$) into H_2O_2. The correlation of H_2O_2 levels is positively correlated with an increase in SOD activity in cells at the beginning of the stress period and decreased in line with the increase in the stress period (Mittler 2002). The accumulation of oxygen singlets is produced through the excitation of chlorophyll at the PS II reaction center as a negative consequence of increased stress (Foyer and Noctor 2003). In this condition, increased photorespiration due to limited CO_2 fixation by RuBP increases the accumulation of H_2O_2 to more than 70% (Mittler 2002; Foyer and Noctor 2003).

Besides SOD, another important antioxidant enzyme is ascorbate peroxidase (APX), an enzyme needed to convert H_2O_2 to oxygen and water (Sofo et al. 2015). This reaction requires ascorbic acid (a non-enzymatic antioxidant) as one of the electron donors (Foyer and Noctor 2003). Based on the data in Table 11 and 12, in low iron stress levels (pyrite 100 ppm), Ciherang as a control susceptible cultivar has the highest APX enzyme activity. This indicated that Ciherang cultivar is classified as an iron susceptible cultivar. In local cultivar, Siam 11 Panjang also has the highest APX enzyme activity compared to other local cultivars (Table 11 and 12). This result indicated that Siam 11 Panjang cultivar is classified as an iron susceptible rice cultivars compared to other local cultivars. Meanwhile, Pandan Ungu cultivar obtained the lowest APX enzyme activity in high iron stress (pyrite 400 ppm) compared to other cultivars, it indicated that Pandan Ungu cultivar is classified as an iron tolerance cultivar.

The tolerance of Kalimantan local rice to iron stress evaluated during the vegetative phase was different between cultivars. Overall, the higher the level of iron stress given, is directly proportional to the changes in antioxidant activity of SOD, APX, and SOD catalysis products in the form of H_2O_2. In these conditions, the plants decreased morphophysiological performance as indicated by a decrease in plant height, length and width of the leaf, leaves number, as well as the levels of chlorophyll and carotenoids. However, this reduction is significantly inversely related to an increase in enzymatic antioxidant activity. In certain cultivars, although physiological performance is shown to decrease, the resulting reduction is smaller than in plants that express relatively low enzymatic antioxidants.

Morphophysiological and biochemical analysis in this experiment resulted significant differences between control (pyrite 0 ppm), low iron stress (pyrite 100 ppm), and high iron stress (pyrite 400 ppm) conditions. The ability of iron stress tolerance was demonstrated by Amas, Pandan Ungu, and Kambang cultivars that have better characteristics for tolerating iron toxicity. The highest levels of chlorophyll and carotenoid contents during iron toxicity conditions were observed in Kambang cultivar. Based on the proline levels, the highest-resistance cultivar was Amas. Antioxidant enzyme analysis showed that cultivars Amas, Pandan Ungu, and Kambang had a high percentage increase in the activity of superoxide dismutase (SOD), hydrogen peroxide (H_2O_2), and ascorbate peroxidase (APX) enzymes when they were exposed to high pyrite conditions. Based on morphophysiological and biochemical enzymatic antioxidant activity, it can be concluded that Amas, Pandan Ungu, and Kambang cultivars have the potential as iron toxicity-tolerant rice.

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