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FRO GENE PROFILE ANALYSIS FOR CHARACTERIZATION OF IRON STRESS-TOLERANT GENOTYPES IN SWAMP RICE (*ORYZA SATIVA* L.)

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SUMMARY

Based on the Central Statistics Agency, Indonesia, rice (*Oryza sativa* L.) production decreased by 2.63 million tons (7.75%) in 2019 compared to 2018, and that could refer to the decrease in rice-growing areas. Therefore, the rice crop area requires enhancement by the utilization of suboptimal tidal swamp lands. However, the heavy iron content in swamp lands is a limiting factor to rice growth. The study aimed to select the iron stress-tolerant rice genotypes by using the effective SSR markers. The arrangement of treatments was a factorial (4×4) completely randomized design with six replications. The first factor was four rice strains (Siam Saba, Siam Tanggung, INPARI 34, and Ciherang), and the second factor was two iron concentrations (0 and 1,600 ppm) of sulfate heptahydrate solution. The ISC analysis showed the rice genotype Siam Saba appeared to be tolerant to Fe_2SO_4 stress (1,600 ppm). The SSR marker amplification revealed the primers RM8213, RM252, and RM335 proved more informative and can be effective for genetic studies.

Keywords: *FRO* gene, genetic study, growth traits, iron tolerance, rice (*O. sativa* L.), SSR markers, swamp lands

Key findings: The concerned study produced the tangible information on the rice (*O. sativa* L.) strains resistance to iron stress based on physiological and genetic characteristics. This information will help the breeders in designing breeding strategies for developing iron-tolerant genotypes for cultivation on tidal swamp lands.

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INTRODUCTION

Rice (*Oryza sativa* L.) is a staple food crop in Indonesia. The national rice field conversion rate is around 96,512 hectares per year, which is an alarming level (Mulyani *et al*., 2016). Overcoming this problem requires the suboptimal and tidal swamp lands' utilization as a substitute for rice fields (Mohanty *et al*., 2017). The tidal swamp lands, with characteristics of shallow groundwater levels, year-round groundwater availability, flat-land topography, and clay and soft soil textures, can support the rice crop growth (Karimian *et al*., 2018). However, the tidal swamp lands also have inhibiting factors for rice growth and production, including their saturated condition with water (aquic). It contains sulfide material, such as pyrite (FeS_2), generally less than pH 4, with extreme soil acidity, and also typically an acid sulfate soil (Kar and Panda, 2018; Li *et al*., 2019).

Rice plants have an Fe homeostatic mechanism that involves the role of various genes, and one of which is the ferric-chelate reductase oxidase (*FRO*) gene (Meena *et al*., 2016). The *FRO* gene plays a vital role in regulating and managing the reductase and Fe transport activities in rice plants. The conduct of rice genotype screening under iron stress is necessary to identify and develop the Fe-tolerant rice lines that can be useful by considering various phenotypic characters. These include leaf yellowness index, root length, plant height, the number of tillers, wet and dry weight of roots, and Fe accumulation in shoots. Those features are the main morphological impact of iron accumulation in plants (Wan *et al*., 2005; Dufey *et al*., 2009).

The identification and development of such genes controlling specific characteristics is an accurate molecular selection for a plant character (Bashir *et al*., 2014). Gene identification can proceed by using the marker-assisted selection (MAS) method, namely employing SSR (simple sequence repeat) and SNP (single nucleotide polymorphism) markers (Inghelandt *et al*., 2010; Jewel *et al*., 2019). The purpose of using markers is to determine the variations in nucleotide bases found in DNA sequences (Chen *et al*., 2014).

Past studies revealed the phenotypic selection of local Kalimantan rice lines tolerant to iron stress took place, with the rice line Siam Saba identified as iron stress tolerant (Thomson *et al*., 2007). However, analysis of the genes related to iron tolerance using SSR markers on the rice strain Siam Saba has not occurred. Therefore, the presented research aimed to determine the growth response of rice genotypes and analyze the *FRO* gene profile, which controls the iron (Fe) tolerance properties of local Kalimantan rice lines, Siam Saba and Siam Tanggung. The study also compares them with the two national rice lines sensitive to iron stress, namely Ciherang and INPARI 34, using SSR markers. Gene profile analysis needs to be made as a first step to support the rice plant breeding program in developing superior rice cultivars resistant to high iron stress to enhance the rice planting efficiency and productivity. The objective of this study was to select the iron stress-tolerant rice genotypes by using the effective SSR markers.

MATERIALS AND METHODS

Breeding material

The genetic material used in this research comprised four rice strains (lNPARI34, Siam Saba, Siam Tanggung, and Ciherang). The equipment used included tile shards, plastic cups, plastic isolation, aluminum foil, a PCR 0.2 ml single tube, a 1.5 ml microcentrifuge tube, and a micropipette tip (size 10–1000 µL). The chemicals used are in the list below: distilled water, chlorox 10%, Yoshida nutrient solution, FeS_2, $FeSO_4.7H_2O$ (iron [II] sulfate heptahydrate), NaOH, HCl, $NH_3Fe(SO_4)_2.12H_2O$, HNO_3, NH_4SCN, acetone 80%, liquid nitrogen, isopropanol, absolute ethanol, TBE 10x, ddH_2O, nuclease-free water, agarose, primer forward SSR, primer reverse SSR, MyTaq™ HS Red Mix 2x (Bioline), Hyper Ladder 1 kb, 100 bp DNA Ladder, Gel Green (Biotium), and Plant Genomic DNA Mini Kit (Geneaid).

METHODS

Preparation of planting material and nutrient solution

The selection of seeds of the local Kalimantan rice lines (Siam Saba and Siam Tanggung) and national rice cultivars (INPARI 34 and Ciherang) used the soaking method (Yoshida, 1981). The rice seeds underwent germination for 3–4 days in a dark room. The Yoshida nutrient solution contains macronutrients in the form of N, P, K, Ca, and Mg and micronutrients in the form of Mn, Mo, B, Zn, Cu, and Fe for plant growth. The iron (II) sulfate heptahydrate's addition to the nutrient solution was according to the treatment. The experiment to measure dissolved ferric iron concentration in nutrient solutions used a randomized complete block design with stratified iron (II) sulfate heptahydrate concentration factors (400, 800, 1,200, and 1,600 ppm) with six replications. The experiment layout in RCBD sought the optimal concentration between those various dosages of iron (II) sulfate heptahydrate, which later served as a variable for the next experiment. The plastic container contains the Yoshida's nutrient solution, being replaced every seven days until the rice plants become 21 days old. Next, the rice plants sustained stress treatment according to the treatment combination for 10 days.

The concentration measurement of dissolved ferric iron in the nutrient solution transpired for absorbance on days 0, 2, 4, 7, and 9 after treatment (Oni *et al.*, 2020). The control solution consisted of mixing only the HNO solution (4M) and NH_4SCN (2M). The blank (control) solution and standard iron solution's analysis used a spectrophotometer to measure the absorbance at a wavelength of 480 nm. The obtained absorbance values served in developing a calibration curve. This calibration curve can help to determine the relationship between the absorbance value and the dissolved iron concentration to obtain a linear equation. Nutrient solution samples for analysis came from days 0, 2, 5, and 9. Then the absorbance value of the samples measured had a wavelength of 480 nm using a spectrophotometer. From the absorbance value obtained, the concentration of dissolved iron in the nutrient solution can be detected by entering the absorbance value into the earlier attained linear equation.

The rice seeds' selection for use in the research proceeded by selecting the seeds with uniform growth during germination. The planting of four rice lines in the container progressed randomly using a completely random design to minimize the experimental error. The 21-day-old rice seedlings sustained iron-stress treatments by adding 1,600 ppm of iron (II) sulfate heptahydrate solution as the iron stress and 0 ppm as the control. The iron-stress application ran for 55 days to give the clear morphological effect of iron poisoning in plants. Replacing Yoshida's nutrient solution in the plastic container ensued every one week.

Measurement of plant growth variables

Score bronzing calculation depended on the percentage of affected leaf area bronzing (Figure 1). Leaf area bronzing indicates the severity of iron poisoning in plants. The severity of iron poisoning measurement followed the Standard Evaluation System (SES) guidelines established by the International Rice Research Institute (IRRI) for genetic evaluation (IRRI, 2013). Measuring plant height (cm) began at 55 days after planting (DAP). Plant height measurements ensued using a measuring tape (Yoshida, 1981). Likewise, root length (cm) estimation occurred 55 DAP. Measurements continued by taking the plant from the container and then appraising it using a tape measure (Yoshida, 1981). The chlorophyll level (mg L^{-1}) assessment also surfaced 55 DAP. The absorbance assessment used a spectrophotometer at the wavelengths of 645, 663, and 652. Total chlorophyll (mg L^{-1}) calculation used the following formula (Yoshida, 1981).

$$\text{Total chlorophyll (TC)} = (8.02 \times D_{663}) + (20.2 \; D_{645})$$

Where D_{645} = absorbance value at a wavelength of 645, 20.2 = absorbance coefficient at wavelength 645, D_{663} =

absorbance value at a wavelength of 663, and 8.02 = absorbance coefficient at wavelength 663.

DNA isolation, amplification, and visualization

Rice DNA isolation commenced after the plants were 55 days from planting. Leaf shoots became samples used for DNA isolation. The total number of leaf samples used was eight samples (two treatments × four lines). Rice leaf DNA isolation followed the Plant Genomic DNA Mini Kit (Geneaid) protocol. Purity and concentration measurements of DNA employed a nanodrop spectrophotometer. The DNA isolation quality also took place using an electrophoresis device. Rice DNA amplification succeeded by applying the method of polymerase chain reaction (PCR), utilizing the simple sequence repeat (SSR) markers in this research. The primers used in the SSR markers refer to Onaga *et al.* (2013) and Chen *et al.* (1997) (Table 1). The concentration of agarose gel used was 1% and got mixed into the TBE (Tris-borate-EDTA) buffer solution.

Figure 1. Percentage and severity of bronzing leaves (IRRI, 2013).

Table 1. The SSR markers used in the study.

SSR Markers	Repetition Motive	Sequence		Temp. (℃)	PCR Product (bp)
RM124	(TC)10	F	ATCGTCTGCGTTGCGGCTGCTG	64	225–328
		R	CATGGATCACCGAGCTCCCCCC		
RM241	(CT)31	F	GAGCCAAATAAGATCGCTGA	52	138
		R	TGCAAGCAGCAGATTTAGTG		
RM8213	(TC)10	F	AGCCCAGTGATACAAAGATG	52	177
		R	GCGAGGAGATACCAAGAAAG		
RM252	(CT)19	F	TTCGCTGACGTGATAGGTTG	54	230–240
		R	ATGACTTGATCCCGAGAACG		
RM261	C9(CT)8	F	CTACTTCTCCCCTTGTGTCG	54	125
		R	TGTACCATCGCCAAATCTCC		
RM307	(AT)14(GT)21	F	GTACTACCGACCTACCGTTCAC	54	174
		R	CTGCTATGCATGAACTGCTC		
RM5473	(AG)20	F	GGAGATAAGACACGAGGGAATTATGC	57	266
		R	AGATTAACTACGCGCGCTCATCC		
RM335	(CTT)25	F	GTACACACCCACATCGAGAAGC	56	89–159
		R	TCCATGGATATACGAGGAGATGC		
RM551	(AG)18	F	CTTACTCCATTGGGCTGGAACC	58	165–215
		R	TGTAGGGTGGTAAGAGATCCACTCC		

RESULTS AND DISCUSSION

Measurement of ferric iron concentration (Fe^{3+}) dissolved in nutrient solution

In the presented study, the iron (II) sulfate heptahydrate became a source of iron stress for the plants of four different rice genotypes. Past research on iron stress usually used the iron (II) sulfate heptahydrate to induce iron stress (Li *et al.*, 2019). Iron (Fe) can form as a ferrous ion (Fe^{2+}) and ferric (Fe^{3+}) (Connorton *et al.*, 2017). Iron found in ferrous form can be directly absorbed by rice plants and used in metabolic processes. In contrast to iron in the ferric form, it typically creates a precipitate of compounds lepidocrocite and goethite, which is directly unusable to the rice plants. Under anaerobic conditions, the redox potential and oxygen diffusion rate become least in water (Tam *et al*., 2021). This causes the oxidation rate of ferrous to ferric to be low, and the ferric concentration on day 0 of treatment was nonsignificantly different when administering various concentrations of iron (II) sulfate heptahydrate.

Apart from that, the low diffusion of oxygen in water also caused the dissolved ferric concentration in the nutrient solution to be lower than the ferrous concentration (Chao *et al*. 2015). Measuring ferric iron (Fe^{3+}) dissolved in the nutrient solution was intentionally the basis for determining the concentration of iron (II) sulfate heptahydrate used in the research. In the concerned experiment, the observed variables were the ferric concentration in the nutrient solution, plant height, and root length. The analysis of these three variables showed a concentration of iron (II) sulfate heptahydrate (1,600 ppm) provides the significant iron stress effect, marked by the least plant height and root length.

Based on the results, the iron concentration contained in iron (II) sulfate heptahydrate was around 321 ppm. In the nutrient solution, the critical limit for iron concentration can be toxic to plants at 300 ppm (Muhammad *et al*., 2018). Thus, an iron (II) sulfate heptahydrate concentration of 1,600 ppm became a source of iron stress in the study.

Measurement of plant growth variables

Rice plants can experience the symptoms of iron poisoning at various growth phases. In this study, the iron poisoning impact on rice plants prevailed at the vegetative phase when the rice plants' age was 55 days from planting. This is because development occurs intensively at the vegetative phase, namely, maximum tiller formation and panicle initiation. Therefore, the vegetative phase is the best phase to serve for rice plant tissue analysis (Tam *et al*., 2021).

In rice genotypes, the growth variables observed were the scores for bronzing leaves, plant height, root length, and chlorophyll content. The percentage of inhibition in each variable helped to determine the iron stress effect on each rice strain. Next, the F-test analysis continued under a control condition to determine the uniformity of growth among the tested rice lines. If growth was not uniform, analysis with the Duncan test will determine the differences between the strain groups. However, if several growth variables showed significant differences in control conditions, determining the level of tolerance and sensitivity of rice plants cannot be applicable using the Duncan's advanced test.

Bronzing leaves and small brown spots on the rice plant leaves were the main symptoms of iron poisoning. The highest score for bronzing leaves with iron (II) sulfate heptahydrate resulted in the line Ciherang (8.00), while the lowest was apparent in the line Siam Saba (2.07). The highest percentage of inhibition was evident in the strain Ciherang (87.50%), with the lowest percentage recorded in the strain Siam Saba (5.72%) (Table 2). A higher percentage of resistance indicates the application of iron (II) sulfate heptahydrate causes more brownish spots on the leaves.

For plant height, the Duncan test analysis showed the plant height in the control plants of line Siam Saba emerged significantly different from the other three rice lines. In the control plots, the maximum plant height was

Table 2. Score of bronzing leaves on rice lines after iron stress treatment for 55 days after planting (DAP).

Strains	Bronzing Leaves Score Average ± SE						Inhibition Percentage (%)
	Control			$FeSO_4$ (1600 ppm)			
Siam Saba	1,00	±	0.000[a]	2.07	±	0.230	51.72
Siam Tanggung	1.00	±	0.000[a]	3.00	±	0.756	66.67
Ciherang	1.00	±	0.000[a]	8.00	±	0.378	87.50
INPARI 34	1.00	±	0.000[a]	4.00	±	0.690	75.00

Table 3. Plant height in rice lines after iron stress for 55 days after planting (DAP).

Strains	Plant Height Average ± SE (cm)						Inhibition Percentage (%)
	Control			FeSO4 (1600 ppm)			
Siam Saba	57.20	±	1.015[a]	56.47	±	0.888	1.27
Siam Tanggung	64.36	±	0.276[b]	62.11	±	1.716	3.49
Ciherang	67.31	±	0.937[b]	61.56	±	0.614	8.55
INPARI 34	73.83	±	1.497[b]	67.87	±	1.114	8.07

Table 4. Chlorophyll level in rice lines after iron stress for 55 days after planting (DAP).

Strains	Chlorophyll Levels Average ± SE (mg L^{-1})						Inhibition Percentage (%)
	Control			$FeSO_4$ (1600 ppm)			
Siam Saba	53.52	±	2.781[a]	50.89	±	5.100	4.91
Siam Tanggung	55.27	±	3.102[a]	42.02	±	7.101	23.96
Ciherang	59.54	±	3.384[a]	5.93	±	0.681	90.04
INPARI 34	57.69	±	3.004[a]	11.77	±	1.610	79.58

notable in the strain INPARI 34 (73.83 cm), with the lowest observed in the line Siam Saba (56.20 cm). In the iron (II) sulfate heptahydrate treatment, the maximum plant height was visible in the line INPARI 34 (67.87 cm), while the lowest plant stature occurred in the line Siam Saba (56.47 cm). The topmost plant height resistance was prominent in the line Ciherang (8.55%), while the lowest manifested in the line Siam Saba (1.27%) (Table 3). The high percentage of inhibition indicates the application of iron (II) sulfate heptahydrate can inhibit the plant height compared with the control.

For the chlorophyll level, in the control treatment, the highest leaf chlorophyll content appeared in the strain Ciherang (59.54 mg L^{-1}), while the lowest content was noticeable in the strain Siam Saba (53.52 mg L^{-1}). In the iron (II) sulfate heptahydrate application, the optimum leaf chlorophyll content resulted in the strain Siam Saba (50.89 mg L^{-1}), with the minimum observed in the strain Ciherang (5.93 mg L^{-1}). The maximum inhibition percentage regarding leaf chlorophyll level existed in the line Ciherang (90.04%), while the least value surfaced in the strain Siam Saba (4.91%) (Table 4). The high percentage of inhibition indicates the administration of iron (II) sulfate heptahydrate was able to reduce the chlorophyll level versus the control treatment.

The F-test analysis showed considerable differences in the variables of plant height and root length under control conditions. However, it was necessary to carry out the testing using a two-way ANOVA to determine the effect of rice strains (S), concentration (C), and their interactions (S × C) on the growth traits of rice plants treated for 34 days. The two-way ANOVA provided the rice strain (S), iron concentration (C), and their interactions (S × C) have significant differences in all growth variables, i.e., bronzing leaves, plant height, root length, and chlorophyll content (Table 5).

Table 5. Two-way ANOVA of growth variables in rice lines after iron stress for 55 days after planting (DAP).

Growth Indicators	Source					
	Strains (S)		Concentration (C)		Interaction (S x C)	
	F	Sig.	F	Sig.	F	Sig.
Leaves Bronzing	21.914	0.000	137.438	0.000	21.914	0.000
Plant Height	44.943	0.000	22.449	0.000	3.109	0.035
Chlorophyll Level	13.037	0.000	113.462	0.000	20.850	0.000

Rice lines (S) displayed substantial variations for plant growth traits, indicating the differences in growth response resulted from the genetic potential of the four rice genotypes tested. Differences were also noteworthy for plant height and root length through the F-test results. Iron concentration showed a remarkable disparity in plant growth, indicating the cause of differences in growth responses came from various environmental conditions. The interaction between rice strains and iron concentration made a significant difference for plant growth, and variations existed in plant growth response by the genotypes under varied environments.

The plant growth response to rice lines and iron concentration (S × C) is an important factor in assembling the genotypes tolerant to iron-stress conditions. Rice lines with a good adaptive response will be able to grow under stressful conditions, even though a decrease in grain yield may occur (Sikuku *et al.*, 2010). Eeuwijk *et al.* (2016) suggested growth variables significantly differing with the interaction between rice lines and iron concentration (S × C) can be desirable as basic variables for further selection. These real differences showed these characters can respond differently to diverse environments.

Amplification with SSR molecular markers

The simple sequence repeat (SSR) molecular markers are widely applicable in rice research. The SSR marker is a codominant marker that can differentiate the homozygotes and heterozygotes (Chrisnawati *et al*., 2021). In this study, the SSR marker can also differentiate between genotypes as affected by a certain stress. Each SSR marker attached to a specific genotype refers to a particular ability to tolerate the stress from the environment. Apart from that, the SSR markings are often useful, having superior information accuracy and reliability. Hence, the use of SSR molecular markers in the presented study. The DNA isolated samples' amplification engaged the SSR markers. The nine SSR markers used in the research appear in Table 1 (Chen *et al.*, 1997; Temnykh *et al.*, 2000).

The selected SSR markers amplify the gene on chromosome number 4. Visualization of DNA amplification can identify the monomorphic and polymorphic DNA fragments (Tam *et al*., 2021). The SSR markers tend to be monomorphic if DNA fragments of a certain size appear in all genotypes. This is different from polymorphic SSR markers, when the DNA fragments of a specific size only appear in a few genotypes (Mattar *et al*., 2016; Utami *et al*., 2020). Based on the visualization of DNA amplification using nine SSR markers, four markers emerged monomorphic, i.e., RM124, RM241, RM261, and RM551. Contrastingly, five polymorphic markers existed, viz., RM8213, RM252, RM307, RM5473, and RM335 (Figure 2).

The results of the electrophoresis visualization, as analyzed, used the Gel Analyzer program to estimate the size of the DNA fragments formed. Based on the results of aligning the samples with the 100 bp DNA ladder in Figure 2, it showed the resulting DNA fragments vary in size from 90 to 830 bp. The DNA pattern and band size data also helped formulate the PIC value. The PIC (polymorphic information content) is the level of marker ability to detect the polymorphism. A greater PIC number indicates the ability of the markers to differentiate between groups of individuals.

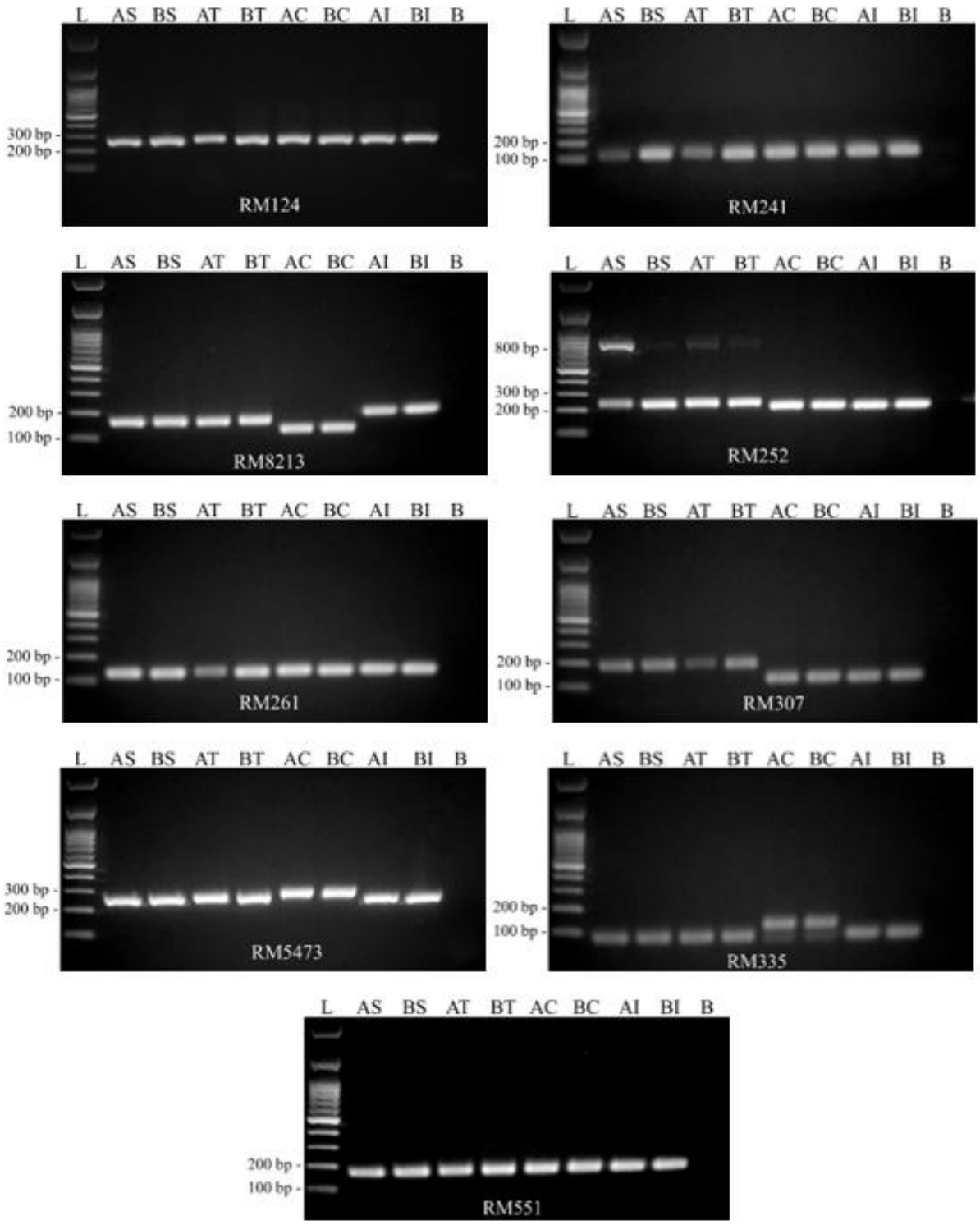

Figure 2. Electrophoresis of rice DNA amplification results using SSR markers: RM124, RM241, RM8213, RM252, RM261, RM307, RM5473, RM335, and RM551. L: DNA Ladder 100 bp, AS: Siam Saba Control, BS: Siam Saba Treatment, AT: Siam Tanggung Control, BT: Siam Tanggung Treatment, AC: Ciherang Control, BC: Ciherang Treatment, AI: INPARI 34 Control, BI: INPARI 34 Treatment, B: Blank.

A marker is ineffective if it cannot be effective to detect genetic differences in a group of individuals (Suman *et al.*, 2020).

For codominant markers, the PIC value ranges from 0 to 1; the value of 0 means the DNA band appears monomorphic, and the value of 1 means the DNA band appears polymorphic. Botstein *et al.* (1980) divided the molecular markers into three groups. These are very informative molecular markers with a PIC value of more than 0.5, somewhat informative molecular markers with a value of 0.25 < PIC < 0.5, and uninformative molecular markers with a PIC value of less than 0. Molecular markers indicated for genetic studies must have a PIC value of more than 0.5 (Table 6).

In this study, the polymorphic SSR markers that were very informative were RM8213, RM335, and RM225. These markers have a PIC value greater than 0.5. In the SSR marker RM8213, the DNA band pattern formed consists of three bands with a size of 163–217 bp. However, it was contrary to the findings of Carsono *et al.* (2020), who reported the marker RM8213 created the DNA band measuring 177 bp. With this DNA band size, the character associated with the SSR marker RM8213 was resistance to brown plant hoppers. The SSR marker RM8213 revealed the PIC value of 0.55, and it can clearly differentiate the four rice genotypes tested. The SSR marker RM8213 can segregate the rice lines into two groups. They are the two rice genotypes tolerant to iron stress (Siam Saba and Siam Tanggung) with a DNA band size of 178 bp, and the two other rice genotypes sensitive to iron stress with a DNA band size of 163 bp (Ciherang) and 217 bp (INPARI 34).

In the SSR marker RM335, the established DNA band pattern comprised three bands with a size of 90 to 150 bp. These results were in accordance with Siddique *et al.* (2014), who reported the DNA band formed measured 89 to 169 bp. The character associated with the SSR marker RM335 was the rice grain weight. The marker RM335 has a PIC value of 0.51; thus, it can differentiate the four rice lines tested. Rice genotypes tolerant to iron stress (Siam Saba and Siam Tanggung) have a DNA band size of 90 bp. Meanwhile, rice genotypes sensitive to iron stress (Ciherang and INPARI 34) have a DNA band size of 130 bp. Apart from that, a distinction between the rice genotypes that were sensitive to iron stress existed, namely, by the formation of a 150 bp DNA band in the line Ciherang.

In the SSR marker RM252, three DNA bands formed had a size of 230 to 830 bp. These results were analogous to the findings of Wan *et al.* (2005), as they reported the marker RM252 created an allele with a size of around 230 to 240 bp. The associated character on the maker RM252 was the bronzing leaves' index and chlorophyll level. The SSR marker RM252 has a PIC value of 0.55, making this marker also effective to distinguish the four rice lines tested. Rice genotypes tolerant to iron stress (Siam Saba and Siam Tanggung) have the DNA band sizes of 240 and 830 bp, respectively. In contrast to rice genotypes found sensitive to iron stress (Ciherang and INPARI 34), they have a DNA band size of 230 bp.

CONCLUSIONS

The main morphological and physiological responses in rice genotypes subjected to iron stress were the bronzing leaves, chlorophyll content, and plant height. The tolerant rice line was Siam Saba, the moderately tolerant rice line was Siam Tanggung, and the sensitive rice lines were Ciherang and INPARI 34. The study also concluded the three SSR markers, viz., RM8213, RM252, and RM335 revealed with the highest level of informativeness. The SSR marker RM252 can differentiate the tolerant and sensitive rice lines with related characteristics like bronzing leaves and chlorophyll level. In the future, the SSR marker RM252 hopefully can be applicable to select the potential rice seed for planting in areas with high concentrations of iron to improve the genetic screening for rice.

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