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Molecular Marker-Assisted Selection of Rice Grain Quality on Rice (*Oryza sativa* L.) Lines Tolerant to Fe Toxicity Stress

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ABSTRACT

The elite rice has been produced, including iron (Fe) tolerant varieties. To get the appropriate rice lines which superior not only Fe tolerant but also have good grain quality needs to be developed selection system, especially in the use of molecular markers. This study was aimed to develop molecular markers for selection the rice grain quality characters of selected rice lines Fe tolerant. A total of 30 selected Fe tolerant rice lines and 5 parents as control lines were used in this research. Characterization of grain quality were quantitatively using the standard. While for genotyping analysis used 19 molecular markers of STS, SSR, Indel and SNP. This study showed that 14 of 19 markers result polymorphic DNA band (DNA markers). Association analysis of genotype and phenotype showed that 10 of 14 markers were significantly ($p < 0.05$) related to high quality of rice grain. Among four types of markers used in this study, STS was the most widely associated significantly with four characters of rice quality. The phenotyping analysis showed that the physical grain and palatability quality which obtained from the total mean of 30 rice lines tested tend to nearly with the parent's value as controls lines. The most of these lines were included in the group IV of National Rice Grain Quality Standard (SNI). The amylose content (AC) showed that the texture was varied from firm and dry (high AC) to soft and sticky (low AC). The association results showed that there were significant ($p \leq 0.05$) markers related with the biosynthesis starch genes, i.e: *sbe1*, *ss1*, *ssiia*, *gpa*, *pul* and *s3cl* which contributed on the character of rice palatability. These selected significant markers could be useful for screening of other population with Fe tolerant and/ or other desired morpho-agronomical traits in support of rice breeding program in Indonesia.

Keywords: *Fe-tolerant rice lines, molecular marker, rice grain quality*

INTRODUCTION

Rice (*Oryza sativa* L.) as a staple food is consumed by more than half the world's population and is the most dominant crop in Indonesia. Rice consumption accounts for 60% of carbohydrates intake [1]. Paddy requires essential micro-nutrients to support electron transport for photosynthesis, such as ferrum or iron (Fe). The nutrient serves as an electron acceptor which is important in the reaction of reduction-oxidation and as an activator of several important enzymes in the metabolic processes of plants [2]. Plants need Fe in small amounts, and in excessive amount it potentially causes plant toxicity, leading to nutrient deficiency, cell damage and water deficiency, thus inhibiting the growth of plants [3].

However, in acid soil, Fe is soluble and available for plants in abundance, which lead to Fe-induced toxicity. Fe toxicity occurs in sub-optimal lands such as marsh, tidal area, some lowland areas with poor drainage and areas of new openings in Indonesia islands [2]. Rising food demand and reduction of farming land due to land conversion are driven factors developing Fe-tolerant rice varieties.

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Various rice lines that are tolerant to environmental stress in both biotic and abiotic have been scrutinized, including Fe-tolerant rice [3]. These rice lines are from multi-crossing population. However, the rice grain quality still needs to be improved, thus it can be an important consideration for consumers as well as for breeders [4]. Breeding is generally intended to improve yield, but on the other hand the obtained varieties are often less acceptable to both farmers and consumers because of undesirable seed quality [5].

High seed quality can be characterized from its physicochemical aspects that include size, shape, texture, color, aroma and taste [6]. The physicochemical is influenced by starch content in the endosperm which is determined by amylose content (AC) and gel consistency (GC) [7]. Molecular markers for selection of high quality seed have been developed by many researchers. Simple sequence repeat markers (SSR), sequence tagged site (STS), and single nucleotide polymorphism (SNP) are common DNA markers for analysis of rice taste and physicochemical properties [6].

Functional markers such as SSR, STS or SNP associated with genes related to the physicochemical properties of rice have been established [6, 7, 8]. Markers used previous studies are *s3cl*, *sbe1*, *ss1*, *ssiia*, *gpa*, *gbss1*, *pul* and *ams* [7]. The markers can be used to assist in selecting rice seed under Fe toxicity. This research was aimed to develop molecular markers for selection the rice grain quality characters of selected rice lines Fe tolerant.

MATERIALS AND METHODS

Plant materials

Paddy from multi crossing population (30 rice lines) was selected for its tolerant to Fe toxicity and 5 parents as control lines. Thirty rice lines tolerant to Fe (bronzing score ≤ 5) [6] and 5 parents as control i.e. IR64 (sticky rice), Inpara 3 (firm rice), Inpara 2 (sticky rice), IR42 (firm rice), and Mashuri (sticky rice) are used for phenotyping analysis of rice physical quality, physicochemical and organoleptic. The same plant materials were analyzed using 19 markers of SSR, STS, SNP, and Indel.

Molecular Marker

The plant material was analyzed by using 19 markers of rice grain quality from 4 markers type including SSR, STS, Indel and SNP (Table 1).

DNA isolation

The DNA isolation was performed using the CTAB method according to Doyle [8]. Plant leaves were ground in liquid nitrogen by tissue lyser. A sample was added with 750 µL Cetyltrimethyl Ammonium Bromide (CTAB) buffer and incubated in 65°C for 30 min. This suspension was then added with 750 µL CI (chloroform : isoamil alcohol = 24 : 1) and centrifuged at 10,000 rpm for 15 minutes. The Supernatant was moved to a new tube and then was added with 50 µL Na acetate 2M pH 5.2 and 1 mL absolute ethanol and incubate overnight at -20°C. After freezing overnight, it was centrifuged at 10,000 rpm for 15 minutes. A pellet was washed with 500 µL 70% alcohol and centrifuged at 10,000 rpm for 5 minutes. A dried pellet was added with 50 µL 1× TE and 10 µL 10 ng/µL RNase and incubated at 37°C for one hour. Inactivation of RNase was performed by incubation in 65°C for 15 minutes. DNA quality was tested by electrophoresis on 0.8% agarose gel in 1× TAE buffer at 100 volts for 60 minutes, then visualized using UV light (BioRad, USA). DNA quantity is determined using the Nano Drop 2000c Spectrophotometer (Thermo Scientific, USA).

PCR analysis

PCR analysis included markers selection and amplification of DNA fragment. In markers selection, PCR analysis was performed in a total volume of 10 µL containing 4 µL DNA (10 ng), 0.5 µL primers-F 2.5 pmol, 0.5 µL primers-R 2.5 pmol, 5 µL of KAPA. PCR conditions were pre-PCR 94°C for 5 minutes, denaturation 94°C for 45 seconds, annealing 55°C for 1 minute, elongation 72°C for a minute, post-PCR 72°C for 7 minutes. PCR was performed for 35 cycles. PCR products were analyzed using electrophoresis on agarose gel 2% in a buffer solution 1× TAE buffer (40 mM Tris-acetate, 1 mM EDTA) at 50 volts for 60 minutes. DNA bands in agarose were visualized with UV light. DNA fragments of selected Fe-tolerant rice (41 populations) were amplified using 14 selected primers related to rice grain quality. PCR reactions were conducted in same procedure as in markers selection, but annealing temperature was adjusted to optimization temperature of each primer for 1 minute. DNA visualization was subsequently measured by GelQuant.NET 3.5 to determine the size of the base of each DNA band

Seed quality

Physical quality of grain and rice was analyzed based on Wibowo *et al.* 2006 [10], modified with SNI 01-6128-1999 [11] and IRRI in 1996 [12]. Cooked rice quality was analyzed using standard methods [13, 14]. Sensory evaluation was conducted by 30 trained pane-

Table 1. A list of previously reported markers and their chromosomal location developed in this study for the evaluation of eating quality on rice indica

Primer	Gene	Marker types[d]	Chr[a]	Sequences	
				Forward (5'-3')	Reverse (5'-3')
EQ1	*ss1*	SSR	6	GATCCGTTTTTGCTGTGCCC	CCTCCTCTCCGCCGATCCTG
EQ2	*sbe1*	SSR	6	ATTTCTTTGGCCACAGGCGA	CCCAGATTCGGAACAAGAAC
EQ3	*sbe1*	STS	6	GAGTTGAGTTGCGTCAGATC	AATGAGGTTGCTTGCTGCTG
EQ4	*ssiia*	SNP	6	CTGGATCACTTCAAGCTGTACGAC	GCCGGCCGTGCAGATCTTAAC
EQ5	*ssiia*	SNP	6	CAAGGAGAGCTGGAGGGGGC	ACATGCCGCGCACCTGGAAA
EQ6	*s3ci*	Indel	7	CCACTCTCATGTCCTTGAAC	GCCATGACATTTGGACAT
EQ7	*treb*	Indel	7	CACTCCAGTTCCTGCTCAAA	CACCTCCAAAACGAATATGG
EQ8	*ams*	SSR	2	CTTCCAAGGACCCCATCCT	CCCAACATCTCCGTCAGAAT
EQ9	*gpa*	SSR	11	CCAAATACGCGGCCTTCT	AGTTTCTGGGCTCGGAGGA
EQ10	*gbss1*	SSR	6	CAAATAGCCACCCACACCAC	CTTGCAGATGTTCTTCCTGATG
EQ11	*ss1*	STS	6	TCTAGATTGCTACACGTGAGAGG	TCTCCACGATAACTTCCACC
EQ12	*sbe3*	STS	2	TCGGTCAATTCGGTTAGTCTCCTC	ACATCCTCTAGCATACTGGCGAC
EQ13	*sssiia*	STS	6	TCTAGATTGCTACACGTGAGAGG	GGAGCCACCTGTAAAGCGTG
EQ14	*isa*	STS	8	CCTGTCTTGCACGTGCGGTA	GCACGGTTCTGATGTACGAGAG
EQ15	*pul3*	STS	4	GGGTTCGCTTTCACAACACAG	GTCACGACATAAGAGAAGCTGC
EQ16	*pul5*	STS	4	AGTTCGCTAGTCATCTGCTCG	CCACATGTCCTTGTCTCCACTT
EQ17	*mad*[b]	STS	12	TAACAACCACGGCCGAGAA	GAGCGTTCTTTTCTTTCGGTA
EQ18	*hp*[b]	STS	3	TGGAGGAGATGTACGTCGAG	GAAGTCGAGGTGGTCCATGA
EQ19	*aglu*[c]	STS	1	CCTCTGGAATCTTGCTATTTAGG	ATCCGCTAGATCACTGACAAA

[a] Chr: Chromosome number

[b] Markers developed from the candidate genes residing QTL regions identified by Wada *et al.* (2008) [15]: MAD: OsMAD20 MADS box family; HP: Homeobox domain containing protein; PP2 (Phosphoserine phosphatase).

[c] Markers developed from randomly chosen candidate genes based on their potential association with palatability by their functions: Aglu (*Acyl UDP N acetylglucosamine O acyltransferase*).

[d] List of markers developed by Liu *et al.* (2004) [16]; Bao *et al.* (2006) [8]; Lestari *et al.* (2015) [6]

lists and assessed according to glossiness, color, aroma, stickiness (level of waxy), and taste. Organoleptic score was presented using ranking (order) to determine the differences and changes in taste or quality of the rice palatability. The ranked average was the average value scored by 30 panelists.

Data analysis

The genotype and phenotype data obtained were used in association analysis using software of Tassel 2.0. Molecular markers (genotype) associated significantly with $p < 0.05$ indicated that the markers were associated with the phenotype.

Markers associated with phenotype response ($p < 0.05$) showed that the markers were associated with genes in the rice grain quality on selected Fe tolerant rice lines. Physical quality of grain and rice was analyzed based on Wibowo *et al.* 2006 [10] but it modified with SNI 01-6128-1999 [11] and IRRI in 1996 [12]. Cooked rice quality was analyzed using AOAC 1984 [13] and Juliano 1985 *et al.* [14].

RESULTS AND DISCUSSION

Character of rice grain quality

Rice grain quality was determined by (1) physical and milling properties, (2) eating and cooking quality and, (3) nutritional quality [17].

Physical and milling rice

According to SNI, maximum moisture content of rice grain is 14%. The moisture content is the ratio of water in the sample to initial sample weight [16]. It is significantly associated with a shelf life of grain which in turn affects the growing of grain. A high 1,000 grains weight is associated with 30 g, while low weight is associated with below 30 g. A 1,000 grains weight is the ratio of the 1000 grain produced by a strain or variety, which is applicable for determination of seed required in

Table 2. Quality of physical and milling on 30 selected Fe tolerant rice lines

Character	Crossing population				
	In3 (n=14)	In2 (n=4)	IR42 (n=3)	IR64 (n=2)	Mhs (n=7)
Level of milling grain (%)	131.57 ± 15.30	141.25 ± 20.55	136.7 ± 9.29	137 ± 25.46	131.6 ± 9.85
Moisture content (%)	11.28 ± 0.20	11.48 ± 0.57	11.70 ± 0.61	11.05 ± 0.67	11.21 ± 0.57
Empty grain+material dirt (%)	3.42 ± 4.07	3.00 ± 2.36	0.47 ± 0.77	1.82 ± 1.08	2.29 ± 2.91
Brown grain (%)	3.81 ± 1.52	3.42 ± 1.86	2.46 ± 0.44	3.59 ± 1.72	3.38 ± 2.06
Red grain (%)	0.09 ± 0.34	0.05 ± 0.08	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.03
Head grain (%)	75.12 ± 10.73	70.43 ± 5.19	67.59 ± 14.5	71.95 ± 16.7	72.63 ± 17.50
Broken grain (%)	23.52 ± 10.42	27.75 ± 4.95	30.22 ± 14.8	26.40 ± 16.9	25.44 ± 16.65
Groats grain (%)	1.36 ± 0.56	1.83 ± 0.28	2.19 ± 0.38	1.65 ± 0.18	1.93 ± 1.13
Chalky green grain (%)	0.34 ± 0.32	0.92 ± 0.41	3.61 ± 5.64	0.45 ± 0.33	0.32 ± 0.20

*n is the number of derived lines on each parent control

*In3= Inpara 3, In2= Inpara 2, Mhs= Mashuri

Table 3. Quality of physical and milling on 5 parents as control

Character	Inpara 3	Inpara 2	IR42	IR64	Mashuri
Level of milling grain (%)	126	137	133	155	130
moisture content (%)	11.35	11.30	11.40	11.30	11.20
Empty grain + material dirt (%)	16.47	2.30	3.79	0.31	8.42
Brown grain (%)	4.19	2.14	5.10	1.08	0.34
Red grain (%)	0.54	6.92	0.00	0.00	0.00
Head grain (%)	73.83	85.84	52.67	92.94	91.52
Broken grain (%)	22.83	13.16	44.64	4.82	7.75
Groats grain (%)	3.35	1.00	2.69	0.26	0.73
Chalky green grain (%)	0.12	0.32	0.16	0.21	0.29

Table 4. Cooking quality of 5 parent as control

Character	Inpara 3	Inpara 2	IR42	IR64	Mashuri
Amylose content (%)	23.32	15.76	22.44	15.89	18.80
Gel melting (mm)	41.00	67.00	41.00	61.50	61.00
Rice texture	Pera	Pulen	Pera	Pulen	Pulen
Protein content (%)	8.61	7.58	7.54	8.79	9.61

Table 5. Cooking quality of 30 selected Fe tolerant rice lines

Character	Crossing population				
	In3 (n = 14)	In2 (n = 4)	IR42 (n = 3)	IR64 (n = 2)	Mhs (n = 7)
Amylose content (%)	20.60 ± 3.17	19.84 ± 1.81	22.00 ± 2.93	15.92 ± 0.11	21.13 ± 4.20
Gel melting (mm)	52.36 ± 13.15	57.38 ± 4.23	46.17 ± 5.86	67.75 ± 3.89	51.36 ± 11.97
Rice texture	1.57 ± 0.51	1.75 ± 0.50	2.33 ± 0.58	1.00 ± 0.00	2.00 ± 0.82
Protein content (%)	8.50 ± 0.73	8.32 ± 0.75	8.42 ± 0.26	8.59 ± 0.12	8.72 ± 0.69

*Numbers in column is Mean ± Standard deviation

*n is the number of derived lines on each parent control

*In3= Inpara 3, In2= Inpara 2, Mhs= Mashuri

1 ha [16].

Based on the percentage of chalky green grains, a total of 28 rice lines was suitable for SNI requirements (grade 1) with 1% of chalky green grain, while the remaining 2 rice lines were in the grade IV. Chalky green grains are preferred by consumers, because it produces

a chalky rice and is susceptible to damage by pests which lead to short shelf life [19]. The percentage of brown grains on 30 rice lines was in grade V (maximum 5%). In percentage of red grains, all lines derived from IR42 and IR64 met grade I (maximum 0%) (Table 2). Furthermore, physical and milling properties of 30 rice lines (Table 2) were almost same as parent control (Table 3), and some characters tend to have a small standard deviation.

Eating and cooking quality

Rice can be grouped by amylose content as waxy (0 –2%), very low (3 – 12%), low (13 – 20%), moderate (21 – 25%), and high (≥ 26%). Cooking quality of 30 rice lines and 5 parents as control was generally low to high level of amylose, which is classified in sticky (waxy) and firm (non-waxy) with an average of soft to medium gel consistency. Gel consistency (GC) enables to indicate the rice texture after cooled. This was consistent with the statement of Juliano *et al.* (1981) [14] that amylose content (AC) is one of the important criteria in rice classification. Medium amylose content is attributed to sticky rice (not too wet or dry), while high amylose is correlated to hard, dry, and firm rice. Low amylose rice has lower water absorption ratio due to differences in the active groups.

Amylose have hydroxyl group which is polar (hydrophilic) and high affinity to water, leading to high wa-

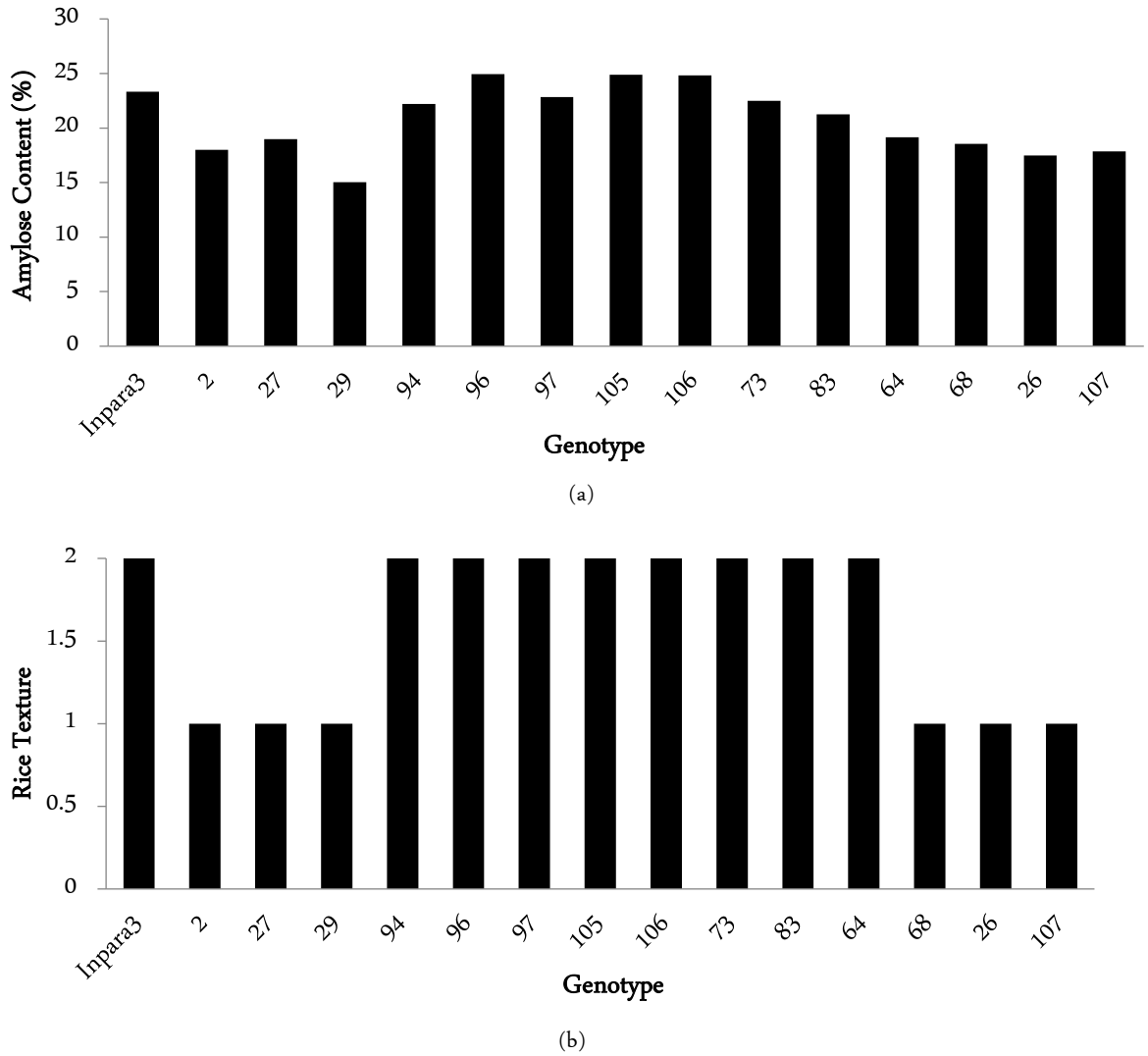

Figure 1. Diagram (a) Percentage of Amylose content dan (b) Rice texure (1.00 = sticky (waxy), 2.00 = firm (non-waxy) to the character of cooking quality of several selected Fe tolerant rice lines with Inpara 3 parent as control

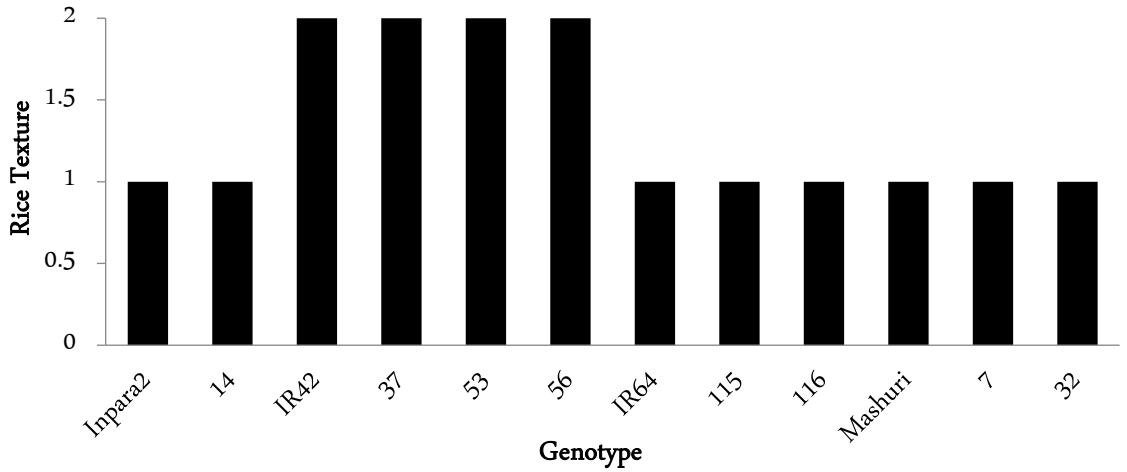

Figure 2. Diagram of Rice texture (1.00 = sticky (waxy), 2.00 = firm (non-waxy)) in derivative lines of parent control (Inpara 2, IR42, IR64, Mashuri) with the texture of the rice same with each parent

ter absorption capability. Therefore, firm rice requires more water for starch granule swelling. Cooking quality values of 30 rice lines (Table 4) were close to parent control (Table 5) and had small standard deviation in several characters. Based on amylose content and rice texture, Fe toxicity-tolerant lines from Inpara 3 were grouped as sticky and firm. Based on Figure 1, 8 of 14 lines showed same firm rice texture as Inpara 3, namely G-94, G-96, G-97, G-105, G-106, G-73, G-73, G-64, and 6 lines were sticky rice texture. Rice lines that were similar to parent had medium amylose content, while 6 rice lines which were different from parent had low amylose content.

In Figure 2, 1 rice line (G-14) of 4 lines derived from Inpara 2 had sticky texture. All rice lines derived from IR42 (G-37, G-53, G-56) was firm texture which is similar to parent and had high amylose. Furthermore, all rice lines derived from IR64 (G-115, G-116) had sticky texture as parent. Only 2 (lines G-7 and G-32) derived from Mashuri was same as parent (Figure 2) with low amylose and sticky texture, while 5 remains had high amylose content and firm texture. Therefore, a total of 16 rice lines tolerant to Fe stress were same as 5 parent controls, while 14 remaining rice lines were not.

Genotypes characteristics using seed quality-related markers

Molecular analysis involved 19 molecular markers that are SSR, STS, Indel and SNP, showed that 14 selected markers (P-EQ1, P-EQ2, P-EQ3, P-EQ 6, P-EQ7, P-EQ8, P-EQ9, P-EQ13, P-EQ14, P-EQ15, P-EQ16, P-EQ17, P-EQ18 and P-EQ19) were polymorphic, while 5 remaining markers were not polymorphic. PCR analysis using P-EQ2 as primer showed that allelic variation ranged from 175 bp to 232 bp (Figure 3). The highest frequency of allele size was at 201 bp (Table 7), indicating that it was conserved in both crossing variety and derived lines. However, minor allele frequency (< 5%) was detected in 175 bp, and 183 bp. The minor allele frequency was specific allele for particular lines. For example, alleles with the size of 175 bp, and 183 bp were only detected in the G-20, G-83, G-97.

Association analysis of phenotype and genotype

To determine significant markers ($p < 0.05$), association analysis was conducted using Tassel 2.0. Some significant markers (Table 6) associated with physical quality of rice grain, physical quality of rice, and organoleptic and cooking quality were detected, which was found in chromosomes 1, 3, 4, 6, 7 and 11. Several significant markers have been acknowledged to mark starch biosynthesis-related genes. The genes are *sbe1*, *gpa*, *pul*, *ss1*, *sssiia* while *s3cl* is involved in sucrose biosynthesis. P-EQ2 which marks *sbe1* gene was significantly associated with physical quality of the rice grain, namely chalky green grain. *sbe1* (Starch branching enzyme 1 or 1.4-alpha-glucan-branching enzyme) was in chromosomes 6. Some SBE1 characteristics in the rice endosperm are responsible for starch structure [19].

P-EQ9 which marks GPA (glucosamine-fructose-6-phosphate aminotransferase) gene was significantly associated with physical quality of the grain, namely red grains. GPA catalyzes the formation of macromolecular precursors containing lots of active sugar, thus the gene is involved in the synthesis of starch, mapped on chromosome 11 [20]. P-EQ15 and P-EQ16 that mark PUL

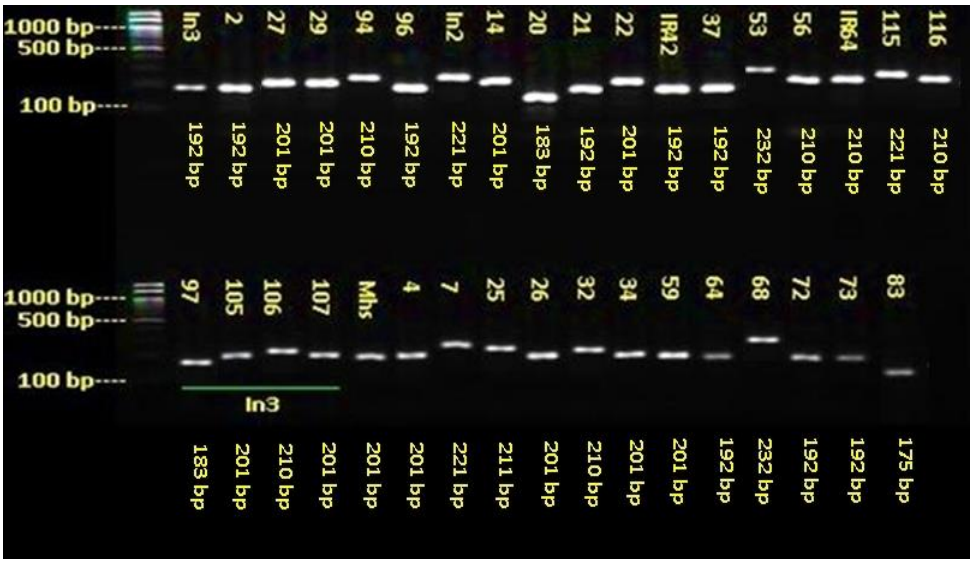

Figure 3. The result of genotyping PCR analysis on 30 selected Fe tolerant rice lines and 5 parents as control using P-EQ2 marker

Table 6. Some markers with significance highest the results of association ($p < 0.05$)

Marker	*Gene*	*Marker Type*	*Chromosome*	*Character*	*Trait*	*P-Value (< 0.05)*
EQ2	*sbe1*	SSR	6		Chalky green grain	7.01×10^{-05}
EQ6	*s3cl*	Indel	7		Chalky green grain	1.70×10^{-02}
EQ9	*gpa*	SSR	11		Chalky green grain	6.00×10^{-03}
EQ9	*gpa*	SSR	11	Physical grain	Red grain	1.35×10^{-10}
EQ9	*gpa*	SSR	11	quality	Red grain	3.90×10^{-02}
EQ15	*pul3*	STS	4		Empty grain + Material dirt	1.41×10^{-05}
EQ6	*s3cl*	Indel	7		Empty grain + Material dirt	2.84×10^{-05}
EQ15	*pul3*	STS	4		Weight of 1000 grain	3.00×10^{-03}
EQ1	*ss1*	SSR	6		Milled rice	1.68×10^{-04}
EQ15	*pul3*	STS	4		Broken grain	3.70×10^{-02}
EQ1	*ss1*	SSR	6	Physical rice	Level of white rice	1.25×10^{-04}
EQ18	*hp* [b]	STS	3	quality	Level of white rice	9.29×10^{-05}
EQ13	*sssiia*	STS	6		Level of sosoh rice	2.00×10^{-03}
EQ13	*sssiia*	STS	6		Transparency	3.70×10^{-02}
EQ16	*pul5*	STS	4		Protein Content	4.10×10^{-02}
EQ17	*mad* [b]	STS	12		Amylose Content	1.50×10^{-02}
EQ19	*aglu* [c]	STS	1	Cooking rice	Amylose Content	2.00×10^{-03}
EQ13	*sssiia*	STS	6	quality	Gel Melting (mm)	4.10×10^{-02}
EQ15	*pul3*	STS	4		Gel Consistency	1.60×10^{-02}
EQ15	*pul3*	STS	4		Rice Texture	1.70×10^{-02}
EQ15	*pul3*	STS	4		Aroma	4.00×10^{-03}
EQ6	*s3cl*	Indel	7		Color	1.00×10^{-03}
EQ18	*hp* [b]	STS	3		Color	1.56×10^{-05}
EQ18	*hp* [b]	STS	3	Organoleptic	Glossy	2.00×10^{-03}
EQ18	*hp* [b]	STS	3		Taste	6.96×10^{-04}
EQ6	*s3cl*	Indel	7		Taste	3.00×10^{-03}
EQ15	*pul3*	STS	4		Stickiness	4.00× 10-03

(pullulanase) gene were significantly associated with the character of the physical quality of rice grain, physical quality of rice, and organoleptic and cooking quality. PUL is a major gene for controlling AC. PUL is mapped on chromosome 4 and has a physiological function to degrade starch in the endosperm and leaf, in grains such as corn and rice. Besides it also degrades starch during seed germination [21]. Furthermore, P-EQ1 marks *ss1* (starch synthase I) gene was significantly related to the character of the physical quality of grain and rice. *ss1* is mapped on chromosome 6. It controls AC adjusted to QTL analysis on rice by physicochemical

P-EQ13 which marks *sssiia* gene (soluble starch synthase IIa) was significantly related to physical and cooking quality of rice, namely milling degree, transparency, and long-melt gel. *sssiia* was mapped on chromosome 6 and plays role in controlling the disintegration of alkali rice grain and in synthesizing starch in the rice endosperm. Besides, *sssiia* also determines eating and cooking quality of rice by affecting amylose content AC and GC. *alk* gene is the major gene that controls GT, but as a minor gene, it affects AC and GC [4].

P-EQ6 that marks S3cl gene (mapped on chromosome 7) was significantly associated with organoleptic character that determines the taste and aroma of the rice. These genes play role in synthesizing sucrose and controlling development and growth of rice plants, in addition to seed filling [22]. Based on association analysis, the genes that often and significantly correlate with seed quality and physicochemical properties of rice were *pul* and *s3cl*, respectively. *pul* and *s3cl* respectively contribute to starch and sucrose synthesis. Both genes are responsible for eating and cooking quality in rice by affecting AC and GC [4].

CONCLUSION

Phenotypic analysis of the physical and cooking quality of rice showed that the 30 rice lines had an average quality score that tend to be close to the value of its parent, which was mostly in grade IV for physical quality. The amylose content was in the range of low to high, whereas cooking quality (rice texture) classified as sticky until firm. Among 30 Fe-tolerant rice lines, 16 rice lines had similar characteristics to parent control based on amylose and rice texture. This study showed that 14 of 19 primers result polymorphic DNA band (DNA markers). Association analysis of genotype and phenotype showed that 12 of 14 markers were significantly (p - Value < 0.05) related to high quality of rice grain. Significant markers related to genes *sbe1*, *ss1*, *sssiia*, *gpa*, *pul*, *s3cl* showed contribution in starch biosynthesis that was critical factor for eating and cooking quality of rice lines or variety. The significant molecular markers are potential to be use as markers of selection for rice grain quality of Fe-tolerant rice line that produced through breeding program.

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