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RICE BACKCROSS POPULATION ASSESSMENT FOR IRON TOLERANCE THROUGH PHENOTYPIC AND GENOTYPIC ANALYSES

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SUMMARY

Iron toxicity has become a serious issue affecting rice (*Oryza sativa* L.) production in many irrigated lowland areas. The selection of Fe^{2+}-tolerant rice cultivars under iron toxicity conditions and the identification of molecular markers are good approaches to obtaining tangible results. This study aimed to identify simple sequence repeat (SSR) markers that were associated with iron tolerance traits in a rice backcross population. A total of 117 seedlings from the backcross (BC_3F_2) of 'OM6830'/'AS996'//'AS996' were phenotyped at the 4-week-seedling stage at Ton Duc Thang University, Ho Chi Minh City, Vietnam. The rice population was screened in Yoshida nutrient medium supplemented with $FeCl_2$ at a concentration of 150 mg L^{-1} under greenhouse conditions. Phenotypic analysis was conducted by scoring two parameters, namely, root length and leaf bronzing. Genotypic analysis was carried out on the BC_3F_2 population by using four markers, i.e., RM6, RM240, RM252, and RM451, for association analysis with iron tolerance. A total of 23 BC_3F_2 lines were selected on the basis of their higher tolerance (score 1) for Fe^{2+} compared with the tolerant parental line 'AS996'. The markers RM6 and RM240 were highly polymorphic and identified different Fe^{2+}-tolerant lines in the BC_3F_2 population. Among the BC_3F_3 progeny derived from the selected 23 BC_3F_2 lines, BC_3F_3-7 was identified as the most Fe^{2+}-tolerant line. BC_3F_3-15 was also found to be Fe^{2+} tolerant. Both lines showed good development capability and provided high yields under stress conditions. These tolerant BC_3F_3 lines could be further screened with additional SSR markers in future breeding programs aiming to increase rice production in iron-contaminated areas of the Mekong Delta, Vietnam.

Keywords: Backcrosses, iron toxicity, phenotypic and genotypic traits, screening, SSR markers, genetic analysis, *Oryza sativa* L.

Key findings: Among the 117 rice lines derived through the backcross method, line BC_3F_3-7 was selected as the most Fe^{2+}-tolerant line by using the two highly polymorphic markers RM6 and RM240. BC_3F_3-15 was found to be the next most Fe^{2+}-tolerant line. The two rice lines displayed good growth and increased yield under Fe^{2+}-stressed conditions.

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INTRODUCTION

Rice (*Oryza sativa* L.) is an important food crop that is consumed by more than half of the global population (Chakravarthi and Naravaneni, 2006). Global rice production reached 506.0 million tons (milled basis) in 2021 (FAO, 2021). Against the background of climate change, rice production is at risk of difficulties due to various environmental factors, such as heat, drought, salinity, flooding, and iron and aluminum toxicity (Dabi and Khanna, 2018). In recent years, the problem of iron toxicity has become a serious issue affecting rice production in irrigated-lowland areas (Sikirou *et al.,* 2018), especially in Southeast Asia and West Asia. The occurrence of iron toxicity is associated with a high concentration of Fe^{2+} in the soil solution. In wetlands, iron toxicity has been reported to reduce rice yield by 20%–100% in accordance with soil iron and varietal tolerance levels (Sahrawat, 2004; Santos *et al.,* 2019).

At low concentrations, iron is an essential micronutrient for plants; however, either a deficiency or an excess of iron inhibits plant growth (Seraj and Rahman, 2018). Acid sulfate soils that cause iron toxicity occupy approximately 1.5 million hectares of the Mekong River Delta, Mekong Delta, Vietnam. They are mainly distributed in Dong Thap Muoi, Tu Giac Long Xuyen, and West Song Hau and are also scattered in some other areas of Vietnam. Rice remains the main crop planted in these areas (Bong *et al.,* 2018). Iron toxicity and aluminum toxicity are often considered to be two limiting factors for crop yield in acid soils. The increase in pH due to the reduction process that occurs when alum soil is submerged in water can limit aluminum toxicity but at the same time exacerbates iron toxicity. Iron toxicity affects growth and grain yield and is particularly severe at the early seedling stage (Liu *et al.,* 2016). Rice breeding for iron toxicity tolerance has placed major emphasis on identifying resistance and harnessing genes for genetic and phenotypic improvement. The identification and development of new rice cultivars with high yield and quality traits are important factors for enhancing and stabilizing rice production in the country. Among the measures taken to limit iron toxicity in rice, the most effective is the selection and cultivation of varieties with high tolerance (Bresolin *et al.,* 2019). Therefore, the selection of Fe^{2+}-tolerant cultivars has been an important strategy and an effective measure to reduce rice yield losses (Stein *et al.,* 2014; Mahender *et al.,* 2019).

Field evaluation and selection are difficult to perform due to several factors, such as G × E interaction effects, and their need for time-consuming and costly trials. Therefore, molecular markers are considered as a tool for assisting the genotyping and selection of tolerant rice cultivars (Platten *et al.*, 2019). Although classic breeding methods have shown efficiency in producing improved cultivars, marker-assisted selection further increases the effectiveness of breeding (Sakiyama *et al*., 2014). Adequate genotyping and phenotyping are important for the success of plant breeding with marker-assisted selection (Sakiyama *et al*., 2014). Large-scale genotyping is becoming faster, cheaper, and more automated (Sakiyama *et al*., 2014). Given that it is unaffected by environmental effects, it shortens the breeding cycle (Lincoln *et al.*, 2018). By complementing phenotypic data, molecular markers improve the capability to compare genotypes even if these genotypes are sampled in different environments, tissue types, or developmental stages (Sakiyama *et al.*, 2014). Genome-wide DNA polymorphisms are detected by using molecular markers (Sakiyama *et al.*, 2014). In rice, many quantitative trait loci (QTL) for iron toxicity have been identified and mapped by using DNA markers (Zhang *et al.,* 2017). Efforts to identify the QTL for iron tolerance have focused on easily measurable traits, such as leaf bronzing index, shoot and root dry weight, tiller number, plant height, root length, and iron accumulation in the shoot (Wan *et al.,* 2003; Dufey *et al.,* 2015). The

combination of QTL mapping with marker-assisted selection has become an effective breeding technique in the precise identification of genotypes (Collard and Mackill, 2007; Boopathi, 2020). DNA markers are used for multiple purposes, such as gene mapping, gene tagging, estimating genetic diversity, differentiating between cultivars, and purity testing (Nagaraju *et al.*, 2002; Boopathi, 2020). Markers and laboratory testing are used in combination with field testing for the identification of traits that are most important to farmers, processors, and consumers to produce new crop varieties that are adapted to climate change and ensure food security. The current study aimed to identify new rice lines in backcrossed populations through a combination of phenotypic analysis and SSR markers related to high iron tolerance. The results of this work will provide a basis for planning the future breeding program of iron-tolerant rice cultivars for the Mekong Delta, Vietnam.

MATERIALS AND METHODS

Plant material

The genotype 'AS996', a Fe^{2+}-tolerant line that was developed from a cross between 'IR64' and *Oryza rufipogon*, has been identified as strongly tolerant to acid-sulfate soils (Buu and Lang, 1997, 2007). The susceptible parent 'OM6830' is a common rice variety collected from Cuu Long Delta Rice Research Institute (CLRRI), Vietnam. The BC_3F_1 population was obtained by backcrossing the hybrid of the cross 'OM6830' × 'AS996' with the tolerant parent 'AS996'. The BC_3F_2 ('OM6830'/'AS996'//'AS996') population was derived by self-pollinating each BC_3F_1 plant. The 117 BC_3F_2 lines and their parents were screened at Genomic Research Institute and Seed, Ton Duc Thang University, Ho Chi Minh City, Vietnam.

Phenotypic analysis

The recommended doses of Fe^{2+} that are used for screening iron toxicity tolerance are 100 mg L^{-1} at 4.0 pH (Fageria and Robelo, 1987) and 200 Fe^{2+} mg L^{-1} at 5.0 pH (Yamaguchi and Yoshida, 1981). However, in the current study, we applied 150 mg L^{-1} Fe^{2+} in the solution for screening the rice population under the assumption that iron toxicity will increase in the future when annual flooding causes serious damage to Vietnam's agriculture and results in sulfate and iron toxicity (Nugroho *et al.*, 2021).

The experiment was arranged in a randomized complete block design with three replications (9 rice seeds/3 holes) and carried out in the greenhouse. In the greenhouse, the temperature was maintained at 32 °C/28 °C light/dark, and the relative humidity was varied from 85% to 90%. The 117 BC_3F_2 lines and their parents were cultured hydroponically for 14 days in Yoshida solution. On the 14th day, 150 mg L^{-1} $FeCl_2$ solution was added to the solution, and the pH was maintained at 5.0. Samples were collected, and phenotypic evaluation was done 14 days after transfer to the Fe^{2+} solution. Root length was measured, and leaf bronzing was evaluated on a 1–9 scale at 4 weeks by following the Standard Evaluation System (SES) of the International Rice Research Institute (IRRI), Los Baños, Philippines (IRRI, 2002) (Table 1). Tolerance and susceptibility assessment was done on the basis of root length and leaf bronzing scores. On the basis of this phenotypic analysis, 23 tolerant lines were selected from 117 BC_3F_2 lines. These lines were subjected to genotypic analysis. Furthermore, the BC_3F_3 population (BC_3F_3-1 to BC_3F_3-23) developed by self-pollinating the selected BC_3F_2 plants that were used to evaluate yield and yield components in an iron-toxic environment.

Table 1. SES for rice (IRRI, 2002).

Score	Symptoms
0	Growth and tillering are nearly normal
1	Growth and tillering nearly normal; reddish-brown spots or orange discoloration on the tips of older leaves
3	Growth and tillering nearly normal; older leaves reddish-brown purple or orange yellow
5	Growth and tillering retarded; many leaves discolored
7	Growth and tillering cease; most leaves discolored or dead
9	Almost all plants dead or dying

Note: Levels of 0–3 on the SES scale are considered "tolerant" and those of 5–9 are "susceptible"

Table 2. Primer sequences of the molecular markers used for identifying the lines tolerant to iron toxicity.

No.	Markers	Primer sequences used for gene detection (5'– 3')	Chromosome
1	RM6	F'GTCCCCTCCACCCAATTC' R'TCGTCTACTGTTGGCTGCAC'	2
2	RM240	F'CCTTAATGGGTAGTGTGCAC' R'TGTAACCATTCCTTCCATCC'	2
3	RM252	F'TTCGCTGACGTGATAGGTTG' R'ATGACTTGATCCCGAGAACG'	4
4	RM451	F'GATCCCCTCCGTCAAACAC' R'GATCCCCTCCGTCAAACAC'	4

Genotypic analysis

The 23 selected BC_3F_2 lines and the parental genotypes ('OM6830' and 'AS996') were used, and DNA was extracted via the CTAB method as described by Lang (2002). Four SSR markers (RM6 and RM240 on chromosome 2 and RM252 and RM451 on chromosome 4) were used to analyze the BC_3F_2 population (Table 2).

Young leaf tissue (50 mg) was crushed by using a mortar and pestle with 400 µL of extraction buffer (100 mM Tris-HCl, 50 mM EDTA, 500 mM NaCl, 1.25% [w/v] SDS, and 3.8 g per L sodium bisulfite). The mixture was added into a 2.2 mL tube and gently mixed with 400 µL of chloroform:isopropanol (24:1) and centrifuged at 13 000 rpm for 1 min. The supernatant was transferred into a new tube (2.2 mL) and added with 800 µL of absolute ethanol. After freezing overnight, the suspension was centrifuged at 13 000 rpm for 5 min. The obtained pellet was washed with 500 µL of 70% ethanol. The pellet was resuspended in 50 µL of TE buffer and incubated at room temperature. DNA quality was tested by electrophoresis on 3% agarose gel in 1× TAE buffer for 7–10 min, and the DNA was visualized on a gel documentation system (Bio-Rad Gel Doc XR™ imaging system, California, USA) under UV light after staining with ethidium bromide.

Polymerase chain reaction (PCR) and simple-sequence repeat (SSR) analyses were performed with four primer pairs by using the template of the genomic DNA in accordance with Panaud *et al.* (1996) with certain modifications. In marker selection, PCR (Alpha Cycler, AC-2, Staffordshire, United Kingdom) was performed by using a total volume of 12.5 µL, which contained 6.25 µL of the master mix (Qiagen), 0.5 µL of primer F (10 mM), 1 µL of DNA (40 ng/µL), and 4.25 µL of H_2O. The PCR conditions were 94 °C for 5 min followed by 29 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min with a final extension cycle of 5 min at 72 °C. The PCR products were separated via

electrophoresis (Mupid One, Takara, Japan) on 8% polyacrylamide gel (2.4 mL of TBE 5×, 3.2 mL of acrylamide, 200 µL of APS, 18 µL of Tedmed, and 6.4 mL of H_2O), and the banding pattern was visualized by staining with ethidium bromide. The stained gels were photographed with a Gel Doc Molecular Imager (Bio-Rad Gel Doc XR^{TM} Imaging System, USA).

Evaluation of yield and yield components

The 23 BC_3F_3 rice lines and the two parental varieties ('AS996' and 'OM6830') were screened in the field. The soil characteristics at the experimental site are shown in Table 3. Data measurements were obtained for yield and yield components: plant height (cm), the number of tillers, panicle length (cm), filled grain, unfilled grain (%), 1000-grain weight (g), and grain yield (g).

Data analysis

The mean root lengths of the genotypes were calculated by using Excel software. The yield and yield parameters of the tolerant rice were analyzed by using the general linear model. Analysis of variance was performed with SPSS software 17.0 (SPSS Inc. Chicago, IL, USA), and Duncan's multiple range test was applied with a significant difference at the 0.05 level.

Table 3. Soil characteristics of the experimental site in O Mon, Can Tho City, Mekong Delta, Vietnam.

Characteristics	Unit	Value
Clay	%	62
Loam	%	33
Sand	%	5
pH (KCl)		3.9
EC	mS/cm	0.37
Total N	%	0.17
Available N	Mg/kg	70
Total P	%	0.06
Available P	Mg/kg	4.64
Total K	%	1.12
K^+	meq/100 g	0.08
Total Fe	Mg/kg	2.67
Available Cu	Mg/kg	33.67
Available Zn	Mg/kg	23.23
Total Ca	%	0.0024
Ca^{2+}	meq/100 g	1.581
Total Mg	%	0.122
Mg^{2+}	meq/10 0g	3.76
Total Mn	%	0.022
CEC	meq/100 g	25

Note: KCl – Potassium chloride; EC – Electrical conductivity; Total N – Total nitrogen; Available N – Available nitrogen; Total P – Total phosphorus; Available P – Available phosphorus; Total K – Total potassium; K^+ – potassium ion; Total Fe – Total ferrite; Available Cu – Available copper; Available Zn – Available zinc; Total Ca – Total calcium; Ca^{2+} – Calcium ion; Mg^{2+} – Magnesium ion; Total Mn – Total manganese; CEC – Cation exchange capacity.

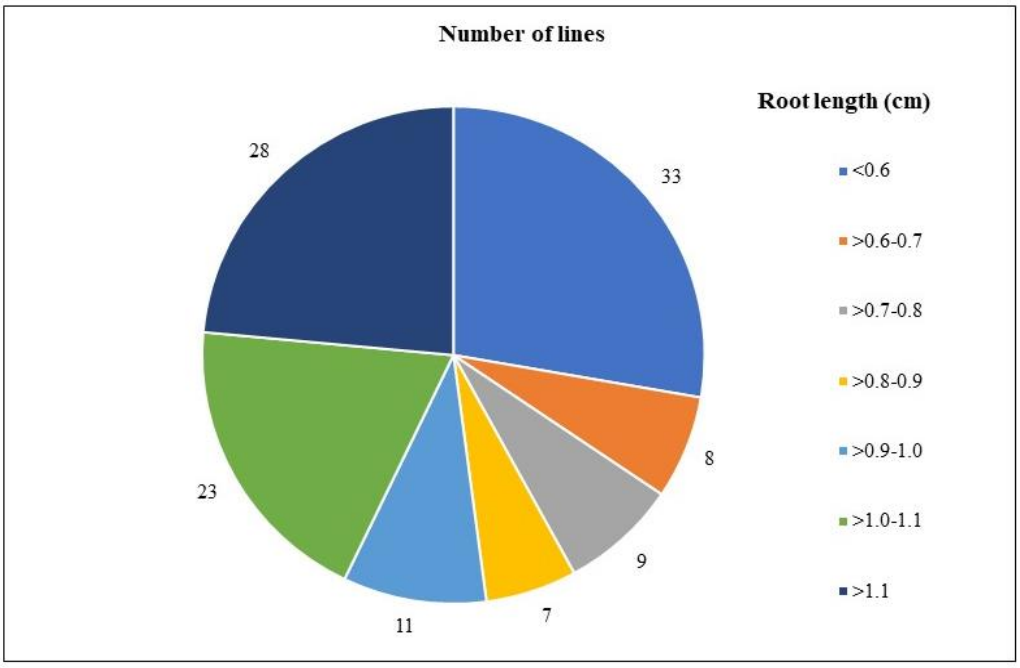

Figure 1. Root length of contrasting lines in the 'OM6830'/'AS996'//'AS996' population treated with 150 mg L^{-1} $FeCl_2$ for 14 days.

RESULTS AND DISCUSSION

Phenotypic analysis

Various traits, such as root growth and leaf bronzing indexes, have been reported to be associated with iron tolerance at the early growth stage of rice and thus can be used as selection criteria (Sahrawat and Audebert, 2000; Becker and Asch, 2005; Wu *et al.,* 2014).

The 119 lines screened for their tolerance to Fe^{2+} at the seedling stage after 4 weeks were categorized into seven groups on the basis of root length (Figure 1). The group with the lowest root length contained 33 rice lines (<0.6 cm), which accounted for 27.7% of the analyzed population. The next most important population of 27 lines and the tolerant parental genotype were in the root length group > 1.1 cm and accounted for 23.5% of the total population. The 23 lines with the root length of 1.0–1.1 cm comprised 19.3% of the total population. Moreover, nine rice lines, including the susceptible parental genotype, were in the root length group of 0.7–0.8 cm, comprising 7.5% of the population of 119 rice genotypes.

In acidic soils, the solubility and high plant availability of iron lead to iron-induced toxicity (Hu *et al.,* 2014). The development of rice cultivars with increased tolerance to Fe^{2+} is an important strategy for improving rice production. Roots play an essential role in iron tolerance in rice. Iron is removed from the roots by parenchymal-derived oxygen or through the enzymatic oxidation of Fe^{2+} into Fe^{3+}, which forms precipitates in the form of iron plaques at the root surface (Wu *et al*., 2014; Stein *et al*., 2019). Roots have the capability to store iron in their tissues and limit iron transfer from the roots to shoots (Stein *et al*., 2014) to avoid the excessive accumulation of iron in leaves and maintain homeostasis (Da-Silveira *et al.,* 2007; Aung and Masuda, 2020).

The results indicated that out of 119 lines, 23 lines and the tolerant parental line 'AS996' possessed high tolerance with the score of 1 (Figure 2). A total of 29 lines had the score of 3, 17

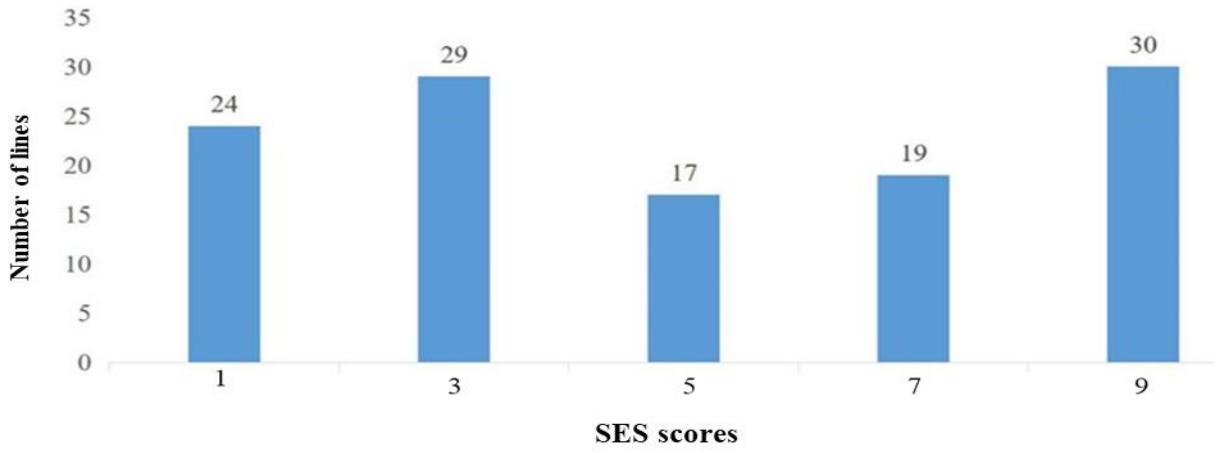

Figure 2. Leaf bronzing score of contrasting lines in the 'OM6830'/'AS996'//'AS996' population treated with 150 mg L^{-1} $FeCl_2$ for 14 days.

lines had the score of 5, and another 17 lines had the score of 7 (Figure 2). The largest number of lines, i.e., 29 lines and the susceptible parental line 'OM6830', showed iron toxicity symptoms with the score of 9. Leaf bronzing is one of the most essential traits for the identification of tolerant genotypes under iron-toxicity conditions (Wu *et al.,* 2014). The tolerant parent 'AS996' used in this study was derived from an 'IR64' × *O. rufipogon* cross. Mendoza *et al.* (2003) screened wild accessions of *O. rufipogon* at the iron concentration of 400 ppm and identified three accessions that were highly resistant and could be a possible resource of useful genes for iron tolerance in rice. The current results verified that the iron responses of the tolerant parental line 'AS996' and the lines of the tolerant groups (SES scores of 1 and 3) were significantly different from those of the susceptible parent 'OM6830' and the lines in the susceptible group (scores of 5, 7, and 9).

Altogether, three phenotypic classes of rice lines were defined on the basis of iron tolerance in the current study: highly tolerant (SES score of 1), less tolerant (SES score of 3), and susceptible (SES score greater than 3). The threshold of toxic iron concentration for rice ranges from 10 mg L^{-1} to 300 mg L^{-1} in accordance with the form of nutrient supply and the tolerance of the rice genotypes (De-Dorlodot *et al.,* 2005; Elec *et al.,* 2013). Solutions with $FeCl_2$ concentrations of 150 mg L^{-1} (Fageria and Robelo, 1987), 200 mg L^{-1} (Yamaguchi and Yoshida, 1981), and 300 mg L^{-1} (Nugraha *et al.,* 2016) have been tested for the screening of rice seedlings for iron toxicity. In this study, the iron concentration of 150 mg L^{-1} was found to be highly suitable for screening Fe^{2+}-tolerant lines. Finally, the 23 most tolerant BC_3F_2 rice lines (1–23) with the score of 1 and the root length of >1.1 cm were selected as the most promising tolerant lines and subjected to genotypic analysis.

Genotypic analysis

Two markers, i.e., RM6 and RM240 on chromosome 2 at 200–210 and 300-310 bp, respectively, were linked to iron toxicity tolerance. Marker RM6 was polymorphic. The susceptible parent 'OM6830' contained allele A with a molecular size of 210 bp, whereas the tolerant line 'AS996' contained allele B with a size of 200 bp. Seven rice lines (2, 7, 12, 13, 14, 15, and 17) exhibited a similar banding pattern as 'AS996' for marker RM6 (Figure 3C). Six lines (3, 7, 8, 11, 15, and 16) displayed similar

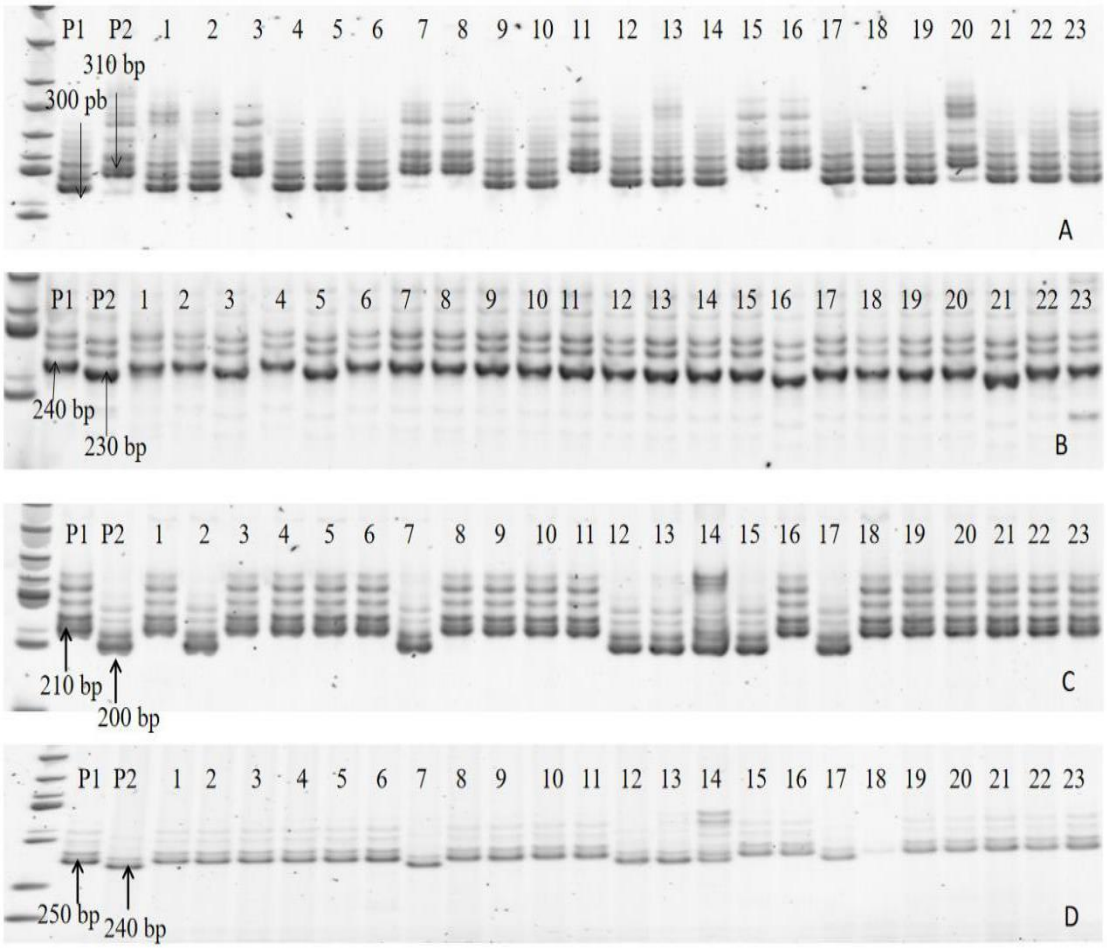

Figure 3. Banding profiles of some lines obtained with primer RM240 (A), RM252 (B), RM6 (C), and RM 451 (D) in the BC_3F_2 population of 'OM6830'/'AS 996//'AS996'. Lane 1: 'OM6830' (A), lane 2: 'AS996' (B), lanes from 4 to 25 are the 23 BC_3F_2 lines, which were named from 1–23.

banding patterns for RM240, indicating that they possessed an iron toxicity tolerance gene with the molecular size of 310 bp that was carried by the parental genotype 'AS996' (Figure 3A).

Rasheed *et al.* (2021) reported that iron toxicity leads to the reduction in the uptake of numerous essential nutrients, such as nitrogen, phosphorous, and potassium. Wan *et al*. (2005) showed that the QTL for leaf bronzing index located in the RM6–RM240 region on chromosome 2 is also linked to the QTL for potassium uptake under potassium deficiency stress in the RZ58–CDO686 region. Furthermore, the locations of *O. nivara*-derived QTL, such as *qFe2.1, qFe3.1, qFe8.2*, and *qZn12.1*, were consistent in both BC_2F_3 mapping populations derived from the crosses of *O. sativa* cv 'Swarna' and two different accessions of *Oryza nivara* (Swamy *et al.*, 2018). QTLs for iron concentration were located in the RM106 and RM6 regions on chromosome 2 in population 1 (Swamy *et al.*, 2018).

In addition to those two markers, two other markers (RM252 and RM451) at 230–240 and 240–250 bp on chromosome 4 were linked to iron tolerance. The parents were polymorphic for the marker RM252. The susceptible parental line 'OM6830' carried allele A with the

molecular size of 240 bp, whereas the tolerant parental line 'AS996' carried allele B with the molecular size of 230 bp. The iron tolerance gene of the four rice lines (3, 5, 16, and 21) was similar to that of tolerant parental line 'AS996' with the molecular size of 230 bp of RM252 (Figure 3B). Five lines (7, 12, 13, 14, and 17) carried an iron tolerance gene that was similar to the iron tolerance gene of the tolerant-parent 'AS996' with the molecular size of 240 bp carried by RM451 (Figure 3D).

QTL for iron toxicity have been identified by using DNA markers (Wan *et al.,* 2003; Zhang *et al.,* 2017). The combination of QTL determination with molecular marker-assisted selection has become effective in many breeding programs through precise genotyping (Boopathi, 2020). Wan *et al.* (2005) studied the F_2 and equivalent F_3 populations derived from 'Longza 8503' × 'IR64' and found that QTL controlling leaf bronzing index are located in the RM6–RM240 region on chromosome 2 and the RM252–RM451 region on chromosome 4. Two markers, namely, RM252 and RM451, on chromosome 4 are identical to the QTL for decreased chlorophyll content on a rice function map (Wan *et al.*, 2005).

In the current study, line BC_3F_2 -7 was identified as the most tolerant genotype with the markers RM240, RM6, and RM451. This line was completely tolerant of Fe^{2+} at the concentration of 150 mg L^{-1}. Thus, the PCR screening of this line was carried out through two consecutive self-pollinated generations (from BC_3F_1 to BC_3F_2). The PCR test results (BC_3F_2 generation) confirmed that tolerance genes had been transferred into the desired rice lines. The genotypic results showed that the 23 tolerant lines and the tolerant parental line 'AS996' can be directly subjected to MAS to identify iron toxicity tolerance. The identification of robust markers for traits is essential for the incorporation of tolerance through MAS breeding. In previous reports, the loci associated with iron toxicity tolerance on chromosome 1 were localized in the same region wherein several QTL had been previously detected in different studies (Wu *et al.,* 2014; Dufey *et al.,* 2015). In the current study, markers RM6 and RM240 on chromosome 2 were quite closely associated with tolerance to iron toxicity in the existing rice population (Figure 3A, C). These results were also consistent with the past findings of QTL analysis for aluminum tolerance in rice via marker-assisted selection (Buu *et al.*, 2010). Therefore, identifying the rice lines containing tolerance genes for further evaluation and selection is possible.

Yield and yield components of the selected rice lines

The 23 developed BC_3F_3 lines were further screened for their genetic potential for yield and yield components (Table 4). The results showed that plant heights ranged from 102 cm to 120 cm. Among the 23 lines, BC_3F_3-7 and BC_3F_3-15 had a higher number of tillers (11 and 10) than their parental genotypes (seven and nine tillers) ($P < 0.05$). Three lines, namely, BC_3F_3 -7, BC_3F_3 -15, and BC_3F_3-19, showed the highest number of filled grains of 122, 107, and 100, respectively, whereas the parental checks had 61 and 85 filled grains. Grain yield ranged from 6 g to 35 g per plant. The line BC_3F_3-7 presented a higher yield (35.4 g) than the parental checks (8.9 and 17 g). The grain yield per plant of BC_3F_3-7 (35.4 g) was 4-fold and 2-fold higher than those of the susceptible (8.9 g) and tolerant (17g) parental lines, respectively. In the current study, at pH < 4.0, the rice lines BC_3F_3-7 and BC_3F_3-15 showed significantly higher number of tillers, 1000-grain weight, filled grain, and grain yield and fewer unfilled grains than other lines. These indexes can be used as selection criteria in future breeding programs.

The selection of iron-tolerant rice lines is a continuous process involving hybridization, strain selection, and adaptation testing in acid-affected areas. Approximately 50% of the area in the Mekong Delta region of Vietnam consists of acid sulfate soil and accounts for nearly 70% of the area's agricultural land (Bong

Table 4. Yield and yield components of the selected Fe^{2+}-tolerant rice lines in the BC_3F_3 population.

Rice genotypes	PH	NT	PL	FG	UFG	W−1000	GY
P_1 ('OM6830')	107^{fg}	7^{de}	23.7^{cdef}	61^{n}	49.2^{g}	20.9^{hijk}	8.9^{kl}
P_2 ('AS996')	105^{hi}	9^{bc}	24.3^{abcde}	86^{f}	26.3^{n}	21.9^{fghijk}	17.0^{cd}
BC_3F_3−1	106^{fgh}	6^{ef}	22.2^{dgh}	81^{i}	37.1^{kl}	22.5^{defghi}	11.0^{ij}
BC_3F_3-2	110^{de}	5^{f}	21.0^{ghi}	54^{q}	33.3^{m}	23.2^{efg}	6.2^{m}
BC_3F_3-3	106^{fh}	6^{ef}	20.7^{hi}	87^{f}	65.1^{b}	24.2^{bcde}	12.6^{fghi}
BC_3F_3-4	113^{bc}	7^{de}	20.0^{i}	86^{f}	64.5^{b}	23.4^{defg}	14.1^{efg}
BC_2F_3-5	112^{cd}	8^{cd}	24.3^{abcde}	78^{j}	23.0^{o}	23.1^{defg}	14.4^{ef}
BC_3F_3-6	118^{a}	9^{bc}	20.5^{hi}	67^{l}	73.0^{a}	20.6^{ijk}	12.4^{hi}
BC_3F_3-7	108^{ef}	11^{a}	24.2^{abcde}	122^{a}	22.3^{o}	26.4^{a}	35.4^{a}
BC_3F_3-8	113^{bc}	8^{cd}	25.7^{ab}	67^{l}	53.5^{e}	23.8^{bcdef}	12.8^{fghi}
BC_2F_3-9	111^{cd}	7^{de}	23.0^{def}	59^{op}	61.0^{c}	22.2^{efghij}	9.2^{kl}
BC_3F_3-10	118^{a}	6^{ef}	25.3^{abc}	84^{g}	50.4^{fg}	20.5^{jk}	10.3^{jk}
BC_3F_3-11	102^{j}	8^{cd}	24.0^{bcdef}	65^{m}	51.6^{f}	22.6^{defgh}	11.7^{hij}
BC_3F_3-12	105^{hi}	5^{f}	22.7^{efg}	68^{l}	45.9^{h}	24.2^{bc}d	8.2^{l}
BC_3F_3-13	112^{cd}	7^{de}	24.7^{abcd}	82^{hi}	43.3^{ij}	22.0^{fghijk}	12.6^{fghi}
BC_3F_3-14	108^{ef}	7^{de}	24.3^{abcde}	83^{gh}	38.2^{kl}	21.8^{ghijk}	12.7^{fghi}
BC_3F_3-15	107^{fg}	10^{ab}	21.0^{ghi}	107^{b}	38.7^{k}	25.6^{ab}	27.4^{b}
BC_3F_3-16	110^{de}	9^{bc}	22.7^{efg}	92^{e}	42.1^{j}	21.7^{ghijk}	18.0^{c}
BC_3F_3-17	113^{bc}	6^{ef}	23.7^{cdef}	98^{d}	43.9^{i}	20.1^{k}	11.8^{hij}
BC_3F_3-18	100^{ij}	8^{cd}	19.2^{i}	58^{p}	53.9^{e}	25.4^{abc}	11.8^{hij}
BC_3F_3-19	103^{ij}	7^{de}	26.0^{a}	100^{c}	22.1^{o}	22.0^{fghijk}	15.4^{de}
BC_3F_3-20	115^{b}	6^{ef}	23.8^{bcdef}	84^{g}	33.7^{m}	23.6^{cdefg}	11.9^{hij}
BC_3F_3 -21	108^{ef}	9^{bc}	26.0^{a}	64^{m}	56.6^{d}	22.4^{defghi}	12.9^{fgh}
BC_3F_3-22	115^{b}	8^{cd}	23.0^{def}	76^{k}	32.1^{m}	22.1^{efghij}	13.4^{fgh}
BC_3F_3-23	120^{a}	9^{bc}	23.0^{def}	60^{no}	36.6^{l}	22.8^{defgh}	12.3^{ghi}
CV%	1.28	13.30	4.32	1.27	2.28	4.40	7.26

Means followed by different trailing letters in the same column show a statistically significant difference ($p < 0.05$). Note: Plant height (PH, cm); Number of tillers (NT); Panicle length (PLL, cm); Filled grain (FG); unfilled grain (UFG, %); 1000-weight (W-1000, g); grain yield (GY, g). P_1, susceptible parent; P_2, tolerant parent.

et al., 2018). Acidic soil damages plants through many factors, including iron and aluminum toxicity. At a very low soil pH (<4.0), the strong reduction activity of Fe^{3+} into Fe^{2+} may increase available iron in the root zone, as well as iron absorption and uptake, thereby resulting in iron toxicity (Rout and Sahoo, 2015). The physiochemical activity of rice plants may decrease drastically, thus weakening root functions (Sahrawat, 2005). Krohling *et al.* (2016) suggested that iron is 1000 times more soluble at pH 3.0 than at pH 6.0.

Efforts in the development of iron-tolerant cultivars for the acidic soils of the Mekong Delta have made initial achievements. The use of molecular markers to aid the selection of tolerant genotypes results in better performance than direct field screening experiments. Various rice genotypes have varying levels of tolerance for certain toxins and growing conditions. In addition, a significant difference exists between laboratory testing and actual adaptation when it comes to the survival and development of a variety because of their dependence on farmers' needs. With the release of numerous tolerant varieties, the breeding program for iron toxicity continues to fulfill farmers' needs while enhancing the characteristics of existing varieties (Sikirou *et al*., 2015). Therefore, tolerant

genotypes were selected and evaluated for yield and yield components to eliminate unwanted genotypes before being assessed in paddy field trials in various ecological regions. The present research showed that the rice lines BC_3F_3-7 and BC_3F_3-15 exhibited enhanced growth and the best performance for yield-related traits in an iron-contaminated area.

CONCLUSIONS

The BC_3F_2 rice population was phenotyped for iron toxicity tolerance by using root lengths and leaf bronzing scores after 4 weeks of growth, including 14 days in nutrient solution, followed by 14 days of exposure to 150 mg L^{-1} Fe^{2+}, a level that is normally toxic to rice. Different rice lines in the BC_3F_2 population that were tolerant to iron toxicity were identified by using the two molecular markers RM6 and RM240 on chromosome 2. BC_3F_3-7 and BC_3F_3-15 were the most tolerant genotypes. They presented increased tillers, 1000-grain weight, filled grain, and grain yield with few unfilled grains under iron contamination conditions in the field. These rice lines could be further studied and used in future breeding programs to increase rice production in the iron-contaminated areas of the Mekong Delta, Vietnam.

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