Agronomy Research **21**(S1), 376–396, 2023
https://doi.org/10.15159/AR.22.078

Resistance of local rice progeny to ferrous iron toxicity between locations, seasons, and salt application in tidal lands

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Received: August 13th, 2022; Accepted: November 27th, 2022; Published: December 7th, 2022

Abstract. Rice is the main food in Indonesia that grows in various agroecosystems. The challenge is ferrous iron toxicity (FIT), wherein adaptive varieties with high yield potential be required to support increased production. The study objectives were to produce tolerant and widely adapted lines of FIT from local parents, to determine the stability of the lines in various environments and seasons in FIT rice fields, and to determine the response of rice lines to salt application. Two local Fe-tolerant parents that is Cekau and Karya, were used as females to produce lines that were tested for FIT. High-yielding lines and early maturity were selected to represent tolerant, quite tolerant, and moderate to FIT. The research was designed according to a randomized complete block design with 3 replications. Most of the local cultivar descent were resistant to FIT and stable at various locations and seasons. There was an interaction between the lines and the environment in the multilocation test, but in the high-Fe field test, there was no interaction between the lines and the season. Sensitive lines gave higher yields in the dry season than in the rainy season, but the tolerant lines are not affected by the seasons. The long dry season followed by high rainfall caused the accumulation of Fe on the soil surface to increase followed by a decrease in yields of moderate and sensitive lines. The addition of 200 kg ha^{-1} of salt increased the productivity of tolerant, quite tolerant, and moderate lines by improving root quality.

Key words: adaptation, climate, high-yielding variety, production, Indonesia.

INTRODUCTION

Rice (*Oryza sativa* L.) is the main food crop consumed by more than 50% of the world's population (de Oliveira et al., 2020). The area for rice planting is 167 million hectares (m ha); most are conventionally grown in continuously flooded lowlands

(Girsang et al., 2019). Prolonged flooding and poor drainage of iron (Fe)-rich soils create a risk of Fe toxicity (van Oort, 2018). Fe toxicity is one of the most widespread mineral disturbances in lowland rice production (Audebert & Fofana, 2009) due to the formation of excess Fe^{2+} in reduced soil which interferes with plant growth and productivity (dos Santos et al., 2019). Cases of Fe toxicity begin with flooding within a few days or more, which reduces Fe^{3+} to Fe^{2+}, which at high concentrations is toxic to plants (Becker & Asch, 2005) through the mechanism of $FeS_2 + 2Fe^{3+} \rightarrow 3Fe^{2+} + 2S$ (Dent, 1986).

In general, yield losses associated with Fe toxicity typically range from 15% to 30%, but total crop failure can occur in response to severe toxicity in early growth stages (Audebert & Sahrawat, 2000; Becker & Asch, 2005). In West African countries, Fe toxicity can cause rice yield losses of up to 50% (Audebert & Fofana, 2009). The critical level of Fe toxicity in plant tissues varies from 300 to 500 mg Fe kg^{-1} dry mass (Dobermann et al., 2000).

In Riau Province, Indonesia, cases of Fe toxicity often occur in tidal areas, low Fe-rich land, newly opened rice fields, or peatlands. The introduction of new high-yielding varieties (HYV) tolerant of Fe toxicity is not always successful because of the high rate of blast disease, leaf spot, and bacterial leaf blight. Riau Province is located on the equator with high temperature and humidity. In addition, the introduced HYV are not in conformity with the environment and local farmers' preferences.

Initially, the Fe-rich tidal rice fields in Riau-Indonesia planted local rice varieties tolerant to various stresses including Fe toxicity, but the yields are low, thus improvements are required. Crosses of local rice cultivars have been implemented to produce Fe tolerant varieties, broad adapted, high yield, and early maturity.

Although yield reductions can be partially avoided by using the super tolerant rice cultivars to excess Fe, progress in developing high-yielding cultivars with adequate tolerance to Fe toxicity has generally been slow due to the large genotype × environment interaction and high field heterogeneity, which renders rice selection ineffective (Sikirou et al., 2015). Breeding rice varieties with Fe toxicity tolerance is the most effective and economical way to minimize yield losses due to Fe toxicity stress. Tolerance to Fe toxicity in rice is a complex genetic trait, and there is a large genotypic variation in the primary rice gene pool (Liu et al., 2016).

The use of local varieties as one of the crossbreeding parents is strongly recommended, in order to obtain specific superior genes and to expand the genetic background of the superior varieties to be produced (Sitaresmi et al., n.d.), to increase the effectiveness of selection because it has adapted specifically to various stresses including tolerance to Fe toxicity. Two of the local varieties of tidal rice grown in Riau are Cekau and Karya. These two varieties generally grow in Fe-rich tidal soils. For the reason, specialized varieties matching individual environments and varieties adapted to a wide range of Fe toxicity environments should be developed. The environmental conditions, timing and level of Fe stress, the screening system, and other factors play crucial roles in determining genotype responses to Fe toxicity (L.B. Wu et al., 2014). Because the screening technique also affects the genotype's response to Fe toxicity, testing necessity to be performed between environments and seasons.

In 2011, several elite lines were produced from the cross between Cekau × Cisantana, which produced B-53, and Karya × Fatmawati which produced A5 and A15. Due to the interaction between various stresses, the Fe test performed in the laboratory or hydroponics is not always representing the stress in the field, which makes the stress suffered by plants become more serious. Therefore, field testing is more representative of all interactions that happens. The results of the study will be able to produce stable lines adapted to the tidal lands with the high Fe accumulation depending on the season. New lines produced by crossing local varieties and new high yielding varieties respond to the challenges of facing climate change. The addition of salt is also expected to be a solution to improving the quality of plants which will ultimately increase rice production. Data shows that the tidal area reaches 23% of the land area or 43 million ha in Indonesia (ICALRD, 2015) with local rice productivity only 2–3 t ha^{-1} (Noor, 2004). This is a challenge in getting superior lines, based on Alihamsyah & Ariza (2006) that the use of new high-yielding varieties was able to produce productivity of 4–6 t ha^{-1}. The study objectives were to produce tolerant and broadly adapted lines of Fe toxicity from local parents, to determine the stability of the lines in various environments and seasons in Fe toxicity rice fields, and to determine the response of rice lines to salt application.

MATERIALS AND METHODS

Experimental site

The cross of the Cekau × Cisantana and Karya × Fatmawati varieties was implemented at the Riau Assessment Institute for Agricultural Technology (AIAT), Pekanbaru City in 2009. F1–F3 seeds were planted in Dramaga Village, Bogor City in 2010–2011 (irrigated rice fields). The planting of F4-F6 was conducted in Sungai Solok Village, Kuala Kampar District, Pelalawan Regency (tidal rice fields) in 2012–2013. Selection of the best family from the F8 generation was implemented in Dadahup Village, Kapuas Regency and Mantaren Village, Pulang Pisau Regency, which are tidal rice fields of Fe poisoning in August - November 2013. From the results of family selection, 10 elite lines grew good in Fe poisoning fields. The ten lines were tested in multiple locations at 8 tidal fields of Parit Senang 1 Village (site 1); Parit Senang 2 Village (site 2); and Sungai Selamat Village (site 3) in Sungai Solok Village; Sungai Upih Village (site 4) in Kuala Kampar District, Pelalawan Regency, Riau Province; Rimba Melintang District, Rokan Hilir Regency, Riau Province (site 5); Petak Batuah Village, Kapuas Murung District, Kapuas Regency, Central Kalimantan Province (site 6); Pulau Petak District, Kapuas Regency, Central Kalimantan Province (site 7); and Bunga Raya Village, Bunga Raya District, Siak Regency (site 8) in 2014 (Table 1).

Futhermore, test for Fe poisoning was conducted at Tamanbogo Experimental Station, East Lampung -5.0053205 S, 105.4871335 E, from June to September 2016. The area used for testing was the rice field which have high Fe content (300–400 ppm), 27 m.a.s.l, low fertile sandy clay loam (Ultisol). Research land belonging to the Ministry of Agriculture which is often used for testing rice resistance to Fe.

To determine the effect of growing season on Fe availability and productivity of rice lines were conducted in newly opened rice fields that were poisoned with Fe in Muara Kelantan Village, Siak Regency which contained 334.38 ppm Fe based on soil analyzed in 2017. Previously this land was swamp land then it was opened for rice cultivation for 5 years, but still Fe poisoning because the drainage system is poor. The lines were planted on Fe toxicity land in the dry season and the wet season in 2017–2020. The experiment to determine the effect of salt will be performed at the same location in 2020.

Table 1. Physical and chemical properties of the research area

No	Soil properties and characteristics	Sites						
		Parit Senang	Sungai Selamat	Dadahup	Pulau Petak	Rimba Melintang	Sungai Upih	Siak
1	Sand (%)	30	21.16	0	0	27	23.66	
2	Silt (%)	26	33.50	44	37	38	52.68	
3	Clay (%)	44	45.34	56	63	35	23.66	
4	C organic (%)	1.34	4.88	2.63	3.35	1.37	6.12	2.30
5	N total (%)	0.11	0.30	0.15	0.21	0.13	0.35	0.13
6	C/N	12	16.27	17.68	16.05	11	17.49	17.00
7	pH	5.1	5.20	3.93	4.07	5.5	4.52	4.60
8	Al^{3+} (me 100 g^{-1})	0.28	5.73	8.71	8.02	0.02	0.20	
9	P_{Bray} (ppm)	11.3	16.56	130.5	62.39	24 (Olsen)	59.45	12.47
10	Ca_{dd} (me 100 g^{-1})	5.69	1.80	0.48	0.07	4.26	1.03	0.05
11	Na_{dd} (me 100 g^{-1})	4.48	0.51	0.21	0.27	1.97	0.26	0.03
12	K_{dd} (me 100 g^{-1})	0.75	0.44	0.31	0.22	0.30	0.37	0.57
13	CEC (me 100 g^{-1})	17.17	10.62	8.92	9.07	9.11	19.31	
14	Base saturation (me 100 g^{-1})	14.07	11.57	11.34	05.42	05.08	18.14	
15	P_2O_5 potential (me 100 g^{-1})	34	80.80	45	58	12	170.3	0.22
16	K_2O potential (me 100 g^{-1})	87	90.77	18	13	21	179.3	77.67
17	Fe (ppm)	70	102	148.04	135.10	27.71	265.43	334.38
18	Pyrite content (mg kg^{-1})	71	71	578	-	-	432	0.02
19	Land type	Tidal Swamp	Tidal type C	Tidal type C	Tidal type B	Rainfed	Rainfed	Tidal type C
20	Type of soil	Peaty alluvial	Ultisols	Acid sulfate	Acid sulfate	Alluvial	Alluvial	Alluvial
21	Texture	Clay	Clay	Silty clay	Clay	Clay loam	Silty loam	

Experimental design and management

The research was conducted through five stages of 1). Crossing local varieties with superior varieties. The local cultivars Cekau and Karya are two local rice that is widely grown by farmers in tidal swampland in Pelalawan Regency. The distribution area of this cultivar contains very high Fe, where other varieties usually suffer from Fe toxicity. The Cekau cultivar was crossed with the Cisantana variety and the Karya cultivar was crossed with Fatmawati. Cisantana and Fatmawati varieties intolerance to Fe toxicity. Fifty F1 seeds were planted and had uniform growth. The results of 50 hills of F1 were selected for the 5 best hills to produce F2 seeds, composited, then planted as a whole. From the segregated F2 population, the best 250 hills were selected. Panicles per hills were planted 1 panicle per row. In the F3 generation the best families were selected and from the best families the best hills were selected until the F6 generation according to the pedigree selection method. Seeds from the F6 generation were harvested and composited to be tested for yield ability. The adaptable progeny to Fe-rich soils and high productivity were selected from the F2 to F8 generations. 2). Screening of Fe toxicity-tolerant lines. A total of 10 tidal rice lines were tested by comparison with Mahsuri (tolerant) and IR64 (sensitive) varieties. Rice seeds were sown until they are 21 days old and then transplanted. Each line/variety was planted in 1-row × 5 m, one seed per hill with a spacing of 0.2 m × 0.2 m. The site received 350 kg fertilizer applied two times by broadcasting 75 kg Urea ha^{-1}, 100 kg TSP ha^{-1}, and 100 kg KCl ha^{-1} at crop establishment and 75 kg urea ha^{-1} at 28 days after planting (DAP). Weeding was done before the second fertilization. Pest and diseases protection was done intensively; 3). Multilocation Test. The research was conducted in 8 locations were Dusun Parit Senang 1 (site 1), Parit Senang 2 (site 2), and Dusun Sungai Selamat (site 3) in Sungai Solok Village, Sungai Upih Village (site 4), Kuala Kampar District, Pelalawan Regency, Riau Province; Rimba Melintang District, Rokan Hilir Regency, Riau Province (site 5); Petak Batuah Village, Kapuas Murung District, Kapuas Regency, Central Kalimantan Province (site 6); Pulau Petak District, Kapuas Regency, Central Kalimantan Province (site 7); and Bunga Raya Village, Bunga Raya District, Siak Regency (site 8) (Table 1). The multi-location test in 2013–2014 was conducted with 10 lines from crosses of local varieties were P1F-KK-A1 (A1), P1F-B-A7 (A7), P1F-B-A15 (A15), P1D-KK-A26 (A26), P1D-KK-A45 (A45), P17E-B-A48 (A48), P1D-KK-A48b (A48b), P1D-KK-A67 (A67), P5E-KK-A5 (A5), P253F-B-53 (B-53), with two control varieties were Batang Piaman and Inpara 2. The study used the following technical cultures: (1) seed aged 21 days after sowing; (2) fertilizer at nursery was Urea 50 kg ha^{-1}, TSP 50 kg ha^{-1}, KCl 25 kg ha^{-1}; (3) plant spacing 0.2 m × 0.2 m; (4) two seedlings per hill; (5) basic application was Urea 100 kg ha^{-1}, TSP 150 kg ha^{-1}, KCl 50 kg ha^{-1}, (6) the second application was Urea 50 kg ha^{-1} and KCl 50 kg ha^{-1} at 35 days after transplanting; (7) weeds were controlled with application 2,4-D dimetil amina 865 g L^{-1}, (9) controlling pests and diseases using the Deltametrin 25 g L^{-1} and propinep 70%. 4). Effect of growing season on Fe availability and productivity of rice lines. The Fe-sensitive varieties, Inpara 5 (Nugraha et al., 2016), Fe-tolerant, quite tolerant, and moderate lines were tested using a randomized complete block design with three replicates. Harvest data were analyzed using PBSTAT 1.2 and 3.1. 5). Effect of salt on decreasing the Fe toxicity. The test was implemented in rice fields which always experience Fe toxicity. Three lines and one variety were grown with treatments of 0, 100, and 200 kg NaCl ha^{-1}. The study was designed using a randomized complete block design with 3 replications. The condition of the soil is mud that is not waterlogged. Seedlings aged 21 days were planted with plant spacing of 0.2 m × 0.2 m. Experimental plots were not irrigated until 10 days. After the soil started to crack, urea, TSP, and KCl were sprinkled with doses of 200 kg ha^{-1}, 100 kg ha^{-1}, and 100 kg ha^{-1}, respectively with water level at 0.3 m and maintained water until seven days after planting. On the next day, the soil is allowed to dry for three days. Then the soil was flooded as high as 0.1 m and sprinkled with salt according to the treatment.

Soil measurements

Multilocation experiment. Soil sampling was conducted at the beginning of the study at a depth of 0–0.3 m from five diagonal points, composited, and analyzed in the laboratory of Kimia Riset Pekanbaru PT. Sarana Inti Pratama. For the experimental effect of the growing season on Fe availability and productivity of rice lines, soil sampling for Fe content analysis was conducted at harvest time in July 2017, February, July, and December 2018, at planting in April and August 2019, and at harvest in January and July 2020. Soil samples were taken from 5 diagonal points at a depth of 0–0.15 m and then composited.

Plant measurements

The grain yield of all experiments was observed from 2.5 m × 2.5 m plots of each replication. The grain is threshed manually and then dried in the sun to a moisture content of 14%. The grain is cleaned of unfilled grains and dirt then weighed and converted to hectares. The variables observed were dry grain yield, adaptability, and stability of lines between locations.

Statistical methods

Observations screening of Fe toxicity-tolerant lines were made on the tolerance of the tested lines with an assessment using the International Rice Research Institute (IRRI) standard (SES, 2014). The experiment of multilocation test was designed according to a randomized complete block design with three replications with an area of each plot of 4 m × 5 m. The data obtained were analyzed by analysis of variance, and the mean difference in treatment was tested by Duncan's statistic at a significant difference level of 5%. Analysis was performed using SAS and PBStat 1.2 and 3.1 software. The adaptability and stability of the lines were tested using stability parameter analysis according to Eberhart & Russell (1966), with a linear model as follows:

$$Y_{ij} = \mu + b_i I_j + \delta_{ij} \qquad I_j = \frac{\Sigma_i Y_{ij}}{t} = \frac{\Sigma_i \Sigma_j Y_{ij}}{ts}$$

Annotation: Y_{ij} = the average yield of the i-cultivar at the j-location; μ = the average of all cultivars at all locations; b_i = regression coefficient of the i-genotype on environmental index, which shows the genotypic response to the environment; I_j = environmental index, i.e. the deviation of the genotypic mean at a season of all averages; t = number of tested genotypes; s = season; δ_{ij} = regression deviation of the i-genotype at the j-location.

A genotype is considered stable if the regression coefficient (bi) is insignificantly different from one and the standard deviation (Sd) is not different from zero based on the student's t-test (Eberhart & Russell, n.d.). The value of bi is approximated by bi = $y_{ij}I_{ij} / I^2_j$, while the value of S_d^2 is obtained by:

$$S_d^2 = \frac{\Sigma\delta_{ij}^2}{1-2} - \frac{S_e^2}{r}$$

where S_e^2 = estimated variance galad.

For the second experiment is effect of growing season on Fe availability and productivity of rice lines and effect of salt on decreasing the Fe toxicity were designed using a randomized block design with 3 replications. Harvest data were analyzed using PBSTAT 1.2 and 3.1.

RESULTS AND DISCUSSION

Results

Soil characteristic within the experimental site is shown in Table 1. Soil conditions at the study site were dominated by high clay content except in the Sungai Upih area with a silty loam texture with pH classified as acidic to very acidic for the seven study sites. Soils that contain high fine clay have highly water retention abilities (Girsang et al., 2020) which tend to have poor drainage which has an impact on high soil acidity (Rendana et al., 2021). Furthermore, the content of organic matter is categorized as low to very high. In terms of the macronutrients such as N is in the low to moderate while P and K are in the low to very high category. The exchangeable nutrient such as Ca, K, and K have a great range from very low to very high with the research land types tidal swamp, tidal type B and C, and rainfed. From the overall soil analysis data that the land in the Sungai Upih is more fertile than other locations, this is supported by CEC data, available P, and base saturation with the main limiting factor being Fe toxicity.

Screening of Fe Toxicity Tolerant Lines

There are variations in resistance to Fe toxicity among breeding lines (Table 2). There were many tolerant, quite tolerant, and moderate lines but only three lines with the highest productivity were selected by farmers, namely: B-53, A15, and A5.

Most of the selected lines were tolerant to moderately sensitive to Fe toxicity, have inherited the character of resistance to Fe toxicity from the female parent. The Cekau cultivar which was used as a female parent to produce B-53 and Karya to produce others lines, was a local cultivar that was Fe toxicity tolerant, while the male parent did not have the character. Of the ten elite lines, there were no sensitively lines to Fe toxicity. Differences in tolerance levels between lines from the same parent indicated the presence of several genes

Table 2. Pelalawan swamp rice lines tested for Fe at KP. Tamanbogo, dry season 2016

No	Lines	Score	Category
1.	A45	4.3	Quite tolerant
2.	A26	3.7	Tolerant
3.	A48b	3.7	Tolerant
4.	A67	3.7	Tolerant
5.	A1	5.0	Moderate
6.	A7	4.3	A bit tolerant
7.	A5	5.0	Moderate
8.	A48	5.7	Quite sensitive
9.	A15	4.3	Quite tolerant
10.	B-53	3.7	Tolerant
11.	MAHSURI (control)	1.7	Very Tolerant
12.	IR64 (control)	9.0	Very sensitive

controlling resistance to Fe toxicity. According to Dufey et al. (2015), rice tolerance to Fe toxicity is a quantitative trait controlled by many genes. The expression is strongly influenced by environmental conditions.

In tidal land, double stress often occurs, so plants must face other stresses besides Fe toxicity, such as nutrient deficiency, waterlogging, salinity, acidity, or disease. Local varieties have adapted to these conditions where the symptoms of Fe toxicity are lighter. Some descent of local cultivars inherit this character, they are more resistant to Fe and secondary stress. Therefore, the assembly of Fe-toxicity tolerant varieties should be site-specific, taking into account other stress.

Multilocation Test on Various Fe Levels in Tidal Land

The lines × environment (L × E) interaction was significant in the multilocation test on rice yield. Interaction means the failure of a line to maintain its rank in different environments. The pattern of the influence of the L × E interaction is clearly seen in Fig. 1. There are lines that are stable in various environments, but there are also lines that are specifically adapted to certain environments. It turned out that not all lines that were declared tolerant to Fe toxicity when tested in the laboratory would be strong when tested in the field. On the other hand, there were moderate lines in the laboratory, very good in the field. The A26 and A48b lines were tolerant to Fe toxicity when screened in the laboratory. But in the field, these lines still showed symptoms of Fe toxicity and low yields.

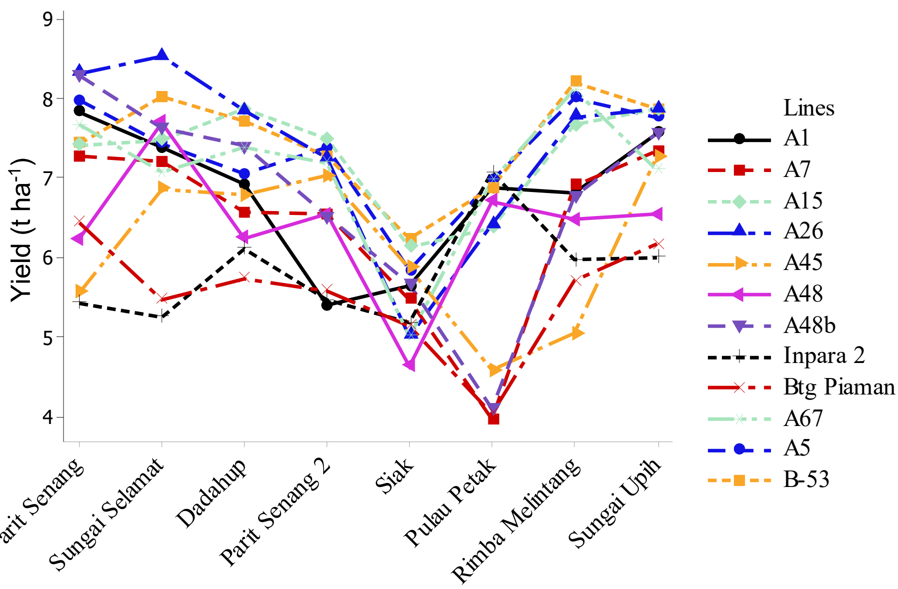

Figure 1. Ranking of lines between locations based on yield.

The A5, A15, and B-53 lines have an average regression coefficient value of the yield on the environmental average (bi) close to 1, their contribution to the genotype × environment interaction (Wi^2) is low, the regression deviation (δi^2) is relatively small (Table 4). This means that these genotypes are stable in eight tidal environments. This stability can also be seen from the AMMI 2 biplot, where the three elite lines are close to the center point (Fig. 2). The three genotypes gave a high average yield.

Despite the higher Fe content, these genotypes produce fairly high yields in more fertile environments such as Sungai Upih, Sungai Selamat, and Parit Senang. There are some lines that are good under pressure or low productivity environments such as Siak and Pulau Petak, namely: A1, A15, A5, and B-53.

The grades of the A15, A5, and B-53 lines did not differ significantly between locations (Table 3, Fig. 1). In this case, it is not clear the effect of moderate, quite tolerant, and tolerant line status on yields in different environments. At the high Fe location in Sungai Upih, the three lines gave no significantly different yields.

The yields of lines A15, A26, A5, and B-53 were all higher than those of control varieties Inpara 2 and Batang Piaman. The average of the four lines across all locations is also quite good. However, especially in Fe-rich regions, such as in Siak, the A5, A15, and B-53 lines produced higher yields than all tested lines/varietals. Up to this point, the yield of existing varieties in the research location is only 3–4 t ha^{-1} dry milled grain (DMG).

Table 4. Parameters of yield stability of elite lines according to Finlay-Wilkinson

Lines/ Varieties	$\bar{y}_{i.}$	b_i	δ_i^2	CV_i	W_i^2
A1	6.80	0.89	2.95	19.70	3.00
A7	6.40	1.52 *	0.68	31.13	3.42
A15	7.28	0.95	0.74	13.14	0.74
A26	7.37	1.73 *	0.37	15.86	0.61
A45	6.12	0.70	4.76	30.45	4.91
A48	6.38	0.84	2.08	15.63	3.28
A48b	6.74	1.74 *	0.79	34.35	5.15
Inpara 2	5.80	0.14	1.17	18.47	6.24
Btg Piaman	5.53	0.84	0.64	23.78	0.97
A67	7.06	1.08	0.88	9.55	1.53
A5	7.30	0.93	0.62	9.65	0.85
B-53	7.45	0.91	0.49	10.45	0.55

Description: * = significantly different from 1; $\bar{y}_{i.}$ = mean genotype yield; b_i = regression coefficient; δ_i^2 = regression deviation; CV_i = coefficient of variance; W_i^2 = contribution to the genotype × environment interaction

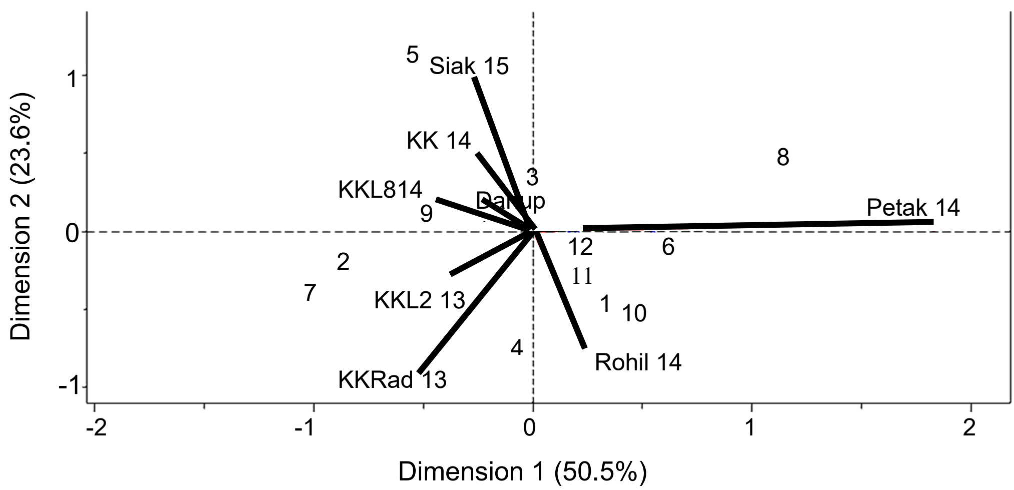

Figure 2. AMMI 2 biplot of grain yields with a conformity level of 50.5 9%.

Note: 1 = A1; 2 = A7; 3 = A15; 4 = A26; 5 = A45; 6 = A48; 7 = A48b; 8 = Inpara 2; 9 = Batang Piaman; 10 = A67; 11 = A5; 12 = B-53; Kkrad13 = Parit Senang; KKL213 = Sungai Selamat; Dahup13 = Dadahup; KK14 = Parit Senang 2; Siak15 = Siak; Petak14 = Pulau Petak; Rohil14 = Rimba Melintang; KKL814 = Sungai Upih.

Table 3. The yield of the dry milled grain of elite lines planted in eight locations

Lines	Locations								Average of lines
	Parit Senang	Sungai Selamat	Dadahup	Parit Senang 2	Siak	Pulau Petak	R. Melintang	Sungai Upih	
A1	7.82- ae	7.38 a-g	6.91 a-j	5.39 b-j	5.63 a-j	6.87 a-j	6.80 a-j	7.57 a-g	6.80 ABC
A7	7.27 a-g	7.20 a-g	6.56 a-j	6.53 a-j	5.47 a-j	3.96 j	6.90 a-j	7.33 a-g	6.40 BCD
A15	7.41 a-g	7.48 a-g	7.87 a-e	7.49 a-g	6.13 a-j	6.37 a-j	7.67 a-f	7.87 a-e	7.28 A
A26	8.31 ab	8.52 a	7.84 a-e	7.25 a-g	5.00 e-j	6.40 a-j	7.77 a-e	7.87 a-e	7.37 A
A45	5.55 a-j	6.85 a-j	6.78 a-j	7.02 a-j	5.87 a-j	4.57 g-j	5.03 e-j	7.27 a-g	6.12 CDE
A48	6.23 a-j	7.69 a-f	6.24 a-j	6.53 a-j	4.63 f-j	6.70 a-j	6.47 a-j	6.53 a-j	6.38 BCD
A48b	8.30 ab	7.63 a-g	7.40 a-g	6.51 a-j	5.67 a-j	4.10 hij	6.77 a-j	7.57 a-g	6.74 ABC
Inpara 2	5.43 b-j	5.25 b-j	6.10 a-j	5.46 b-j	5.17 c-j	7.07 a-i	5.97 a-j	6.00 a-j	5.80 DE
Btg Piaman	6.43 a-j	5.47 a-j	5.73 a-j	5.58 a-j	5.13 d-j	4.03 ij	5.70 a-j	6.17 a-j	5.53 E
A67	7.67 a-f	7.07 a-i	7.38 a-g	7.19 a-g	5.00 e-j	6.97 a-j	8.10 a-d	7.10 a-h	7.06 AB
A5	7.97 a-e	7.43 a-g	7.04 a-i	7.38 a-g	5.83 a-j	6.97 a-j	8.00 a-e	7.77 a-e	7.30 A
B-53	7.43 a-g	8.02 a-e	7.71 a-e	7.27 a-g	6.23 a-j	6.87 a-j	8.20 a-c	7.87 a-e	7.45 A
Avg of locations	7.15	7.17	6.96	6.63	5.48	5.91	6.95	7.24	

Note: Numbers followed by the same lowercase or uppercase letters in columns and rows mean that not significantly different according to the Tukey 0.05.

Some tolerant lines still showed symptoms of Fe toxicity of varying severity. But the A5 line, which is moderately to Fe toxic, grows well in Siak and Pulau Petak, although these two sites are more toxic than others. Meanwhile, the Fe-resistant A67 line performed poorly at the Fe-toxic site of Siak.

Although Dadahup had a soil pH of 3.93 and Pulau Petak had a soil pH of 4.07 (very acid), the yields of the three lines were insignificantly different and the average yield was quite good. Soil acidity is not the only factor that suppresses productivity. According to Azura Azman et al. (2014), the optimal pH for rice is 6.

The adverse effects of high Fe concentrations in Pulau Petak, Dadahup, and Siak were greater due to low sodium (Na). The presence of Na in Parit Senang, Sungai Selamat, and Rimba Melintang due to seawater intrusion caused no symptoms of Fe toxicity in the lines seen in Dadahup, Pulau Petak, and Siak. This fact indicates that fluctuation in the toxic effect of Fe in the soil are related to the Na concentration that is important for the Fe balance of plants.

The three lines selected by the farmer were stable between locations with different levels of Fe toxicity, although two of them were only moderately and quite tolerant. This fact shows that the use of parents from the same agroecosystem as the target location for varietal development will produce progeny that adapts to the agroecosystem.

Effect of the Growing Season on Fe Availability and Productivity in Rice Lines

The growing season in Indonesia is divided into two seasons, the dry season (S1) and the rainy season (S2). Generally, S1 occurs in April-September and S2 takes place from October to March. However, in Riau Province which is on the equator, the difference between the S1 and the S2 is often not extreme, which causes the dynamics of Fe in the soil to be similar in the two seasons.

Differences in Fe status in different environments in the multi-site assay did not define the tolerant, slightly tolerant, and moderate characteristics of the three lines. It is suspected that cross-season testing at the location of high Fe can clarify this by testing at S1 and S2. According to X. Wu et al. (2016), environmental conditions affect the level of toxicity.

In a multi-location test, there is an interaction between the line and the environment (L × E). But in the field of high Fe, there is no interaction between lines and seasons. ANOVA of 4 genotypes at 8 planting times showed that both season ($P < 0.000$) and genotype ($P < 0.000$) had very significant effects on rice yield, but their interaction was not significant. The absence of interaction between lines and seasons suggests that performance and genotype ranks are stable between seasons. For example, the B-53 rating has been consistently high from season to season. This is supported by the Finlay-Wilkinson stability analysis (Table 5), where the correlation coefficient values are close to 1. However, the season has a big effect on the concentration of Fe in the tillage layer, Fe is only a small part of the environment that affects plants, and its toxic effects depend on other environmental components, it is site-specific. This also means that the medium and sensitive lines will not outperform the tolerant lines even under favorable growing season conditions, but their yields will be nearly the same in the dry season.

Table 5. Yield stability in the dry season and rainy season in 2017–2020 according to Finlay-Wilkinson Stability Analysis for yield

Genotype	Yield	b_i
A15	4.95	1.10
B-53	5.99	1.13
A5	4.15	0.84
Inpara 5	4.12	0.93

There are variations in Fe content on the soil surface in the S1 and the S2. The Fe content in the tillage layer is very high when the rice fields were flooded after a long S1. The long S1 causes many and deeper soil fractures, leading to extensive oxidation. When rice fields are flooded, Fe^{3+} which is not absorbed by plants turns into Fe^{2+} which is toxic. This can be seen in the high levels of rust deposited on the soil surface when farmers started growing rice at the beginning of S2. At very highconcentrations, Fe on the so il surface can cause damage to seedlings that are just a few days old. At lower Fe concentrations, plants can survive to the reproductive stage but become poisoned during flowering. This fact happened in 2020 when Fe accumulation occurred in the root layer. The leaves of the bottom plants turn orange-yellow in color, bronzing, and the freshness of the leaves is reduced even if the paddy fields are flooded, and the panicles are unfilled and dry.

The difference between S1 and S2 was insignificant in 2017 and 2019 but was significant in 2018 and 2020. However, the ranking of all lines is stable across all seasons and years. In the S2, which was preceded by a long S1, as happened in 2018, yields of sensitive, moderate, and quite tolerant lines fell sharply compared to tolerant lines (Tables 6a, 6b, 6c, 6d, 6e). This continued into 2019 as soil surface Fe levels increased due to heavy rainfall (Fig. 3). The combined analysis of yield data for 2017–2020 shows that the average yield is higher in the S1 and is significantly different from the rice yield in the S2 (Table 6e).

The influence of the S2 and the S1 in 2017 is insignificant due the rainfall is almost flat and is not high every month. The rice fields have not flooded for a long time, there are insignificantof Fe poisoning in the S2. But in 2019, the average rainfall was high and evenly distributed throughout the year, causing waterlogging in S1 and S2, resulting in an increase in Fe on the soil surface, which caused yields to fall. In 2020, heavy rainfall and strong drainage reduced Fe content in the soil surface,

Table 6a. Grain yields of lines/variety in 2017

Lines/ variety	Season 1	Season 2	Average of lines
A15	6.16	5.51	5.84^{b}
B-53	7.45	7.29	7.37^{a}
A5	5.22	4.46	4.84^{c}
Inpara 5	5.51	4.74	5.12^{bc}
LSD 5%	-	-	0.90
Avg of seasons	6.08	5.50	

Table 6b. Grain yields of lines/variety in 2018

Lines/ variety	Season 1	Season 2	Average of lines
A15	7.45	3.45	5.45^{a}
B-53	7.56	5.13	6.35^{a}
A5	5.08	2.76	3.92^{b}
Inpara 5	5.38	2.51	3.94^{b}
LSD 5%	-	-	1.06
Avg of seasons	6.37^{a}	3.46^{b}	

Table 6c. Grain yields of lines/variety in 2019

Lines/ variety	Season 1	Season 2	Average of lines
A15	3.54	3.20	3.37^{ab}
B-53	4.67	3.33	4.00^{a}
A5	2.99	2.64	2.82^{b}
Inpara 5	3.15	2.50	2.83^{b}
LSD 5%	-	-	0.68
Avg of seasons	3.59	2.92	

Table 6d. Grain yields of lines/variety in 2020

Lines/ variety	Season 1	Season 2	Average of lines
A15	5.14	5.11	5.12^{b}
B-53	5.67	6.84	6.26^{a}
A5	4.98	5.05	5.02^{b}
Inpara 5	4.40	4.80	4.60^{b}
LSD 5%	-	-	0.76
Avg of seasons	5.05^{b}	5.45^{a}	

leading to an increase in crop yields. A significant difference between S1 and S2 was found in 2018 and 2020 (Table 6b, 6d). The lines A5, A1, and B-53 did not show a change in tolerance level in the S2 but all became as if tolerant in the S1 with high yields (Table 6a, 6c, 6d) where the distribution of rainfall is almost flat between S1 and S2.

At specific locations were always Fe poisoned, it was only seen that the tolerant lines were always ranked 1, moderate lines were ranked 2, and the sensitive line was ranked 3 in the S1 and S2. This indicates that the selection of varieties is very important in Fe toxicity areas depending on the growing season and the previous S1. If planting is done in the S2 and water cannot be drained, farmers should plant alternative Fe tolerant varieties with attention to how severe the previous S1 was.

Table 6e. Combined data analysis of grain yields, 2017–2020

Lines/ variety	Season 1	Season 2	Average of lines
A15	5.57	4.32	4.95[b]
B-53	6.34	5.65	5.99[a]
A5	4.57	3.73	4.15[c]
Inpara 5	4.61	3.64	4.12[c]
LSD 5%	-	-	0.80
Avg of seasons	5.27[a]	4.33[b]	

Note: Numbers followed by the same letter in the same column or row insignificantly different based on the 5% level *LSD* test.

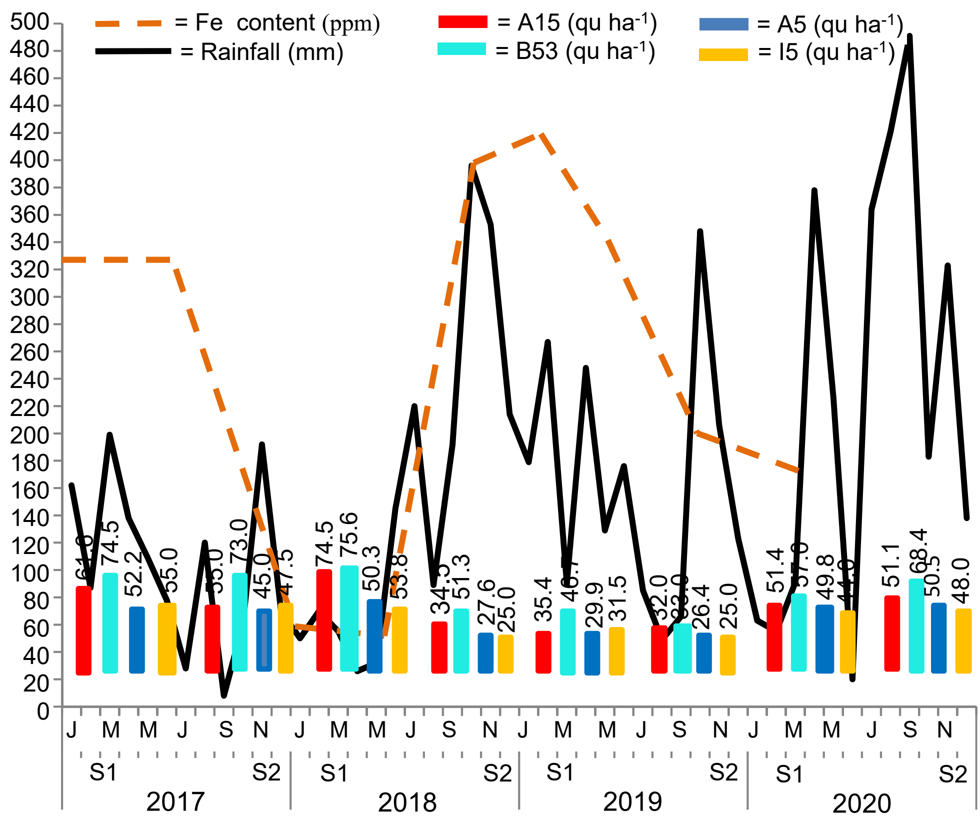

Figure 3. Effect of rainfall on soil surface iron content and rice yields. S1 = dry season, S2 = rainy season.

After a rather long S1, there is a spike in Fe on the soil surface if followed by high rainfall and long-standing water in the rice fields. Along with the increase in Fe, there was a decrease in the yield of non-tolerant lines.

Low rainfall from 2017 to mid-2018 gave high yields for all varieties tolerant, moderate, and sensitive to Fe. In that season, the rice fields are rarely flooded, but the soil is still moist enough for growth even though the soil has cracked. Fe toxicity is minimal because the concentration of Fe on the soil surface is still low, at 70.9 ppm.

A drought that was quite extreme from February to May 2018, did not affect crop yields because rice plants were already in the ripening phase. But the soil fractures are quite numerous, wide, and deep. This caused a spike in Fe on the ground surface during the S2 2018 which caused crop yields to drop drastically and continue into 2019.

In the fourth quarter of 2019 (S2), rainfall was high at transplanting to the vegetative phase, and low before harvest but could not increase yields. The level of toxicity in plants began to decrease because the water was no longer stagnant in the fields due to the disposal of stagnant water through the small canal in the rice field. This significantly reduces soil Fe concentrations and increases crop yields in S1 2020.

Yield changes due to seasonal changes were quite large in A5 and Inpara 5, although their regression coefficient (b_i) is close to 1 (Fig. 2). A15 and B-53 responded to the improvement of the growing environment (bi > 1) although they were tolerant of Fe toxicity, while A5 and Inpara 5 did not respond (bi < 1).

Effect of Salt on Decreasing the Fe Toxicity

The minimum symptom of Fe toxicity in rice plants in coastal areas is the idea in this study. The scarcity of site-specific Fe toxicity tolerant varieties is a limiting factor for farming in Fe-toxicity rice fields during the rainy season. Farmers must cope with environmental improvements wherein Fe toxicity is not too severe. The material that can be used is sea salt (NaCl).

The salt treatment and variety had a significant effect ($P = 0.00$) on the yield, but the salt × variety interaction had no significant effect ($P = 0.2434$) on the yield. Fe-tolerant lines and Fe-sensitive lines react differently to salt treatment. The B-53 line gave a positive response to a NaCl dose of 200 kg ha^{-1} with an increase in yield of 1.41 t ha^{-1} DMG compared to 0 kg ha^{-1} NaCl (Table 7). Fe-sensitive varieties A5 and Inpara 5, were still depressed due to Fe even though they were given salt as a suppress or of the effect of Fe. It is suspected that salt only slightly reduces the effect of Fe.

Table 7. The yield of tolerant and sensitive varieties to Fe toxicity in the salt treatment

Varieties	Salt dose (kg ha^{-1})			Varieties
	0	100	200	avg.
A15	4.20	4.64	5.01	4.62^{b}
A5	3.25	3.75	3.95	3.65^{c}
B-53	5.26	5.47	6.67	5.80^{a}
Inpara 5	3.70	3.56	3.55	3.60^{c}
LSD 5%	-	-	-	0.51
Salt average	4.10^{b}	4.36^{b}	4.80^{a}	

Note: Numbers followed by the same letter in the same column or row insignificantly different based on the 5% level *LSD* test.

The effect of varieties to control the toxic effects of Fe is very important. In the existing conditions, the tolerant line B-53 can produce 5.26 t ha^{-1} DMG, 1–2 t higher than other lines. Its tolerance to salinity causes yields to increase with the addition of salt and on the other hand, salt suppresses the toxic effects of Fe. The increase in yield due to improved environmental conditions showed that the character of tolerance to Fe in the

B-53 line seemed to be inductive. B-53 can adapt to their stressed natural environment, as well as be able to develop their genetic characteristics in a better environment.

Salt treatment reduced the symptoms of Fe toxicity in all varieties. Moderate and sensitive lines to Fe toxicity showed bronzing symptoms in the vegetative to generative phase with mild-moderate intensity. The results of this study indicate the importance of adding salt to areas where Fe toxicity is very low in Na content. Low-dose salt can be implemented when Fe levels increase, usually after a long dry season followed by a rainy season.

Fe plaque that covered the roots was very easy to find in Fe-rich and Na-low locations but was not found in the roots of plants grown in Na-rich locations even though Fe was abundant. Under conditions of high Na concentration in the soil, the rice roots are white but the penetration is shallow and the roots are rather brittle. This causes the plants to be easily removed.

The roots are rarely covered by Fe plaque and have the ability to regenerate the roots, where they can still absorb water and nutrients well are characteristics of Fe toxicity tolerant varieties. This can be seen from the number of roots of plants that tolerate Fe toxicity compared with sensitive plants. In dry conditions, sensitive lines also grow new roots on the soil surface, but cannot keep up with the activity of tolerant varieties.

The Fe plaque covering the roots is believed to be the reason for the low output of farmers. Fe plaque inhibits the absorption of nutrients and water.

The roots of Fe-sensitive lines were brownish-black from the base to the tip of the roots (4a), while the tolerant lines still had plaque-free roots about 1/3 of the root length from the tips in high Fe areas (4b). The root color of the sensitive (4c) and tolerant (4d) lines became white with the addition of 200 kg ha^{-1} of salt.

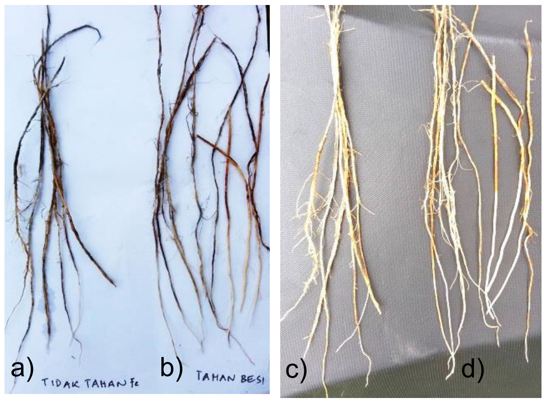

Figure 4. Performance of rice roots sensitive (a) and tolerant (b) to Fe toxicity without NaCl, sensitive (c) and tolerant (d) to Fe toxicity in NaCl treatment.

Discussion

Fe plays an important role in various metabolic processes of organisms (Grillet & Schmidt, 2019), biosynthesis of chlorophyll, carotenoids, and many proteins (Adamski et al., 2012). However important Fe is in life, Fe is toxic to most plants and animals at higher concentrations (White & Brown, 2010). Various levels of plant response to Fe indicate the diversity of controlling genes. Understanding the tolerance level of varieties to Fe toxicity is very important to choose the right variety in the dry season and the rainy season preceded by a long dry season

Breeding rice varieties tolerant to Fe toxicity is more effective and economical to reduce yield losses. Tolerances to Fe toxicities in rice are genetically complex traits; there is large genotypic variation in the primary rice gene pool (Liu et al., 2016), and they are inherited quantitatively. The existence of resistance categories such as sensitive, moderate, and tolerant to Fe toxicity due to differences in the genes that control it. Therefore, the assembly of high yielding varieties tolerant to Fe toxicity should use parents from the target location because they have adapted to the environment.

The number of genes involved in resistance to Fe toxicity causes different resistance categories to be very tolerant, tolerant, moderately tolerant, moderate, and sensitive to Fe toxicity. Furthermore, according to L.B. Wu et al. (2014), environmental conditions, timing and levels of Fe stress, screening systems, and other factors play important roles in determining genotype responses to Fe toxicity. Noor et al. (2022) reported that the yield of medium tolerant and fully tolerant genotypes treated with organic matter will increase due to a decrease in Fe toxicity.

When the above resistance categories are combined with environmental conditions and soil Fe content, interactions will be more complicated and more important to screen for site-specific genotypes. According to Becker & Asch, (2005), toxicity levels depend on the region, the soil type, the cropping season, and the severity and duration of Fe-toxicity occurrence; genotypes strongly differ in their response patterns and their ability to cope with excess amounts of Fe^{2+}. The finding of QTL in Fe stress conditions and without Fe-stress conditions (Zhang et al. (2017), indicated that the expression of most of the genes was constitutional and inductive. The constitutional expression causes the yield of the varieties not to increase even though they are grown under conditions without Fe stress because some of the energy is used to form secondary metabolites.

There are quite a number of genes controlling the resistance of rice plants to Fe toxicity with varying strengths, so it is necessary to use QTL pyramiding to develop Fe resistant lines in rice (Rasheed et al., 2020). Varietal assembly or gene pyramiding can be done by selecting genes that are only expressed during Fe-stress. Zhang et al. (2017) reported that 29 QTL were identified in the Fe stress experiment, including four detected only in a control condition, 12 detected only under Fe stress condition, and 13 commonly detected under both control and Fe stress conditions

However, the introgression of tolerance traits into high-yielding germplasm has been slow-owing to the complexity of tolerance mechanisms and large genotype-by-environment effects (Kirk et al., 2022). There were wide variations in Fe-toxicity tolerance responses on rice lines, which depended on stress duration, strength, and plant development stage. Some genotypes might show contrast performances depending on how and the location of experiments were accomplished. The exposure of Fe^{2+} excess affected roots and continued to severe reduction of chlorophyll concentration in the leaf, as will be shown as the bronzing spot. Nevertheless, there was no reduction in the tolerant one (Stein et al., 2019). The use of local parents in this study has minimized the interaction of genotype $^{\times}$ environment with a large number of lines that adapt and tolerate Fe poisoning.

The continuously flooded may be able to break the defense system of the rice genotype which has only a few genes controlling toxicity to Fe. Fe toxicity in rice plants can reduce plant height, dry weight, number of productive tillers, number of panicles, number of filled grains, delaying flowering and ripening (Audebert & Fofana, 2009), decrease plant production (Amnal, 2009). One of the weaknesses of traditional swampland is no water system where the land is flooded during the rainy season.

A significant negative correlation between grain yield and leaf bronzing score (LBS) at 60 DAS was found in Fe treatments (Sikirou et al., 2016). On average, in Fe-toxic soils, one unit increase in LBS was related to a yield decrease of about 20%. In the field situations, it is reported that an increase in LBS score by one unit reduced yield by 390 kg ha^{-1} (Audebert & Fofana, 2009).

Fe^{2+} concentration up to 3.2 mM did not damage rice roots while induced IP formation obviously (Fu et al., 2018). Under excess Fe were detected (i) nutritional deficiencies, especially of calcium and magnesium in leaves; (ii) negligible changes in grain nutritional composition, independently of Si application; (iii) decreases in net photosynthetic rates, stomatal conductance, and electron transport rate, in parallel to decreased grain yield components, especially in the Fe-sensitive cultivar (dos Santos et al., 2020). Fe^{2+} concentrations of 300 to 400 ppm were highly toxic to rice and resulted in low plant nutrient availability (Ikehashi & Ponnamperuma, 1978).

Fe toxicity can also occur in normal soils at low soil pH when harmful organic acids and hydrogen sulfide accumulate (Liu et al., 2016. Excess Fe can cause direct poisoning and nutritional imbalances, during the vegetative and reproductive stages (Sahrawat, 2004; Müller et al., 2015), and P, K, Ca, Mg, and Mn deficiency (Olaleye et al., 2001; Audebert & Fofana, 2009; Stein et al., 2009). Genotypes with enhanced Fe storage in roots and stems may be better suited to such conditions (Kirk et al., 2022). The application of fertilizers significantly decreased average shoot Fe concentrations partly due to Fe exclusion favored by enhanced root plaque formation (Rakotoson et al., 2019). Excessive S supply (60 and 120 mg kg^{-1}) significantly decreased IP on the root surface during flooded conditions (Yang et al., 2016).

In the vegetative phase, the toxic effect of Fe on rice plants has been associated with a decrease in the net CO_2 (A) assimilation rate due to the limitations of stomata and non-stomata photosynthesis (Pereira et al., 2013). At the reproductive stage, excess Fe causes a significant decrease in the number of tillers and grain fertility, which in turn reduces grain yield. This loss may be substantial, depending on the cultivar, time of Fe poisoning, stress intensity, and management strategy (eg, mineral fertilization) (Olaleye et al., 2001; Audebert & Fofana, 2009; Stein et al., 2009).

In cultivation techniques, there are ways to eliminate the effects of Fe toxicity, for example choosing rice varieties tolerant to Fe toxicity (Becker & Asch, 2005), water management, amelioration, application of fertilization, and use of high yielding varieties of Fe toxicity tolerant (Khairullah et al., 2021), the addition of salt, application of Si (dos Santos et al., 2020), or liming (Suriyagoda et al., 2017). However, none of those options is universally applicable or efficient under the diverse environmental conditions where Fe toxicity is expressed (Becker & Asch, 2005).

Fe bioavailability to plants is reduced in saline soils, but salting should not be excessive because plants will be faced with two major challenges for poor crop productivity: high salinity and Fe deficiency (Sultana et al., 2021). The concentration of plant-available Fe in saline-alkaline soils is often low due to immobilization (Li et al., 2016). In alkaline soils, Fe occurs mainly in the form of insoluble hydroxides and oxides, limiting its bioavailability for plants (Li et al., 2016).

The use of Si can be recommended as an effective management strategy to reduce the negative impact of Fe toxicity on the photosynthetic performance of rice and crop yields with no effects on the grains (dos Santos et al., 2020). Application of dolomite to lowland rice fields affected by Fe^{2+} toxicity can increase grain yield while reducing the negative impacts of Fe^{2+} toxicity. The magnitude of these positive responses would vary depending on variety, season, and soil conditions (Suriyagoda et al., 2017).

The intermittent water system showed dominance in increasing soil pH at planting 0, 7, and 14 days after application of the water system. The planting time of 14 days showed the lowest soil pH. The intermittent water system and planting time of 14 days and 21 days after application of the water system showed the lowest soil Fe content. Intermittent water management increased rice growth and yield higher than flooded continuously by 13.6% and/or flushing system by 13.2%. An intermittent system after one week was accompanied by a delay in planting time of 14 days to 21 days after being flooded (Khairullah et al., 2021).

CONCLUSIONS

1. The use of local varieties from Fe toxicity areas as parents for crosses resulted in offspring that were tolerant to Fe toxicity and were stable between locations and seasons.
2. The change from the dry season to the wet season caused the yield of sensitive varieties to decrease more than the moderate and tolerant lines. Sensitive varieties and moderate lines gave higher yields in the drough season than in the wet season.
3. The long dry season followed by high rainfall caused the accumulation of Fe on the soil surface followed by a decrease in yields of moderate and sensitive lines.
4. The addition of 200 kg ha^{-1} of salt increased the productivity of tolerant, quite tolerant, and moderate lines by improving root quality.
5. The stability in the multi-location test can be used as a reference for stability in inter-seasonal fluctuations of soil Fe content.

ACKNOWLEDGEMENTS. Thank you to the Pelalawan Government Regency for funding research in 2013-2014 through the Improvement of the Quality and Character of Pelalawan Local Rice and the Indonesian Agency for Agricultural Research and Development of the Ministry of Agriculture of the Republic of Indonesia through TTP in 2017–2018 and VUB Rice Demonstration Activities in 2019–2020.

AUTHOR CONTRIBUTIONS. Parlin Halomoan Sinaga: Design research, formal analysis, methodology, supervision, writing original draft, interpretation; Elfiani: data collection, validation, interpretation; Rachmiwati Yusuf: writing-review, interpretation; Nurhayati: methodology, formal analysis; Dwinita Utami: conceptualization, formal analysis; Rossa Yunita: Interpretation and discussion; Setia Sari Br. Girsang: Interpretation, discussion, and writing-review.

DATA AVAILABILITY STATEMENT. The data presented in this study are available upon request from the corresponding author. The data are not publicly available yet but will be in due course.

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