BIODIVERSITAS
Volume 23, Number 7, July 2022
Pages: 3738-3743

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d230750

Trait selection and screening of Indonesian local rice accessions for iron stress tolerance

KHAIRUNNISA LUBIS[1], LISNAWITA[1], FRANS MAATHUIS[2], IRDA SAFNI[1,♥]
[1]Department of Agrotechnology, Faculty of Agriculture, Universitas Sumatera Utara. Jl. Prof. A. Sofyan No. 3, Medan 20155, North Sumatra, Indonesia. Tel.: +62-61-8213236, Fax.: +62-61-82111924, ♥email: irda@usu.ac.id
[2]Department of Biology, University of York. Heslington, York YO10 5DD, United Kingdom

Manuscript received: 14 February 2022. Revision accepted: 28 June 2022.

Abstract. *Lubis K, Lisnawita, Maathuis F, Safni I. 2022. Trait selection and screening of Indonesian local rice accessions for iron stress tolerance. Biodiversitas 23: 3738-3743.* Iron (Fe) poisoning in rice causes changes in both morphological and physiological characteristics of the plants, but the stress response is often genotype-dependent. This research used a screening method based on nutrient culture media to evaluate 20 local Indonesian genotypes for tolerance to Fe stress by studying the response of root morphology, vegetative growth, and biomass. Trait selection for abiotic stress tolerance is essential to achieve a successful breeding program. This research aimed to identify cultivars that were highly resistant to excess Fe from 20 Indonesian local rice accessions. The research was conducted in a greenhouse from May to June 2019 and used a randomized complete block design with two factors and three replications. The first factor consisted of 20 local Indonesian rice accessions. The second factor was the concentration of Fe in the nutrient media (0, 100, 200, and 300 ppm Fe). The results showed that high Fe concentration in the nutrient solution decreased root growth, vegetative growth, and biomass in a genotype-specific manner. The application of >200 ppm Fe in the medium resulted in inhibition of plant growth characterized by yellowing and dry leaves, a decrease in the number of root branches, root length, number of tillers, leaf length, and shoot/root biomass. Several tolerant cultivars were discovered, including Inpara 9, Lipigo 2, and Sigambiri Merah, which could be useful in tolerance breeding. Evaluation of all parameters identified shoot dry weight and number of leaves as reliable traits to assess Fe toxicity tolerance in rice.

Keywords: Indonesia growth parameters, iron stress, rice, screening, trait selection

INTRODUCTION

Rice grown on acid sulfate soils suffer from low pH and Al^{3+} and/or Fe^{2+} toxicity, and considerably lowers yields. The critical pH and Al concentration for rice growth are 6 and 15-30 uM respectively (Shamshuddin et al. 2017). This translates into around 300 mg/L of water-soluble Fe and is generally considered the basic constraint for the cultivation of lowland rice due to soils becoming infertile (Li et al. 2016; Shamshuddin et al. 2017).

The soils of Indonesia are dominated by Inceptisols, Ultisols, and Oxisols, which makes soils in Indonesia mostly acidic. The three soil orders are distributed mainly in Sumatra, Kalimantan, Sulawesi, and Papua Islands occupying 73% of the land area (Suwardi 2019). Those soils are classified as marginal with some being used for agricultural activities while others are forested. Acid sulfate soils are characterized by a low pH (around 3) and the presence of sulfuric horizons, overlaying sulfidic materials, which are mainly due to pyrite oxidation and usually have low macro and micronutrient content (Shamshuddin et al. 2014). North Sumatra has quite extensive acid soils that have the potential to be used as agricultural land, especially to increase rice production, which is the staple food crop in Indonesia.

One of the main constraints in rice production on acid soils is the high content of Fe in soil. Fe overload in the soil can damage the root uptake system an d adversely affect the acquisition of other nutrients, such as phosphorus, zinc and magnesium, leading to reduced growth and yield loss and even plant death. Furthermore, Fe toxicity inhibits cell division, elongation of the primary roots and also the growth of lateral roots (Li et al. 2015). Soil acidity stimulates the release of Al, Fe, and Mn from their soil-bound forms into bioavailable Al^{3+}, Fe^{2+}, and Mn^{2+}. The anaerobic conditions in paddies promote reduction of ferric (Fe^{3+}) to ferrous (Fe^{2+}) iron. The latter is more soluble and hence can readily cause iron toxicity (George et al. 2012). Fe toxicity often occurs in rice grown in submerged paddy fields with low pH, leading to considerable increases in ferrous ion concentration, disrupting cell homeostasis and impairing growth and yield. (Aung and Masuda 2016; Onyango et al. 2019).

To cope with Fe excess, plants have evolved complex adaptive responses (Li et al. 2016). Fe poisoning of rice plants causes changes in both morphological and physiological properties that are to a large extent genotype-specific, depending on the level of tolerance (Müller et al. 2015). Heavy Fe toxicity in rice plants causes inhibition of vegetative growth at the nursery stage. Furthermore, a reduced number of tillers causes low production and can cause crop failure during the reproductive period. However, rice roots are able to excrete organic acids when in the presence of high concentrations of Al and/or Fe, which further reduces the availability of Al and Fe in the water (Kariali et al. 2012; Shamshuddin et al. 2017).

Several reports showed that due to high content of Fe^{2+} in acid sulfate soils, toxicity symptoms in plants can be observed during the whole growth period, and the yield losses range from 40% to 100% (Wu et al. 2016). Typical visual symptoms related to Fe toxicity in rice are the occurrence of bronzing or yellowing in leaves followed by the drying of plants (Dorothy et al. 2019). The severity of leaf bronzing depends on the intensity and concentration of Fe in plants (Elec et al. 2013). This leaf damage causes stunted plant growth, a low number of tillers, and underdeveloped root systems with few and coarse roots (Li et al. 2016).

A sustainable approach to overcome the problems of marginal soils is to cultivate crops with inherent tolerance to the prevailing environmental conditions such as high Fe content. Screening programs can be carried out to select positive traits and identify genotypes with Fe tolerance. In particular, using hydroponic methods, which are straightforward, cost-effective, and extremely useful in pinpointing tolerant germplasm, can be used to inform the subsequent phase of field testing, which is often laborious and expensive. Furthermore, hydroponics allows easy access to underground tissues such as roots that are essential for the absorption of nutrients and water.

Indonesia has many indigenous rice varieties. These are typically adapted to local environments after many generations of cultivation, showing resistance to biotic and abiotic stresses occurring in specific agro-ecosystems. However, these varieties often have low yield potential, making them less economically viable. Thus, it is imperative to identify germplasm that shows both high levels of Fe toxicity tolerance and high yield potential. Several Indonesian indigenous rice varieties have been selected that have potential yield and biotic (pests and diseases) stress resistance, namely Gamapadi-2 and Gamapadi-4 which were resistant to brown plant hoppers and bacterial leaf blight (Aristya et al. 2021). A number of Indonesian local rice varieties that have potential biotic (pests and diseases) and abiotic (drought, high salinity, low temperature) safety resistant genes have been listed in an inventory of Indonesian local rice collection (Chaniago 2019).

In this study, 20 Indonesian local rice accessions were studied for various morphological properties. The goal was to get a selection character related to the underlying trait mechanisms of rice tolerance to Fe toxicity and screening for recommended breeding targets. We used a hydroponic approach to collect data on a number of growth and morphological parameters, to select convenient traits for Fe toxicity screening. By combining different phenotypic screening methodologies on abiotic properties and continuing with biotic properties, it provides good prospects for engineering superior rice varieties that are resistant to Fe and biotic stress.

MATERIALS AND METHODS

Plant growth conditions

This research was conducted in a greenhouse with a temperature of 27-30ºC and RH 80-85% from May to June 2019. The experimental media were based on a modified Yoshida nutrient solution with various concentrations of Fe and pH. The macro and micronutrient concentrations of the standard medium were as follows: 40 ppm N (NH_4NO_3), 10 ppm P ($NaH_2PO_4{\cdot}2H_2O$), 40 ppm K (K_2SO_4), 40 ppm Ca ($CaCl_2$), 40 ppm Mg ($MgSO_4{\cdot}7H_2O$), 0.5 ppm Mn ($MnCl_2{\cdot}4H_2O$), 0.05 ppm Mo ($(NH_4)6{\cdot}Mo_7O_{24}{\cdot}4H_2O$), 0.2 ppm B ($H_3BO_3$), 0.01 ppm Zn ($ZnSO_4{\cdot}7H_2O$), 0.01 ppm Cu ($CuSO_4{\cdot}5H_2O$), and 2 ppm Fe ($FeSO_4.7H_2O$) (Yoshida 1981).

As planting containers Styrofoam boxes (from fruit box waste) were used with a volume of ±1500 mL (20 cm wide, 10 cm high, and 100 cm long), filled with a 1,000 mL Yoshida nutrient solution and Fe according to the treatments (0, 100, 200 and 300 ppm). Forty holes were perforated in the lid of the box to plant the seedlings that were germinated for 7 days in the germination medium. The surface of the box was closed by plastic to minimize oxygen ingress and evaporation of the medium. The volume was adjusted every two days. Rice seeds were sown in germination tubs with husk medium and transferred to a nutrient solution at pH 4.5 according to treatment after seven days of seeding (DAS), where the seed already has roots and the coleoptile has emerged. The pH of the solution was maintained at pH 4.5 by adding KOH or HCl every two days to maintain stress conditions. The degree of acidity of the solution was determined before the addition of KOH or HCl. The Fe concentration treatment used was derived from the $FeSO_4$ $7H_2O$ compound, with the treatment of Fe concentrations (0, 100, 200 and 300 ppm).

Experimental design

The experiments were carried out using a randomized complete block design (RCBD) with two factors and three replications. The first factor consisted of the 20 local rice genotypes of Sumatra and Java, namely Inpara 5, Inpara 8, Inpara 9, Inpari 17, Inpara 30, Inpari 4, Inpari 32, Inpari 33, Sigambiri Putih, Inpago 9, Inpago 10, Inpago Unsoed 1, Lipigo 2, Sigambiri Merah, Inpari 34, Towuti, Inpari 39, Mekongga, Situ Bagendit, and Rindang. The second factor was the Fe concentration in the medium solution (0, 100, 200, 300 ppm Fe).

Morphological observations

Morphological observations of the plants were made four weeks after transplantation. The observed parameters included plant height (cm), number of leaves, number of tillers, root length (cm), number of root branches, root dry/wet weight (g), and shoot dry/wet weight (g). The shoot and root dry weights were observed after drying in an oven at 70°C for 24 hours.

Data analysis

The analysis of variance was done using ANOVA, followed by Duncan's test. The experimental data was sorted in MS Excel 2010 and was analyzed using IBM SPSS Statistics 21.0. Means were compared using the least significant range at the 0.05 significance level to determine the differences between the means of each treatment. The P-Pearson correlation test was used to observe the correlation between the observed characters.

RESULTS AND DISCUSSION

Morphological and growth phenotypes in response to varying levels of Fe

Iron (Fe) is an essential microelement but is highly toxic when it is in excess. Plants have evolved complex adaptive responses that include morphological and physiological modifications. The results of the analysis of variance showed that the concentration of Fe, genotype and their interactions had a significant effect on all the observed characters, namely number of leaves, number of tillers, root length, shoot wet/dry weight, root wet/dry weight and number of root branches (Table 1). Figure 1 shows examples of plant morphology after treatment. In general, higher Fe concentrations in the growth solution increased toxicity symptoms.

Visually, the morphological appearance of the leaves in the third week included browning or yellowing and partially dried tissues. This seems to be the result of Fe poisoning. Absorption of Fe^{2+} by rice roots and its translocation into leaves results in an increased production of toxic oxygen radicals which can damage the structural components of cells and damage the physiology process. Typical visual symptoms associated with this process are "bronzing" of rice leaves and substantial yield loss. The severity of leaf bronzing depends on the intensity and concentration of Fe in plants (Elec et al. 2013; Li et al. 2016; Onyango et al. 2019).

Table 1. Results of analysis variance of rice genotypes characteristic

Character	Fe concentration MS	Genotypes MS	Fe concentratrion x genotype MS
Number of leaves (pcs)	244.67*	43.39*	16.55*
Number of tillers (pcs)	14.63*	2.77*	0.78*
Root length (cm)	863.61*	1952.19*	86.24*
Shoot wet weight (g)	10.16*	2.75*	0.79*
Shoot dry weight (g)	0.52*	0.18*	0.04*
Root wet weight (g)	0.14	0.38*	0.18*
Root dry weight (g)	0.04*	0.03*	0.004*
Number of root branches (pcs)	999.22*	894.25*	73.28*

Note: *significantly different α <5%, MS: Means of Square

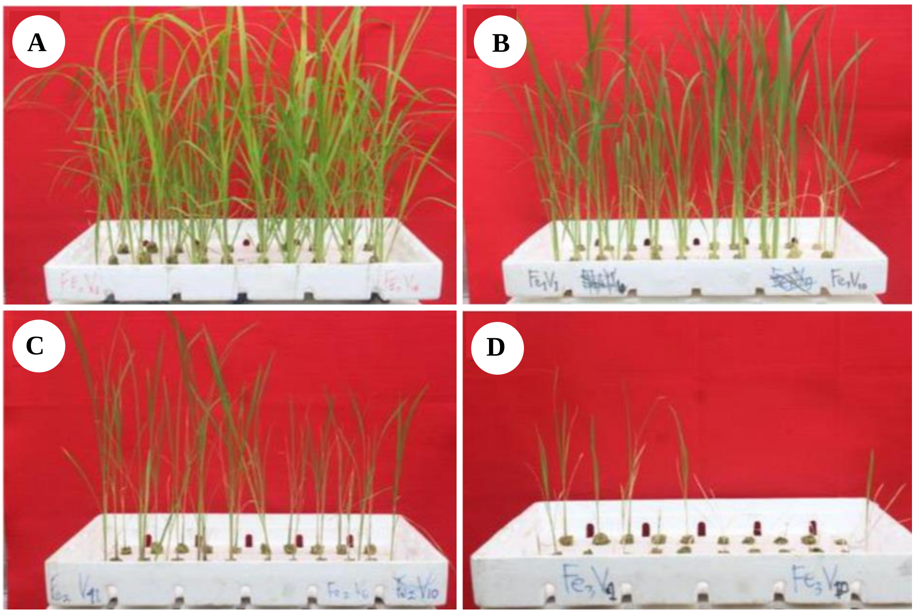

Figure 1. Morphology of rice plants was observed at the age of 3 weeks after transplanting the plants to treatments. A. Rice plant under control condition (Fe 0 ppm); normal leaf and root growth, B. Morphology of rice plants at 100 ppm Fe concentration; leaf growth was normal but the number of leaves was reduced, C. Morphology of rice plants at 200 ppm Fe concentration; the leaves of the plant began to turn yellow (bronzing) and initial root damage occurs, D. The morphology of the rice plant at a concentration of Fe 300 ppm; plants began to dry out and some non-tolerant varieties began to die

Table 2. shows how the shoot-related parameters, i.e., the number of tillers, number of leaves, and shoot dry weight, varied in response to different levels of Fe. In general, increasing Fe led to a reduction in all three parameters. The data obtained for the number of tillers showed a clear decrease when comparing values at 100 ppm to those at the control, but further increases in Fe produced only slight responses, making this trait less useful in screening assays.

Table 3 gives the quantitative data for three root-related parameters: root branches, root length, and root dry weight. As seen for shoot parameters, different genotypes responded in different ways. The root character can also be used as a suitable marker to test the tolerance for Fe toxicity, because it has a high correlation with the shoot/root dry weight production character (Table 4). The values for root length increased at 100 ppm (23.63 cm) and at 200 ppm (27.93 cm) compared to control values (19.61 cm), No overall reduction was observed when plants were exposed to 300 ppm Fe (19.89 cm) when values were comparable to that of the control (19.61 cm). The values for number of root branches increased at 200 ppm (18.38 cm) and at 300 ppm (19.89) compared to control (17.63 cm), but an overall reduction was found when plants were exposed to 300 ppm Fe (10.13 cm), thus ruling it out as a reliable predictor of Fe toxicity tolerance. The average root dry weight was higher at 100 ppm (0.148 g) than observed at the control (0.142 g), though these data were skewed by the behavior of the Mekongga variety at 100 ppm.

The root system is an effective selection character to determine the ability of plants to manage the effects of Al/Fe stress (Mahender et al. 2019). The effects of Fe excess on root system architecture are poorly understood. Li et al. (2016) showed that excess Fe treatment in *Arabidopsis* not only directly impairs primary root growth but also arrests lateral root formation by acting at the tip of the growing primary root. Such a change was believed to assist root system architecture adjustment and to restrict excessive Fe absorption in the part of the rhizosphere subject to acute toxicity while maintaining the absorption of other nutrients in the less stressed components of the root system.

Fe toxicity significantly reduces the growth and metabolism of rice varieties (Onyango et al. 2019). Morphological factors that are disturbed by the Fe toxicity levels include the shoot length, root length, and the number of lateral roots. Physiological processes such as photosynthesis, stomatal functioning, and transpiration rate can also often be affected. (Kobayashi and Nishizawa 2012; Briat et al. 2015; Li et al. 2019; Onyango et al. 2019).

Conventionally, the traits selection must consider several factors, including ease to observe and reveal the results early, high diversity, high heritability, and traits that are positively correlated with yield (Breseghello and Coelho 2013).

Table 2. Effect of Fe concentration on the average number of tillers, number of leaves, and shoot dry weight of 20 Indonesian local rice genotypes

Genotypes	Number of leaves				Number of tillers				Shoot dry weight			
	0 ppm	100 ppm	200 ppm	300 ppm	0 ppm	100 ppm	200 ppm	300 ppm	0 ppm	100 ppm	200 ppm	300 ppm
Inpara 5	**13.67 a**	7.89 fgh	5.33 j-o	1.56 uv	**3.00 a**	2.00 b	1.11 e-h	1.00 fgh	**0.59 ab**	0.34 g-l	0.26 j-r	0.07
Inpara 8	7.78 fgh	5.67 i-n	5.22 k-o	0 w	1.67 bcd	1.33 c-f	1.11 e-h	0 i	0.51 bcd	0.30 h-n	0.31h-m	0 w
Inpara 9	3.89 o-t	4.78 l-p	3.00 q-u	2.22 tuv	1.00 fgh	1.11 e-h	1.00 fgh	1.00 fgh	0.14 s-v	0.18 o-v	0.13 s-v	0.11t-w
Inpari 17	9.11def	5.44 j-o	4.33 n-s	3.28 q-u	2.00 b	1.33 c-f	0.78 gh	0.78 gh	0.46 c-f	0.29 i-o	0.28 i-p	0.18 o-v
Inpara 30	10.67 bcd	7.00 hij	3.89 o-t	4.33 n-s	1.67 bcd	1.56 b-e	0.67 h	0.67 h	0.47 cde	0.35 f-k	0.21m-t	0.23l-s
Inpari 4	7.67 fgh	2.67 s-v	2.89 r-u	2.22 tuv	1.44 c-f	1.00 fgh	1.00 fgh	1.00 fgh	0.24 k-s	0.10 t-w	0.20 m-u	0.10 t-w
Inpari 32	**13.44 a**	5.33 j-o	4.56 m-r	2.00 uv	**2.67 a**	1.11 e-h	1.00 fgh	1.00 fgh	**0.64 a**	0.21m-t	0.23 l-s	0.08 vw
Inpari 33	10.00 cde	6.33 h-l	3.00 q-u	2.56 s-v	1.67 bcd	1.44 c-f	1.00 fgh	1.00 fgh	0.46 c-f	0.30 h-n	0.16 q-v	0.15 r-v
Sigambiri putih	4.33 n-s	4.67 m-q	0 w	0 w	0.87 fgh	1.00 fgh	0 i	0 i	0.19 n-v	0.09uvw	0 w	0 w
Inpago 9	10.33 cde	5.67i-n	5.11k-p	0 w	1.67 bcd	1.11 e-h	1.11 e-h	0 i	**0.62 ab**	0.41 d-h	0.41d-h	0 w
Inpago 10	7.22 ghi	2.22 tv	2.89 q-u	2.56 s-v	1.33 c-f	1.00 fgh	1.00 fgh	1.00 fgh	0.45 c-g	0.16 q-v	0.19 n-v	0.11t-w
Inpago Unsoed	8.89 efg	6.56 h-k	5.33 j-o	2.00 uv	1.78 bc	1.33 c-f	1.33 c-f	1.00 fgh	0.38 e-i	0.38 e-i	0.37 e-j	0.13 s-v
Lipigo 2	6.33 h-l	4.89 k-p	4.11n-s	0 w	1.22 d-g	1.00 fgh	1.00 fgh	0 i	0.51bcd	0.34 g-l	0.30 h-n	0 w
Sigambiri merah	5.22 k-o	3.44 p-u	2.11uv	1.78 uv	1.00 fgh	1.00 fgh	1.00 fgh	1.00 fgh	0.13 s-v	0.24 k-s	0.16 q-v	0.14 s-v
Inpari 34	12.22 ab	4.22 n-s	0 w	0 w	1.50 b-e	1.00 fgh	0 i	0 i	0.28 i-p	0.08 vw	0 w	0 w
Towuti	11.00 bc	5.78 i-n	3.44 p-u	3.22 q-u	2.00 b	1.11 e-h	1.00 fgh	1.00 fgh	**0.56 abc**	0.27 i-q	0.17 p-v	0.20 m-u
Inpari 39	**13.22 a**	3.78 o-t	0 w	0 w	1.44 c-f	1.00 fgh	0 i	0 i	0.32 h-m	0.08 vw	0 w	0 w
Mekongga	4.56 m-r	9.89 cde	4.78 l-p	3.33 p-u	1.00 fgh	1.67 bcd	1.00 fgh	1.00 fgh	0.40 d-h	0.22 m-t	0.14 s-v	0.14 s-v
Situ Bagendit	0 w	0 w	0 w	0 w	0 i	0 i	0 i	0 i	0 w	0 w	0 w	0 w
Rindang	4.44 m-r	0 w	3.00 qu	1.78 uv	1.00 fgh	0 i	1.00 fgh	1.00 fgh	0.28 i-p	0 w	0.13 s-v	0.08 vw
Average	6.65	5.32	3.58	2.02	1.16	0.72	0.23	0.07	**0.33**	**0.19**	**0.17**	**0.10**

Note: Numbers followed by the same letter in the columns in the treatment are not significantly different results based on the DMRT test at a level of 0.05

Table 3. Effect of Fe concentration on the average root length and root branches of 20 Indonesian local rice genotypes

Genotypes	Root length				Number of root branches				Root dry weight			
	0 ppm	100 ppm	200 ppm	300 ppm	0 ppm	100 ppm	200 ppm	300 ppm	0 ppm	100 ppm	200 ppm	300 ppm
Inpara 5	24.74 c-j	30.72 b-h	26.50 c-j	9.77 kl	29.56 ab	19.89 e-m	28.44 a-d	6.22 s-u	0.22 bc	0.16 b-f	0.14 c-h	0.01 mn
Inpara 8	26.33 c-j	32.24 b-g	30.87 b-h	0 l	24.89 a-f	17.44 f-p	28.44 a-d	0 u	0.16 b-f	0.12 e-i	0.13 d-h	0 n
Inpara 9	24.74 c-j	32.36 b-f	24.08 e-j	19.18 ijk	10.11 o-s	13.89 i-s	24.67 a-g	9.11 q-t	0.14 c-h	0.13 d-h	0.10 f-k	0.04 k-n
Inpari 17	24.23 e-j	28.44 b-i	30.38 b-h	28.66 b-i	20.33 d-k	17.44 f-p	16.22 h-r	13.67 i-s	0.17 b-f	0.11 f-j	0.11 f-j	0.13 d-h
Inpara 30	23.74 f-j	32.39 b-f	**49.93 a**	30.19 b-h	23.44 a-h	18.22 e-o	16.00 h-r	13.89 i-s	0.19 b-e	0.14 c-h	0.11 f-j	0.14 c-h
Inpari 4	23.57 f-j	23.97 e-j	24.34 e-j	21.48 g-j	20.00 e-m	10.22 o-s	14.22 i-s	9.44 p-t	0.06 i-n	0.06 i-n	0.08 g-l	0.05 j-n
Inpari 32	23.54 f-j	25.91 c-j	31.98 b-g	16.58 jk	22.67 a-h	25.00 a-f	16.56 g-q	10.44 o-s	0.13 d-h	0.07 h-m	0.09 g-l	0.05 j-n
Inpari 33	24.23 e-j	30.86 b-h	23.54 f-j	22.26 f-j	21.67 b-i	28.78 abc	17.56 f-p	10.22 o-s	0.14 c-h	0.09 g-l	0.10 f-k	0.07 h-m
Sigambiri putih	26.06 c-j	32.00 b-g	0 l	0 l	11.33 l-s	25.00 a-f	0 u	0 u	0.08 g-l	0.04 k-n	0 n	0 n
Inpago 9	20.59 h-k	29.77 b-i	24.68 d-j	0 l	23.11 a-h	19.22 e-n	16.56 g-q	0 u	0.15 b-g	0.20 b-d	0.22 bc	0 n
Inpago 10	21.46 g-j	22.51 f-j	28.16 b-i	23.21 f-j	22.56 a-h	13.11 j-s	11.78 m-s	12.33 k-s	0.08 g-l	0.07 h-m	0.08 g-l	0.05 j-n
Inpago Unsoed	24.98 c-j	30.61 b-h	34.68 bcd	16.90 jk	25.67 a-f	26.44 a-e	29.22 abc	11.89 l-s	0.17 b-f	0.13 d-h	0.15 b-g	0.08 g-l
Lipigo 2	28.19 b-i	35.29 bcd	31.97 b-g	23.00 f-j	29.00 abc	16.00 h-r	18.33 e-o	11.00 n-s	0.17 b-f	0.13 d-h	0.13 d-h	0 n
Sigambiri merah	25.28 c-j	**37.40 b**	35.52 bc	29.41 b-i	19.22 e-n	11.78 m-s	19.11 e-n	8.44 rst	0.03 l-n	0.09 g-l	0.06 i-n	0.06 i-n
Inpari 34	24.33 c-j	29.89 b-i	0 l	0 l	16.75 g-q	15.65 h-r	0 u	0 u	0.31 b	0.05 j-n	0 n	0 n
Towuti	29.08 b-i	32.81 b-f	29.66 b-i	27.03 b-i	**30.11 a**	20.11 e-l	21.22 c-j	13.33 j-s	0.14 c-h	0.09 g-l	0.10 f-k	0.11 f-j
Inpari 39	24.35 c-j	29.85 b-i	0 l	0 l	26.30 a-e	18.75 e-n	0 u	0 u	0.12 e-i	0.14 c-h	0 n	0 n
Mekongga	23.38 f-j	35.80 bc	20.86 h-k	26.93 b-i	17.33 f-p	23.00 a-h	18.00 e-o	13.67 i-s	0.18 b-e	0.85 a	0.06 i-n	0.05 j-n
Situ Bagendit	0 l	0 l	0 l	0 l	0 u	0 u	0 u	0 u	0 n	0 n	0 n	0 n
Rindang	25.69 c-j	0 l	20.64 h-k	15.26 jk	12.67 k-s	0 u	15.89 h-r	13.11 j-s	0.07 h-m	0 n	0.03 l-n	0.03 l-n
Average	19.61	23.63	27.93	19.89	17.63	14.31	18.38	10.13	0.142	0.148	0.11	0.07

Note: Numbers followed by the same letter in the columns in the treatment are not significantly different results based on the DMRT test at a level of 0.05

Table 4. Correlation between the number of leaves, number of tillers, root length, number of root branches, shoot dry/wet weight, and root dry/wet weight

Characters	Number of leaves	Number of tillers	Root length	Number of root branches
Number of leaves	-			
Number of tillers	0.64**	-		
Root length	0.22*	0.28*	-	
Number of root branches	0.37*	0.46**	0.53**	-
Shoot dry weight (SDW)	**0.51****	**0.59****	0.44**	**0.56****
Shoot wet weight (SWW)	**0.51****	**0.59****	0.14*	0.43**
Root dry weight (RDW)	0.38*	0.46**	0.48**	**0.52****
Root wet weight (RWW)	0.16	0.21*	0.35*	0.32*

Note: *significant correlation α<5%, **significant correlation α<1%

The results of shoot-related traits indicated that the number of leaves, number of tillers and shoot dry/wet weight showed good correlations with the stress levels and thus may be useful for screening purposes (correlation >0.50). The root-related traits appeared to be suitable predictors of Fe toxicity tolerance too. This is indicated by number of root branches which have a good correlation with shoot dry weight and root dry weight (Table 4).

Screening for rice tolerance to Fe toxicity using the shoot dry weight, number of leaves and number of root branches

Tables 5-7 show the absolute values of each parameter for each condition, and the 20 cultivars for which data were obtained. Table 5-7 also lists the stress-induced change with respect to the values obtained in the control condition. The latter, expressed as percentage reduction of the respective parameters, provides a better measure of relative tolerance and avoids potential mislabeling of cultivars as tolerant or sensitive based on innate differences in vigor. Each cultivar and treatment condition were assigned a tolerance ranking based on the percentage reduction values.

Table 5 shows that the ranking across treatments was fairly consistent. Where the shoot dry weight is concerned, V14 (Sigambiri Merah) reproducibly appeared as the most tolerant cultivar, ranking first in all three stress treatments (i.e., 1, 1, 1). Similarly, V3 (Inpara 9), ranking 2, 3, 2 in 100, 200, and 300 ppm Fe, scored highly across treatments. The relatively sensitive cultivars included V10 (Inpago 9), ranking 15, 12, 11, and V7 (Inpari 32), ranking 16, 13, 12, while intermediate cultivars included V4 (Inpari 17), ranking 8, 6, 5, and V1 (Inpara 5), ranking 10, 11, 13.

As observed for the shoot dry weight, tolerance ranking according to the number of leaves parameter generally showed excellent reproducibility between stress treatments (Table 6). Consistent results for tolerance were detected for cultivar V18 (Mekongga), ranking 1, 1, 1, and V3 (Inpara 9), ranking 2, 2, 2, and possibly V13 (Lipigo 2), ranking 4, 5, though the latter lacks the data for the 300 ppm treatment. V1 (Inpara 5) consistently emerged as a middle-ranking cultivar with scores of 11, 11, 13, while V7 (Inpari 32) showed a reproducibly high ranking of 14, 14, 12, pointing to relative Fe toxicity sensitivity.

In combination, and taking both parameters into account, cultivar V3 (Inpara 9) emerged with a consistently high tolerance score (2, 2, 2, 2, 2, 2). Moderate tolerance was found in V13 (Lipigo 2), ranking 6, 8, 4, 5, though here too, the data for the highest stress treatment was missing, while moderate sensitivity was apparent in V1 (Inpara 5) ranking 10, 11, 13, 11, 11, 13. V7 (Inpari 32) showed sensitivity according to both parameters (ranking 16, 13, 12, 14, 14, 12). In a number of lines, ranking differed greatly, depending on the trait used. A prime example is V14 (Sigambiri Merah), which was found to be the most tolerant line according to shoot dry weight (scoring 1, 1, 1) but only ranked moderately tolerant on the basis of the number of leaves (scoring 7, 9, 7). Vice versa, V18 (Mekongga), which was identified as the most tolerant cultivar when assessing the number of leaves (scoring 1, 1, 1), had a low tolerance ranking (11, 14, 7) on the basis of the shoot dry weight.

Table 5. Genotype ranking on the basis of reduced shoot dry weight

Genotypes	Reduced shoot dry weight									
	0 ppm	100 ppm	% reduction	Ranking	200 ppm	% reduction	Ranking	300 ppm	% reduction	Ranking
Inpara 5	0.59	0.34	42.37	10	0.26	55.93	11	0.07	88.14	13
Inpara 8	0.51	0.30	41.18	9	0.31	39.22	7	0	100	#
Inpara 9	0.14	0.18	-28.57	2	0.13	7.14	3	0.11	21.43	2
Inpari 17	0.46	0.29	36.96	8	0.28	39.13	6	0.18	60.87	5
Inpara 30	0.47	0.35	25.53	5	0.21	55.32	10	0.23	51.06	3
Inpari 4	0.24	0.10	58.33	14	0.20	16.67	5	0.10	58.33	4
Inpari 32	0.64	0.21	67.19	16	0.23	64.06	13	0.08	87.50	12
Inpari 33	0.46	0.30	34.78	7	0.16	65.22	15	0.15	67.39	9
Sigambiri Putih	0.19	0.09	52.63	13	0	100	#	0	100	#
Inpago 9	0.45	0.41	8.89	4	0.41	8.89	4	0	100	#
Inpago 10	0.45	0.16	64.44	15	0.19	57.78	12	0.11	75.56	11
Inpago Unsoed	0.38	0.38	0	3	0.37	2.63	2	0.13	65.79	8
Lipigo 2	0.51	0.34	33.33	6	0.30	41.18	8	0	100	#
Sigambiri Merah	0.13	0.24	-84.62	1	0.16	-23.08	1	0.14	-7.69	1
Inpari 34	0.58	0.08	86.21	18	0	100	#	0	100	#
Towuti	0.56	0.27	51.78	12	0.17	69.64	16	0.20	64.29	6
Inpari 39	0.32	0.08	75.00	17	0	100	#	0	100	#
Mekongga	0.40	0.22	45.00	11	0.14	65.00	14	0.14	65.00	7
Situ Bagendit	0	0	NA	NA[1]	0	NA	NA	0	NA	NA
Rindang	0.28	0	100	#[2]	0.13	53.57	9	0.08	71.43	10

Note: [1]NA: data not available, [2]#: yield reduction of 100%

Table 6. Genotype ranking on the basis of reduction in the number of leaves

Genotypes	Number of leaves (pcs)									
	0 ppm	100 ppm	% reduction	Ranking	200 ppm	% reduction	Ranking	300 ppm	% reduction	Ranking
Inpara 5	13.67	7.89	42.28	11	5.33	61.01	11	1.56	88.59	13
Inpara 8	7.78	5.67	27.12	6	5.22	32.90	3	0	100	#
Inpara 9	3.89	4.78	-11.44	2	3.00	22.88	2	2.22	42.93	2
Inpari 17	9.11	5.44	40.28	10	4.33	40.28	7	3.28	63.99	5
Inpara 30	10.67	7.00	34.39	8	3.89	63.54	13	4.33	59.42	3
Inpari 4	7.67	2.67	65.18	15	2.89	62.32	12	2.22	71.06	9
Inpari 32	13.44	5.33	60.34	14	4.56	66.07	14	2.00	85.12	12
Inpari 33	10.00	6.33	36.70	9	3.00	70.00	16	2.56	74.40	10
Sigambiri Putih	4.33	4.67	-7.85	3	0	100	#	0	100	#
Inpago 9	10.33	5.67	45.11	12	5.11	50.53	8	0	100	#
Inpago 10	7.22	2.22	69.25	17	2.89	59.97	10	2.56	64.54	6
Inpago Unsoed	8.89	6.56	26.21	5	5.33	40.04	6	2.00	77.50	11
Lipigo 2	6.33	4.89	22.75	4	4.11	35.07	5	0	100	#
Sigambiri Merah	5.22	3.44	34.09	7	2.11	59.58	9	1.78	65.90	7
Inpari 34	12.22	4.22	65.47	16	0	100	#	0	100	#
Towuti	11.00	5.78	47.45	13	3.44	68.73	15	3.22	70.73	8
Inpari 39	13.22	3.78	71.41	18	0	100	#	0	100	#
Mekongga	4.56	9.89	-116.88	1	4.78	-4.82	1	3.33	26.97	1
Situ Bagendit	0	0	NA[1]	NA	0	NA	NA	0	NA	NA
Rindang	4.44	0	100	#[2]	3.00	32.43	4	1.78	59.91	4

Note: [1]NA: data not available, [2]#: yield reduction of 100%

Table 7. Genotype ranking on the basis of reduced number of root branches

Genotypes	Number of root branches (pcs)									
	0 ppm	100 ppm	% reduction	Ranking	200 ppm	% reduction	Ranking	300 ppm	% reduction	Ranking
Inpara 5	29.56	19.89	32.71	13	28.44	3.78	7	6.22	78,95	14
Inpara 8	24.89	17.44	29.93	12	28.44	-14.26	3	0	100	15
Inpara 9	10.11	13.89	-37.38	2	24.67	-144.01	1	9.11	9.89	2
Inpari 17	20.33	17.44	14.21	8	16.22	20.21	9	13.67	32.75	4
Inpara 30	23.44	18.22	22.26	10	16.00	31.74	14	13.89	40.74	5
Inpari 4	20.00	10.22	48.90	18	14.22	28.90	12	9.44	52.80	7
Inpari 32	22.67	25.00	-10.27	5	16.56	26.95	10	10.44	53.94	10
Inpari 33	21.67	28.78	-32.81	3	17.56	18.96	8	10.22	52.83	8
Sigambiri Putih	11.33	25.00	-120.65	1	0	100	#	0	100	#
Inpago 9	23.11	19.22	16.83	9	16.56	28.34	11	0	100	#
Inpago 10	22.56	13.11	41.88	16	11.78	47.78	16	12.33	45.34	6
Inpago Unsoed	25.67	26.44	-2.99	6	29.22	-13.82	4	11.89	53.68	9
Lipigo 2	29.00	16.00	44.82	17	18.33	36.79	15	11.00	62.08	13
Sigambiri Merah	19.22	11.78	38.70	15	19.11	0.57	6	8.44	56.08	12
Inpari 34	16.75	15.65	6.56	7	0	100	#	0	100	#
Towuti	**30.11**	20.11	33.21	14	21.22	29.52	13	13.33	55.72	**11**
Inpari 39	26.30	18.75	28.70	11	0	100	#	0	100	#
Mekongga	17.33	23.00	-32.71	4	18.00	-3.86	5	13.67	21.11	3
Situ Bagendit	0	0	NA	NA	0	NA	NA	0	NA	NA
Rindang	12.67	0	100	#	15.89	-25.41	2	13.11	-3.47	1

Note: [1]NA: data not available, [2]#: yield reduction of 100%

Since rice generally shows a good correlation between biomass and grain yield (Matsubara et al. 2016; Li et al. 2019), the values obtained for the shoot dry weight in the control condition (0 ppm) should be indicative of yield performance. According to Tables 5 and 6, Sigambiri Merah, a rather slow-growing cultivar with a shoot dry weight of only 0.13 g, was the most promising line based on the shoot dry weight. Similarly, Inpara 9, which showed good tolerance for either shoot dry weight and number of leaves, only yielded 0.14 g biomass. However, Lipigo 2, which showed good tolerance, also produced relatively high biomass (0.51 g); hence, it is likely to yield well in both control and high Fe conditions.

Based on the root parameters, excess Fe has been shown to inhibit initiation of root branching, root length and root dry weight development, and these inhibitory effects are seen in newly grown roots. However, the effect is different for each genotype (Table 7.)

This shows that excess Fe also arrests growth by decreasing both cell elongation and division. Changes in the overall root system architecture determine root plasticity and allow plants to efficiently acclimate to environmental constraints and restrict the excessive accumulation of nutrients and toxicants. In fact, plants can respond to the heterogeneous availability of nutrient resources by flexibly, and relatively rapidly (Garcia-Palacios et al. 2015; Li et al.2016).

Based on the root and shoot parameters, number of leaves, number of tillers, number of root branches and shoot dry weight, were identified as useful traits to assess Fe toxicity tolerance in rice. Using these four easily accessible parameters should give enhanced confidence with respect to the identification of genuine toxicity resilience. High correlation between growth components and yield provides an optimal response to the selection of the desired trait (Table 4). Using the above metrics, several tolerant cultivars which could be useful in tolerance breeding, such as Inpara 9, Lipigo 2, and Sigambiri Merah, were identified. Furthermore, the Fe resistant line, Lipigo 2, performed well in terms of biomass production, making it the ideal germplasm for further (field) tests and cultivations in high Fe environments.

ACKNOWLEDGEMENTS

The authors gratefully thank the Newton Fund research project by the British Council (ref 414140726) for financial support.

REFERENCES

AristyaVE, Trisyono YA, Mulyo JH, Taryono T. 2021. Participatory varietal selection for promising rice lines. Sustainability 13 (12): 6856. DOI: 10.3390/su13126856.

Aung MA, Masuda H. 2020. How does rice defend against excess iron?: Physiological and molecular mechanisms. Front Plant Sci 11: 1102. DOI: 10.3389/fpls.2020.01102.

Breseghello F, Coelho ASG. 2013. Traditional and modern plant breeding methods with examples in rice (*Oryza sativa* L.). J Agric Food Chem 61 (35): 8277-8286. DOI: 10.1021/jf305531j.

Briat JF, Dubos C, Gaymard F. 2015. Iron nutrition, biomass production, and plant product quality. Trends Plant Sci 20: 33-40. DOI: 10.1016/j.tplants.2014.07.005.

Chaniago N. 2019. Potensi gen-gen ketahanan cekaman biotik dan abiotik pada padi lokal Indonesia: A Review. Agriland: Jurnal Ilmu Pertanian 7 (2): 86-93. [Indonesian]

Elec V, Quimio CA, Mendoza R, Sajise AGC, Beebout SE, Gregorio GB, Singh RK. 2013. Maintaining elevated Fe^{2+} concentration in solution culture for the development of a rapid and repeatable screening technique for iron toxicity tolerance in rice (*Oryza sativa* L.). Plant Soil 372 (1): 253-264. DOI: 10.1007/s 11104-013-1739-4.

García-Palacios P, Maestre FT, Bardgett R, De Kroon H. 2012. Plant responses to soil heterogeneity and global environmental change. J Ecol 100 (6): 1303-1314. DOI: 10.1111/j.1365-2745.2012.02014.x.
George E, Horst WJ, Neumann E. 2012. Adaptation of plants to adverse chemical soil conditions. Marschner's Mineral Nutrition of Higher Plants. Academic Press, Cambridge.
Kariali E, Sarangi S, Panigrahi R, Panda BB, Mohapatra PK. 2012. Variation in senescence pattern of different classes of rice tillers and its effect on panicle biomass growth and grain yield. Am J Plant Sci 3 (8): 1047. DOI: 10.4236/ajps.2012.38125.
Kobayashi T, Nishizawa NK. 2012. Iron uptake, translocation, and regulation in higher plants. Annu Rev Plant Biol 63: 131-52. DOI: 10.1146/annurev-arplant-042811-105522.
Li G, Kronzucker HJ, Shi W. 2016. Root developmental adaptation to Fe toxicity: Mechanisms and management. Plant Signal Behav 11 (1): e1117722. DOI: 10.1080/15592324.2015.1117722.
Li R, Li M, Ashraf U, Liu S, Zhang J. 2019. Exploring the relationships between yield and yield-related traits for rice varieties released in China from 1978 to 2017. Front Plant Sci 10: 543. DOI: 10.3389/fpls.2019.00543.
Li G, Song H, Li B, Kronzucker HJ, Shi W. 2015. Auxin resistant1 and PIN-FORMED2 protect lateral root formation in *Arabidopsis* under iron stress. Plant Physiol 169 (4): 2608-2623. DOI: 10.1104/pp.15.00904.
Mahender A, Mallikarjuna Swamy BPM, Anandan A, Ali J. 2019. Tolerance of iron-deficient and-toxic soil conditions in rice. Plants 8 (2): 31. DOI: 10.3390/plants8020031.
Matsubara K, Eiji Y, Nobuya K, Takuro I, Junichi T, Hiroshi T, Satoshi Y, Osamu M, Jun-ichi Y, Ritsuko M, Toshio Y, Hiroshi K, Masahiro Y. 2016. Improvement of rice biomass yield through QTL-based selection. PLoS ONE 11 (3): e0151830. DOI: 10.1371/journal.pone.0151830.
Müller C, Kuki KN, Pinheiro DT, de Souza LR, Siqueira Silva AI, Loureiro ME, Oliva MA, Almeida, A.M., 2015. Differential physiological responses in rice upon exposure to excess distinct iron forms. Plant Soil 391:1 23-138. DOI: 10.1007/s11104-015-2405-9.
Onyango DA, Entila F, Dida, MM, Ismail AM, Drame KN. 2019. Mechanistic understanding of iron toxicity tolerance in contrasting rice varieties from Africa: 1. Morpho-physiological and biochemical responses. Funct Plant Biol 46 (1): 93-105. DOI: 10.1071/fp18129.
Shamshuddin J, Azura AE, Shazana MARS, Fauziah CI, Panhwar QA, Naher UA. 2014. Properties and management of acid sulfate soils in Southeast Asia for sustainable cultivation of rice, oil palm, and cocoa. Adv Agron 12: 91-142. DOI: 10.1016/B978-0-12-800138-7.00003-6.
Shamshuddin J, Panhwar QA, Alia FJ, Shazana MARS, Radziah O, Fauziah CI. 2017. Formation and utilisation of acid sulfate soils in Southeast Asia for sustainable rice cultivation. Pertanika J Trop Agric Sci 40 (2): 225-246.
Suwardi. 2019. Utilization and improvement of marginal soils for agricultural development in Indonesia. IOP Conf Series Earth and Environ Sci 383: 012047 DOI: 10.1088/1755-1315/383/1/012047.
Wu LB, Shhadi MY, Gregorio G, Matthus E, Becker M, Frei M. 2014. Genetic and physiological analysis of tolerance to acute iron toxicity in rice. Rice (NY) 7 (1): 8. DOI: 10.1186/s12284-014-0008-3.
Yoshida S, Forno DA, Cock JH, Gomez KA. 1976. Laboratory Manual for Physiological Studies of Rice (3rd ed.). International Rice Research Institute, Manila.