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Original Research Article

Morpho-physiological responses of rice genotypes and its clustering under hydroponic iron toxicity conditions

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Abstract

The acid soil area covers major topics land where Iron (Fe) toxicity is one of a limiting factor for rice production which can be overcome by planting the tolerant variety. The information of morpho-physiological characters and the genetic variation of tolerant genotypes is very important. Here we study the variation of root and shoot growth as well as physiological responses to iron toxicity between ten rice genotypes under hydroponic conditions with agar addition. Growth parameters, leaf bronzing score, Fe content in the shoot and root plaques, total chlorophyll, carotenoids, and malondialdehyde (MDA) were observed in this study. Based on morpho-physiological data related to iron toxicity, ten rice genotypes were clustered into three groups which the best performance genotypes were Pokkali and Hawara Bunar. Leaf bronzing score showed correlated with Fe content in the shoot, but tolerant and sensitive genotypes could be differentiate based on this character because it showed non significant Fe content between those two groups. Our study found that the pattern in morpho-physiological characters variation could be useful for selection of desirable genotypes for Fe tolerant rice.

Keywords: Iron toxicity, Leaf bronzing score, MDA, Morpho-physiological characters, Tolerance

Introduction

Iron (Fe) is a micronutrient that participates in photosynthesis, the respiratory chain (in cytochromes), and as a cofactor in various enzymes (Marschner, 1995). However, excess of Fe can be toxic to plants, its abundance inside the cell must be tightly controlled. The critical Fe concentration that causes toxicity for rice is about 250-500 ppm (Yoshida et al., 1976).

Iron toxicity is a symptom associated with high Fe concentration inside the cell, which is dangerous for plants because it leads to oxidative stress (Kampfenkel et al., 1995). The general symptom associated with iron toxicity in the plant is brown spot (bronzing) developed in leaf blades (Takehisa and Sato, 2007). Iron toxicity causes a decrease in growth of some crop rice (Audebert and Fofana, 2009; Nugraha et al., 2016a), Australian hexaploid wheat (Khabaz-Saberi et al., 2010), and tobacco (Nicotiana plumbaginifolia) (Kampfenkel et al., 1995).

Iron toxicity in rice affects the regulation of Fe homeostasis through protein transporters, ROS generation, carbohydrates, hormones, and secondary metabolisms (Quinet et al., 2012). Iron excess in the plant cells generates reactive oxygen species (ROS),

which cause lipid peroxidation of the cell membranes and it indicated with malondialdehyde (MDA) production (Fang et al., 2001; Polit 2007; Hamim et al., 2017). ROS production in Iron excess condition through two chemical reactions, named oxidation and Fenton reaction. In the oxidation reaction, ferrous (Fe^{2+}) can react with oxygen then produce superoxide radical. Meanwhile, in the Fenton reaction, ferrous (Fe^{2+}) can react with hydrogen peroxide then produce hydroxyl radical (Marschner, 1995).

There are many information on hypothetic tolerance strategies in plant when it is exposed to iron excess (Engel et al., 2012; Nugraha and Rumanti, 2017), but it can be simplified in the exclusion and inclusion strategies that involve complex physiological processes. For excluder plants, roots improve the oxidation power in the rhizosphere area to oxidize Fe^{2+} to become Fe^{3+} in the root surface layer, which causes plaque accumulation (Nugraha et al., 2016b). For the inclusion strategies the plant may accumulate the Fe inside the cell with a compartmentation strategy in the vacuole and produce ferritin molecules (Onaga et al., 2016) or through ROS detoxification, which involves enzymes and metabolite antioxidants (da Silveira et al., 2009; Kang et al., 2011; Kabir et al., 2016). However, the tolerance level of plant to iron toxicity stress depends on plant development stage, stress intensity, stress duration, and climatic conditions (Engel, 2009).

Many traits in plants has been known affected by iron toxicity, these include LBS and morpho-physiological characters. LBS is a key secondary trait to differentiate the tolerance level of plants to iron toxicity, especially in rice (Sikirou et al., 2015). However, the LBS did not always correlate with the tolerance level of rice (Becker and Asch, 2005; de Dorlodot et al., 2005; Nugraha et al., 2015). Enhanced tolerance to iron toxicity in breeding program of rice needs the information of morpho-physiological characters (Nugraha et al., 2016c).

Morpho-physiological of some Indonesian rice varieties in responses to iron toxicity may varied that could be tolerant-excluder type or tolerant-includer type. Morpho-physiological analysis has been success to determinate the adaptation pattern of durum wheat (Annicchiarico et al., 2008), genetic diversity on heat tolerance of tall fescue (Festuca arundinacea Schreb.) accessions (Sun et al., 2015) and tolerance level to Aluminum toxicity in rice varieties of North East India (Awasthi et al., 2017). Based on those some previous papers, morpho-physiological characters might be also used in the determination of iron toxicity tolerance level in rice. In this early investigation, we use morpho-physiological characters from two sensitive and eight tolerant genotypes to iron excess to investigate and classify their tolerance type. This paper reports the variations in rice root and shoot growth as well as physiological responses to iron toxicity in ten rice genotypes.

Material and Methods

Plant materials

The plants used in this research were Fe-sensitive (IR64 and Inpara 5) and Fe-tolerant rice genotypes (Mahsuri, Pokkali, Inpara 2, Inpara 6, Danau Gaung, Indragiri, Hawara Bunar, IRH108) provided by Indonesian Center for Rice Research and Plant Physiology and Molecular Biology Laboratory, Department of Biology, Bogor Agricultural University (Table 1).

Analysis of growth and physiological responses of ten rice genotypes to iron toxicity

This experiment aimed to evaluate the variation of tolerance-type of 10 rice genotypes to iron toxicity. The seeds were surface sterilized using sodium hypochlorite 1 % (v/v) for 15 minutes and rinsed with sterile distillate water. The seeds were germinated in the incubator (27 ˚C) for three days. Uniform seedlings were transferred to a sheet-holed styrofoam floating on 9 L plastic trays (35 x 28.5 x 12) cm^3 filled with 8 L half-strength Yoshida's solution until the plants reach two-weeks of age with pre-culture solution renewal every 7 days. Two-weeks old seedlings were then grown on 800 ml plastic pot (Ø: 8.5 cm; height: 15 cm) containing 750 ml HSYA or nutrient culture solution were prepared by dissolving 0.2% agar powder in Yoshida's solution (Yoshida et al., 1976). The Fe treatment followed the procedure conducted by Nugraha et al. (2015). The Fe was added to every nutrient culture solution with two different Fe levels of concentration i.e. 0 (control) and 400 ppm in the form of $FeSO_4{\cdot}7H_2O$. The experiment was arranged as complete randomized design with three replications. Leaf bronzing score at the 10^{th} DAS (LBS10), maximum root length, shoot height, root- and shoot elongation rate, relative growth rate, iron plaque content, and shoot iron content were measured in this experiment.

The LBS10 were determined at 2^{nd}, 3^{rd}, and 4^{th} leaves (Shimizu et al., 2005) with scoring index scale from 1

(no bronzing symptom on the leaves) to 10 (the plant died) according to IRRI (2013).
Root- and shoot elongation rates (RER and SER) were measured on two plants in each pot before and after 10 days of treatment. The dry biomass of root and shoot of both stressed and control plants were determined after being dried at 80 °C for 72 hours. The dry biomass of root and shoot used for relative growth rate (RGR) determination.
Fe content of root plaque and shoot tissues were determined according to Nugraha et al. (2015) and Engel et al. (2012) at 10th DAS respectively. Fe content was analyzed using atomic absorption spectrometry (AAS) (Agilent 200 Series AA Systems, Agilent Technologies, Inc, USA).

Evaluation of physiological responses between tolerance-type to iron toxicity
This experiment aimed to analyze the difference of physiological responses between tolerance-type to iron toxicity. The condition of the experiment was similar to the previous experiment as described above. The experiment was arranged as a complete randomized design with three replications. Three rice genotypes were used in this experiment, IR64, Inpara 2, and Pokkali. The Fe was added by two different Fe concentrations, 0 (control) and 400 ppm in HSYA solution.
Total chlorophyll (Chl) and carotenoid extraction were determined according to Quinet et al. (2012) and were calculated according to Lichtenthaler (1987). The lipid peroxidation level was detected using malondialdehyde (MDA) quantification in the root and leaf organs. MDA extraction was carried out according to Muller et al. (2015) with a small modification. The MDA concentration was calculated according to Wang et al. (2013).

Statistical analysis
The collected data were analyzed by Duncan's Multiple Range Test (α=5%) for comparison among means of morpho-physiological data using SPSS version 16. The average data of morpho-physiological data were subjected by correlation analysis using SPSS version 16 and principal component analysis (PCA) and clustering analysis using PAST version 3.06 (Hammer et al., 2001). The unstandardized squared Euclidean distance and the unweighted pair group arithmatic averaging (UPGMA) were imputed in a cluster analysis to classify the genotypes based on their tolerance type to iron toxicity according to Annicchiarico et al., (2008).

Results

Ten rice genotypes shows different reaction of morpho-physiological under iron toxicity
Iron toxicity reduced both shoot and root growth of 2-week-old rice seedlings in both Fe-tolerant and -sensitive genotypes. Shoot height of Fe-tolerant genotypes was reduced between 16.0-22.7 %, while the shoot height of Fe-sensitive genotypes was reduced between 22.5-22.7 % (Table 2). Shoot elongation rate was also reduced in both Fe-tolerant and -sensitive genotypes with reduction ranges of 41.8-61.6 % and 80.6-87.3 %, respectively (Table 2). Iron toxicity also decreased shoot relative growth rate (SRGR) between 9.5-25.3 % in all genotypes (Table 2).
Iron toxicity inhibited the root growth, which the highest growth inhibition was shown by Inpara 5, and the lowest one was shown by Inpara 6. The percentage of maximum root length was reduced between 12.7-26.5 % (Table 2). Root elongation rate was also inhibited between 72.2-75.9 % and 32.8-54.4 % in sensitive tolerant genotypes, respectively (Table 2). Root relative growth rate (RRGR) of 10 rice genotypes were decreased significantly (23.8-61.3 %) in both sensitive and tolerant genotypes under iron toxicity (Table 2).
When the rice plants were exposed to Fe treatment, almost all the plants showed bronzing symptom in the leaf blades. There was a variation in bronzing symptom among the genotypes. LBS10 (leaf bronzing score at 10th DAS) showed that score in sensitive genotypes was higher than that of the tolerant genotypes. LBS10 data showed that the highest bronzing score occurred in Inpara 5 and IR64 (10), while the lowest score was in Mahsuri (3.7) (Figure 1).
Fe content in shoot tissue and root plaque using atomic absorption spectrometry (AAS) showed that in shoot tissue ranged 8.8-11.1 mg.g^{-1} DW (Table 3). The Fe content in shoot tissue of sensitive genotypes was higher than that in Fe-tolerant genotypes. AAS data in this research also showed that the Fe content in root plaque was varied among genotypes, but Fe content in root plaque of tolerant genotypes was not always higher than that of sensitive genotypes. Fe content in root plaque of tolerant genotypes was 3.6-5.3 mg.g^{-1} DW, while in sensitive genotypes it was 4.9-5.1 mg.g^{-1} DW (Table 3). This study also showed the variation

of Fe content in both shoot tissues and root plaques (Table 3). Average Fe content in the shoot of sensitive genotypes (10.2 mg.g^{-1} DW) was higher than that of tolerant genotype (9.9 mg.g^{-1} DW) (Table 3). The highest Fe content in shoot is showed by Hawara Bunar (11.1 mg.g^{-1} DW) and the lowest one is showed by Inpara 2, (8.8 mg.g^{-1} DW).

Physiological responses between rice genotypes and tolerance type to iron toxicity

Iron toxicity caused a significant decrease in total chlorophyll content in leaves of all genotypes in comparison with control plants, which decreased between 27.3-34.4 % (Table 4). In addition, total carotenoid content in leaves of three genotypes were also significantly reduced under iron toxicity between 23.5-28.6 % (Table 4). There was no particular pattern among genotypes regarding chlorophyll and carotenoid content.

Malondialdehyde data indicated that lipid peroxidation occurred both in roots and leaves after being exposed to iron toxicity for 10 days. MDA content significantly increased in leaves of IR64, Inpara 2, and Pokkali in comparison with the control plants (Table 4). The increase of leaves MDA content in sensitive genotype (IR64) was higher than that of both tolerant genotypes (Inpara 2 and Pokkali). However, MDA content did not always significantly increase in roots of those genotypes in response to iron toxicity. The increase of MDA content in roots of both tolerant genotypes (Inpara 2 and Pokkali) did not significantly increase while MDA content in IR64 roots showed significant increased under iron toxicity (Table 4).

The relationship among morpho-physiological characters in rice were demonstrated using Pearson's correlation. A significant positive correlation was found between MDA content in leaves and LBS10, while significant negative correlations were found between MDA content in roots, shoot and root elongation rate to LBS10 (Table 5).

Clustering of tolerance in ten genotypes based on morpho-physiological characters

To know the variation among morpho-physiological characters on ten rice genotypes, the PCA was carried out. In this study, out of total nine principal components (PC), two PC were have Eigen value >1 and it contributed 78.8% of total variation among the rice genotypes observed for nine morpho-physiological characters (Table 6). The PC1 has highest contributed to the variation (59.9%) followed by PC2 (18.9%). PC1 was positively and strongly associated with LBS10, shoot height, shoot extention rate, root length, and root extention rate. Shoot relative growth rate, Fe content in shoot tissues, and Fe content in root plaques have most important contribution in PC2 (Table 6). PCA result showed clear differentiation between sensitive- and tolerant genotypes (Figure 2).

The cluster analysis demonstrated using UPGMA method to classify ten rice genotypes based on their morpho-physiological characters to iron toxicity. Using nine morpho-physiological characters, the classification results showed that cluster 1 comprised of 2 genotypes, cluster 2 comprised of 6 genotypes, and cluster 3 comprised of 2 genotypes (Figure 3). The genotypes in cluster 1 have high inhibition on root relative growth rate, high Fe in shoot and root plaque. The genotypes in cluster 2 have low inhibition on root relative growth rate. The genotypes in cluster 3 have high inhibition in all morpho-physiological characters observed in this study. This study also demonstrated that three belong to the total clorophyll, carotenoid, and malondialdehyde (MDA) content of the rice leaves of the representative genotypes were analyzed in this study. IR64, Inpara 2, and Pokkali were the representative genotypes of Cluster 1, Cluster 2, and Cluster 3, respectively.

Discussion

Varied response demonstrated in the tolerant genotypes according their tolerance level to iron toxicity (Table 2). Iron toxicity in previous studies reduced shoot height between 38-62% under 300 ppm iron stress for 4 weeks (Suryadi, 2012). We noted in this study that the root elongation rate and maximum root length inhibited more than shoot elongation rate and shoot height. The data showed that the root relative growth rate inhibited more than the shoot, and it was consistent between Fe-sensitive and -tolerant genotypes (Table 2). According to Li et al. (2016) iron toxicity decreases both root cell elongation and division and inhibits lateral root initiation process because of direct contact between root tips and Fe.

The variation of Fe content in both shoot tissues and root plaques also showed in this study (Table 3). The small amount of Fe content in shoot tissues of tolerant genotypes such Inpara2 is an indication of the Fe exclusion strategy through oxidation of Fe in the rhizosphere, which is an efficient strategy of Fe

tolerance mechanism (Kang et al., 2013). In contrast to the Fe in the shoot, we also observed that the Fe content in root plaques showed inconsistent pattern between Fe-sensitive and -tolerant genotypes (Table 3). Several tolerant rice genotypes include Mahsuri, Indragiri, Inpara 6, Inpara 2, and IRH108, showed less Fe content in root plaques than that of sensitive genotypes (Inpara 5 and IR64), while the other three tolerant genotypes (Hawara Bunar, Pokkali, and Danau Gaung) showed Fe content in root plaques higher than that of sensitive genotypes. This research noted that the Hawara bunar has the highest capability to accumulate Fe in root plaques, while Indragiri has the lowest one.

The tolerant genotypes to iron toxicity have the ability to oxidize Fe^{2+} on the surface of the roots. Plants with high ability to develop aerenchyme cells are able to receive more O^2 in the roots area. However, highly reduced Fe in roots area will react with O^2, which leads to the Fe plaque formation on the root surface (Harahap et al., 2014). Based on Fe content in root plaques and leaf bronzing score, Pokkali and Hawara Bunar have a strategy to tolerate iron toxicity through Fe exclusion, which is in agreement with the previous research that suggests Pokkali is an excluder type (Engel et al., 2012). In addition, this research also showed that Pokkali and Hawara Bunar genotypes have inclusion strategy based on their Fe content in shoots (Table 3). We suggested Pokkali and Hawara Bunar have double mechanisms to tolerate iron toxicity, which involved both exclusion and inclusion strategies. Pokkali and Hawara Bunar indicated the best performance in tolerate to iron excess condition compared with other tolerance genotypes. Both genotypes have good strategy to accumulate Fe in the shoot and root plaque and their growth performance was not affected significantly by the iron stress condition.

The PCA and cluster analysis were performed for grouping ten rice genotypes based on their morpho-physiological responses to iron toxicity. Based on both analyzes, the sensitive genotypes showed clearly separate from the tolerant genotypes (Figure 2 and 3). There are two sub-group in tolerant genotypes, which is illustrated by Cluster 1 and 2. Both clusters were differentiated by root relative growth rate (RRGR) character. The genotypes in Cluster 1 have low root growth activity, while the genotype in Cluster 2 have high growth activity (Table 2; Figure 3).

Cluster analysis grouped ten rice genotypes into 3 group tolerance-type. Cluster 1 was categorized as tolerance group, Cluster 2 was categorized as moderate tolerance group, and Cluster 3 grouped sensitive genotypes. Several previous classifications of tolerance-type was carried out by Engel et al., (2012), Harahap et al., (2014), Nugraha et al., (2016b) rice, but they grouped the rice genotypes only based on the LBS and iron fate in the tissues. In this study, we classify tolerance-type to iron toxicity in rice based on their morpho-physiological characters. In this research, we showed Mahsuri, IRH108, Danau Gaung, Indragiri, Inpara 2, and Inpara 6 categorized as moderate tolerance group (Figure 3). This group is separated from the other two groups based only on root relative growth rate.

Interestingly, LBS10 did not show correlation with Fe content in the roots and shoot (Table 5). This corresponds to the reports of Becker and Asch (2005) and de Dorlodot et al. (2005) who demonstrated LBS did not always correlate with the tolerance level of rice. Based on our findings, rice genotype selection under Fe excess condition could be based on not only LBS but also based on root relative growth rate character (Table 2).

To know the physiological responses among tolerance-type of rice genotypes to iron toxicity, the total chlorophyll, carotenoid, and MDA content were analyzed in the representative genotypes of sensitive (IR64), moderate (Inpara2), and tolerant (Pokkali) to iron toxicity. Decrease in the chlorophyll content under iron toxicity suggested with the decrease of photosynthesis rate and chlorophyll biosynthesis (Quinet et al., 2012).

The study also showed different pattern of lipid peroxidation between roots and shoots under iron toxicity based on MDA content (Table 4). IR64 has the highest increase in MDA content under iron toxicity indicated that IR64 experienced highest stress than that of the other two genotypes. In this present study, Inpara 2 and Pokkali had differ MDA content. Those two genotypes suggested had differ in their tolerance-type to iron toxicity. Inpara 2 classified as the tolerant-includer type, meanwhile Pokkali as the tolerant-excluder type (Engel et al., 2012; Nugraha et al., 2016b). The MDA content indicates the level of lipid peroxidation of the cell membranes caused by ROS and oxidative stress (Polit, 2007). Iron toxicity could produce ROS in many organelles and parts of the cell, such as chloroplast, endoplasmic reticulum, peroxisomes, mitochondria, plasma membrane, cell wall, and apoplast (Sharma et al., 2012). ROS molecules also damage essential molecules inside the

cell, i.e.: DNA, RNA, and protein (Mittler, 2016). The tolerant genotypes have some strategies to tolerate iron toxicity through increasing oxidation power in the rhizosphere area, ROS detoxification, and compartmentation in the apoplasm, chloroplas and vacuole (Onaga et al., 2016).

ROS detoxification involves both enzymatic and non-enzymatic mechanisms. Inpara 2 and Pokkali have significantly decrease the carotenoid content compared to the control plant (Table 4), but the Pokkali has a small decrease in carotenoid content compared to IR64, which is a sensitive genotype to iron toxicity. Carotenoids will protect the chloroplasts as photosynthetic organelles by inhibiting the formation of triplet chlorophyll (3Chl*) and excited chlorophyll (Chl*) to prevent the production of $^1O_2$ (Sharma et al., 2012; Puthur, 2016). According to Kabir et al., (2016) increased catalase enzyme activity (CAT), peroxidase (POD), glutathione reductase (GR), and superoxide dismutase (SOD) as well as increased ascorbic acid content, glutathione and amino acids (cysteine, methionine, proline) in the Pokkali are suggested to be strong antioxidant defenses of tolerant plant when iron toxicity occurs. The study demonstrated total chlorophyll and carotenoid content did not significant correlation with MDA content in the shoots. However, chlorophyll content had significant correlation with Fe content in root plaque, while the carotenoid content did not significant correlation with Fe content in root plaque. Based on our findings it was suggested that leaf chlorophyll and carotenoid content did not directly relate with tolerance strategy to iron toxicity. The decrease in total chlorophyll suggested as an impact of stress condition in leaves, which is demonstrated with the high MDA content. On the whole results of our study suggested that the morpho-physiological characters could be used to predict the tolerance level to iron toxicity in rice. This study supports the genetic improvement strategies in rice to obtain rice genotype tolerant to iron toxicity. Further analysis on basic genetic, molecular, and physiological processes underlying the tolerance mechanism of rice to iron toxicity is still required to be investigated.

Table 1: The tolerance level of ten rice genotypes in this present study

Geno types	Tolerance level to iron toxicity	Reference
Inpara 5	Sensitive	Nugraha and Rumanti (2017)
IR64	Sensitive	Nugraha and Rumanti (2017)
Mahsuri	Tolerant	Nugraha and Rumanti (2017)
Pokkali	Tolerant	Engel et al., (2012)
Inpara 2	Tolerant	Suprihatno et al., (2010)
Inpara 6	Tolerant	Suprihatno et al., (2010)
Danau Gaung	Tolerant	Suprihatno et al., (2010)
Indragiri	Tolerant	Suprihatno et al., (2010)
Hawara Bunar	Tolerant	Amnal (2009)
IRH108	Tolerant	Kolaka (2016)

Table 2. Shoot and root growth of 10 rice genotypes after exposed to 400 ppm Fe for 10 days.

Genotypes	Percentage of growth decreasing (%)					
	SH	SER	RRGR	RL	RER	RRGR
Inpara 5	22.5^{bc}	80.6^{c}	18.5^{bc}	26.5^{d}	72.2^{d}	50.9^{c-e}
IR64	22.7^{c}	87.3^{c}	25.3^{c}	25.1^{d}	75.9^{d}	61.3^{e}
Hawara Bunar	18.8^{a-c}	44.2^{ab}	9.5^{a}	17.2^{bc}	40.3^{ab}	51.6^{c-e}
Pokkali	16.2^{a}	61.6^{b}	15.4^{ab}	16.4^{a-c}	35.9^{ab}	57.6^{de}
Mahsuri	16.0^{a}	53.8^{ab}	14.5^{ab}	17.1^{bc}	42.8^{ab}	23.8^{a}
Danau Gaung	17.9^{a-c}	47.0^{ab}	14.1^{ab}	14.5^{ab}	37.7^{ab}	36.8^{a-c}
Indragiri	16.2^{a}	41.8^{a}	18.5^{bc}	18.7^{bc}	44.1^{a-c}	45.0^{b-d}
Inpara 6	19.1^{a-c}	49.6^{ab}	16.4^{b}	12.7^{a}	32.8^{a}	34.7^{ab}
Inpara 2	17.3^{a-c}	43.8^{ab}	16.8^{b}	18.6^{bc}	44.5^{bc}	36.1^{a-c}
IRH108	17.2^{ab}	59.9^{ab}	13.8^{ab}	19.3^{c}	54.4^{c}	28.9^{a}

$^{a-e}$Different letters in the same column show significant differences ($p<0.05$) according to Duncan's Multiple Range Test. LBS10=Leaf bronzing score at 10th DAS; SH=Shoot height; SER=Shoot extension rate; SRGR=Shoot relative growth rate; RL=Root length; RER=Root extension rate; RRGR=Root relative growth rate.

Table 3: Fe concentration in shoot tissues and root plaques of 10 genotypes under iron toxicity. Data were taken on 10th day after stress.

Genotypes	Fe content in shoot tissues (mg.g^{-1} DW)	Fe content in root plaques (mg.g^{-1} DW)
Inpara 5	10.4^{a}	5.1^{de}
IR64	10.0^{a}	4.9^{de}
Hawara Bunar	11.1^{a}	5.3^{e}
Pokkali	9.9^{a}	5.0^{de}
Mahsuri	9.5^{a}	4.3^{c}
Danau Gaung	9.4^{a}	5.1^{de}
Indragiri	9.7^{a}	3.6^{a}
Inpara 6	10.9^{a}	4.1^{bc}
Inpara 2	8.8^{a}	3.8^{ab}
IRH108	9.6^{a}	4.7^{d}

$^{a-d}$Different letters in the same column show significant differences ($p<0.05$) according to Duncan's Multiple Range Test.

Table 4: Physiological responses of IR64, Inpara 2, and Pokkali were grown under control and iron toxicity hydroponic conditions.

Characters	Genotypes					
	IR64		Inpara 2		Pokkali	
	C	++Fe	C	++Fe	C	++Fe
Total chlorophyll content (mg.g^{-1} FW)	306.5^{c}	215.2^{ab}	347.2^{d}	185.2^{a}	340.8^{cd}	246.1^{b}
Carotenoid content (mg.g^{-1} FW)	84.9^{c}	62.9^{ab}	95.4^{c}	55.3^{a}	92.2^{c}	70.8^{b}
MDA content in leaves (µmol.g^{-1} FW)	0.2^{a}	1.8^{c}	0.2^{a}	1.5^{ab}	0.2^{a}	1.3^{b}
MDA content in roots (µmol.g^{-1} FW)	0.3^{b}	0.7^{c}	0.1^{a}	0.1^{b}	0.2^{ab}	0.2^{ab}

$^{a-d}$Different letters in the same row show significant differences ($p<0.05$) according to Duncan's Multiple Range Test. Control=0 ppm $FeSO_4 \cdot 7H_2O$; ++Fe= 400 ppm $FeSO_4 \cdot 7H_2O$.

Table 5. Correlation analysis among morpho-physiological characters under iron toxicity of ten rice genotypes

	LBS10	SRGR	RRGR	FeS	FeR	CHL	CAR	SMDA	RMDA	SER	RER
LBS10	1.000	-0.515	-0.377	0.123	0.195	-0.249	-0.207	0.716*	0.857**	-0.816**	-0.876**
SRGR		1.000	0.816**	-0.226	-0.734*	-0.231	-0.221	-0.250	-0.598	0.768*	0.382
RRGR			1.000	-0.111	-0.813**	-0.495	-0.511	-0.219	-0.381	0.526	0.155
FeS				1.000	0.329	0.083	-0.240	0.189	0.531	-0.231	-0.222
FeR					1.000	0.768*	0.671*	-0.026	0.379	-0.477	-0.137
CHL						1.000	0.942**	-0.514	-0.035	-0.051	0.247
CAR							1.000	-0.518	-0.137	-0.054	0.258
SMDA								1.000	0.502	-0.393	-0.687*
RMDA									1.000	0.285	0.717*
SER										1.000	0.668*
RER											1.000

**=Correlation is significant at the 0.01 level (2-tailed); *=Correlation is significant at the 0.05 level (2-tailed). . LBS10=Leaf bronzing score at 10th day after stress; SRGR=Shoot relative growth rate; RRGR=Root relative growth rate; FeS=Fe content in shoot tissues; FeR=Fe content in root plaques; CHL=Total chlorophyll content; CAR=Carotenoid content; SMDA=MDA content in leaves; RMDA=MDA content in roots; SER=Shoot elongation rate; RER=Root elongation rate.

Table 6: Loading score of two principal components, Eigen value, % variance and % cumulative variance on inhibition percentage (%) of morpho-physiological characters of genotypes evaluated in this study

Characters	Loading score of principal component	
	PC1	PC2
LBS10	0.42	-0.06
Shoot height	0.38	0.16
Shoot elongation rate	0.39	-0.01
Shoot relative growth rate	0.31	-0.41
Root length	0.38	-0.17
Root elongation rate	0.40	-0.17
Root relative growth rate	0.28	0.27
Fe content in shoot tissues	0.10	0.61
Fe content in root plaques	0.17	0.55
Eigen value observed	5.39	1.70
Proportion of total variance (%)	59.9	18.9
Cumulative proportion of total variance (%)	59.9	78.8

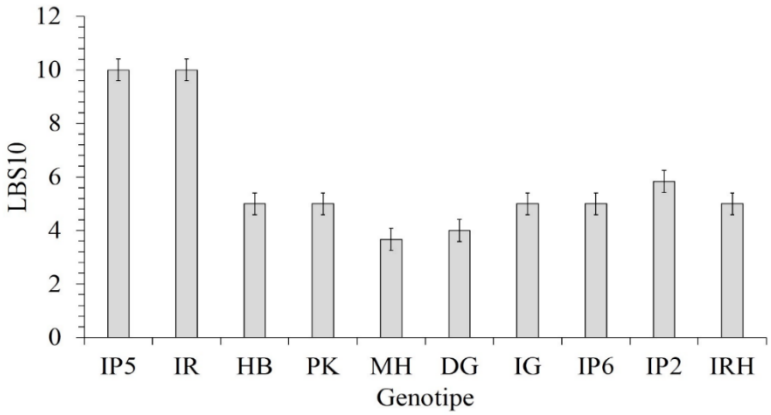

Figure 1: Leaf bronzing score at 10th DAS (LBS10) of ten rice genotypes under iron toxicity. Data were taken on 10th DAS. IP5=Inpara 5; IR=IR64; HB=Hawara Bunar; PK=Pokkali; MH=Mahsuri; DG=Danau Gaung; IG=Indragiri; IP6=Inpara 6; IP2=Inpara 2; IRH= IRH108. Bars indicate the standard error.

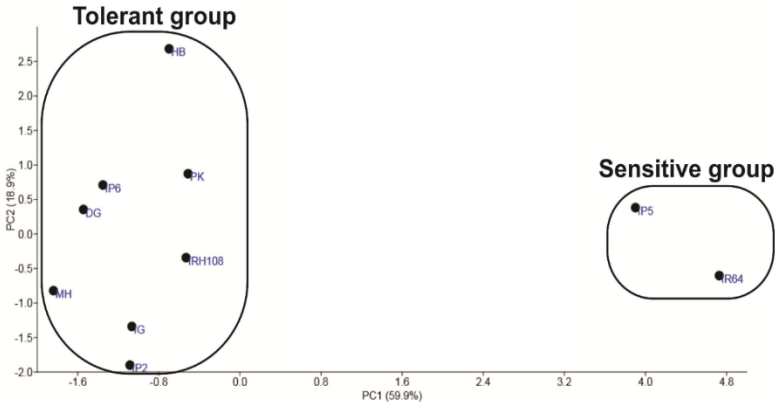

Figure 2: Principal component analysis (PCA) of 10 rice genotypes under iron toxicity. IP5=Inpara 5; IR=IR64; HB=Hawara Bunar; PK=Pokkali; MH=Mahsuri; DG=Danau Gaung; IG=Indragiri; IP6=Inpara 6; IP2=Inpara 2; IRH= IRH108.

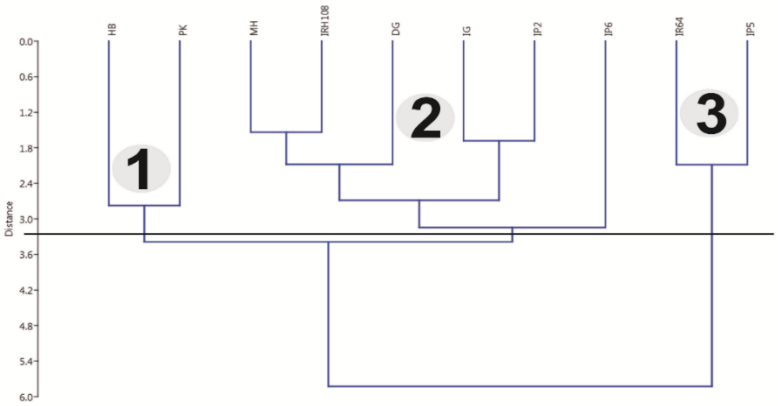

Figure 3: Cluster analysis of ten rice genotypes under iron toxicity. IP5=Inpara 5; IR=IR64; HB=Hawara Bunar; PK=Pokkali; MH=Mahsuri; DG=Danau Gaung; IG=Indragiri; IP6=Inpara 6; IP2=Inpara 2; IRH= IRH108.

Conclusion

The present study concluded that iron toxicity significantly affect morphological and physiological characters on rice genotypes. PCA and cluster analysis using those characters demonstrated there are three groups in related to iron toxicity tolerance level. The sensitive group consisted of IR64 and Inpara 5. The moderate group consisted of Mahsuri, IRH108, Danau Gaung, Indragiri, Inpara 2, and Inpara 6. The tolerant group consisted of Hawara Bunar and Pokkali. In addition, iron toxicity decreased chlorophyll and carotenoid concentration and increased the lipid peroxidation level in both the root and shoot. Rice var. Pokkali and Hawara Bunar showed the best growth performance under the iron toxicity condition in comparison with other genotypes. This valuable finding will be useful to improve iron toxicity tolerance in rice through selection strategy in breeding programs.

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