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Original article

Determination of Some Paddy Varieties Resistant to Iron Toxicity

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Abstract

The aim of this study is to determine some paddy varieties resistant to iron toxicity. Two different nutrient solutions were applied in the form of iron sulphate (FeSO4.7H2O) (Fe concentrations of I) 45 µM Fe (sufficient Fe), II) 3.50 mM Fe (toxic Fe) to paddy cultivars grown in sand media. Among the paddy cultivars grown at toxic iron level (3.50 mM Fe), the closest paddy cultivars in terms of investigated traits were identified as Hamzadere and Edirne cultivars, while the furthest cultivars were identified as Biga incisi and Ronaldo cultivars. Present findings revealed that Biga incisi and Edirne paddy cultivars were tolerant to toxic iron levels and Ronaldo paddy cultivar was the most susceptible to iron toxicity. Biga incisi and Edirne paddy cultivars formed a group and the best traits of these cultivars designating iron toxicity were identified as iron ratio transported to shoot, tolerance index to toxic iron level, shoot total iron content and leaf relative peroxidase activity. According to biplot analysis, Ronaldo paddy cultivar formed a different group and the best traits of this cultivar at toxic iron level were identified as iron ratio remained in roots and root cold-extractable Fe/Zn ratio.

Keywords: Paddy cultivar, Sensitive and resistant to iron toxicity, Iron toxicity traits

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INTRODUCTION

Rice, grown under inundated conditions, uptakes excessive quantities of Fe^{+2} ion. Up taken iron then transported to other plant organs through transpiration. Free Fe^{+2} ions react with hydrogen peroxide (H_2O_2) produced in cell components including chloroplasts and mitochondria (Becana et al., 1998). Hydroxyl radicals are highly reactive and are not able to be removed by plant antioxidants (Apel and Hirt, 2004). Reactive radicals generate irreversible damages on lipids, proteins and nucleotides. Such damages ultimately result in cellular deaths (Quinet et al., 2012). Photosynthetic activity of paddy plants recesses due to stomal and non-stomal limitations under toxic iron conditions (Pereira et al., 2013). As related to increasing phenol oxidase activity and accumulation especially of oxidized polyphenols, typically “bronzing” is encountered in paddy leas as visual iron toxicity symptom. Bronzing symptoms initiate with the emergence of small brown spots spreading from tip to the base of fully developed old leaves. In further stage of the symptoms, leaf tips get orange-yellow color and leaves may dry out in paddy cultivars. In severe toxicity cases, entire leaf turns from brown into rusty brown or purple-brown (Fairhurst and Witt, 2002). These symptoms are especially encountered in old leaves with high transpiration rates (Yamanouchi and Yoshida, 1981).

Iron toxicity symptoms may emerge in different growth stages of the plant. Iron toxicity may influence paddy plants in vegetative development stage, early and late generative stages. Bronzing takes place in plant leaves based on growth stages. Toxicity emerged in seedling stage significantly shortens tillers, then plant gets a dwarf posture (Abraham and Pandey, 1989). Iron toxicity encountered in vegetative development stage results in reduced plant height and dry matter accumulation (Abu et al., 1989). Shoots are more influenced from iron toxicity than the root biomass (Fageria, 1988). In case of iron toxicity in late vegetative or early vegetative stages, less number of combined panicles are formed on plant top head (Singh et al., 1992) and an increase is also seen in spikelet sterility (Virmani, 1977). Besides, 20-25 days delay may be encountered in flowering and ripening. There may be no flowering at all in highly sensitive paddy cultivars (Ayotade, 1979). Typical symptoms related to Fe-toxicity may emerge as “bronzing” in paddy leaves (Ponnamperuma et al., 1955). Excessive soil iron levels may generate damages on plant root system (Figure 1). Additionally, excessive iron levels may negatively influence uptake of the other nutrients like phosphorus, zinc and copper (De Dorlodot et al., 2005). Depending on paddy cultivar, soil iron concentration and paddy growth stages, Fe toxicity induced yield losses were reported as between 12–100 % (Audebert and Fofana, 2009).

Figure 1. Paddy leaves and roots influenced by Fe toxicity (Wu, 2016) [(A) Different bronzing symptoms, healthy (top), moderate bronzing (mid) and high level bronzing (bottom) on paddy leaves; paddy roots grown in (B) non-toxic (top), moderately toxic (mid) and severely toxic (bottom) soils]

The factors inducing iron toxicity include; release of iron from parent material into soil solution, reduction in oxidation reduction potential, increase in ionic strenght, low soil fertility, low soil pH, soil organic matter content, microbial activities, interactions of iron with the other elements and plant genetic diversity (Fageria et al., 2008).

Paddy plants grown in iron-toxic soils develop tolerance mechanisms against excessive iron quantities through morphological and physiological avoidance means to sustain vital functions. These mechanisms play an important role in selection of tolerant and adaptive paddy genotypes. Various adaptation strategies could be distinguished including “Inclusion” and “exclusion”, as well as “avoidance” and “tolerance” mechanisms. Strategy-I plants keep Fe^{+2} out at plant root level and in this way plant shoot tissue is not damaged. Strategy-II plants take Fe^{+2} into the roots, but plant tissue damage is prevented through compartmentation (immobilization of active iron in discharge sites, for instance old leaves or photosynthetically less active leaves) or exclusion from symplast (immobilization in leaf apoplast) (Becker and Asch, 2005).

Efficient measures to prevent Fe^{+2} toxicity in plants include; periodical surface drainage for oxidization of reduced Fe^{+2}, lime treatments on acidic soils, sufficient and balanced use of fertilizers and selection of iron toxicity-tolerant plant genotypes. Iron extraction capability of paddy plants decrease in P, K, Ca and Mg deficiencies (Obata, 1995). Especially K deficiency easily induce Fe toxicity (Fageria et al., 2003a). Among these management practices, use of tolerant cultivars is the most economic and environment-friendly practice (Fageria et al., 2008).

The aim of this study is to determine some paddy varieties resistant to iron toxicity.

MATERIALS AND METHODS

Paddy cultivars grown in sand media under greenhouse conditions were supplied from Black Sea Agricultural Research Institute. These cultivars were: Biga incisi, Osmancık-97, Hamzadere, Ronaldo and Edirne paddy cultivars.

Experiment

Seed sterilization was achieved through keeping paddy seeds in 5.0 % (v/v) sodium hypochlorite solution for 15 minutes. Seeds were then washed through deionized water and germinated in moist cloth bags. Germinated seeds were transferred to perlite-filled containers (40 x 25 x 5 cm dimensions) and grown there for 10 days to get paddy seedlings. Paddy seedlings were transplanted into plastic pots (12 x 12 cm) filled with 1 kg of quartz sand as to have 10 seedlings in each pot.

Two different iron treatments were applied to paddy cultivars as of: in the form of iron sulphate ($FeSO_4 \cdot 7H_2O$); I) 45 µM Fe (sufficient Fe), II) 3.50 mM Fe (toxic Fe). Nutrient solutions were applied to 5 different paddy cultivars at equal quantities as to have 3 cm water head over the sand in experimental pots. Nutrient solution pH was adjusted at 5.5 with the use of dilute HCl or KOH solution. Experiment lasted for 50 days. As specified by Zhang et al. (1998), following non-iron containing nutrient solutions containing essential nutrients were used:

500 µM NH_4NO_3; 60 µM $NH_4H_2PO_4$; 230 µM K_2SO_4; 210 µM $CaCl_2$; 160 µM $MgSO_4 \cdot 7H_2O$; 2.5 µM $MnCl_2$; 0.75 µM $(NH_4)_6Mo_7O_{24}$; 3.2 µM H_3BO_3; 0.1 µM $CuSO_4$; 2.0 µM $ZnSO_4 \cdot 7H_2O$

At the end of the experiments, roots and above-ground parts of paddy plants were separated and dried in an oven at 65 ºC. Then, dry root and shoot weights were determined.

Phenological observations were made and iron toxicity was visually assessed at 2-5 leaf stage of paddy plants in accordance with IRRI (2002). Portable SPAD meter device (Konica Minolta SPAD-502 Plus) was used to get SPAD readings from the middle of the leaves.

At the end of experiments, fresh roots of paddy plants were extracted with dithionite-citrate-bicarbonate (DSB) solution and iron and zinc concentrations of the resultant extract were measured with the use an atomic absorption spectrophotometer device. Following the performance of relevant calculations, cold-extractable iron and zinc concentrations of fresh roots were expressed in % (Taylor and Crowder, 1983). Fe/Zn ratio of fresh roots was also calculated.

Fresh leaf samples were taken from the paddy cultivars before the harvest and chlorophyll and carotenoid analyses were conducted on these samples (Arnon, 1949; Witham et al., 1971).

Active iron of dry leaf samples was determined with use of AAS device (Oserkowsky, 1933).

Root and shoot total iron and zinc contents were determined with the use of an atomic absorption spectrophotometer (AAS) device in accordance with Kacar and İnal (2008).

In fresh leaf samples, Ascorbate peroxidase (APX) activity was determined in accordance with the method specified in Amako et al., (1994); Catalase (CAT) activity with the method specified in Dhindsa et al. (1981b); Peroxidase (POD) activity with the method specified in Wakamatsu et al. (1993); Glutathione reductase (GR) activity with the method specified in Jiang et al. (2002). Proline content of fresh leaves was determined in accordance with the method specified in Bates et al. (1973).

The tolerance index for iron toxicity in paddy varieties was calculated as follows (Fageria et al., 2008):

Relative value (Toxicity tolerance index), % = (A / B) × 100

where;

A = Measurement values of the plants grown at toxic iron (3.50 mM Fe) concentration

B = Measurement values of the plants grown at sufficient iron (45 µM Fe) concentration

The iron ratio transported to shoot of paddy cultivars (%) was calculated with the use of the following equation:

Iron ratio transported to plant shoot (%) = (A / B) × 100

A = Plant shoot iron uptake, µg Fe / pot

B = Plant shoot + root iron uptake, µg Fe / pot

The remaining iron ratio in roots of paddy cultivars (%) was calculated with the use of the following equation:

Remaining iron ratio in plant root (%) = (A / B) × 100

A = Plant root iron uptake, µg Fe / pot

B = Plant shoot + root iron uptake, µg Fe / pot

Statistical Analyses

Cluster or similarity test was conducted to determine closeness and distance of 5 different paddy cultivars to each other in terms of iron toxicity traits. Cluster analysis was conducted in accordance with Ward Method with the use of JMP.5.0 statistical software. Biplot analysis was conducted for classification of paddy cultivars based on iron toxicity levels, variation of paddy cultivars in terms of these levels and to determine the best traits of each cultivar.

RESULTS and DISCUSSION

Comparison of Paddy Cultivars Grown under Iron Toxicity Conditions

The relative values for 15 traits investigated for grouping, closeness-distance status and the best traits of paddy cultivars grown at toxic iron level (3.50 mM Fe) are provided in Table 1.

Table 1. Relative values for 15 traits investigated for grouping, closeness-distance status and the best traits of paddy cultivars grown at toxic iron level (3.50 mM Fe)

Cultivars	I	II	III	IV	V	VI	VII	VIII	IX	X
Biga incisi	3	49.4	1553	5282	0.8	1.43	0.05	28.4	20.7	79.2
Osmancık-97	5	42.5	1598	5214	0.94	2.04	0.08	24.6	20.9	79.0
Hamzadere	7	39.3	1516	5297	1.09	1.55	0.07	22.2	20.5	79.4
Ronaldo	9	31.8	1610	5216	1.1	1.93	0.04	38.9	25.1	74.8
Edirne	7	39.9	1463	5063	1.14	1.69	0.06	27.9	21.8	78.2

Cultivars	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX
Biga incisi	1.36	82.5	76.9	80.8	105.0	88.8	101.6	136.1	85.4
Osmancık-97	0.97	65	80.7	60.2	73.9	144.7	86.5	207.5	88.8
Hamzadere	0.88	98.5	98.0	93.5	116.7	95.3	103.5	104.9	101.0
Ronaldo	0.75	115.7	117.3	66.3	101.1	101.9	125.8	170.2	101.3
Edirne	1.01	78.9	90.3	93.3	54.4	95.3	72.1	124.9	91.9

The information about the characteristics of the roman numbers in the table is given below.

Iron toxicity grade (I); Toxicity tolerance in shoot (II); Active iron in shoot (III); Total iron content in shoot (IV); Total iron content in root (V); Cold extractable iron in the root (VI); Cold extractable zinc in the root (VII); Cold extractable Fe / Zn ratio in the roots (VIII); The proportion of iron remaining in the root (IX); The ratio of iron carried to the shoot (X); High iron dose tolerance index (XI); Relative total chlorophyll [by sufficient level] (XII); Relative carotenoid [by sufficient level] (XIII); Relative Peroxidase (XIV); Relative Catalase (XV); Relative Glutathione reductase (XVI); Relative Ascorbate peroxidase (XVII); Relative Proline (XVIII); Relative SPAD (XIX)

[*] Relative value was calculated as % = [value determined at toxic iron (3.50 mM Fe) level / value determined at sufficient iron (45 µM Fe)] × 100.

The values indicating closeness and distance levels of paddy cultivars grown at toxic iron level (3.50 mM Fe) to each other are provided in Table 2.

Table 2. Closeness and distance levels of paddy cultivars grown at toxic iron level to each other

Level	Number of groups	Similarity level	Distance level	Linkage between the groups		New groups	Number of cultivars in each group
1	4	65.4002	21.6184	3	5	3	2
2	3	48.7380	32.0291	1	3	1	3
3	2	41.8127	36.3561	1	2	1	4
4	1	0.7760	61.9964	1	4	1	5

The dendrogram for grouping of paddy cultivars grown at toxic iron level (3.50 mM Fe) is presented in Figure 2.

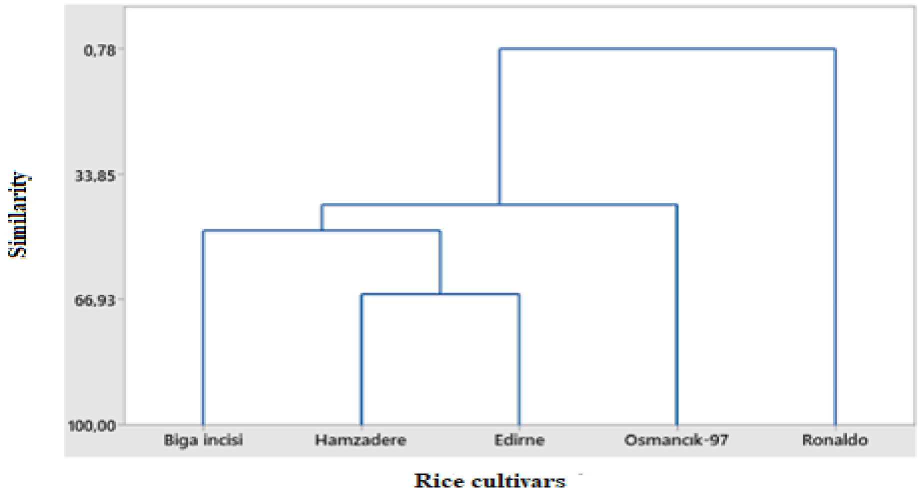

Figure 2. The dendrogram generated with the use of Ward method, explaining closeness and distance of paddy cultivars to each other based on the traits determined at toxic iron level

As can be inferred from Table 2 and Figure 2, 5 different paddy cultivars grown at toxic iron level (3.50 mM Fe) were assessed in terms of investigated 15 traits. According to these assessments, Hamzadere and Edirne cultivars constituted a group and these cultivars were found to be close to Biga incisi cultivar. Additionally, Biga incisi was also found to be close to Osmancık-97 cultivar in terms of investigated traits; on the other hand, Ronaldo cultivar was found to be closer to Osmancık-97 cultivar. The closest paddy cultivars grown at toxic iron level (3.50 mM Fe) in terms of investigated traits were identified as Hamzadere and Edirne cultivars (level of distance 21.61); on the other hand, the furthest cultivars were identified as Biga İncisi and Ronaldo cultivars (level of distance 61.99).

Classification of paddy cultivars grown at toxic iron level based on mean values of investigated 19 traits and variations of cultivars in terms of investigated traits are presented in Figure 3. According to Biplot analysis, two principal components explained 76,9 % of total variation (the first principal component, PC1 explaining 44.5 % and the second principal component, PC2 explaining 24.5 %). As can be inferred from Figure 3, investigated traits and distribution of cultivars varied with the cultivars. According to present analyses, Biga incisi and Edirne paddy cultivars grown at toxic iron level (3.50 mM Fe) constituted a group and the best traits for this group of cultivars were identified as ratio of iron transported to shoot, tolerance index to toxic iron level, shoot total iron content and relative peroxidase activity. The ratio of iron transported to shoot at toxic iron level was identified as 79.28 % in Biga incisi cultivar and 78.20 % in Edirne cultivar, these were considered to be high. Relative SPAD value was identified as the best trait of Osmancık-97 and Biga incisi cultivars grown in lime-supplemented sand media under zinc deficiency conditions. Tolerance index at toxic iron level was identified as 1.36 in Biga incisi cultivar and 1.01 in Edirne cultivar (Table 1). In other words, Biga incisi and Edirne cultivars were found to be tolerant to toxic iron level. Shoot total iron content at toxic iron level (3.50 mM Fe) was identified as 5282 mg kg^{-1} in Biga incisi cultivar and 5063 mg kg^{-1} in Edirne cultivar, these values were also considered to be high. Relative peroxidase activity at toxic iron level was identified as 80.83 % in Biga incisi and 93.33 % in Edirne cultivar (Table 1). These values indicated that Biga incisi and Edirne cultivars were tolerant to toxic iron level.

According to Biplot analysis, Hamzadere paddy cultivar constituted a different group and the best trait of this cultivar at toxic iron level was identified as relative peroxidase activity. Relative peroxidase activity of Hamzadere cultivar at toxic iron level was identified as 93.45 %. It could be stated that Hamzadere cultivar was tolerant to toxic iron level. Chukupee (2015) conducted a study for selection of paddy cultivars tolerant to iron toxicity in continuously inundated soils and grouped the paddy cultivars based on iron toxicity tolerance traits. Researchers identified CK801 and ARICA8 paddy cultivars as tolerant to iron toxicity. It was also indicated that these cultivars (CK801 and ARICA8) resistant to iron toxicity could be used in paddy breeding programs to be conducted to increase productions in paddy fields with iron toxicity.

According to Biplot analysis, Osmancık-97 paddy cultivar alone formed another group and the best trait of this cultivar at toxic iron level was identified as relative glutathione reductase activity. Relative glutathione reductase activity of Osmancık-97 cultivar as 144.63 %) (Table 1). It could be stated based on these findings that Osmancık-97 cultivar was tolerant to toxic iron level. Li et al. (2019) reported that S-nitrosoglutathione reductase increased root tolerance to Fe toxicity in plants by inhibiting iron-induced nitrosative and oxidative cytotoxicity via nitric oxide.

Biplot analysis revealed that Ronaldo paddy cultivar alone constituted a different group and the best traits of this cultivar at toxic iron level were identified as iron ratio remained in roots and cold-extractable Fe/Zn ratio. The iron ratio remained in roots was identified as 25.12 % and cold-extractable Fe/Zn ratio was identified as 38.93 % (Table 1). These values of Ronaldo cultivar were greater than the values of the other cultivars. High iron ratio remained in roots indicated that Ronaldo cultivar was tolerant to toxic iron level (3.50 mM Fe).

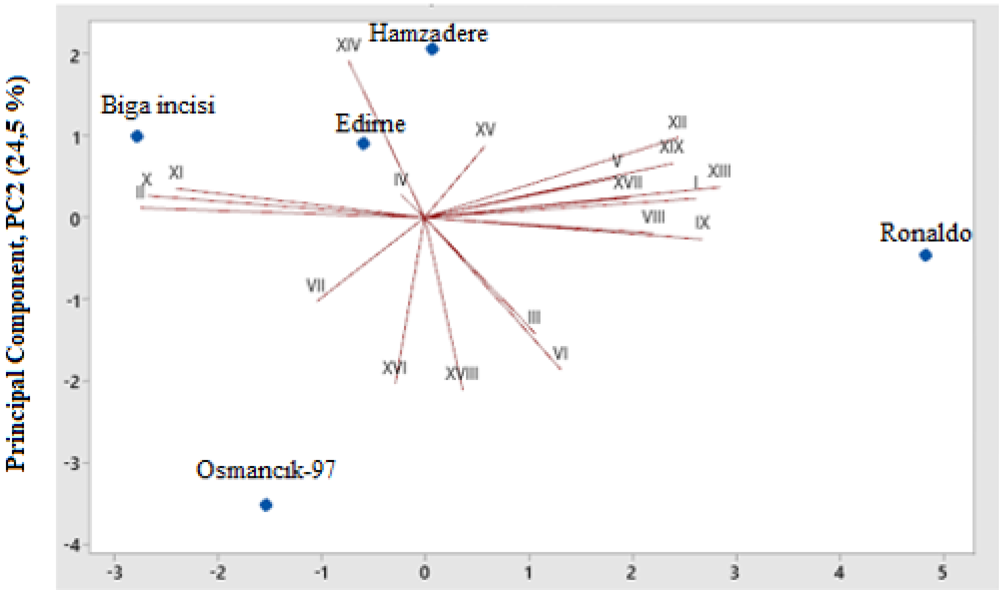

Figure 3. Biplot grouping of paddy cultivars at toxic iron level based on investigated traits and best traits of the cultivars

CONCLUSION

Among the paddy cultivars grown at toxic iron level (3.50 mM Fe), the closest paddy cultivars in terms of investigated traits were identified as Hamzadere and Edirne cultivars; on the other hand, the furthest cultivars were identified as Biga incisi and Ronaldo cultivars. Biga incisi and Edirne paddy cultivars were identified as tolerant to toxic iron level. On the other hand, Ronaldo paddy cultivar was identified as the least resistant to iron toxicity. Biga incisi and Edirne paddy cultivars constituted a group and the best traits of these cultivars designating iron toxicity were identified as iron ratio transported to shoots, tolerance index to toxic iron level, shoot total iron content and relative peroxidase activity. According to Biplot analysis, Ronaldo paddy cultivar formed a different group and the best traits at toxic iron level were identified as iron ratio remained in roots and root cold-extractable Fe/Zn ratio.

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