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# Novel Strategies for Confirmation of Extreme Lines for Ferrous toxicity in Reciprocal Introgression Lines of Rice

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**Abstract:**

*The flooded soils for longer period of time as well as low lands under rice cultivation are usually contaminated with ferrous toxicity. Therefore, this study had been conducted to record the effects of ferrous* ($Fe^{+2}$) *toxicity on the growth parameters of rice and ferrous content in their respective parameters as well. Two parents MH63 indica and 02428 japonica along with six reciprocal introgression lines of each highly tolerant and highly susceptible from both of the respective backgrounds to ferrous stress brought under study in green house conditions. Ferrous in the form of* $FeSO_4.7H_2O$ *at the rate of 300 ppm and control was consequently applied to the rice seedlings for 21 and 26 days in hydroponic culture. A decrease in all parameters of the treated seedlings was observed. The tolerant lines had more ferrous content than highly susceptible lines had lower weights and lengths than highly tolerant lines. However, the phenotypic performance of tolerant RI lines was better than highly susceptible RI lines. The leaf*

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*bronzing was also observed in treated plants. More interestingly to note that the roots of treated plants obtained brown color and the roots of untreated plants were found colorless. Therefore, it is suggested for rice plants that these may be brought under further study to know its complete mechanism for ferrous toxicity tolerance.*

**Key words:** Green house, Extreme lines, Ferrous toxicity, Reciprocal Introgression lines, Rice

## Introduction

Ferrous iron toxicity is one of the most important constraints affecting primarily in lowland rice grains on acid flooded soils that are rich in reducible iron (Sahrawat 2004). High concentration of ferrous iron in plant tissues is known to generate hydroxyl radicals, which may cause lipid per oxidation and consequently membrane injury, protein degradation, enzyme inactivation, pigment bleaching in disruption of DNA strands in chloroplasts (Bode et al. 1995). Under anaerobic conditions, the ferric iron is reduced into its ferrous form which is soluble in water and readily taken up by plants (Gross et al., 2003). High concentration of Fe (II) in the soil solution also causes imbalance of nutrient uptake by reducing considerably the absorption of the other nutrients especially P, K, Ca, Mg, Zn and Mn (Genon et al., 1994). Yield reduction due to iron toxicity generally ranges from 15 to 30%, but losses up to 100% can be observed, depending on the rice genotype, growing stage of the plant, soil fertility status and severity of the stress (WARDA 2002; Asch et al. , 2005; Becker and Asch, 2005).

Ferrous iron Fe (II) is produced in reductive soils and has been shown to be one of the important factors responsible for the suppression of seedling emergence and the establishment of rice (Hagiwara and Imura, 1993). When organic materials are applied ferrous iron in the soil solution can suppress the growth of the weeds and rice plants. Although rice is mainly transplanted in Japan, weed seeds are placed in or on flooded soils, this condition can be beneficial for rice under Fe (II) stress/toxicity, because, Fe (II) toxicity can selectively suppress weed emergence (Takuhito et al., 2009).

In order to minimize the effect of iron toxicity several resistance mechanisms developed by the plants have been well documented in the literature, i.e. oxidation of $Fe^{2+}$ in the rhizosphere to $Fe^{3+}$ using oxygen transported in the aerenchyma of the roots (Doran et al. , 2006). Different strategies can be applied to maintain a good productivity in rice fields submitted to iron stress. The improvement of soil, water and nutrient management practices may considerably reduce the impact of iron toxicity in the rice crop (Audebert (a) et al., 2000; Sahu et al., 2001). However, it remains very costly for low-income farmers in developing countries, which will be reluctant to adopt such practices. The other promising approach to improve the yield under iron toxicity conditions is the selection of more resistant rice cultivars (Neue et al. 1998; d Dorlodot et al. 2005; Audebert 2006). Keeping in view the importance of this element on the overall susceptibility of the plants, studies were conducted to find out the highly susceptible and highly tolerant Reciprocal Introgression Lines of rice under iron toxicity conditions which would be helpful in knowing the mechanisms involved and methodology used for finding out of ferrous toxicity tolerance in rice crop.

## Materials and methods

### Production of plant material

Two sets of introgression lines (ILs) were developed from a cross between MH63 an *indica* variety and 02428 a japonica variety. The F1 hybrids simultaneously back crossed two times with both parents to produce two BC2F1 populations. The two BC2F1 were allowed to self-produce for BC2F2:8 generation. Finally 2 sets of reciprocal introgression lines (RILs) were developed, consisting of 200 lines in the MH63 background and 200 lines in 02428 background. These lines were used for following genotypic and phenotypic study. Out of these 200 RILs, 6 from each highly tolerant and highly susceptible RILs were selected from earlier experiments based on their root and shoot weight as well as root and shoot length.

## Phenotypic evaluation of $Fe^{2+}$ toxicity tolerant-related traits

A series of experiments was conducted in the green house at the Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, China. The ICS provided all the materials and other facilities to conduct the experiments. The following methodology was applied.

The seeds were surface sterilized with 1% hypochlorite solution for 10 minutes and rinsed well with distilled water. Then seeds were soaked in distilled water in the dark at 30 $^{o}$C for 48 hours. The most uniform 10 emerged seeds from each RI line per replication were directly sown into perforated Styrofoam sheets covered with nylon net at the bottom. For each experimental condition (i.e. treated and untreated control) most uniform 10 emerged seeds from parents MH63 and 02428 were also sown in each container randomly (Ines et al., 2009). The Styrofoam sheets were allowed to float on water up to 7 days and then transferred to Yoshida culture solution (Yoshida et al. 1976) without applying suitable concentrations of $Fe^{2+}$ stresses for first 15 days. On the 16th day of seeding, at 3rd leaf stage, the ferrous in the form of $FeSO_4.7H_2O$ at the rate of 0 and 300 ppm was applied for 21 days and 26 days.

The pH of the solution on alternative day was adjusted to 5.0 with 1 *N* NaOH/HCl. The solution was renewed every fifth day. The experimental materials were laid out in two replications for all treatments (treated and untreated) under green house conditions at around 32/25 $^{o}$C in day/night, 70-75% of relative humidity and average 12 hours photoperiod. The largest root and shoot length were recorded after 21 days of the treatment. Then the samples were kept in oven for 72 hours (3days) at maximum 65$^o$C (Jian lin, et al., 2003). Finally, dry weight of root and shoot was recorded. On the basis of root dry weight, root length, shoot dry weight, and shoot length, six highly susceptible RILs and six highly tolerant RILs from both backgrounds MH63 indica and 02428 japonica were brought for further experimentation. The same above methodology was applied to confirm and reconfirm the extreme lines in RILs. Therefore, for confirmation and reconfirmation of extreme lines, the experiment was conducted for two different durations under stress conditions, one stress was applied for 21 days, and the other stress was applied for 26 days. Two replications for each ferrous stress and control under two durations were kept. The largest root and shoot length and fresh root and shoot weight were recorded after 21 days and 26 days of the treatment. Then the samples were kept in oven for 72 hours (3 days) at maximum 65 $^o$C (Jian lin, et al., 2003). Dry weight of root and shoot was recorded to get the standard value for concentration of ferrous per line.

## Tissue content analysis for ferrous concentration in extreme RI line populations of MH63 *indica* and 02428 japonica backgrounds (21 and 26 days after stress)

For knowing the concentration of $Fe^{2+}$ from each extreme lines under all conditions ($Fe^{2+}$ stress and control) the maximum 2.0 gms of oven dried roots and shoots samples were taken in glass tubes, each glass tube had a single sample. An acid solution of nitric acid ($HNO_3$) 80 % and per chloric acid (HCL) 20 % at the rate of 3.75 ml/tube was put in it. Then these tubes were kept in hot plate at the maximum temperature of 146 $^o$C for 90 minutes for digestion/dissolution. In order to exhaust the acid molecules from inside to outside, the hot plate was kept in fume-hood by turning on fume-hood. After the complete digestion of samples, these digested solutions were kept in conical flask and the homogeneous solution of each conical flask was maintained at the rate of 100 ml by adding the sterilized water. Then 2 ml of each sample was derived into micro tubes. Finally, the samples were brought under the process of spectrophotometry in available spectrophotometer in laboratory of Department of Rice Molecular Breeding, ICS.

The relative variation among all traits root length (RRL) and shoot height (RSH) as well as root dry weight (RDW) and shoot dry weight (SDW) were calculated by comparison between plants under control and treated conditions according to the following formula:

1-Relative variation of length=
[(Length of treated plant-Length of control plant/Length of control plant)]X100
2-Relative variation of dry weight=
[(DW of treated plant-DW of control plant/DW of control plant)]X100 (Ines et al., 2009)

## Results

### Phenotypic Performance of extreme RI line populations from MH63 *indica* and 02428 japonica backgrounds (21 days of stress)

The two parents were similar in all traits of control conditions; however, 02428japonica parents had significantly higher root length than that of MH63 parents (Table.1). Under $Fe^{2+}$ stress condition, there were similar trends between Parent 1 and Parent 2 except root length trait for which 02428 had significantly longer root length than that of MH63 *indica* parents, and in terms of shoot dry weight, MH63 *indica* parents had significantly higher SDW than that of 02428japonica parents. For $Fe^{2+}$ relative value %, 02428 parents had higher values in all traits with significantly higher root length and root dry weight; however, MH63 *indica* parents indicated significantly higher values than that of 02428 parents. When losses were compared between MH63 parents under control conditions to tolerant RI lines in MH63 *indica* background under control; and 02428 parents with those of tolerant RI lines under control, tolerant RI lines had got higher trends at all traits TRL, TSH, and TSW except TRW, where both had similar values respectively, and tolerant RI lines gained TRL 5.75%, TSH 5.97%, TSW 22.63%; TRL 19.97%, TSH 5.96%, and TSW 16.96% respectively. When losses and gains were compared between the MH63 *indica* parents under control with those of susceptible RI lines under control and 02428 parents under control with those of susceptible RI lines under control, almost all the susceptible RI lines had smaller values than respected parents in both backgrounds, susceptible RILs lost SRL 7.16, SRW 20%, SSH 1.94%, and SRW 4.08%; SRL 17.46%, SRW 25% (significantly lesser), SSH 0.09%, and SSW 7.74% respectively. While losses and gains were compared between MH63 *indica* parents with tolerant RI lines in MH63 backgrounds under ferrous stress and 02428 parents with those of tolerant RI lines under ferrous stress, all the tolerant RILs had got higher trends in both of the backgrounds than those of relative parents under $Fe^{2+}$ stress, MH63 and 02428 parents lost TRL 30.71% (significant loss), TRW 33.33% (significant loss), TSH 9%, and TSW 2.47%; TRL 11.67%, TRW 33.33% (significant loss), TSH 12.67% and TSW 20.99% respectively. In terms of comparison of losses between susceptible RI lines and their respective parents in MH63 *indica* and 02428japonica, susceptible RI lines lost SRL 23.86% (significant loss), SRW 0%, SSH 3.66%, and SHW 7.59%; SRL19.71%, SRW 0%, SSH 4.82% and SSW 3.13% respectively. [Note: The values for MH63 *indica* and 02428japonica had been shown separately by putting sign of semicolon (;)] The ratio of Reciprocal Introgression lines showed transgressive segregations for all $Fe^{2+}$ related traits and showed continuous variations in MH63 *indica* and 02428japonica backgrounds (Table.1).

## Tissue content analysis for ferrous concentration in extreme RI line populations of MH63 *indica* and 02428japonica backgrounds (21 days after stress)

The two parents MH63 *indica* and 02428 japonica were similar in all traits of control and ferrous stress conditions while compared the level of significance; however, 02428 japonica parents had higher concentration of ferrous at all traits than those of MH63 parents except $Fe^{2+}$ relative value %, at which MH63 *indica* parents had significantly higher values at shoot height. While the concentration of ferrous in MH63 *indica* and 02428 japonica parents under control was compared with those of tolerant RILs under control, the tolerant RILs in MH63 background showed significantly higher concentration in root and shoot than MH63 parents, the MH63 parents lost for ferrous concentration in root (FCR) 25.95%, for ferrous concentration in shoot (FCSh) 43.33%; while roots and shoots of tolerant RILs in 02428 japonica RILs had less concentration of ferrous than 02428 japonica parents, the 02428 japonica tolerant RILs had lost 15.06% for FCR, 14.29% for FCSh. The concentration of ferrous in susceptible RILs in MH63 and 02428 japonica backgrounds showed higher values than those of their respective parents, the MH63 *indica* parents had lost 5.93% for FCR, and for FCSh 37.03% (Significant difference); FCR 14.12%, FCSh 12.5%. In comparison of concentration of ferrous in MH63 *indica* and 02428 japonica parents under ferrous stress to tolerant RILs under ferrous stress, the tolerant RILs in MH63 *indica* background had higher concentration in root and shoot traits than MH63 *indica* parents, the MH63 *indica* parents lost 2.58% for FCR, 12.72% for FCSh; while 02428 japonica parents under ferrous stress had higher values than those of tolerant RILs in 02428 japonica background under ferrous stress, the tolerant RILs lost 3.78% for FCRT, 17.84% for FCShT. In case of comparison between concentration of ferrous in MH63 *indica* and 02428 japonica parents under ferrous stress and susceptible RILs in MH63 *indica* and 02428 japonica under ferrous stress, the MH63 parents had gained 9.14 to FCRT and lost 9.71% for FCShT; the 02428 japonica parents under ferrous stress had higher concentration in roots and shoots than those of 02428 japonica susceptible RILs under ferrous stress, the susceptible RILs lost 7.16 for FCRS and 1.54% for FCShS. While the concentration of ferrous in root of tolerant lines of MH63 *indica* and 02428 japonica backgrounds under ferrous stress was compared with those of susceptible lines under ferrous stress, mostly the averages for roots of the tolerant lines under ferrous stress are higher than those of roots of susceptible lines under ferrous stress with the mean 32.11mg/L and range from 20.21 to 40.47; with the mean of 33.34 mg/L and range from 20.003 to 46.28 respectively. In case of concentration of ferrous in shoot under ferrous stress, the shoots of tolerant lines in MH63 showed higher concentration than those of susceptible lines with the mean of 18.01 mg/L and range from 11.89 to 26.62, whereas, in 02428japonica background the case is reverse, because in 02428 japonica background, shoots of susceptible lines under ferrous stress showed higher values than those of tolerant lines with the mean of 19.81 and range from 12.95 to 30.43. [Note: The values for MH63 *indica* and 02428 japonica had been shown separately by putting sign of semicolon (;)]

The ratio of Reciprocal Introgression lines showed transgressive segregations for all $Fe^{2+}$ related traits and showed continuous variations in MH63 *indica* and 02428japonica backgrounds.

## Phenotypic Performance of extreme RI line populations in MH63 *indica* and 02428 japonica backgrounds (After 26 days of stress)

The two parents were similar in all traits of control, ferrous stress and relative value % conditions; however, MH63 *indica* parents had significantly higher root weight for tolerant lines than that of 02428japonica parents (Table 3.5). For $Fe^{2+}$ relative value %, 02428 parents had higher values in all traits with significantly higher root length and root dry weight. When losses were compared between MH63 parents under control condition to tolerant RI lines in MH63 *indica* background under control; and 02428 parents with those of tolerant RI lines under control, tolerant RI lines had got higher trends at all traits TRL, TSH, and TSW except TRW, where all parents and RILs had similar values respectively, and tolerant RILs gained TRL 8.83%, TRW 7.69%, TSH 6.82%, TSW 37.14% (significantly higher); TRL 2.74%, TRW 27.91% (significantly higher), TSH 3.64%, and TSW 15.89% respectively. When losses and gains were compared between the MH63 *indica* parents under control with those of susceptible RI lines under control and 02428 parents under control with those of susceptible RI lines under control, almost all the susceptible RI lines had smaller values than those of related parents in both backgrounds, susceptible RILs lost SRL 2.85%, SRW 25% (significant loss), SSH 4.47%, and SRW 17.61%; SRL 4.35%, SRW 0%, SSH 6.38%, and SSW 7.78% respectively. While losses and gains were compared between MH63 *indica* parents under ferrous stress with tolerant RI lines in MH63 backgrounds under ferrous stress and 02428 parents under ferrous stress with those of tolerant RI lines under ferrous stress, all the tolerant RILs had got higher trends in both of the backgrounds than those of relative parents under $Fe^{2+}$ stress, MH63 and 02428 parents lost TRL 23.96% (significant difference), TRW 0%, TSH 9.88%, and TSW 9.21%; TRL 11.38%, TRW 13.33%, TSH 8.71% and TSW 19.79% respectively. In case of comparison of losses between MH63 *indica* and 02428japonica parents under ferrous stress with those susceptible RI lines under ferrous stress , susceptible RI lines lost TRL 0.36%, TRW 21.86% (significant difference), TSH 0%, and TSW 14.49%; TRL 43.07%, TRW 0%, TSH 1.58%, and TSW 5.19%. [Note: The values for MH63 *indica* and 02428japonica had been shown separately by putting sign of semicolon (;)]

The ratio of Reciprocal Introgression lines showed transgressive segregations for all $Fe^{2+}$ related traits and showed continuous variations in MH63 *indica* and 02428japonica backgrounds.

## Tissue content analysis for ferrous concentration in extreme line populations under MH63 indica and 02428 japonica backgrounds (after 26 days of stress)

The two parents MH63 indica and 02428japonica were similar in all traits of control and ferrous stress conditions while compared the level of significance; however, 02428japonica parents had significantly higher concentration of ferrous in shoots than that of MH63 parents. For $Fe^{2+}$ relative value %, 02428japonica had higher trends at root parameter and in shoot parameter MH63 indica parents had significantly higher values than those of 02428japonica. While the concentration of ferrous in shoot and root of MH63 indica and 02428japonica parents under control was compared with those of tolerant RILs in MH63 indica and 02428japonica backgrounds under control, the tolerant RILs had higher values than those of MH63 indica and 02428japonica parents, the MH63 indica parents under control had lost 7.37% to FCRT, 36.37% for FCShT (significantly different); 14.61% to FCRT, and 13.33% to FCShT respectively. In case of comparison between MH63 indica and 02428japonica parents under control with those of susceptible RI lines under control, all the susceptible RI lines had higher concentration in roots and shoots than their respective parents, the MH63 indica parents lost 7.37% to FCRS, 53.72% to FCShS (significant difference); 02428japonica parents lost 58.46% to FCRS (significantly lower), and 9.30% to FCShS. While the concentration of ferrous in roots and shoots of MH63 indica and 02428japonica parents under ferrous stress were compared with those of tolerant RILs under ferrous stress, the roots of tolerant RILs in MH63 indica and 02428japonica had higher values than those of their respective parents, and shoots of

MH63 indica and 02428japonica parents had significantly higher trends than those of tolerant RILs, for CFRT the MH63 indica and 02428japonica parents lost 5.74% and 8.44% respectively, for CFShT the MH63 indica and 02428japonica tolerant RILs lost 35.05%; 24.62% respectively. When concentration of ferrous in roots and shoots of MH63 indica and 02428japonica parents under ferrous stress was compared with those of susceptible RILs under ferrous stress, the roots of susceptible RILs had trends and shoots had smaller trends than those of their respective parents, for FCRS the MH63 indica and 02428japonica parents lost 8.68%; 11.23% respectively; for FCShS the susceptible RILs lost 22.16% (significantly different); 18.69% respectively.

While concentration of ferrous in root of tolerant lines of MH63 indica and 02428 japonica backgrounds under ferrous stress was compared with those of susceptible lines under ferrous stress, mostly the averages for roots of the susceptible lines under ferrous stress are higher than those of roots of tolerant lines under ferrous stress with the mean 52.19 mg/L and range from 38.04 to 82.70; with the mean of 50.23 mg/L and range from 27.3 to 68.51 respectively. In case of concentration of ferrous in shoot under ferrous stress, the shoots of susceptible lines in MH63 indica and 02428japonica showed higher concentration than those of tolerant lines with the mean of 15.10 mg/L and range from 9.97 to 23.65; with the mean of 16.18 and range from 9.80 to 24.75 respectively. It indicated that as the days under stress increased, the absorption of ferrous by susceptible lines also increased, this might be due to the higher absorption of ferrous for toxicity period under stress. It might be said that as the duration of ferrous toxicity under stress went on to the 26 days, the absorption of ferrous by tolerant lines decreased as compared to the susceptible lines. Hence phenotypic growth parameters of tolerant lines had higher trends than that of susceptible lines (shown in Table 3). One other important to note that concentration of ferrous in roots of tolerant and susceptible lines is significantly higher than those of tolerant and susceptible shoots in both of the backgrounds indicated that ferrous is less mobile, so for this, less accumulated to the upper parts of the plant body. [Note: The values for MH63 indica and 02428japonica had been shown separately by putting sign of semicolon (;)].

The ratio of Reciprocal Introgression lines showed transgressive segregations for all $Fe^{2+}$ related traits and showed continuous variations in MH63 indica and 02428 japonica backgrounds.

## Discussion

The results of this study showed that the phenotypic data and ferrous concentration in RILs and parents indicated that all the growth parameters under this study, such as, root length, shoot height, root dry weight and shoot dry weight are highly important to know the visible effect of $Fe^{2+}$ stress. That is why, mostly the plants under control conditions achieved significantly higher trends at all related traits than the plants under $Fe^{2+}$ stress conditions. The symptoms of bronzing were observed in plants under ferrous toxicity which were also consistent with the study of Jian Lin Wan et al., (2003) and I. Dufey at al., (2012) in their experiments. In comparison among ferrous treated plants with ferrous untreated plants the concentration of ferrous was pre-expected was higher in ferrous treated plants. These findings are resembling with those of I. Dufey et al., 2012.

In case of extreme lines (highly tolerant and highly susceptible), the phenotypic performance indicated that even though all the other environmental conditions (such as containers, Styrofoam sheets, solution, concentration of ferrous stress, pH, time of solution renewal, temperature and humidity in glass house e.t.c.) were same for both highly tolerant and highly susceptible lines. However, growth and growth parameters of tolerant lines had higher trends than those of susceptible lines. It indicated that even though the tolerant lines in both of the background populations absorbed higher amount of ferrous than susceptible lines, but their root growth was better than susceptible lines (showed in phenotypic results above). One other important to note that concentration of ferrous in roots of tolerant and susceptible lines is significantly higher than those of tolerant and susceptible shoots in both of the backgrounds under 21 and 26 days of stress. It indicated that ferrous is less mobile to the upper parts of the plant body. Hence, it is accumulated in roots, where it could produce its physiological and morphological harmful effects to the plants, However, I. Dufey et al., 2012 had also noticed that all morphological, physiological and agronomic parameters were different in both sensitive and resistant lines except ferrous concentration in root. They also observed toxicity symptoms more pronounced in sensitive lines. In 2012, Priyanga Samaranayake et al., had also recorded that ferrous sensitive varieties had significantly decreased root and shoot weight than tolerant varieties. They also noted that ferrous content in treated plants was significantly higher than untreated plants.

## Conclusion

The results of this study showed that all the traits under this study such as root length, shoot height, root dry weight and shoot dry weight were highly susceptible to $Fe^{2+}$ stress. It was because of that the plants under control conditions achieved higher trends than those of all related trends under stress. However, it was noted that as MH63 indica and 02428 japonica RI lines had some new highly ferrous toxicity tolerant lines/varieties. In the light of this study, it is suggested that these two backgrounds MH63 indica and 02428 japonica should be avoided to cultivate in ferrous contaminated soils until and unless, new highly tolerant varieties/lines to that toxicity could be evolved and released for further replication into the farmers fields.

## REFERENCES

Asch, F., Becker, M., Kpongor, D. S. 2005. "A quick and efficient screen for resistance to iron toxicity in low land rice". Journal of Plant Nutrition and Soil Science (168):764-773.

Audebert, A. 2006b. "Diagnostic of risk and approaches to iron toxicity management in low land rice farming". In: Audebert A Narteh LT, Kiepe P, Millar D, Beks B (eds), Iron toxicity in rice – based system in west Africa. Africa Rice Center (WARDA), Contonou, Benin, 6-17.

Audebert, A. and Sahrawat, K. L. 2000a. "Mechanisms for iron toxicity tolerance in low land rice". Journal of Plant Nutrition (23): 1877-1885.

Becker, M., Asch, F. 2005. "Iron toxicity in Rice- conditions and management concepts". Journal of Plant Nutrition and Soil Science (168): 249-255.

Bode, K., Doring, O., Luthje, S., Neue, H. U., Bottger, M. 1995. "The role of Active Oxygen in Iron tolerance of Rice (Oryza sativa L.)". Protoplasm, 184, 249-255.

de, Dorlodot, S., Lutts, S., Berin, P. 2005. "Effects of ferrous iron toxicity on growth and mineral composition of an interspecific rice". Journal Of Plant Nutrition, 28, 1-20.

Doran, G., Eberbach, P., Helliwell, S. 2006. "The impact of Rice plant roots on the reducing conditions in flooded rice soil". Chemosphere 63, 1892-1902.

Dufey Ine`s, Hakizimana Patrice, Draye Xavier, Lutts Stanley , Bertin Pierre. "QTL mapping for biomass and physiological parameters linked to resistance mechanisms to ferrous iron toxicity in rice". *Euphytica*, 2009, 167: 143–160

Genon, J. G., de, Hepcee, N., Dufey, J. E., Delvaux, B., Hennebert, P. A. 1994. "Iron toxicity and other chemical soil constraints to rice in high land swamps of Burundi". Plant Soil 166, 109-115.

Gross, J., Stein, R. J., Fett-Neto, A. G., Fett, J. P. 2003. "Iron homeostasis related genes in Rice". Genet Molecular Biology26(4), 477-497.

Hagiwara, M. and Imura, M. 1993. "Seedling emergence and establishment of direct-sown Paddy rice in soils incorporated with substances produced in reductive paddy soil", Japanese Journal of Crop Sciences, 62, 609-613.

I. Dufey, M. P. Hiel, P. Hakizimana, X. Draye, S. Luttus, B. Kone, K. N. Drame, K. N. Konate, M. Sie, and P. Bertin. 2012. "Multienvironment Quantitative Trait Loci Mapping and Consistency across Environments of Resistance Mechanisms to Ferrous Iron Toxicity in Rice". Crop Science, 52:539–550, doi: 10.2135/cropsci2009.09.0544

Jian lin Wan, Hu qu Zhai, Jian Min Wan, and H. Iqihashi. 2003. "Detection and analysis of QTLs for ferrous iron toxicity tolerance in rice, *Oryza sativa* L". *Euphytica* 131: 201–206,

Neue, H. U., and R. S., Lantin. 1994. "Micronutrient toxicities and deficiencies in rice". In. AR Yeo, TJ Flowers, (Ed), Soil Mineral Stresses: Approaches to Crop Improvement. Springer-Verlag, Berlin, 175-200.

Priyanga Samaranayake, Bonifaced. Peiris and Sirisena Dssanayake. 2012. "Effect of Excessive Ferrous (Fe2+) on Growth and Iron Content in Rice (*Oryza sativa*)". International Journal of Agriculture & Biology, ISSN Print: 1560–8530; ISSN Online: 1814–9596, 11–351/AWB/2012/14–2–296–298.

Sahrawat, K. L. 2004. “Iron toxicity in wetland Rice and the role of other Nutrients”. Journal of .Plant Nutrition, 27(8), 1471-1504

Takuhito, Nozoe, Masaki, Tachibana, Akira, Uchino, and Narifumi, Yokogami. 2009. “Effect of Ferrous iron (Fe) on the germination and root elongation of Paddy rice and weeds”. Weed Biology and Management, 9, 20-26.

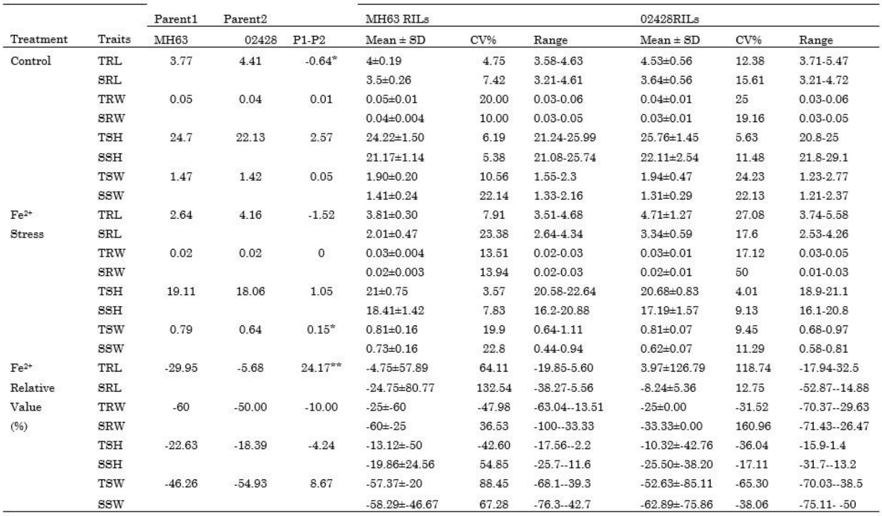
Wan J L, Zhai H.Q., Wan J.M., Ikehashi H. 2003. “Detection and analysis of QTLs for ferrous iron toxicity tolerance in rice (Oryza sativa L.)”. Euphytica, 2003. 131. 201-206

WARDA(West Africa Rice Development Association). 2002. “Painting the Rice Red: Iron toxicity in the lowlands”. Annual Report, 2001-2002, 29-37.

Yoshida, S., Forno, D. A., Cock, J. A., and Gomez, K. A. 1976. *Laboratory manual for plant physiological studies of rice.* 3rd edn. International Rice Research Institute, Manila, The Philippines

**Table 1. Phenotypic Performance of extreme RI line populations from MH63 *indica* and 02428japonica backgrounds (21 days of stress)**

1.



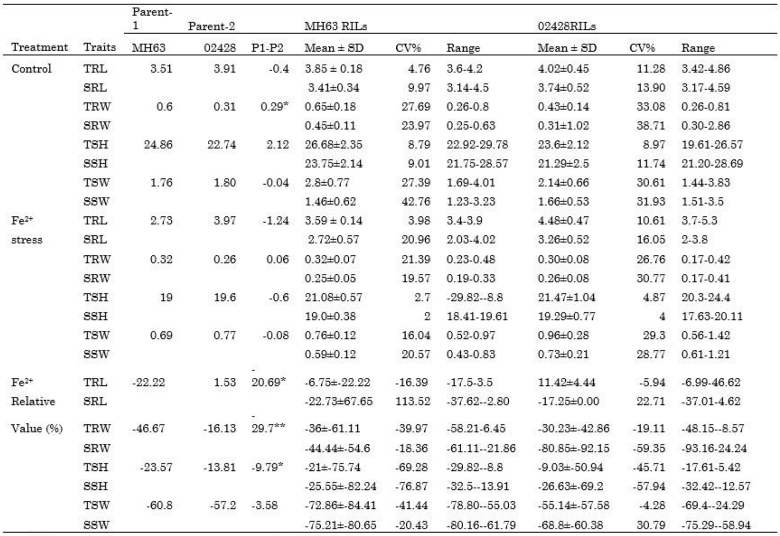
| | | Parent1 | Parent2 | | MH63 RILs | | | 02428RILs | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | Traits | MH63 | 02428 | P1-P2 | Mean ± SD | CV% | Range | Mean ± SD | CV% | Range |
| Control | TRL | 3.77 | 4.41 | -0.64* | 4±0.19 | 4.75 | 3.58-4.63 | 4.53±0.56 | 12.38 | 3.71-5.47 |
| | SRL | | | | 3.5±0.26 | 7.42 | 3.21-4.61 | 3.64±0.56 | 15.61 | 3.21-4.72 |
| | TRW | 0.05 | 0.04 | 0.01 | 0.05±0.01 | 20.00 | 0.03-0.06 | 0.04±0.01 | 25 | 0.03-0.06 |
| | SRW | | | | 0.04±0.004 | 10.00 | 0.03-0.05 | 0.03±0.01 | 19.16 | 0.03-0.05 |
| | TSH | 24.7 | 22.13 | 2.57 | 24.22±1.50 | 6.19 | 21.24-25.99 | 25.76±1.45 | 5.63 | 20.8-25 |
| | SSH | | | | 21.17±1.14 | 5.38 | 21.08-25.74 | 22.11±2.54 | 11.48 | 21.8-29.1 |
| | TSW | 1.47 | 1.42 | 0.05 | 1.90±0.20 | 10.56 | 1.55-2.3 | 1.94±0.47 | 24.23 | 1.23-2.77 |
| | SSW | | | | 1.41±0.24 | 22.14 | 1.33-2.16 | 1.31±0.29 | 22.13 | 1.21-2.37 |
| $Fe^{2+}$ | TRL | 2.64 | 4.16 | -1.52 | 3.81±0.30 | 7.91 | 3.51-4.68 | 4.71±1.27 | 27.08 | 3.74-5.58 |
| Stress | SRL | | | | 2.01±0.47 | 23.38 | 2.64-4.34 | 3.34±0.59 | 17.6 | 2.53-4.26 |
| | TRW | 0.02 | 0.02 | 0 | 0.03±0.004 | 13.51 | 0.02-0.03 | 0.03±0.01 | 17.12 | 0.03-0.05 |
| | SRW | | | | 0.02±0.003 | 13.94 | 0.02-0.03 | 0.02±0.01 | 50 | 0.01-0.03 |
| | TSH | 19.11 | 18.06 | 1.05 | 21±0.75 | 3.57 | 20.58-22.64 | 20.68±0.83 | 4.01 | 18.9-21.1 |
| | SSH | | | | 18.41±1.42 | 7.83 | 16.2-20.88 | 17.19±1.57 | 9.13 | 16.1-20.8 |
| | TSW | 0.79 | 0.64 | 0.15* | 0.81±0.16 | 19.9 | 0.64-1.11 | 0.81±0.07 | 9.45 | 0.68-0.97 |
| | SSW | | | | 0.73±0.16 | 22.8 | 0.44-0.94 | 0.62±0.07 | 11.29 | 0.58-0.81 |
| $Fe^{2+}$ | TRL | -29.95 | -5.68 | 24.17** | -4.75±57.89 | 64.11 | -19.85-5.60 | 3.97±126.79 | 118.74 | -17.94-32.5 |
| Relative | SRL | | | | -24.75±80.77 | 132.54 | -38.27-5.56 | -8.24±5.36 | 12.75 | -52.87--14.88 |
| Value | TRW | -60 | -50.00 | -10.00 | -25±-60 | -47.98 | -63.04--13.51 | -25±0.00 | -31.52 | -70.37--29.63 |
| (%) | SRW | | | | -60±-25 | 36.53 | -100--33.33 | -33.33±0.00 | 160.96 | -71.43--26.47 |
| | TSH | -22.63 | -18.39 | -4.24 | -13.12±-50 | -42.60 | -17.56--2.2 | -10.32±-42.76 | -36.04 | -15.9-1.4 |
| | SSH | | | | -19.86±24.56 | 54.85 | -25.7--11.6 | -25.50±-38.20 | -17.11 | -31.7--13.2 |
| | TSW | -46.26 | -54.93 | 8.67 | -57.37±-20 | 88.45 | -68.1--39.3 | -52.63±-85.11 | -65.30 | -70.03--38.5 |
| | SSW | | | | -58.29±-46.67 | 67.28 | -76.3--42.7 | -62.89±-75.86 | -38.06 | -75.11- -50 |

**Table 2. Concentration of ferrous in extreme RI line populations of MH63 *indica* and 02428japonica backgrounds (21 days after stress)**

| | | Parent-1 | Parent-2 | | MH63 RILs | | | 02428RILs | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | Traits | MH63 | 2428 | P1-P2 | Mean ± SD | CV% | Range | Mean ± SD | CV% | Range |
| Control | FCRT | 2.54 | 2.92 | -0.38 | 3.43±0.76 | 22.05 | 2.19-4.57 | 2.48±0.88 | 35.54 | 1.22-4.16 |
| | FCRS | | | | 2.70±0.83 | 30.59 | 1.81-4.001 | 3.40±1.02 | 29.9 | 1.63-4.79 |
| | FCShT | 0.17 | 0.28 | -0.11 | 0.30±0.22 | 74.5 | 0.04-0.76 | 0.24±0.16 | 64.31 | 0.027-0.52 |
| | FCShS | | | | 0.27±0.19 | 70.65 | 0.014-0.64 | 0.32±0.23 | 72.78 | 0.09-0.72 |
| $Fe^{2+}$ | FCRT | 31.28 | 34.65 | -3.37 | 32.11±9.45 | 29.43 | 15.93-47.29 | 33.34±10.60 | 31.81 | 16.50-50.91 |
| Stress | FCRS | | | | 28.42±7.23 | 25.44 | 20.21-40.47 | 32.17±7.38 | 22.93 | 20.003-46.28 |
| | FCShT | 15.72 | 20.12 | -4.4 | 18.01±5.32 | 29.56 | 11.89-26.62 | 16.53±4.57 | 27.67 | 11.45-24.52 |
| | FCShS | | | | 17.41±4.16 | 23.91 | 10.26-27.20 | 19.81±5.70 | 28.78 | 12.95-30.43 |
| $Fe^{2+}$ | FCRT | 1131.5 | 1086.64 | 44.86 | 836.15±1143.4 | 33.47 | 292.38-1429 | 1244.35±1104.6 | -10.50 | 593.32-1958.38 |
| Relative | FCRS | | | | 952.59±771.08 | -16.84 | 564.82-1646 | 846.18±623.53 | -23.31 | 516.55-1951.13 |
| value% | FCShT | 9147.1 | 7085.71 | 2061.35* | 5903.33±0.22 | -60.32 | 1537.7-36963.3 | 6787.5±2756.25 | -56.97 | 2318.27-90393.73 |
| | FCShS | | | | 6348.14±2089.5 | -66.15 | 2118.13-84980 | 6090.63±2378.3 | -60.46 | 2300.16-36388.22 |

**FCRT**= Ferrous concentration in root of tolerant line, **FCRS**= Ferrous concentration in root of susceptible line, **FCShT**= Ferrous concentration in shoot of tolerant line, **FCShS**= Ferrous concentration in shoot of susceptible line

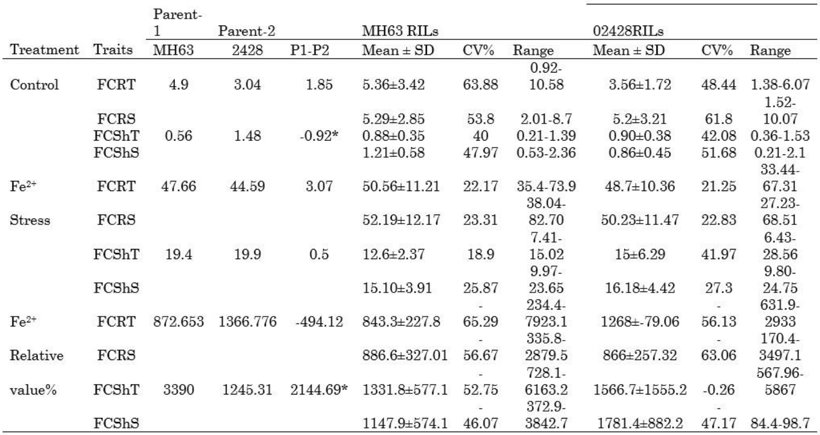
**Table 3. Phenotypic Performance of extreme RI line populations from MH63 *indica* and 02428japonica backgrounds (After 26 days of stress)**



| | | Parent-1 | Parent-2 | | MH63 RILs | | | 02428RILs | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | Traits | MH63 | 02428 | P1-P2 | Mean ± SD | CV% | Range | Mean ± SD | CV% | Range |
| Control | TRL | 3.51 | 3.91 | -0.4 | 3.85 ± 0.18 | 4.76 | 3.6-4.2 | 4.02±0.45 | 11.28 | 3.42-4.86 |
| | SRL | | | | 3.41±0.34 | 9.97 | 3.14-4.5 | 3.74±0.52 | 13.90 | 3.17-4.59 |
| | TRW | 0.6 | 0.31 | 0.29* | 0.65±0.18 | 27.69 | 0.26-0.8 | 0.43±0.14 | 33.08 | 0.26-0.81 |
| | SRW | | | | 0.45±0.11 | 23.97 | 0.25-0.63 | 0.31±1.02 | 38.71 | 0.30-2.86 |
| | TSH | 24.86 | 22.74 | 2.12 | 26.68±2.35 | 8.79 | 22.92-29.78 | 23.6±2.12 | 8.97 | 19.61-26.57 |
| | SSH | | | | 23.75±2.14 | 9.01 | 21.75-28.57 | 21.29±2.5 | 11.74 | 21.20-28.69 |
| | TSW | 1.76 | 1.80 | -0.04 | 2.8±0.77 | 27.39 | 1.69-4.01 | 2.14±0.66 | 30.61 | 1.44-3.83 |
| | SSW | | | | 1.46±0.62 | 42.76 | 1.23-3.23 | 1.66±0.53 | 31.93 | 1.51-3.5 |
| Fe$^{2+}$ stress | TRL | 2.73 | 3.97 | -1.24 | 3.59 ± 0.14 | 3.98 | 3.4-3.9 | 4.48±0.47 | 10.61 | 3.7-5.3 |
| | SRL | | | | 2.72±0.57 | 20.96 | 2.03-4.02 | 3.26±0.52 | 16.05 | 2-3.8 |
| | TRW | 0.32 | 0.26 | 0.06 | 0.32±0.07 | 21.39 | 0.23-0.48 | 0.30±0.08 | 26.76 | 0.17-0.42 |
| | SRW | | | | 0.25±0.05 | 19.57 | 0.19-0.33 | 0.26±0.08 | 30.77 | 0.17-0.41 |
| | TSH | 19 | 19.6 | -0.6 | 21.08±0.57 | 2.7 | -29.82--8.8 | 21.47±1.04 | 4.87 | 20.3-24.4 |
| | SSH | | | | 19.0±0.38 | 2 | 18.41-19.61 | 19.29±0.77 | 4 | 17.63-20.11 |
| | TSW | 0.69 | 0.77 | -0.08 | 0.76±0.12 | 16.04 | 0.52-0.97 | 0.96±0.28 | 29.3 | 0.56-1.42 |
| | SSW | | | | 0.59±0.12 | 20.57 | 0.43-0.83 | 0.73±0.21 | 28.77 | 0.61-1.21 |
| Fe$^{2+}$ Relative Value (%) | TRL | -22.22 | 1.53 | -20.69* | -6.75±-22.22 | -16.39 | -17.5-3.5 | 11.42±4.44 | -5.94 | -6.99-46.62 |
| | SRL | | | | -22.73±67.65 | 113.52 | -37.62--2.80 | -17.25±0.00 | 22.71 | -37.01-4.62 |
| | TRW | -46.67 | -16.13 | -29.7** | -36±-61.11 | -39.97 | -58.21-6.45 | -30.23±-42.86 | -19.11 | -48.15--8.57 |
| | SRW | | | | -44.44±-54.6 | -18.36 | -61.11--21.86 | -80.85±-92.15 | -59.35 | -93.16-24.24 |
| | TSH | -23.57 | -13.81 | -9.79* | -21±-75.74 | -69.28 | -29.82--8.8 | -9.03±-50.94 | -45.71 | -17.61-5.42 |
| | SSH | | | | -25.55±-82.24 | -76.87 | -32.5--13.91 | -26.63±-69.2 | -57.94 | -32.42--12.57 |
| | TSW | -60.8 | -57.2 | -3.58 | -72.86±-84.41 | -41.44 | -78.80--55.03 | -55.14±-57.58 | -4.28 | -69.4--24.29 |
| | SSW | | | | -75.21±-80.65 | -20.43 | -80.16--61.79 | -68.8±-60.38 | 30.79 | -75.29--58.94 |

**Abbreviations are as same as for Table1.**

**Table 4. Concentration of ferrous in extreme Reciprocal Introgression line populations of MH63 indica and 02428 japonica backgrounds for related-traits in rice (after 26 days of stress)**



| | | Parent-1 | Parent-2 | | MH63 RILs | | | 02428RILs | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | Traits | MH63 | 2428 | P1-P2 | Mean ± SD | CV% | Range | Mean ± SD | CV% | Range |
| Control | FCRT | 4.9 | 3.04 | 1.85 | 5.36±3.42 | 63.88 | 0.92-10.58 | 3.56±1.72 | 48.44 | 1.38-6.07 |
| | FCRS | | | | 5.29±2.85 | 53.8 | 2.01-8.7 | 5.2±3.21 | 61.8 | 1.52-10.07 |
| | FCShT | 0.56 | 1.48 | -0.92* | 0.88±0.35 | 40 | 0.21-1.39 | 0.90±0.38 | 42.08 | 0.36-1.53 |
| | FCShS | | | | 1.21±0.58 | 47.97 | 0.53-2.36 | 0.86±0.45 | 51.68 | 0.21-2.1 |
| Fe$^{2+}$ Stress | FCRT | 47.66 | 44.59 | 3.07 | 50.56±11.21 | 22.17 | 35.4-73.9 | 48.7±10.36 | 21.25 | 33.44-67.31 |
| | FCRS | | | | 52.19±12.17 | 23.31 | 38.04-82.70 | 50.23±11.47 | 22.83 | 27.23-68.51 |
| | FCShT | 19.4 | 19.9 | 0.5 | 12.6±2.37 | 18.9 | 7.41-15.02 | 15±6.29 | 41.97 | 6.43-28.56 |
| | FCShS | | | | 15.10±3.91 | 25.87 | 9.97-23.65 | 16.18±4.42 | 27.3 | 9.80-24.75 |
| Fe$^{2+}$ Relative value% | FCRT | 872.653 | 1366.776 | -494.12 | 843.3±227.8 | -65.29 | 234.4-7923.1 | 1268±-79.06 | -56.13 | 631.9-2933 |
| | FCRS | | | | 886.6±327.01 | -56.67 | 335.8-2879.5 | 866±257.32 | -63.06 | 170.4-3497.1 |
| | FCShT | 3390 | 1245.31 | 2144.69* | 1331.8±577.1 | -52.75 | 728.1-6163.2 | 1566.7±1555.2 | -0.26 | 567.96-5867 |
| | FCShS | | | | 1147.9±574.1 | -46.07 | 372.9-3842.7 | 1781.4±882.2 | -47.17 | 84.4-98.7 |

**Abbreviations are as same as for Table 1**