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Effect of Excessive Ferrous (Fe^{2+}) on Growth and Iron Content in Rice (*Oryza sativa*)

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ABSTRACT

Ferrous toxicity tends to occur in rice soils that remain flooded for extended periods. The effect of excess ferrous ions (Fe^{2+}) on the growth and iron content of the rice plant was investigated in this study. Two varieties of rice were used; variety Bw 272-6b was susceptible to Fe^{2+} stress, and variety Bw 267-3 was resistant. Ferrous stress was imposed on 21 days old plants by adding 250 mg Fe^{2+} L^{-1} as $FeSO_4$ to the rice culture solution. Plants were harvested 14 days after the imposition of Fe^{2+} stress. A relative decrease of the dry weight was observed in the shoots of all rice plants. However, the relative decrease of shoot dry weight was 10 times greater in Bw 272-6b than Bw 267-3. Root dry weights of both varieties remained unaffected by the ferrous stress. Under stress conditions the iron contents of the roots of both varieties were higher than that of their respective controls, and roots of susceptible variety showed a significantly higher value compared to the resistant variety. The iron content of the shoots of both varieties under stress was significantly higher than that of their respective controls. However, the iron content of the shoots of the two varieties under stress condition was not significantly different from each other. Although the iron content of the shoots of susceptible and resistant varieties was not significantly different, the leaf symptoms were severe in the susceptible variety. Two possible reasons are suggested for this behavior of the rice plant.

Key Words: Rice; Iron toxicity; Ferrous stress; Bronzing

INTRODUCTION

Rice is the only cereal that can be grown for extended periods in the flooded soil. Slow diffusion of atmospheric oxygen into flooded soils may cause oxygen deficient soil conditions. Decreasing availability of soil oxygen causes hypoxia and eventually anoxia. This will create a chemically reduced state in soils causing reduction of ferric (Fe^{3+}) to ferrous (Fe^{2+}) form. Therefore, when the soil is submerged, ferrous ion concentration in the soil solution increases. This condition occurs only in flooded soils (Becker & Asch, 2005). Increased levels of Fe^{2+} in the soil are toxic to rice (Bush *et al*., 1999).

Higher plants have two mechanisms to acquire iron from the soil. Dicotyledons and non-graminaceous monocotyledons absorb iron by acidification and ferric reduction, while it is normally absorbed as ferric chelates in graminaceous monocotyledons including rice. Since ferrous ion is easily absorbed by plants, the uptake is not controlled by above mechanisms when Fe^{2+} level is high. Thus, excessive Fe^{2+} uptake is eventually the main cause of iron toxicity, which arises not only from the high iron concentration of the solution, but also from certain soil status such as highly reduced conditions and low pH of the soil solution (Guerinot & Yi, 1994).

Iron toxicity is a major problem in rice cultivation. It has been reported from Sri Lanka, India, Vietnam, Malaysia, Philippines, West Africa, Brazil, Colombia and Madagascar (Sahrawat & Singh, 1979). The objective of the present study is to investigate the effect of excessive Fe^{2+} on the growth and iron content of the rice plant.

MATERIALS AND METHODS

Rice varieties and growth conditions: Two varieties of rice (*Oryza sativa* L.) were used in these experiments. One variety namely Bw 272-6b was susceptible to Fe^{2+} stress whereas the other variety Bw 267-3 was resistant. The culture solution described by Bower and Tamimi (1979) was used in this study to grow the rice plants hydroponically.

Seeds were surface sterilized by immersing in 0.1% solution of mercuric chloride for 1 minute and then washed thoroughly with demineralized water. Seeds were soaked for 24 h in demineralized water and then transferred to petridishes containing moist filter paper for germination. Two days later, the germinated seeds were transferred to plastic pots containing 4 L of half strength culture solution. The pH of the culture solution was adjusted daily to 5.0 with either 1N NaOH or 1N HCl. Seven day old seedlings were

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transferred to plastic pots containing 4 L of full strength culture solution. Seedlings were grown for 14 more days under these conditions. The pH of the solution was adjusted daily to 5.0 and the culture solution was changed once in every 3 days.

Ferrous stress was imposed by adding 250 mg Fe^{2+} L^{-1} as $FeSO_4$ to the culture solution when the plants were 21 days old (7 days in the nursery & 14 days in full strength culture solution). The pH of the solution was adjusted to 4.5 daily. For the control pots no Fe^{2+} was added, and the pH was adjusted to 5.0 daily (Wu *et al.*, 1997). Three pots were allocated for each treatment and each pot contained three plants. Plants in the control pots as well as in the treatment pots were grown for 14 more days and then harvested for the determination of shoot and root dry weights and iron content. The average day and night temperatures during the experimental period were 31°C and 29°C respectively. The average relative humidity during the period was 75%.

Evaluation of iron toxicity: Plants were visually scored for iron toxicity symptoms using a scale of 1 - 7 based on the Standard Evaluation System of International Rice Resarch Institute (1988) for rice. A score of 1 indicates normal growth and 7 indicates that almost all the leaves are dead or dying.

Determination of shoot and root dry weight: For dry weight determination, the shoots were separated from the roots and washed thoroughly in deionized water. The samples were then dried in an oven at 80°C for 48 h until a constant weight was obtained. Based on these measurements, relative decrease in shoot dry weight (RDSDW) was calculated using the following formula:

$$RDSDW = \frac{(\text{SDW in the control} - \text{SDW under } Fe^{2+} \text{ stress})}{\text{SDW in the control}} \times 100$$

SDW = shoot dry weight

Determination of iron contents of shoots and roots: Determination of the iron content of shoot and root samples was carried out separately using the Atomic Absorption Spectrophotometer (GBC model 903, GBC Scientific Equipment, Australia).

Statistical analysis: The analysis of variance followed by student t-test ($p = 0.05$) was used to establish significant differences between the treatments.

RESULTS AND DISCUSSION

Ferrous stress has adversely affected the shoot dry weights of both Fe^{2+} resistant and susceptible varieties. However, a significant reduction in the shoot dry weight was observed only in the Fe^{2+} susceptible Bw 272-6b. The relative decrease of shoot dry weight (RDSDW) was 10 times greater in Bw 272-6b than in Bw 267-3 (Table I). These results confirm the susceptibility of Bw 272-6b over Bw 267-3 towards Fe^{2+} stress. However, root dry weights of both varieties were not significantly affected by the Fe^{2+} stress imposed in this experiment (Table I). This showed an organ specific response of the two varieties towards iron stress. Siegel *et al.* (1988) reported that maize root growth has enhanced under 0.32% salts, but shoot growth has retarded. Relative decrease of root dry weights were not calculated because there were no significant differences in root dry weights in any variety under stress and control conditions.

Under control condition, the iron content of the roots of the two varieties did not vary significantly. This indicated that when the iron content of the medium is not in excess, the absorption by varieties differing in resistance towards Fe^{2+} is similar. Under stress condition the iron content of the roots of both varieties were significantly higher than their respective controls (Table II). This showed that, independent of the resistance to Fe^{2+} stress, absorption of Fe^{2+} by both varieties increased significantly with the increase of the iron content in the medium. However, the iron content of the roots of resistant variety was significantly lower than that of the susceptible variety. This indicated that the roots of Fe^{2+} resistant rice varieties were capable of excluding excess Fe^{2+}, whereas the excluding power in the susceptible varieties was low as it has been previously suggested by Peng and Yamauchi (1993). Further, this is in agreement with the fact that the susceptibility of the rice plant to iron toxicity is increased if the roots are cut, as this facilitates entry of iron into the plant (Tanaka *et al*., 1966).

The iron content of the shoots of both varieties under stress was significantly higher than that of their respective controls. However, the iron content of the shoots of the two varieties under stress condition was not significantly different from each other (Table II). Presence of a significantly different iron contents in the roots and the absence of a significantly different iron contents in the shoots under stress condition suggest that the translocation of iron from the roots to shoots has not depended on the iron content of the root as well as the varietal sensitivity to ferrous stress.

Although there were no significant difference between the iron contents of the shoots of two varieties under stress, when the leaf symptoms were evaluated, susceptible variety recorded a score of 7 in the visual scale, whereas the resistant variety recorded only 1 (Table III).

Two possible reasons could be suggested regarding this observation. Firstly, the shoot system of the resistant variety may have a mechanism of partitioning iron in their tissues without causing cell damage, whereas the susceptible variety does not possess such a mechanism. Different levels of iron distribution in different parts of the rice plant and lack of any iron barrier in verities very sensitive to iron toxicity have been reported (Audebert *et al.*, 2006). Secondly, the leaf symptoms may be linked to a chemical signal transmitted by the root system. Having significantly greater iron content in the root system, the signal transmitted by the roots of the susceptible variety may be stronger than the signal transmitted by the low iron containing root

Table I: Shoot and root dry weights and relative decrease of shoot dry weight of Bw 272-6b and Bw 267-3 under Fe^{2+} stress and control conditions

Variety	Treatment	SDW[a] (g)	RDW[b] (g)	RDSDW[c] (%)
Bw 272-6b	Control	2.76 (±0.06)[d]	0.43 (±0.06)	
(Susceptible)	Stress	1.54 (±0.32)	0.44 (±0.06)	44.15
Bw 267-3	Control	2.05 (±0.11)	0.37 (±0.06)	
(Resistant)	Stress	1.96 (±0.24)	0.38 (±0.06)	4.62

[a.]SDW=Shoot dry weight; [b.]RDW=Root dry weight; [c.] RDSDW=Relative decrease of shoot dry weight; [d.] values reported in the table are mean of 9 plants. SD is given in parenthesis

Table II: Iron content of the shoots and roots of Bw 272-6b and Bw 267-3 underFe^{2+} stress and control conditions[a]

Variety	Treatment	Shoot iron content ($mg.g^{-1}$)	Root iron content ($mg.g^{-1}$)
Bw 272-6b	Control	0.270 (±0.004)	0.836 (±0.216)
(Susceptible)	Stress	3.621 (±0.357)	138.958 (±15.323)
Bw 267-3	Control	0.382 (±0.090)	0.682 (±0.208)
(Resistant)	Stress	3.275 (±0.072)	82.812 (±15.446)

[a]Each value represents the mean of 9 plants. SD is given in parenthesis

Table III: Iron toxicity scores of Bw 272-6b and Bw 267-3[a]

Variety	Treatment	Toxicity score
Bw 272-6b	Control	1
(Susceptible)	Stress	7
Bw 267-3	Control	1
(Resistant)	Stress	1

[a]Toxicity score was determined by observing nine plants after 11 days from imposition of stress

system of the resistant variety. However, further investigations are required to understand the mechanism of this important phenomenon.

CONCLUSION

Rice varieties showed organ specific response to ferrous stress. Under stress conditions, the increment of the iron content of the roots was independent of the stress resistance. The translocation of iron from root to shoot does not depend on the iron content of the root or the resistance towards ferrous stress. Development of leaf symptoms depended more on the susceptibility of the variety than leaf iron content.

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