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EFFECTS OF FERROUS TOXICITY ON SEEDLING TRAITS AND ION DISTRIBUTION PATTERN IN UPLAND AND LOW LAND RICE UNDER HYDROPONIC CONDITIONS

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Graphics abstract

Abstract

Environmentally, nutritional disorders are the major constraints due to the change in soil pH for rice crop cultivated under different irrigation system. Iron toxicity is one of the famous nutritional disorders caused by the excessive uptake of ferrous ion in low pH under aerobic conditions. This research study was focused to measure the effect of different levels of ferrous stress on seedling traits, distribution of ferrous, potassium ions in two upland rice and three lowland rice varieties. The mean comparison of growth parameters were formulated using least significant differences, as well as the experimental treatments were compared by analysis of variance. Varieties showed significant differences for growth parameters; however, lowland rice varieties like *Pokkali* and *Firat* exhibited the minimum deterioration in growth parameters. The root length was significantly reduced under ferrous stress in all varieties. Ferrous accumulation was high in roots compared to aerial plant parts and flag leaves exhibited the minimum accumulations. Reduction of potassium reflected in all plant parts when an increased in stress was imposed. Upland rice varieties were found sensitive compared to lowland rice varieties. It is therefore; concluded that the growth parameters would be a reflecting index for ferrous toxicity in rice. Furthermore, the availability and uptake of potassium is improved, it may minimize the effects of ferrous toxicity.

Keywords: Up Land Rice, Ferrous Toxicity, ions distribution, Seedling traits

1.0 INTRODUCTION

Rice is very important staple food, more than 90% rice consumed and grown in Asia. Rice *Oryza sativa L.* is cultivated on approximately 128 million hectares of irrigated and rainfed lowlands (Maclean *et al.*, 2002) and about 55% of the rice-growing area are due either toxicity or nutrient deficiency (Chérif *et al.*, 2009). Yield production in these rice producing lands has been decreased approximately 100 million hectares (Becker and Asch, 2005). Iron (Fe) toxicity is one of most important constraint in acid soil for rice production along with Zn deficiencies that Fe and Zn are soluble ions in low pH damage the root system and reduce the growth and production of rice. In Asia, South America and Africa, a huge area of land suited for agriculture but remain uncultivated due to the iron toxicity.

The iron stress causes the typical leaf-bronzing symptoms and affects the rice growth. The reduction in height and dry matter has a related with rice production and ultimately affect the rice yield. Iron predominates as ferric ion (Fe^{3+}) in the soil. Fe^{3+} is insoluble thus largely unavailable to be absorbed. The soil lower pH results the reduction of Fe^{3+} to Fe^{2+}. Fe^{2+} is soluble and able to absorb by the rice plant (Becker and Asch, 2005). Plants show iron toxicity when absorb high Fe^{2+} by root and transported to leaves via xylem flow. Excessive uptake of Fe^{2+} cause the production of radicals, and lead to an accumulation of oxidized polyphenols (Yamauchi and Peng,1993) which can cause the irreversible cell damage, DNA and plants proteins and (Dorlodot,2005). Therefore, increase level of iron in soil is toxic to plants (Bush *et al*., 1999).

Iron toxicity is a main concern in rice cultivation. It has been reported in Malaysia, Philippines, India, Madagascar, Vietnam, West Africa, Brazil, Colombia and Sri Lanka (Samaranayake, Peiris, and Dssanayake 2012). The objective of the present study is to investigate the effect of excessive Fe^{2+} on the seedling growth, iron content and distribution of ferrous and potassium ions in two upland rice and three lowland rice varieties.

2.0 MATERIALS AND METHODS

2.1 Plant Materials and Growth Condition

Five varieties of rice cultivar from upland rice (*SK 1*, *Panderas*) and lowland rice (*MR211*, *Firat* and *Pokkali*) were used in this experiment. The seeds were obtained from International Rice Research Institute, Pakistan Rice Research Institutes, Philipphines Rice Research Institute and Malaysian Agricultural Research and Development Institute. The seeds treated with surface sterilization using 70% (v/v) ethanol and 5% Clorox and followed by rinsing with distilled water.

Seeds were soaked for 24 h in demineralized water and then transferred to petridishes containing moist filter paper for germination, 7 days later; the germinated seeds were transferred into half strength hydroponic system for 7 days consist 4 liter of Yoshida solution. The pH of the Yoshida solution for the whole experiment was retained at pH 5.0 (Samaranayake *et al*., 2012).

Ferrous stress was imposed by adding 500 and 750mg/L Fe^{2+}as FeCl3 to the culture solution when the plants were 21days old. For the control no Fe^{2+} was added, and the pH was adjusted to 5.0 daily (Wu *et al*., 2006). Plants in the control pots as well as in the treatment pots were grown for 14 more days. The plant height, number of leaves and flat leaf length were measured, then harvested for the determination of shoot and root dry weights and for iron potassium ions analysis. The average day and night temperatures during the experimental period were 31°C and 29°C respectively.

2.2 Evaluation of Iron Toxicity

Leaf bronzing was scored for iron toxicity. Iron toxicity symptoms using a scale of 1–5 according to Standard Evaluation System of International Rice Research Institute (1988) for rice. A score of 1 indicates normal growth and 5 indicates that almost all the leaves are dead.

2.3 Determination of Dry Weight Tissue Parts

For dry weight, the plants were washed thoroughly with deionized water and allowed to dry in an oven at 70°C for 48 hours until a constant weight was obtained. Based on these measurements, relative decrease in shoot, leaf flag leaf and root dry weight calculated.

2.4 Determination of Iron Potassium Ions Distribution

Determination of the iron potassium ions in leaves flag leaf shoot and root samples were carried out separately using the Atomic Absorption Spectrophotometer (AA-7000, Shimadzu).

2.5 Statistical Analysis

The Mean comparison followed by Duncan's multiple range (p = 0.05) was used. To establish significant Analysis of variance (One-way ANOVA), analysis of correlation had used. The significance of the values were considered at a level of a= 5%. The statistical analysis had done with version 16.0 of IBM-SPSS statistics.

3.0 RESULTS AND DISCUSSION

As shown in Table 1, the relative plant height, Leaf bronzing, dry shoot weight, dry root weight, dry leaf and dry flag leaf were significantly decreased. Iron stresses have adversely effect on root leaf and shoots dry biomass of Fe^{2+} resistant and susceptible varieties ($P<5\%$) For root dry biomass, iron stress resulted in 8.24 % to 32.82% reduction in five varieties. Deposition of Fe^{2+} on roots occurred when Fe was oxidized by roots (Li *et al*., 2001). However, a significant root dry mass weight was observed in *SK1* (V1) and *Panderas* (V4), the results that showed these are Fe^{2+} susceptible varieties. The decreased in relative root dry biomass was 10 times greater than *Pokkali* (V5) (Table 1 a and b). However, shoot dry weights; flag leaf biomass and leaf biomass of all varieties were not significantly affected by the Fe^{2+} stresses imposed in the experiment (Table 1). In maize the root has enhanced 0.32% when exposed to salt stress but shoot growth has stunted (Samaranayake, Peiris, and Dssanayake 2012).

excess iron ions penetration in to plants through roots (Engel *et al*., 2012).

Table 1(a) Growth parameters of five rice varieties genotypes under control condition Fe^{2+} stress

Varieties	Treatment (mg/L)	PH(cm)	LB	FLL (cm)	RDB (g)	FLDB (g)	LDB (g)	SDB (g)
	Control	65.60a	1.00a	44.50a	1.39a	0.42a	0.78a	1.02a
SK1	500	58.47b	2.33b	40.47b	1.10b	0.37b	0.66b	0.67b
	750	56.85b	3.00c	39.23b	0.95b	0.33c	0.56c	0.65b
	Control	62.22a	1.00a	41.53a	1.75a	0.38a	0.43a	0.75a
Panderas	500	50.63b	2.67b	38.78b	0.74b	0.36a	0.36b	0.63b
	750	46.63c	4.00c	35.90c	0.72b	0.31b	0.36b	0.53c
	Control	48.69a	1.00a	30.68a	1.52a	0.38a	0.47a	0.69a
MR211	500	45.02b	2.00b	30.19a	0.86b	0.36b	0.43b	0.59b
	750	44.23b	4.00c	30.59a	0.62c	0.31c	0.36c	0.53c
	Control	54.75a	1.00a	36.60a	2.38a	0.37a	0.57a	0.88a
Firat	500	46.73b	2.00b	33.31b	1.05b	0.34b	0.55b	0.76b
	750	42.33c	2.00b	30.44c	0.67c	0.32b	0.45c	0.70c
	Control	85.65a	1.00a	57.52a	1.93a	0.32a	0.87a	1.35a
Pokkali	500	77.23b	2.00b	52.02b	1.49b	0.31a	0.85b	1.15b
	750	75.21b	2.00b	51.49b	1.36b	0.29b	0.81b	1.06b

Same letters are not significantly different at probability ($p<5\%$) error by Duncan multiple range
PH=Plant height, LC=Leaf counting, LB=Leaf bronzing, FLL=Flag leaf length, RDB=root dry biomass, FLDB=Flag leaf dry biomass, LDB=Leaf dry biomass, SDB=Shoot dry biomass.

High iron ion content was observed in roots and lower concentration in Flag leaf of all 5 varieties (Table 1 b).Excessive amount of Fe^{2+} adversely affected growth and development of root, which influences uptake and retention of nutrients (Aboa and Dogbe, 2006). The significantly less percentage reduction in seedling height was recorded in all five varieties, from 2.20 % to 17.14%. *Panderas* was recorded as the highest percentage reduction in seedling height whereas *Pokkali* was recorded as lowest reduction in plant height (Figure 1).

One way ANOVA was done to evaluate the effect of iron stress on growth and Fe^{2+} and K^+ ion uptake (Table 2 a and b). ANOVA revealed significant effect at 0.01 and 0.05 levels on values of the growth parameters analysed. Factor varieties significantly affect all parameters except Fe^{2+} quantity in root and K quantity in flag leaf. Similar results were observed in interaction between varieties and iron stress. A significant effect of interaction between varieties and Fe^{2+} stresses was determined by ANOVA indicating a differential response of varieties to iron stress. The iron quantity of the root, flag leaf and leaf of five varieties under stress levels was significantly higher than that of their relevant controls (Table 1 b). However, the iron ion quantity of the roots in five varieties under stress condition was highly significant (1.48**) (Table 2 b). Similarly, highly significant difference between the iron ion contents and K^+ quantity of the leaf and flag leaf of five varieties under stress (Table 2 b). The roots of rice crops have potential of excluding Iron which preventing the content in the root system, the iron ion translocation by the roots of the susceptible variety may be stronger than the low iron ion containing root. Secondly, shoot system of resistant varieties may have mechanisms which partitioned the iron ions in their tissue without any damage whereas sensitive varieties don't have such defensive mechanism high K^+ content in leaf and flag leaf indicated that K^+ ions reduce the Fe^{2+} accumulation. Similar results were observed by Lie *et al* (2001) in hybrid rice. Various levels of iron distribution in different parts of the rice plant and without any iron barrier in varieties very susceptible to iron toxicity (Audebert *et al*., 2006).

Table 1(b) Physiological parameters of five rice varieties genotypes under control and Fe^{2+} stress condition

Varieties	Treatment (mg/L)	Fe-quantity in root(mg)	Fe-quantity in Leaf(mg)	Fe-quantity in flag leaf(mg)	K-quantity in Root(mg)	K-quantity in Leaf(mg)	K-quantity in Flag Leaf (mg)
Sk1	Control	1.23c	0.02c	0.01c	0.14a	2.76a	2.56a
	500	1.26b	0.46b	0.02b	0.09b	2.40b	2.43b
	750	1.73a	0.63a	0.03a	0.08b	2.20c	2.03c
Panderas	Control	1.10c	0.02c	0.01c	0.04a	2.23a	2.76a
	500	1.13b	0.29b	0.18b	0.07b	2.13b	2.36b
	750	1.23a	0.39a	0.38a	0.07b	1.63c	1.93c
MR211	Control	1.10b	0.01c	0.01c	0.18a	2.60a	2.86a
	500	1.13b	0.28b	0.22b	0.10b	2.43b	2.40b
	750	1.30a	0.51a	0.50a	0.09c	2.16c	2.10b
Firat	Control	0.90c	0.01c	0.01c	0.19a	2.73a	2.76a
	500	1.16b	0.41b	0.01b	0.13b	2.33b	2.63a
	750	2.00a	0.28a	0.02a	0.09c	1.96c	2.36b
Pokkali	Control	0.94c	0.01c	0.01b	0.18a	2.93a	2.93a
	500	1.20b	0.22b	0.02a	0.08b	2.46b	2.53b
	750	1.50a	0.32a	0.02a	0.06c	2.20b	2.00c

Same letters are not significantly different at probability (p<5%) error by Duncan's multiple range.

Table 2(a) Analysis of variance of all parameters measured on five rice varieties cultivated in hydroponics

Source of variable	df	MS						
		PH	FLL	LB	RDB	FLDB	LDB	SDB
Variety	4	1489.21*	660.61**	10.31**	2.641**	0.079**	0.29**	0.44**
Stress	2	412.52*	75.36**	3.94**	14.961**	0.00	0.03**	0.27**
Variety * Stress	8	14.31*	7.81	14.649**	0.88**	0.01*	0.006*	0.01**
Error	29	3.509	4.81	0.046	0.107	0.00	0.005	0.02
Total	43							

MS= mean squares, * and** represent the levels of significance at P< 0.05 and 0.01, respectively. PH=Plant height, LC=Leaf counting, FLL=Flag leaf counting, RDB=root dry biomass, FLDB= Flag leaf dry biomass, LDB=leaf dry biomass, SDB=Shoot dry biomass. df. Degree of freedom

Table 2(b) Analysis of variance of Iron and potassium content in root, leaves and flag leaf measured on five rice varieties cultivated in hydroponics

Source of variable	df	MS					
		Fe-quantity in root	Fe-quantity in leaf	Fe-quantity in flag leaf	K-quantity in root	K-quantity in leaves	K-quantity in flag leaf
Variety	4	0.10	0.04**	0.02**	0.32**	0.37**	0.16
Stress	2	1.48**	0.67**	0.29**	2.50**	1.37**	1.26**
Variety * Stress	8	0.09	0.02**	0.05**	0.07**	0.02**	0.04
Error	29	0.03	0.00	0.001	0.01	0.02	0.04
Total	43	0.10	0.04	0.02	0.32	0.37	0.16

MS= mean squares, *and** represent the levels of significance at P< 0.05 and 0.01, respectively. df.=Degree of freedom

Figure 1 Five varieties were screened after stresses 500 and 750 mg L−1 Fe^{2+}. Leaf bronzing score after 2-days-treatment, leaf bronzing score after 5-days-treatment, relative root, leaf flag leaf and shoot dry weight after 14-days-treatment

4.0 CONCLUSION

Two low land varieties *Sk1* and *Panderas* showed sensitivity to iron stress whereas up land varieties *Pokkali* and *Firat* showed resistance to iron stress.

Under stress conditions, the accumulation of the iron content of the roots was dependent of the stress resistance. Flag leaves have low iron accumulation, indicating that all five varieties have mechanism to protect the flag leaf. Moreover, the availability and uptake of potassium may minimize the effects of ferrous toxicity

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