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COMPARATIVE STUDY OF PHYSIOLOGICAL AND GROWTH PARAMETERS FOR SODIUM AND IRON TOXICITIES IN UPLAND AND LOWLAND CULTIVARS OF *ORYZA SATIVA* L.

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Abstract

Abiotic stresses are common limitations to rice productivity worldwide. Sodium and iron toxicities affect the rice crop yield adversely. The aim of this study was to compare the effects of these toxicities on seedling growth and ionic distribution in (two upland: *SK1* and *Panderas* and three lowland: *Pokkali*, *MR211* and *Firat*) rice varieties. Sodium and iron toxicities (NaCl: 0, 80mM and 120mM and $FeCl_2$: 0, 3.5mM and 7.5mM) were imposed separately, to three weeks old seedling for two weeks, using a hydroponic system. Results showed a significant adverse effect on growth traits with an increase in iron and sodium toxicities. Ionic distribution analysis of roots, leaves and flag leaves revealed that an increase in Na^+ and Fe^{2+} ions reduced K^+ uptake. *Pokkali* showed low accumulations of Na^+ and Fe^{2+} in root, leaves and flag leaf in all stress levels of these toxicities. *Firat* showed high accumulation of Na^+ and low accumulation of Fe^{2+} in high stress levels of these toxicities, respectively. *MR211*and *Panderas* showed high stress injury scores in both toxicities. Therefore, it is concluded that the tolerance for sodium and iron toxicities may be independent mechanisms as reflected by physiochemical analysis. However, ability to uptake potassium would help the seedling to ameliorate the effects of these toxicities.

Key words: *Oryza sativa* L., Abiotic Stresses, toxicity, ion distribution.

Introduction

Rice (*Oryza sativa* L) is the major nutrition source for about 40% of the world's population, including many of the people living in the developing countries (Greenland, 1997). It is cultivated on approximately 128 million hectares of irrigated and rain fed lowlands (Maclean *et al*., 2002) and about 55% of rice cultivated fields are affected due to toxic elements like sodium and iron toxicities (Chérif *et al*., 2009). Excess of iron and sodium in soil are the major agricultural constrains that strongly influencing the rice crop productivity (Engel *et al*., 2012; Borsani *et al*., 2003). They are the most important toxicities that can directly effect on plant growth and development (Galvani, 2007; Lauchli & Grattan, 2007, Arshad *et al*., 2012).

The reduction in height and dry matter has a related with rice production and ultimately affect the rice yield. The salt injury is the main symptom of sodium toxicity whereas, iron toxicity causes the typical leaf-bronzing symptoms and these toxicities effect the rice growth. The excess ions up take, nutrient disorder and water deficiency are reported as major constraints for plants grown in sodium toxicity.

Plants possess different mechanisms to maintain the physiological concentrations of essential ions when they are exposed to high levels of toxicities (Hall 2002). Rice varieties have different level of tolerance to toxicities which have been developed by plant breeders (Sahrawat *et al*., 1996).

Many studies have been conducted to identify the genetic and biochemical mechanisms helping plants to overcome with toxicities of toxic substance like sodium and iron ions. The mechanisms of plants play very crucial role to exclude, detoxify or compartmentalize the toxic substance toxicity for the protection of plant in a contaminated environment (Lin *et al*., 2012).

The main objective of this study was to observe the effects of sodium and iron toxicities on plant growth and development and to screen the rice varieties possessing tolerance mechanism when exposed to high levels of sodium and iron toxicities.

Materials and Methods

Rice varieties and growth conditions: Five rice varieties, two upland (*Sk1* and *Panderas*) and two lowland rice *(MR211, Firat* and *Pokkali*), were used to investigate the effects of sodium and iron toxicities. Seeds were soaked for 24 h in demineralized water and then transferred to Petridishes containing moist filter paper for germination. Seven days later, the germinated seeds were transferred into half strength hydroponic system for 7 days consisting of 4 liters of Yoshida solution (Samaranayake *et al*., 2012). The pH of the Yoshida solution for the whole experiment was retained at pH 5.0-5.5 (Wu *et al*., 2006). Sodium and ferrous toxicities were imposed after one week (Sodium: omM, 80mM and 120mM Iron: 0mM, 3.5mM and 7.5mM, respectively). Solution pH and nutrient solution were adjusted as described by Wu *et al*. (2006). Toxicities were imposed for two weeks.

Growth traits: Then seedlings were harvested to record the data for growth traits. For dry weight, the plants were washed thoroughly with deionized water and allowed to dry in an oven at 70ºC for 48 hours until a constant weight was obtained. Based on these measurements, the relative decrease in shoot and root dry weight was calculated using the following formula:

$$\frac{\text{Mean weight in control - Mean weight under stresses}}{\text{Mean weight in control}} \text{x100}$$

Leaf bronzing and salt injury score were computed by the effects reflected by the leaves under sodium and iron toxicities, respectively. Toxicity symptoms were converted to a scale of 1–5 as described by Standard Evaluation System of International Rice Research Institute (Anon., 1988). A score of 1 indicates normal growth and 5 indicates that all the leaves are dead.

Physio-chemical parameters: From each sample, 0.3 grams of ash in the test tube were dissolved in aqua regia (HCl+ HNO_3), 6ml: 2ml and kept in the aluminium block for digestion at 180°C. The samples were digested to cool and dry and were diluted with 20ml of 10% HCl, till the transparent colour appears.

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

Statistical analysis

Data of the experiment was subjected to statistical analysis with version 16.0 of IBM-SPSS statistics. Mean and standard deviation of data were calculated.

Results and Discussion

The results of hydroponic study indicated that both Na^+ and Fe^{2+} toxicities severely influenced the growth and physiological parameters in all varieties. However, decrease in the growth trait and physiological parameters were more pronounced at 120mM NaCl and 7.5mM $FeCl_3$ concentrations in the hydroponic growth medium for all rice varieties.

Effect of Na and Fe toxicities on growth parameters

Root dry Biomass: Data (Fig. 1), regarding the reduction percentage of root dry biomass showed the comparison Na^+/Fe^{2+} toxic effect on all rice varieties of different Na^+ and Fe^{2+} toxicity levels. In control, maximum root dry weight was recorded in *Pokkali* (3.93mg ± 0.01, and 3.92mg±0.00) followed by *Sk1* (3.89mg±0.56) and *Firat* (2.46 mg± 0.08) in both experiments. The comparison of rice cultivars response at low levels of Na^+ toxicity (80mM) *Pokkali* and *MR 211* showed a minimum reduction 38.73% and 37.83%, respectively, followed by *Panderas* (34.25%) and variety *Firat* showed the lowest dry root biomass (14.61%). Whereas, at low levels of Fe^{2+} toxicity (3.5mm) showed the minimum reduction percentage in root dry biomass in varieties *Firat* (41.74%) and *Panderas* (40.60%) followed by *MR211* (39.27%) and Pokkali (33.73%) while the lowest root dry weight was observed in *SK1* (24.19%). At the highest concentration of Na^+ toxicity, varieties *MR211* (28.50%) and *Pokkali* (26.02%) showed minimum reduction while varieties *SK1* (11.54%) and the *Firat* (9.33%) were showed a maximum reduction in root dry weight. However, at high concentration of Fe^{2+} toxicity (7.5mM) varieties *Panderas* (39.12%), *MR211* (32.62%) and *Pokkali* (30.62%) performed better than other varieties, whereas *SK1* yielded lowest root dry mass. The results are in confirmation with Aboa & Dogbe (2006), Costa *et al*. (1995).

Shoot dry biomass: The results of the shoot dry biomass at different levels of Na^+ and Fe^{2+} gevarieties *MR 211* (33.09%) and *Panderas* (34.08%) at high toxicity of sodium are shown in Fig. 2. Var. *Pokkali* yielded highest (46.15%) and var. *Firat* produced lowest (28.29%) shoot dry weight at high (120mM) sodium toxicity. While in Fe^{2+} toxicities showed that maximum shoot dry weights 86.08%, 77.26%, 76.43%, 75.69% were produced by *MR211* and *Pokkali* in 3.5mM and 7.5mM of Fe^{2+} toxicities, respectively. Whereas, the variety *SK1* yielded 64% and 53% the lowest shoot dry mass. Similar results were also found by Purnendu *et al*., (2004), Maiti *et al*., (2006), Audebert *et al*., (2006) under iron and salinity stresses. Munns & Tester, (2008) also reported that the stresses might directly or indirectly inhibit cell division and enlargement during plant growing period. As a result, shoot and leaves of the affected plants appeared stunted.

Leaf bronzing and salt injury score: As shown in results (Fig. 3) leaf injury score in Na^+ toxicity and leaf bronzing in Fe^{2+} toxicity and were significantly high in all rice varieties. At high toxicity level of Na^+, *Pokkali* (36.29%) and *SK1*(44.37%) showed the lowest leaf injury score while *Firat* (57.28%) and *Panderas* (51.67%) showed the highest leaf injury score as represented in Fig. 5. However, at the highest level of Fe^{2+} toxicity (7.5mM) *Firat* (57.28%) and *Pokkali* (65.67%) showed minimal leaf bronzing while *Panderas* (84.75%), *MR211* (83.56%) and *SK1*(81.76%) showed maximum leaf bronzing as shown in Fig. 4.

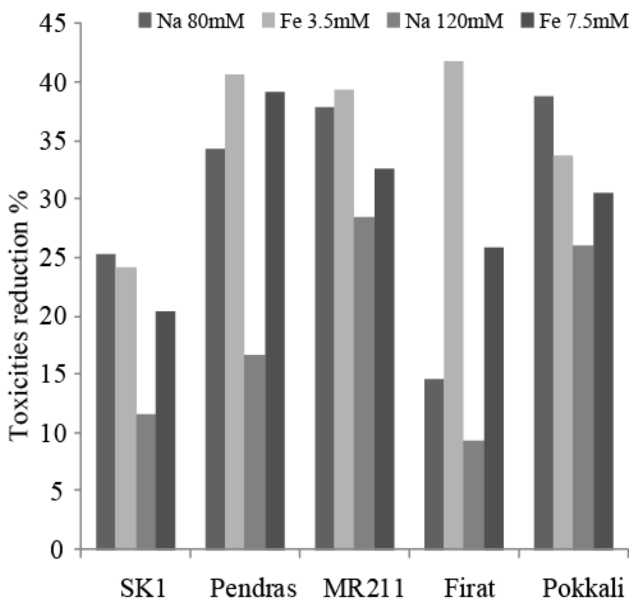

Fig. 1. Root dry weight as affected by sodium and iron toxicities in upland and lowland rice varieties.

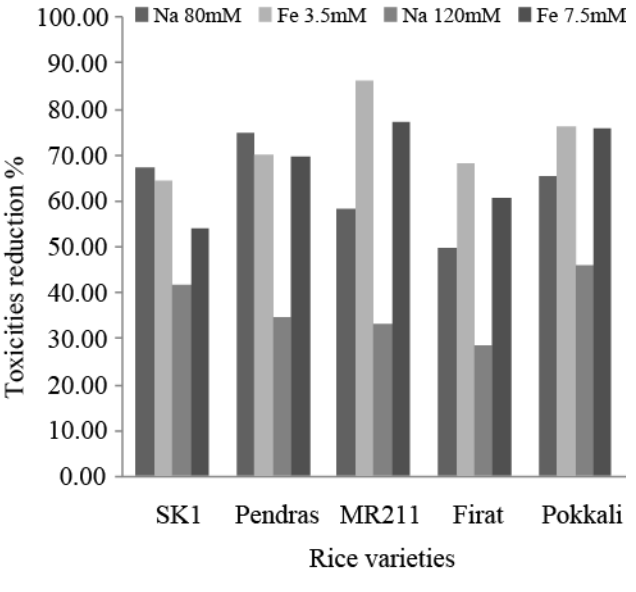

Fig. 2. Shoot dry biomass as affected by sodium and iron toxicities in upland and lowland rice varieties.

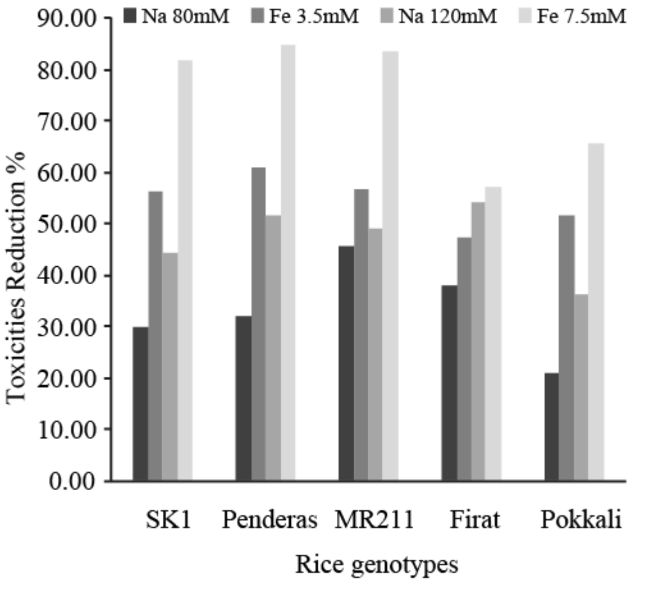

Fig. 3. Leaf bronzing and leaf injury score as affected by sodium and iron toxicities in upland and lowland rice varieties.

Fig. 4. Leaf brozing score in (Left to right) SK1, Panderas, MR211, Firat, Pokkali under iron toxicity

Fig. 5. Salt injury score in (left to right) SK1, Panderas, MR211, Firat, Pokkali under sodium toxicity

Physio-chemical response of varieties under sodium and iron toxicities: The Na^+ and Fe^{2+}ions quantities of the root, leaves and flag leaves of all varieties were significantly different than that of their relevant control. The Na^+/ Fe^{2+} ions distribution in the root, leaves and flag leaf at various rice varieties at different toxic levels is given in Table 1a, b. The Na^+ and Fe^{2+} quantities are significantly higher than their relevant controls. The highest accumulations of Na^+ and Fe^{2+} were found at 120mM and 7.5mM concentrations.

The comparison of rice varieties at different levels showed that minimum Fe^{2+} accumulations 1.5ppm, 0.32 ppm and 0.23 ppm were found in root, leaf and flag leaf respectively, in *Pokkali*. Whereas maximum Fe^{2+} accumulations 1.86ppm and 1.7ppm were recorded in the roots of *Panderas* and *SK1* and 0.63ppm and 0.51ppm were obseved in the leaves of *SK1* and *MR211* while 0.50ppm and 0.36ppm were found in the flag leaves of *MR211* and *Sk1* respectively. Akhtar *et al*., (2001) and Samaranayake *et al*. (2012) reported the similar findings in cotton and rice, respectively. *Pokkali* retained most of absorbed Fe^{2+}in the roots and stopped or slow the transport of Fe^{2+} to leaves and flag leaf .

K^+/Fe^{2+} and K^+/Na^+ Ratio: Table 1 (a) and (b) describes the K^+ concentration in the rice varieties at different toxicity levels of Fe^{2+} and Na^+. On an overall mean indicated that increase in Na^+ / Fe^{2+}decreased K^+ concentration significantly at all levels of stresses, however, the decrease was more pronounced at 7.5mM $FeCl_3$ and 120mM NaCl concentration in growth medium. The comparison of rice varieties at 80mM and 120 mM level of Na^+, 3.5mM and 7.5mM of Fe^{2+} showed the maximum K^+ concentration of the minimum K^+ concentration in different tissues of rice varieties. Decreased in K^+ concentration with increasing Fe^{2+}/Na^+ was reported in rice (Aslam *et al.,* 1993; Li *et al*., 2001) and in cotton (Akhtar *et al*., 2001). At high K^+ supplies, increased root oxidizing power may have increased K uptake by decreasing Fe^{2+}/Na^+ uptake. The interaction between K and Fe^{2+}/Na^+ was reported concentration dependent (Li,*et al*., 2001).

Conclusion

The varieties showed a variable response to sodium and iron toxicities. *Pokkali* may possess both mechanisms for tolerance to iron and salinity toxicities; whereas, *Sk1* and *Firat* exhibited opposite responses to these toxicities as shown by growth traits and ion distribution patterns. These results reflected that tolerance mechanisms to these toxicities would be independent of each other. Uptake of K^+ is reduced with an increase in toxicity of sodium and iron. Therefore, availability of potassium and ability of the plant to uptake K^+ would minimize the effects of these toxicities.

Table 1. (a) Physio- chemical parameters of all rice varieties as affected by Na^+ toxicities

Variety	Stress	Physio-chemical parameters (ppm)					
		Na-R	Na-L	Na-FL	K-R	K-L	K-FL
SK1	Control	0.20±0.0	0.10±0.0	0.6±0.7	2.06± 0.0	3.20±0.0	3.42±0.0
	80mM	1.40±0.01	0.50±0.0	1.45 ± 0.3	1.21± 0.0	1.79±0.0	2.17±0.0
	120mM	1.80±0.0	1.47±0.0	1.45± 0.0	1.09± 0.0	1.30 ±0.0	1.84± 0.0
Pendras	Control	0.14±0.1	0.25±0.0	0.92±0.0	1.83±0.0	2.50±0.0	3.14±0.2
	80mM	0.97±0.0	1.7±0.2	1.46±0.1	1.02±0.0	1.39±0.0	1.85±0.0
	120mM	1.01±0.0	2.38±0.0	1.63±0.1	0.69±0.0	0.98±0.0	1.26±0.1
MR211	Control	0.18±0.0	0.09±0.0	0.06±0.0	1.79±0.0	2.68±0.0	2.56±0.2
	80mM	1.28±0.0	1.17±0.1	0.50±0.0	0.97±0.0	1.78±0.0	1.58±0.0
	120mM	1.59±0.0	1.90±0.1	0.85±0.0	0.48±0.0	1.43±0.1	1.02±0.3
Firat	Control	0.30±0.0	0.15±0.0	0.05±0.0	1.70±0.0	2.63±0.1	3.40±0.2
	80mM	0.39±0.0	0.68±0.0	0.58±0.0	1.39±0.0	1.75±0.0	2.20±0.1
	120mM	0.44±0.0	1.84±0.0	1.34±0.0	0.99±0.0	1.15±0.0	1.59±0.1
Pokkali	Control	0.65±0.0	0.06±0.0	0.03±0.0	1.34± 0.0	2.56±0.0	3.64±0.1
	80mM	0.81±0.0	0.48±0.0	0.16±0.0	1.11±0.0	1.96±0.0	2.50±0.0
	120mM	0.95±0.0	1.36±0.0	0.48±0.1	1.05±0.0	0.85±0.0	1.80±0.2

Mean ± Standard deviation. Na^+--R= Na^+--quantity in root, Na^+--L= Na^+-quantity in leaf, Na^+--FL= Na^+--quantity in flag leaf, K^+-R= K^+ quantity in root, K^+-L=K^+ quantity in leaf, K^+- FL=K^+ quantity in flag leaf.

At Na^+ toxicity level 120mM maximum Na^+ accumulations were 1.84ppm and 1.59 ppm in roots of *Sk1* and *MR211*, 1.63ppm and 1.45ppm in flag leaf of *Panderas* and *Sk1*, 2.38ppm and 1.9ppm in leaf *Panderas* and *MR211* respectively. While the lowest accumulations of Na^+ were 0.95ppm in root, 0.48ppm in flag leaf and 1.36ppm in leaf found in variety *Pokkali*. Increase in a Na^+ concentration resulting increase in toxicity was confirmed by findings of Nawaz *et al*. (1998) in wheat and Claudia *et al*. (1995) in rape seed.

Table 1. (b) Physio-chemical parameters of all rice varieties affected by Fe^{2+}

		Physio-chemical parameters (ppm)					
Variety	**Stress**	**Fe^{2+}-- R**	**Fe^{2+}--L**	**Fe^{2+}--FL**	**K^+-R**	**K^+-L**	**K^+-FL**
SK1	Control	1.23±0.2	0.02±0.0	0.01±0.0	1.43±0.2	2.76±0.0	2.56±0.4
	3.5mM	1.26±0.0	0.40±0.0	0.27±0.1	0.91±0.0	2.40±0.1	2.43±0.2
	7.5mM	1.70±0.1	0.63±0.1	0.36±0.1	0.87±0.0	2.20±0.1	2.03±0.1
Panderas	Control	1.13±0.1	0.02±0.0	0.01±0.0	1.40±0.2	2.23±0.1	2.70±0.0
	3.5mM	1.23±0.1	0.29±0.0	0.18±0.0	0.78±0.0	2.13±0.2	2.30±0.1
	7.5mM	1.86±0.3	0.39±0.0	0.38±0.0	0.70±0.0	1.63±0.0	1.93±0.0
MR211	Control	1.13±0.4	0.01±0.0	0.01±0.0	1.83 ±0.0	2.00±0.3	2.86±0.2
	3.5mM	1.31± 0.1	0.28±0.0	0.22±0.0	0.93±0.1	2.60±0.2	2.40±0.1
	7.5mM	1.41±0.1	0.51±0.0	0.50±0.0	0.94±0.0	2.10±0.1	2.10±0.2
Firat 1	Control	0.90±0.1	0.01±0.0	0.01±0.0	1.90±0.1	2.70±0.3	2.76±0.2
	3.5mM	1.16±0.1	0.20±0.0	0.17±0.0	1.30 ±0.0	2.30±0.0	2.60±0.3
	7.5mM	1.23±0.3	0.40±0.3	0.29±0.0	0.66±0.0	1.96±0.1	2.36±0.1
Pokkali	Control	0.94±0.0	0.01±0.0	0.01±0.0	1.18±0.1	2.93±0.3	2.93±0.0
	3.5mM	1.20±0.0	0.20±0.0	0.17±0.0	0.84±0.0	2.46±0.2	2.53±0.0
	7.5mM	1.50±0.2	0.32±0.0	0.23±0.0	0.62±0.0	2.22±0.1	2.50±0.1

Mean ± Standard deviation. Fe^{2+}--R= Fe^{2+}--quantity in root, Fe^{2+}--L= Fe^{2+}-quantity in leaf, Fe^{2+}--FL= Fe^{2+}--quantity in flag leaf, K^+-R= K^+ quantity in root, K^+-L=K^+ quantity in leaf, K^+- FL=K^+ quantity in flag leaf

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