



EFFECT OF POTASSIUM CHLORIDE ON GROWTH AND ACCUMULATION OF METAL MICRONUTRIENTS IN RICE SEEDLINGS GROWN HYDROPONICALLY

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Abstract: A greenhouse experiment was conducted to observe the effect of potassium chloride (KCl) on rice (*Oryza sativa* L. cv. Hitomebore). The plants were grown up to 14 days after treatments (DAT), with 0, 10, 20 and 30 mM KCl. It was observed that the rice seedlings were highly sensitive to KCl salinity, especially on producing toxicity symptoms and growth reduction. Iron concentration though increased significantly in shoot with increasing KCl concentration in the medium, it however, decreased in the root at the 30 mM KCl treatment. Manganese and Cu concentration decreased both in shoot and root at the 20 and 30 mM KCl treatments, however, Zn concentration decreased in shoot at the 20 and 30 mM KCl treatments. The concentration sensitivity of the metal micronutrients in shoot followed the general trends of Mn > Cu > Zn > Fe at the 20 and 30 mM KCl treatments. The K concentration increased as compared to the metal micronutrients with the increasing KCl in the medium.

Key words: Accumulation, metal concentration, necrosis, translocation

Introduction

Next to nitrogen (N), potassium (K) is considered as the 2nd largest mineral nutrient required by plants and 3rd most likely after N and phosphorus (P) to limit plant productivity (Brady and Weil, 2002). Potassium requirement for optimal plant growth is in the range of 2-5% of the plant dry weight (dw) of vegetative parts, fleshy fruits and tubers (Marschner, 1998). When the soil does not contain sufficient amount of K, KCl is the mostly used K fertilizer, which is known as muriate of potash to the common farmers. Generally, K remains as K⁺ ion in cell sap and acts as an activator for cellular enzymes. Over 80 different enzymes are activated for enzyme metabolism including starch synthesis, nitrate reduction, and photosynthesis. When the K supply is abundant 'luxury consumption' of K often occurs, which deserves attention for its possible interference with the uptake and physiological availability of Ca and Mg including other nutrient elements like Fe, Mn, Zn and Cu (Mengel and Kirkby, 2001).

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Potassium is the most important cation species regarding its concentration and physiological and biochemical functions in plant tissue. Generally, K^+ is taken up by root and translocated to the aerial parts of the plants. Root accumulates K^+ by plasma-membrane transporters, which are able to adjust quickly to the wide range of K^+ in soil solution (Maathuis and Sanders, 1997). This rapid rate of K^+ uptake is due to various K^+ uptake systems, mainly specific channels in the plasma-lemma and tonoplast of plant tissues. Hille (1996) reported that the K^+ channels are structurally the simplest in the super family of cation channels and are characterized by a short section in the pore region, which is responsible for the K^+ selectivity. Both outward and inwardly directing channels are present in which the K^+ flux follows the electrical potential gradient (Colombo and Cerana, 1991; Pottosin and Andjus, 1994). The activation of various enzymes is the main physiological function of K^+ . For example, the activity of starch synthetase is strongly activated by K^+ followed by Rb^+ , Cs^+ and NH_4^+ in sweet corn. The activity of K^+ is maximum in the concentration range of 40 to 80 mol m^{-3} in a *vitro* experiment (Mengel and Kirkby, 2001).

Chloride (Cl^-) ion is ubiquitous in nature and is highly soluble in water. It is thus, rarely deficient for plants in normal condition. Along with Mn, Cl^- is required for the oxygen evolving reactions in photosynthesis. The physiological function of Cl^- is to maintain electrical neutrality across the membranes and it is one of the principal somatically active solutes in the vacuole. It is also required for cell division in both leaves and shoots (Hopkins, 1995). Among the micronutrients, next to Fe, Cl is absorbed in large quantities by most agricultural crop plants (Brady and Weil, 2002). Beneficial effects of Cl on plant growth are common. This element helps to control several plant diseases such as stalk root in corn and "take all" in wheat. Toxic effects of Cl have also been noted. This element is an obligatory component of the most widely used K fertilizer (KCl). Consequently, when high rates of K are required for optimum plant growth, equally high rates Cl are applied. Such high levels are sometimes toxic to some plants, especially if they are of a Solonaceae family (tomato and the like). It is well known that when the soil solution contains high concentration of KCl it causes salinity problem. Salinity is the greatest problem in the coastal region of Bangladesh as the salinity is being increased gradually in Khulna and Chittagong Division of the country. Salinity has harmful effect on soil structure and plant growth. For example, when salinity is caused by K salt, it may remove other cations from the absorption sites by competition. There are many reports related to salinity and plant growth but the effect of elevated concentration of KCl on hydroponic rice has not been well documented. Therefore, the present research has been conducted to observe the effect of KCl on growth and metal micronutrients response of rice seedlings.

Materials and Methods

Seedling preparation and plant culture: Rice seeds were (*Oryza sativa* L. cv. Hitomebore) surface sterilized with 2% (w/v) chlorinated lime [$Ca(OCl)_2$] for 45 minutes rinsed with tap water continuously for 1 hour and soaked between moistened towels covered with wrapping paper at 25 °C for 72 hours in an electric incubator. Germinated seeds were placed on plastic net in the seed box containing 2 mM $CaCl_2$ solution in the greenhouse.

After 7 days, the chlorinated solution in the seed box was replaced with 1/2-strength modified Hoagland-Arnon nutrient solution for rice (Hewitt and Smith, 1975). This was done 2 times before starting the treatments. A total of 24 days were required for the seedling preparation. All plants were allowed to grow until 5th full leaf and some plants also contained 6th leaf at emergence stage. After 24 days, the seedlings were transplanted in bunches (5 plants were wrapped with sponge rubber to make one bunch) and 8 bunches were placed in one pot (capacity 5 liters) filled with full strength rice solution containing 10 μM Fe^{3+} -citrate. At this stage of the seedlings, the nutrient deficiencies or inhibitory effects were likely to be most apparent and therefore differences among the treatments would be easiest to observe. The treatments of KCl were 0, 10, 20 and 30 mM. The plants were grown in the greenhouse and the pH of the solutions was 5.5 at the time of solution preparation. In this pH (5.5) iron concentration together with other mineral elements are easily available for plant uptake. We did not adjust the pH and also did not maintain the water levels daily in the pot. The nutrient solutions were renewed every week during the study (July 22, 2005 to August 28, 2005).

Composition of full-strength nutrient solution: The composition of the full-strength nutrient solution were 1 mM NH_4NO_3 , 1 mM K_2SO_4 , 0.8 mM MgSO_4 , 0.5 mM CaCl_2 , 0.5 mM NaH_2PO_4 , 10 μM MnSO_4 , 1 μM CuSO_4 , 1 μM ZnSO_4 , 3 μM H_3BO_3 , 0.05 μM H_2MoO_4 and 10 μM Fe^{3+} -citrate.

Sample collection and preparation: After 14 DAT, 3 bunches were taken from each treatment for determining some growth parameters and Fe, Mn, Zn and Cu. The plants were washed with deionized water properly and separated into shoot and root with sterilized scissor and were dried for 48 hours at 55-60°C in an electric oven. The samples were cut into small pieces and digested with nitric-perchloric acid mixture (Piper, 1942) and analyzed.

Chemical analysis: Iron, Mn, Zn and Cu were determined with the Hitachi 170-30 atomic absorption spectroscopy (AAS).

Experimental design: The experiment was arranged in a completely randomized block design with three replications. Data were analyzed by analysis of variance (Anon, 1988).

Calculation procedure: Concentration of elements in plant tissue refers to the element present in one g of sample (mg or μg of the element g^{-1} dw) while accumulation refers to the specific element present per plant shoot or root (mg or μg of the element plant^{-1} shoot or root). Uptake is being considered as the total accumulation (accumulation in shoot plus accumulation in root). Translocation (%) is expressed in nutrient accumulation in shoot $\times 100 /$ total accumulation (shoot + root). Translocation factor (TF) is defined as the ratio of concentration of an element in shoot to that of root (Tu *et al.*, 2002).

Results

Visible symptoms: Necrotic symptom (burning of leaf tips and death associated with discoloration and dehydration of leaves) was visualized in the oldest leaves at the 30 mM KCl treatment. Younger leaf tip died and the severity was higher at the higher KCl treatments. The youngest leaves failed to unfold, leaf size (length and width) decreased

drastically and visible growth reductions were found at the 30 mM KCl treatment. Curling of the younger leaves followed by shriving occurred. The most common symptom, however, was growth reduction. Root length reduced significantly at the 30 mM KCl treatment, but increased at the 10 mM KCl.

Dry matter yield: Shoot and root growth decreased at the highest KCl treatment. However, at 10 mM KCl, root growth increased as compared to the highest KCl solution. The highest shoot dry weight was recorded for the control and the lowest was for the 30 mM KCl treatments, respectively. Shoot dry matter decreased by 6, 19, and 27% in the 10, 20 and 30 mM KCl treatments, respectively.

Effect of KCl on K concentration in plant tissues: Potassium concentration values were 35.9, 62.8, 76.9 and 92.9 mg g⁻¹ dw in shoot, but in root the values were 15.8, 17.8, 27.2 and 24.3 mg g⁻¹ dw in the 0, 10, 20 and 30 mM KCl treatments, respectively.

Effect of KCl on Fe concentration, accumulation and translocation: Iron concentration increased in shoot significantly at the 10, 20 and 30 mM KCl treatments as compared to control (Table 1). Iron accumulation in shoot was not much affected by the KCl treatments (Fig. 1a). However, the

Table 1. Concentration ($\mu\text{g g}^{-1}$ dw) of different micronutrients in rice seedlings grown in different levels of KCl solution. Translocation (%) of nutrients from root to shoot of rice seedlings grown in nutrient solution with different levels of potassium chloride.

Treatment mM KCl	Fe		Mn		Zn		Cu	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
0	102 c	716 b	442 b	44.4 a	99.5 b	92.8 b	28.9 a	82.5 a
10	128 b	612 c	491 a	51.2 a	111 a	85.9 b	25.3 b	60.9 c
20	131 b	804 a	327 c	36.3 b	91.7 c	100 a	21.3 c	70.9 b
30	142 a	647 c	271 d	31.2 c	84.6 d	99.2 b	21.3 c	55.1 c

^a Means followed by the different letters in each column are significantly different ($p < 0.05$).

accumulation decreased in root at the 30 mM KCl treatment. Potassium chloride increased the Fe translocation in the highest concentration in this experiment (Table 2).

Effect of KCl on Mn concentration, accumulation and translocation: Manganese concentration increased in shoot at the 10 mM KCl treatment (Table 1). However, this concentration did not significantly increase the accumulation in the 10 mM KCl treatment (Fig. 1b). However, opposite results were obtained with the 20 and 30 mM KCl treatments in both shoot and root in this experiment, indicating high concentration of KCl decreased Mn concentration. Potassium chloride did not have any clear effect on Mn translocation (Table 2).

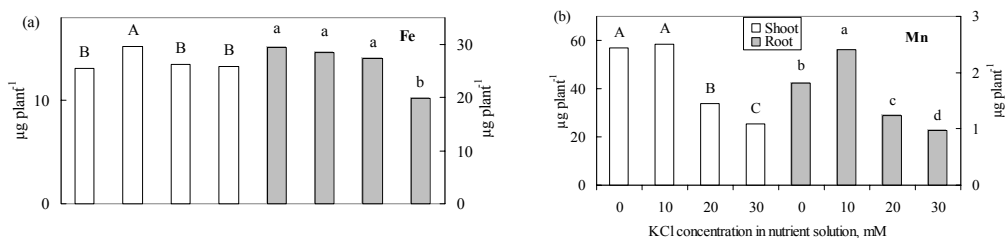


Fig. 1. Effect of KCl on the accumulation of (a) Fe and (b) Mn in shoot and root of rice seedlings grown in nutrient solution. Bars with the different letters are significantly different ($p < 0.05$) according to a Ryan-Einot-Gabriel-Welsch multiple range test.

Effect of KCl on Zn concentration, accumulation, and translocation: The concentration of Zn significantly decreased in shoot at the 20 and 30 mM KCl treatments, however, the concentrations were not much affected in root (Table 1). The accumulation of Zn was also decreased at the 20 and 30 mM KCl treatments (Fig. 2a) in both shoot and root. Potassium chloride did not have any significant effect on Zn translocation (Table 2).

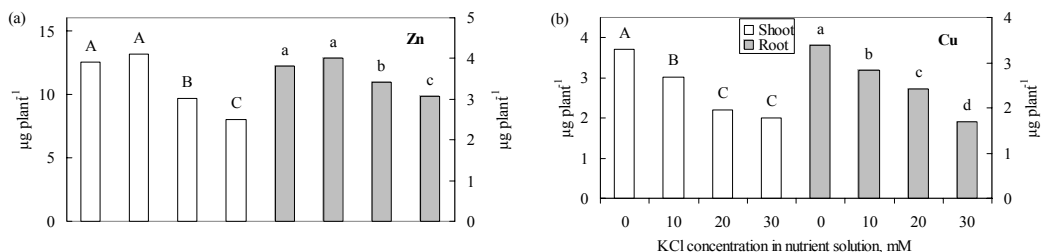


Figure 2. Effect of KCl on the accumulation of (a) Zn and (b) Cu in shoot and root of rice seedlings grown in nutrient solution. Bars with the different letters are significantly different ($p < 0.05$) according to a Ryan-Einot-Gabriel-Welsch multiple range test.

Effect of KCl on Cu concentration, accumulation and translocation: Potassium chloride decreased the concentration of Cu significantly both in shoot and root at all KCl treatments (Table 1). The highest and the lowest values were recorded in the control and in the highest KCl concentration in the medium, respectively. Not only concentration but also accumulation (Fig. 2b) was decreased at elevated KCl concentration. Potassium chloride did not have any significant effect on Cu translocation (Table 2).

Table 2. Translocation (%) of nutrients from root to shoot of rice seedlings grown in nutrient solution with different levels of potassium chloride.

Treatment mM KCl	Fe	Mn	Zn	Cu
0	31 b	97 a	77 a	52 a
10	35 b	96 a	77 a	51 a
20	33 b	96 a	74 a	48 a
30	40 a	96 a	72 a	54 a

^a Means followed by the different letters in each column are significantly different ($p < 0.05$).

Discussion

Visible symptoms: The necrotic and curling of leaf tip symptoms might be due to the inhibition of water movement from root to shoot at the higher concentration of KCl in the medium. Lack of water in the leaf tip might be associated with the burning of leaf tip. Discoloration of roots appeared and fell slippery to the touch, might be due to deterioration of the middle lamella by excess KCl, leading to root function disruption. Potassium chloride increased the root growth most probably due to the effect of chlorine (Brady and Weil, 2002) at low concentration. The result of the present study indicated that higher concentration of element resulted harmful physiological reactions in the plant cell.

Dry matter yield: The result indicated that the shoot of rice plants was more sensitive to KCl than that of root. It has already been reported that the enzymes of glycophytes and halophytes (Greenway and Osmond, 1972; Greenway and Munns, 1980) are inhibited by salt *in vitro* equally, resulting growth decreased. Only extremely halophylic bacteria have enzymes that are tolerant to high salinity. In glycophytes, Na⁺ salts are more toxic than K⁺ salts, while in halophytes, toxicity follows the general pattern: K₂SO₄ > KCl >

$\text{Na}_2\text{SO}_4=\text{NaCl}$ (Egan and Ungar, 1998). Protein synthesis in the leaves of soybean plants growing in NaCl saline condition is reduced might be due to chloride toxicity in sensitive species (Marschner, 1998).

Effect of KCl on K concentration in plant tissues: The increase of potassium concentration was most probably due the increase of KCl concentration in the medium. The result suggested that at elevated KCl concentration, the rice seedlings took up much amount of nutrients for osmoregulation.

Effect of KCl on Fe concentration, accumulation, and translocation: Increase in iron concentration in shoot might be concentration effect as the growth decreased much. Similarly, the increment of Fe concentration in root with the 20 mM KCl treatment was also concentration effect. Potassium might not compete with Fe during the translocation in the shoot tissues. Results reported on salinity effects on Fe are inconsistent; salinity increased the Fe concentration in the shoot of pea, tomato, soybean, squash and rice and decreased its concentration in the shoot of barley and corn (Grattan and Grieve, 1999). In this experiment, the concentration ratio of K/Fe increased with the increasing KCl concentration in the rooting medium (Table 3), indicating K concentration increased much more than the Fe. The KCl might decrease the concentration of active Fe in shoot, resulting lower chlorophyll formation. This speculation needs to be investigated in the future.

Table 3. Concentration ratio of K with metal micronutrients in shoot and in root of rice seedlings grown in nutrient solution with different levels of potassium chloride.

Treatment mM KCl	K/Fe		K/Mn		K/Zn		K/Cu	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
0	352 d	22 c	81 d	356 b	368 d	170 c	1242 d	192 d
10	491 c	29 b	128 c	348 b	566 c	207 b	2482 c	292 c
20	587 b	34 ab	235 b	749 a	821 b	272 a	3610 b	384 b
30	654 a	38 a	343 a	779 a	1098 a	245 ab	4362 a	441 a

^a Means followed by the different letters in each column are significantly different ($p=0.05$).

Effect of KCl on Mn concentration, accumulation, and translocation: Our experimental result suggested that KCl hindered Mn absorption but could not show antagonistic effect during translocation through xylem tube. The hydrated K ion (K^+) has a radius of 0.331 nm (Marschner, 1998) or 2.350~2.66Å (Biggin *et al.*, 2001). However, Mn^{2+} has the radius of 0.075 nm (Marschner, 1998) and it is therefore, possible that K can substitute or compete with Mn^{2+} in various reactions strongly as K has greater radius and both are cations, resulting much decrease of Mn concentration in shoot and root in the higher KCl treatments. Manganese uptake occurs in the form of Mn^{2+} presumably by facilitated diffusion across the plasmalemma (Fox and Guerinet, 1998) which might be affected by high KCl treatments resulting lower Mn concentration. The concentration ratio of K/Mn increased with the increasing KCl concentration in the medium (Table 3), indicating that K decreased Mn concentration.

Effect of KCl on Zn concentration, accumulation, and translocation: The increase in Zn concentration was most probably due to the concentration effect because the growth decreased much. Zinc is transported as Zn^{2+} across the plasma membrane of plant root (Mengel and Kirkby, 2001). Potassium is also taken up as K^+ cation; therefore, there is a high possibility to show antagonistic relationship between them during absorption and

translocation. Potassium and Zn are chemically similar and may be able to substitute Zn in many metabolic processes inside the plant body. Further, Cu^{2+} strongly inhibits Zn^{2+} uptake because these two ions species compete for the same uptake system (Mengel and Kirkby, 2001). However, in this experiment Cu was not added in high concentration without high KCl concentration in the medium. Therefore, the reduction of Zn concentration was most probably due to higher KCl concentration. For most plant species Zn concentrations in leaves below 10 to 15 $\mu\text{g Zn g}^{-1}$ dw are indicative of Zn deficiency and concentrations in the range of 20 to 100 $\mu\text{g Zn g}^{-1}$ are sufficient (Boehle and Lindsay, 1969). In this context, our experimental plants contained sufficient Zn in shoot tissues. The concentration ratio of K/Zn increased with the increase of KCl concentration (Table 3), indicating that K decreased Zn concentration.

Effect of KCl on Cu concentration, accumulation, and translocation: There is ample evidence to indicate the existence of a P-Cu interaction regarding the availability and uptake of Cu by the plant root (Loneragan and Asher, 1967). It is also well known that Cu absorption could be influenced by Zn cation (Bowen, 1969). In the experiment we did not use P or Zn in excess concentrating without KCl, therefore, the reduction of Cu concentration was most probably due to excess KCl concentration in the medium. We found not only concentration but also accumulation (Figure 3b) and translocation (Table 2) decreased by the excess KCl concentration. Reduction of Cu accumulation was most probably due to growth reduction by the excess KCl in the medium. It has already been reported that the concentration of Cu decreased in salt-stressed maize in soil (Rahman *et al.*, 1993) and in hydroponic (Izzo *et al.*, 1991) culture. However, opposite result was also obtained for tomato in hydroponic culture (Grattan and Grieve, 1999). These dissimilarities were most probably due the difference of plant species. The concentration ratio of K/Cu increased with the increasing KCl concentration (Table 3), indicating that KCl decreased Cu concentration in rice.

Conclusion

Potassium chloride toxicity produced severe necrotic symptoms in the old leaves and discoloration in the youngest leaves of rice seedlings in hydroponic culture. Discoloration produced by excess KCl might be due to lack of chlorophyll formation in leaves although potassium chloride increased Fe concentration in shoot, which might be due to inactivation of Fe in shoot by the elevated KCl. The concentrations and accumulations of Mn, Zn and Cu decreased in shoot at high KCl concentrations in the medium used in this experiment. The translocation of Fe increased from root to shoot while Mn, Zn, and Cu translocation did not significantly change with increasing KCl concentration.

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