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TISSUE CONCENTRATION AND TRANSLOCATION RESPONSES OF RICE CULTIVARS TO APPLIED Cd IN HYDROPONIC STUDIES

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ABSTRACT

Four rice varieties, IRRI-6, KS-282, Super basmati and Shaheen basmati were investigated for growth response to Cd application and its translocation from roots to shoots. The test varieties included. The studies were conducted under hydroponic conditions with Cd application rates of 0, 10, 14, 18, 22 and 26 ppm. The cv. Shaheen Basmati produced statistically the highest root and shoot weights followed by KS-282, IRRI-6 and Super Basmati. Shoot Cd was low in KS-282 and Shaheen basmati. The translocation factor of Cd was recorded to be higher in Super basmati than the other cultivars.

Keywords: cadmium contamination, rice, cultivar, tissue concentration, translocation factor.

INTRODUCTION

The sewage water contains heavy metals like Cd, Pb, Cr, Ni, Hg and Fe, continuous application of which is contaminating soils, crops and surface as well as ground waters that warrant an immediate attention (Yagdi *et al.*, 2000). Cadmium is one of the most toxic metal pollutants in waters, soils and plants (Sanita di Toppi and Gabrielli, 1999). Generally, Cd is generated in waste material from metal mining, manufacture and disposal, use of Cd-containing rock phosphate based fertilizers, pesticides, sewage sludge and smelter dust (Angelova and Atanassov, 1993). Plants often accumulate large quantities of Cd without toxicity symptoms (Lehoczky *et al.*, 1998), which enters into food chain that may endanger human health. Its high mobility in soil-water-plant system does help easy Cd entry into food chain.

Rice is the staple food after wheat in the human diet but, it is an important cash crop in Pakistan. Out of 76.61 mha of geographic area of Pakistan, rice is grown on about 2.3 mha with production of about 318.2×10^6 tons. In many regions, paddy rice is heavily exposed to Cd. The human renal tubular dysfunction is related to soil Cd in subsistence rice farm families in Asia (Chaney *et al.*, 2005) which is currently considered risk for food safety.

In many polluted agricultural lands, demand and pressure to produce foodstuff are so high that farmers can not afford to fallow agricultural soils for remediation. Thus, using cultivars those have low metal accumulation potential could provide an option for farmers to cope with such a risk, and to reduce the influx of pollutants into human food chain. Many attempts have been made to identify genotypes that could have potential for growing on metal contaminated soils with low metal contents in economical produce (Belimov *et al.*, 2003; Cheng *et al.*, 2006; Yu *et al.*, 2006). Since, cultivars of the same crop species differ widely in their response to heavy metal stress, the most appropriate option is to select one having the genetic architecture to accumulate low Cd in edible parts. Be selected (Liu *et al.*, 2003, 2007; Yu *et al.*, 2006). Genetic differences among plant types for Cd absorption and accumulation in grains have been observed recently (Liu *et al.*, 2007). Thus solution culture studies were conducted with the objective to investigate the varietal differences for Cd concentration and translocation in rice, so that the most appropriate ones could be referred to farmers and plant breeders.

MATERIALS AND METHODS

These studies were conducted in the greenhouse, Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, during June 2007 and 2008. Four varieties of rice, IRRI-6, KS-282, Super Basmati and Shaheen Basmati were tested in this study. The seeds of the above mentioned crop varieties were grown in polythene lined trays containing acid pre-washed sand. Nursery was irrigated with distilled water. Two-week old seedlings of uniform size were transplanted into tubs containing foam-plugged holes in thermo pore sheets floating on continuously aerated half strength Johnson's solution. The Cd treatments were applied once 3 weeks after transplanting. Cadmium concentration created in growth medium was 0, 10, 14, 18, 22 and 26 mg L^{-1} using $CdCl_2.2.5\ H_2O$ salt. The pH of solution in all the tubs was maintained daily at 6.0 ± 0.5 with HCl or NaOH. Plants were harvested after 14-18 days of treatment application. Plant root and shoot samples were washed with acidified water followed by washing with distilled water. After being ground in a Wiley mill fitted with stainless steel blades, plant (roots and shoots) samples were subjected to Cd determination using standard procedure (AOAC, 1990).

Samples were oven dried to constant weight at 65°C in an oven. Then root dry matter (RDM) and shoot dry matter (SDM) were recorded. The diacid plant extract was used to measure Cd concentration in plant roots and shoots with the help of atomic absorption spectrophotometer having Cd lamp in place. Translocation factor for Cd in plants was determined by using the ratio of shoot- to root- Cd concentration (Liu *et al.* 2007). The data were analyzed following analysis of variance technique and Least Significant Difference (LSD) test (Steel and Torrie, 1980) was applied, using M-STATC

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version 1.10 computer software packages, to differentiate the varietal growth responses, Cd concentration and translocation.

RESULTS AND DISCUSSIONS

Root dry weight

Cadmium application rates (T) and varieties ((V) influenced the root dry weight significantly but interaction, T x V remained statistically similar (Table-1).

The rates of Cd application had inverse relation with root dry weight. Maximum root dry weight was recorded with the control treatment (1.442 g plant^{-1}) which gradually decreased with Cd application @ 10, 14, 18, 22 and 26 mg L^{-1}. Shaheen Basmati produced the highest dry root weight followed by KS-282, IRRI-6 and Super Basmati.

Table-1. Dry weight of rice roots (g plant^{-1}) as affected by Cd application rates in nutrient solution.

Cd (mg L^{-1})	**Variety**				**Mean**
	IRRI-6	**KS-282**	**Super basmati**	**Shaheen basmati**	
0	1.431	1.441	1.428	1.470	1.442 A
10	1.091	1.157	1.029	1.144	1.105 B
14	1.057	1.105	1.002	1.070	1.058 B
18	0.970	0.939	0.853	0.960	0.930 C
22	0.775	0.868	0.789	0.918	0.838 D
26	0.631	0.667	0.584	0.813	0.673 E
Mean	0.992 BC	1.030 AB	0.947 C	1.062 A	

LSD: Treatment (T) = 0.06, variety (V) = 0.05, T x V = NS

Toxicity to rice roots might have resulted from the binding of Cd to sulphydryl groups in proteins leading to an inhibition of activity or disruption of cell walls (Van Assche and Clijters, 1990). Root growth against Cd stress has been considered a reasonably good parameter to study the relative tolerance or sensitivity of plants (Benavides *et al.*, 2005). Root growth of rice has been reported as more sensitive point of metal availability than chlorophyll assays (Morgan *et al.*, 2002). The applied Cd could easily penetrate into roots through cortical tissues (Yang *et al.*, 1998). In all the soil conditions, like other ions Cd enters first into roots, and consequently roots experience Cd damage first (Sanita di Toppi and Gabrielli, 1999). However, despite the different mobility of metal ions in plants, Cd concentration is generally greater in rice roots than in the above ground tissues (Ramos *et al.*, 2002). In the present studies, reduction in growth of rice roots with increasing application rate of Cd indicates sensitivity to Cd stress as reported by Liu *et al.* (2004, 2007), Diao *et al.* (2005) and Yu *et al.* (2006). However, relating root growth to heavy metal tolerance (Macnair, 1990) it was concluded that it is difficult to distinguish the interaction of root growth genes with genes inducing tolerance to heavy metal in genotypes.

Shoot dry weights

Cadmium application rates and varieties affected significantly the shoot dry weight but their interaction effect was not statistical (Table-2).

Table-2. Dry weight of rice shoots (g plant^{-1}) as affected by application rates of Cd in nutrient solution.

Cd (mg L^{-1})	**Variety**				**Mean**
	IRRI-6	**KS-282**	**Super basmati**	**Shaheen basmati**	
0	4.167	4.500	4.100	4.700	4.367 A
10	3.667	4.133	3.600	4.000	3.850 B
14	2.800	3.567	2.667	3.800	3.208 C
18	2.700	3.200	2.533	3.667	3.025 C
22	2.067	2.967	1.933	3.567	2.633 D
26	1.533	2.200	1.567	2.700	2.000 E
Mean	2.822 C	3.428 B	2.733 C	3.739 A	

LSD: Treatment (T) = 0.23, variety (V) = 0.18, T x V = NS

Maximum weight was recorded for the control treatment (4.367 g plant^{-1}) which decreased with Cd application @ 10, 14, 18, 22 and 26 mg L^{-1}. Shaheen Basmati produced statistically the highest shoot dry weight followed by KS-282, IRRI-6 and Super Basmati.

Stomatal opening, transpiration and photosynthesis are mostly affected adversely by cadmium absorbed by plants (Benavides *et al.*, 2005). Cadmium could also interfere with the uptake, transport and use of several other essential elements (Ca, Mg, P, K) and water by plants (Das *et al.*, 1997), even could reduce absorption of nitrate, its transport from roots to shoots, inhibit nitrate reductase activity in shoots (Hernandez *et al.*, 1996), alter functioning of membranes by inducing lipid peroxidation (Fodor *et al.*, 1995), and disturb chloroplast metabolism by inhibiting chlorophyll synthesis and reduce the activity of enzymes involved in CO_2 fixation (De Filippis and Ziegler, 1993). The adverse effects of Cd on these functions of plants combine to adversely affect the shoot mass production as has been recorded in the present studies.

Literature unveils a fascinating point that good root growth of rice against Cd may be a good feature but at the same time, good root activity may lead to more Cd absorption (Macnair 1990; Wu *et al.* 1999), which could be translocated and accumulated in shoots that might be lethal. In other words, good root growth under Cd stress is not enough for a plant or cultivar to be regarded as tolerant/resistant to Cd but in reality it is the plant control on Cd movement to above ground edible parts for safe food production. Shaheen Basmati and KS-282 showed good relative root growth under Cd stress (Table-1) but attained better shoot growth as well (Table-2) as compared to other two varieties.

Root Cd concentration

Varieties, application rates of Cd and interaction between T x V significantly affected the root Cd concentration (Table-3).

Table-3. Root Cd (mg kg^{-1}) in rice as affected by application rates of Cd in nutrient solution.

Cd (mg L^{-1})	Variety				Mean
	IRRI-6	KS-282	Super basmati	Shaheen basmati	
0	3.427 j	3.263 j	3.717 j	3.747 j	3.538 F
10	2059.0 g	1820.0 h	1483.0 i	2104.0g	1866.0E
14	2453.0 f	2663.0cdef	1674.0 hi	2213.0g	2251.0D
18	2597.0def	2640.0def	2493.0 ef	2517.0ef	2562.0C
22	2821.0 cd	2891.0 bc	2587.0def	2706.0cde	2751.0B
26	3080.0 b	3654.0 a	2703.0cde	3076.0b	3128.0A
Mean	2169.0 B	2278.0 A	1824.0 C	2103.0B	

LSD: Treatment (T) =103.70, Variety (V) = 84.64, T x V = 207.00

Minimum root Cd (3.538 mg kg^{-1}) was recorded for the control treatment which increased with Cd application @ 10, 14, 18, 22 and 26 mg L^{-1}. At 14 mg Cd L^{-1} or higher rates, root Cd concentration was recorded in the decreasing order of KS-282 > IRRI-6 > Shaheen Basmati > Super Basmati.

Wu *et al.* (1999) narrated that the amount of Cd absorbed by rice had significant positive relationship with the biomass and the grain yields of rice. Liu *et al.* (2003) discovered that Cd tolerant rice varieties initially absorbed Cd into roots because of their higher root activity and more water-consumption per gram of grain. Later the tolerance character controlled translocation and accumulation of less Cd in the above ground (edible) parts. Similar results were reported by Liu *et al.* (2004, 2007), Diao *et al.* (2005) and Yu *et al.* (2006).

Shoot Cd concentration

The Cd application, rice varieties and the interaction T x V affected statistically the shoot Cd concentration (Table-4).

Table-4. Shoot Cd concentration (mg kg^{-1}) in rice as affected by application rates of Cd in nutrient solution.

Cd (mg L^{-1})	Variety				Mean
	IRRI-6	KS-282	Super basmati	Shaheen basmati	
0	3.840 k	3.792 k	3.793 k	4.952 k	4.094 F
10	107.1 j	106.6 j	110.4 j	102.6 j	106.6E
14	144.8 h	130.7 i	150.4 h	126.8 i	138.2D
18	199.5 e	183.9 f	207.1 de	168.0 g	189.7 C
22	223.9 c	215.4cd	220.2 c	201.5 e	215.3 B
26	272.0 a	253.5 b	270.8 a	219.6 c	254.0 A
Mean	158.5 A	149.0 B	160.4 A	137.2 C	

LSD: Treatment (T) = 4.38, variety (V) = 3.58, T x V = 8.76

Minimum Cd was recorded in control plants which increased with Cd application @ 10, 14, 18, 22 and 26 mg L^{-1}. At 10 mg Cd L^{-1} application, there was no significant difference among varieties. But at higher Cd application rates, shoot Cd was the highest in Super Basmati followed by IRRI6, KS-282 and Shaheen Basmati.

The Cd concentration in roots and shoots (Tables 3 and 4) of rice varieties help to conclude that KS-282 and Shaheen Basmati initially absorbed more Cd from the same contaminated solutions due to their better root growth (Table-1) but later these varieties allowed comparatively less Cd translocation into shoots than that in Super Basmati and IRRI-6. Another inference can be derived that salt tolerant nature of Shaheen basmati might have also helped acquire less shoot Cd than Super basmati. This aspect needs more investigations before drawing final conclusion.

Translocation factor for Cd

Translocation of Cd varied statistically among rice varieties, Cd rates and their interaction (Table-5).

Table-5. Translocation factor of Cd in rice as affected by application rates of Cd in nutrient solution.

Cd (mg L^{-1})	Variety				Mean
	IRRI-6	KS-282	Super basmati	Shaheen basmati	
0	1.120 b	1.167 b	1.017 c	1.323 a	1.157 A
10	0.052 d	0.060 d	0.074 d	0.049 d	0.059 B
14	0.059 d	0.049 d	0.090 d	0.057 d	0.063 B
18	0.077 d	0.067 d	0.083 d	0.067 d	0.074 B
22	0.079 d	0.075 d	0.085 d	0.074 d	0.078 B
26	0.088 d	0.069 d	0.100 d	0.071 d	0.082 B
Mean	0.244 B	0.248 B	0.241 B	0.274 A	

LSD: Treatment (T) = 0.03, Variety (V) = 0.02, T x V = 0.05

Rates of Cd application among themselves differed nonsignificantly for each cultivar. Among rates of Cd applications, it was the lowest with Cd @ 10 mg L^{-1} which increased with application rate. As Cd application increased from 10 to 26 mg L^{-1}, Super Basmati shoots acquired more Cd followed by IRRI-6, Shaheen Basmati and KS-282. At 26 mg Cd L^{-1}, the translocation factor was minimum in KS-282 followed by Shaheen Basmati, IRRI-6 and Super Basmati.

It may be concluded that applied Cd stress brought about decrease in root and shoot weights of all the four rice cultivars in the decreasing order of Super Basmati > IRRI-6 > KS-282 > Shaheen basmati. Regarding the root Cd concentration, rice varieties ranked in the decreasing order of KS-282 > IRRI-6 > Shaheen basmati > Super Basmati. Super Basmati trans located more Cd to its shoots resulting in maximum shoot Cd concentration of 160.4 mg kg^{-1} followed by IRRI-6 (158.5 mg kg^{-1}), KS-282 (149.0 mg kg^{-1}) and Shaheen basmati (137.2 mg kg^{-1}). The information seem useful for rice breeders. It is inferred that shoots of salt tolerant Shaheen basmati (Shabbir *et al.*, 2001) and KS-282 (Khan and Abdullah, 2003) contained less shoot Cd perhaps through controlling its translocation compared to salt sensitive Super basmati (Ali *et al.*, 2006).

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