Pak. J. Bot., 42(4): 2747-2754, 2010.

EFFECT OF CADMIUM AND SALINITY STRESSES ON ROOT MORPHOLOGY OF WHEAT

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Abstract

A hydroponics experiment was conducted in the University of Zhejiang Hangzhou, China to investigate the effect of salinity (NaCl) and cadmium (Cd) on root morphology of different wheat cultivars differing in salt tolerance. Cd and NaCl stress inhibited shoot and root dry weight, number of root tips (lateral roots), total root length, root average diameter and total root volume. Increasing concentration of Cd and NaCl had negatively affected root morphology of all cultivars under study. The combined effect of Cd and NaCl on these parameters was more than both Cd and NaCl alone. There was an obvious difference in response to both stresses among the three cultivars with Bakhtawar-92 being less affected.

Introduction

Salinity reduces productivity of agricultural soils in large areas of the world. Salt stress affects plant physiology at both whole plant and cellular levels through osmotic and ionic stress (Hasegawa *et al*., 2000; Muranaka *et al*., 2002; Ranjbarfordoei *et al*., 2002). Salinity stress is known to affect various growth processes including photosynthesis, ion regulation, water relations etc. (Ashraf, 2004). It has been found that Cd uptake is enhanced when plants were grown in soils with a higher NaCl content (Weggler-Beaton *et al*., 2000). However, the detailed mechanism of interaction between salinity and heavy metals including Cd is not completely understood. The various physiological processes which are severely affected by salinity includes changes in plant growth, mineral distribution, membrane instability resulting from calcium displacement by sodium, membrane permeability (Gupta *et al*., 2002) and decreased efficiency of photosynthesis (Hasegawa *et al.,* 2000; Munns, 2002; Ashraf & Shahbaz, 2003; Kao *et al*., 2003; Sayed, 2003). Salt stress, in addition to the known components of osmotic stress and ion toxicity, also result in an oxidative stress (Hernandez & Almansa, 2002).

It has been well documented that NaCl and Cd stress in combination caused higher plasma membrane permeability and enhanced the production of oxygen radicals and H_2O_2 in wheat (Muhling & Lauchli, 2003). It has been found that Cd uptake is enhanced when plants were grown in soils with a higher NaCl content (Weggler-Beaton *et al*., 2000). In addition to salinity, heavy metal pollution of soils and surface water has increased especially in developing countries (Helal *et al*., 1999). Cadmium ion (Cd^{2+}) is taken up by plants through the root system, which thus is probably the first site of negative influence of these ions. The reaction of plants to heavy metal stress is also firstly expressed in root system (Kollmeier *et al*., 2000), and root system take the leadership in reacting to the heavy metal stress through modifying root system morphological and physiological characteristics. In soil the root system is the direct injured part caused by salinity and cadmium. Under these conditions root system morphology and physiology show the adaptive characteristics and behaviors of the plant in effectively absorbing and using nutrients in the soil. The rooting test is normally used to asses the influence of cadmium and salinity on wheat plants. Root growth was particularly sensitive to metals and salinity. The present research was initiated to investigate the effect of Cd and NaCl on root morphology of three cultivars differing in salt tolerance.

Materials and Methods

The experiment was conducted in the greenhouse of Huajiachi campus, Zhejiang University, Hangzhou, China, during 2007 using three wheat cultivars viz., Bakhtawar-92, Pir Sabak-85 and Khyber-87. Seeds were firstly surface sterilized with 0.1% H_2O_2 for 20 min, rinsed thoroughly with deionized water, then soaked overnight in sterile water at room temperature and germinated in sterilized moist quartz sand. At two leaf stage, plants were selected for uniformity and transplanted into plastic trays containing 30 L nutrient solution (Hoagland & Arnon, 1950), covered by a polystyrol plate with 48 evenly distributed holes with two seedlings per hole. Cadmium in the form $CdCl_2$ was added into the solution to obtain three Cd levels; 0, 2 and 4 μM and salinity was added as NaCl to three levels, 0, 75 and 150 mM. The experiment was arranged as a split split plot design with Cd as main plot factor, salinity as sub plots factor and cultivars as sub sub plot factor. There were three replications for each treatment. The nutrient solution was continuously aerated for 28 days and renewed after every 7 days. After 28 days treatment, plant was taken out from the plastic trays, and then washed with distilled water three times. These plants were then divided into below and the above ground parts. Fresh samples were put into the oven at 105ºC for 30 minutes, and then kept at 80ºC for 24 h to get constant weight and measured the dry weight. Root tips (number of lateral roots), total root length, root average diameter and total root volume were determined with a root automatic scan apparatus (MIN MAC, STD I600), equipped with a commercial software package 4.1 Win RHIZO (Arsenault et *al*., 1995) offered by Regent Instruments, 2000 Company, USA.

Statistical analysis: All data are presented as mean values of three replicates. Data were analyzed statistically for analysis of variance (ANOVA) following the method described by Gomez & Gomez (1984). MSTATC computer software was used to carry out statistical analysis (Russel & Eisensmith, 1983). The significance of differences among means was compared by using Least Significant Difference (LSD) test (Steel & Torrie, 1980).

Results

Dry weight of shoot and root: The effect of Cd and NaCl in the nutrient solution resulted in significant ($p \leq 0.05$) reduction in dry weight of shoot and root relative to their control (Table 1). However, among cultivar difference in root dry weight was non-significant. Shoot and root dry weight decreased with increasing levels of Cd and NaCl in the nutrient solution. Non significant difference in shoot and root dry weight was observed between both levels of Cd (2, or 4 μM) when compared with significant ($p \leq 0.05$) difference between the two levels (75 or150 mM) of NaCl. Our results also indicated that the combined stresses of NaCl + Cd resulted in further reduction of shoot and root dry weight when compared with the individual effect of Cd and NaCl. Non significant ($p \geq 0.05$) difference was observed due to the treatment effect of cultivars on root dry weight when compared with the significant ($p \leq 0.05$) effect on shoot dry weight. However, Bakhtawar-95 was least affected due to its exposure to cadmium and salinity when compared with other cultivars.

Table 1. Shoot and root dry weight (g) of wheat cultivars as affected by cadmium and salinity.

Treatment		Shoot dry weight (g plant^{-1})			Root dry weight (g plant^{-1})		
Cd (µM)	NaCl (mM)	Bakhtawar-92	Pir Sabak-85	Khyber-87	Bakhtawar-92	Pir Sabak-85	Khyber-87
0	0	0.87	0.44	0.46	0.45	0.32	0.34
	75	0.44	0.31	0.41	0.20	0.25	0.16
	150	0.30	0.27	0.27	0.17	0.13	0.14
2	0	0.29	0.29	0.25	0.12	0.12	0.10
	75	0.26	0.24	0.19	0.11	0.11	0.09
	150	0.21	0.17	0.18	0.09	0..08	0.07
4	0	0.25	0.18	0.21	0.11	0.10	0.09
	75	0.19	0.15	0.24	0.10	0.08	0.08
	150	0.14	0.12	0.13	0.08	0.06	0.07
LSD (p≤o.o5)		0.091			0.090		
Between cultivars		s			ns		
Cd + NaCl		s			s		

s, ns represent significance and non significance at 5% probability level

Table 2. Root tips and total root length of wheat cultivars as affected by cadmium and salinity.

Treatment		Root Tips (no. of lateral roots)			Total root length (cm)		
Cd (µM)	NaCl (mM)	Bakhtawar -92	Pir Sabak-85	Khyber-87	Bakhtawar -92	Pir Sabak-85	Khyber-87
0	0	3008	2132	2747	1889	1579	1821
	75	1253	944	924	1125	1094	1027
	150	893	812	883	800	322	578
2	0	915	834	794	599	555	537
	75	712	716	673	578	452	465
	150	566	538	586	562	441	459
4	0	889	707	714	552	542	505
	75	566	538	613	437	384	417
	150	550	364	536	389	285	366
LSD (p≤0.05)		298.00			110.00		
Between cultivars		s			s		
Cd + NaCl		s			s		

s represent significance at 95% probability level

Root tips and total root length: Cd and NaCl significantly ($p \leq 0.05$) reduced number of root tips and total root length when compared with control (Table 2). Similarly, significant ($p \leq 0.05$) difference was also observed for these parameters among the three cultivars under study. Root tips and total root length decreased significantly with increasing concentration of Cd and NaCl when compared with control treatment. The interactive effect of Cd and NaCl was more pronounced when compared with the effect of Cd and NaCl alone. Bakhtawar-92 ranked first in term of highest number of root tips (lateral roots) and total root length when compared with Pir Sabak-85 and Khyber-87.

Table 3. Root average diameter and total root volume of wheat cultivars as affected by cadmium and salinity.

Treatment		Root average diameter (mm)			Total root volume (cm^3)		
Cd (µM)	NaCl (mM)	Bakhtawar-92	Pir Sabak-85	Khyber-87	Bakhtawar-92	Pir Sabak-85	Khyber-87
0	0	0.580	0.533	0.776	4.43	2.75	4.88
	75	0.543	0.531	0.548	2.65	2.42	3.91
	150	0.525	0.498	0..491	1.33	1.13	1.52
2	0	0.541	0.495	0.509	1.21	1.07	0.95
	75	0.513	0.483	0.457	1.17	0.84	0.91
	150	0.505	0.440	0.455	1.08	0.68	0.77
4	0	0.421	0.398	0.397	0.99	0.67	O.90
	75	0.373	0.410	0.395	0.69	0.55	0.82
	150	0.406	0.365	0.374	0.61	0.45	0.53
LSD (p≤o.o5)		0.052			0.374		
Between cultivars		s			s		
Cd + NaCl		s			s		

S represents significance at 95% probability level.

Root volume and average diameter: Exposure of wheat plants to Cd and NaCl stress resulted in significant ($p \leq 0.05$) decline in root average diameter and root volume (Table 3). The total root volume and root average diameter were evidently different for all the three cultivars at different levels of Cd and NaCl. The exposure of plants to Cd and NaCl showed significant ($p \leq 0.05$) effect when their concentration was increased from moderate to high level. Similarly, the combined effect of Cd and NaCl was more pronounced on total root volume and root average diameter when compared with the exposure of plants to Cd and NaCl alone. The effect of cultivars on total root volume and root average diameter was also significant ($p \leq 0.05$). Bakhtawar-92 and Khyber-87 showed almost similar response in term of high root volume and high root average diameter when compared with the Pir sabak-85.

Discussion

Dry weight of shoot and root: In this study, 28 days treatments with 2 µM, 4 µM Cd and 75 mM, 150 mM NaCl reduced dry weight (DW) of shoot and root of wheat cultivars. Root morphological changes under varied environmental conditions bring direct influences to the plant population structure, above ground parts and their biomass composition (Zhang *et al*., 2002; Liu *et al*., 2006). Increased NaCl and Cd stress probably caused increased portioning of assimilates towards the leaf at the expense of stem (Sifola & Postiglione, 2002). Our results are also consistent with those reported by research workers with other plants (Gohar *et al*., 2003). On the average, shoot dry weight reduced by almost two times in Cd and NaCl treated plants respectively. Lin & Kao (2001) attempted to determine the cost of salinity resistance, and assumed that it led to slower growth rate and lower biomass production, which is therefore a disadvantage compared with non resistant plants growing on uncontaminated soil. Plant growth is ultimately the direct result of massive and rapid expansion of the young cells produced by meristematic divisions. Moreover, cell expansion in both roots and leaves can be inhibited by salinity

(Hussain *et al*., 2004). The detrimental effects of Cd and NaCl on dry weight of shoot may be due to the direct effect on photosynthesis (Parida *et al*., 2003). NaCl and Cd caused reduction in photosynthesis through its adverse impact on gas exchange parameters such as stomatal conductance and photosynthetic rate. Our results are in agreement with earlier reports on halophyte as well as non halophytes (Netondo *et al*., 2004; El-Hendawy *et al*., 2005). Salinity has been identified as an important determinant of Cd in crops. It was suggested that the formation of chlorocomplexes increases the toxic effects of Cd with a subsequent effect on shoot and root dry weight (Weggler-Beaton *et al*., 2000). Cadmium and salinity generate oxidative stress in plants and the surrounding environment, leading to growth inhibition and even plant death. Oxidative stress is the phenomenon implicated as one of the main causes of cellular damage in all organisms exposed to a wide variety of stress conditions (Liu *et al*., 2006; Lin & Kao, 2001).

Root tips and total root length: Root tips and total root length decreased significantly with increasing concentration of Cd when compared with control treatment. In response to Cd and NaCl toxicity, roots can also respond *via* changes in surface area, volume and diameter, production, inhibition of lateral roots, root tips, total root length and variation in other morphological parameters. It has been revealed that Cd caused serious damage to the root tips in *Arabidopsis*, corroborating not only our results but also observation from other plant species (Schutzendubel *et al*., 2001). Ammonium inhibits the root growth of many plants. NaCl was effective in stimulating the accumulation of ammonium in roots and that accumulation of ammonium in roots preceded inhibition of root growth caused by NaCl (Jbir *et al*., 2001). NaCl inhibited root growth of rice seedlings is associated with increase in H_2O_2 level and cell wall POD activity (Lin & Kao, 2001) reported that $NaCl^-$ induced cell wall stiffening is a possible mechanism of NaCl inhibiting root growth of rice seedlings. Addition of both Cd and NaCl to nutrient solution caused severe toxicity to wheat plants when compared with the individual stress of Cd and NaCl. The combined stress of Cd and NaCl resulted in reduced biomass of shoots, roots and adverse impact on root morphological characteristics (total root length, root tips etc.). Significant difference in number of root tips (lateral roots) and total root length was due to cultivars. Bakhtawar -92 ranked first in term of highest number of root tips (lateral roots) and total root length when compared with Pir Sabak-85 and Khyber-87. Our results showed that roots of Bakhtawar-92 have great potential to tolerate Cd and NaCl toxicity from growth medium. However, an improvement in the root morphological parameters such as total root length and root tips of Bakhtawar-92 might be due to stimulated increased synthesis of cell wall polysaccharides to detoxify the higher Cd concentration *via* aggregating it mainly into cell walls. It was reported by An, (2004) that *Cucumis sativus* could retain greater amount of metals in the roots due to its root morphology.

Root average diameter and total root volume: The treatment of Cd and NaCl showed significant effect on root average diameter and total root volume. In response to toxicity of cadmium and salinity, roots responds through changes in term of root average diameter, root volume and other morphological parameters. These findings are consistent with the findings of Siroka *et al*., (2004). Growth inhibition was concentration dependant and exhibited a positive correlation with the reduction in the viability of root cells. The inhibition of root growth in term of average diameter and volume can be attributed in part to the inhibition of mitosis, reduced synthesis of cell wall components, damage to golgi apparatus and changes in the polysaccharide metabolism, while browning is caused by suberin deposits (Berkelaar & Hale, 2000). In many plant species, it has been observed that although cell division did not cease as the result of Cd treatment, the growth of cell was inhibited. The inhibition of cell growth after Cd treatment appeared to be caused by the formation of stronger cross binding between pectin molecules in the cell wall and by a reduction in the size of the intercellular space. Various types of stress, including of Cd, induce the incorporation of lignin in to cell wall of maize roots, with the result that cell wall rigidity increases and cell wall expansion is reduced (Degenhardt & Gimmler, 2000). Other specific effect of Cd is the microtubule damage observed in maize root cells, resulting in the inhibition of cell division (Eun *et al*., 2000). A decrease in root length density, root volume and root diameter under saline conditions may be due to decreased availability of photosynthates from the shoots and water stress and ion toxicity due to salts around the roots. A decrease in root length under saline conditions has also been reported earlier by Gohar *et al*., (2003). Hassan *et al*., (2008) reported that root of rice retained higher Cd concentration than stem, which resulted in reduced root weight. In maize Cd was found to inhibit the formation of lateral roots, while the roots became brown, rigid and twisted with reduced root diameter. There was also a reduction in coleoptile extension, accompanied by chlorosis and necrosis (Nocito *et al*., 2002).The effect of Cd and NaCl on root volume and root diameter was more pronounced for the cultivar of Pir Sabak-85. Roots of Bakhtawar-92 to some extent can tolerate Cd and NaCl and its root length, root volume and root diameter is a positive indicator. These results are consistent with the findings of Arisi *et al*., (2000) and Schutzendubel *et al*., (2001). They reported that Cd induced the generation of reactive oxygen species (ROS) and affected various toxicities in the cells, resulting in inhibition of plant growth and severely suppressed root elongation and root diameter. The ranking of three cultivars for Cd and NaCl concentration differed among different plant parts, suggesting genotypic difference in Cd and NaCl distribution. Cd and NaCl concentration inhibited root growth in term of shoot and root dry matter, total root length, root tips (# of lateral roots), root average diameter and total root volume. The combined stresses of NaCl and Cd has resulted further reduction in shoot and root dry matter, total root length, root tips (# of lateral roots), root average diameter, and total root volume when compared with stress of Cd and NaCl alone. The Cd and NaCl tolerated capacity of Bakhtawar-92 was higher than Khyber-87 and Pir Sabak-85. It is suggested that for future research, more cultivars should be selected for test and the range of NaCl and Cd concentration should be enlarged.

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(Received for publication 29 March 2009)