The interaction of salinity and chromium in the influence of barley growth and oxidative stress

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ABSTRACT

The effect of chromium and NaCl on growth and anti-oxidative enzymes in two barley genotypes differing in salt tolerance was investigated in a hydroponic experiment. Salinity stress reduced plant growth, photosynthetic rate and stomatal conductance, while increased SOD and POD activities, and MDA content in barley plants. CM72, a salt-tolerant genotype was less affected by salinity stress than Gairdner, a salt-sensitive genotype. The effect of Cr on plant growth and anti-oxidative enzymes varied with Cr level. Under low Cr level (10 μmol), plant growth inhibition and oxidative stress caused by salinity stress were generally alleviated, in particular for Garidner. The combined stress of high Cr level (50 μmol) and NaCl stress caused more severe oxidative stress, resulting in further reduction of plant growth parameters, photosynthetic rate and stomatal conductance as compared to two stresses alone.

Keywords: anti-oxidative enzymes; photosynthesis; genotype; plant biomass; lipid peroxidation

Salinity and chromium problem can arise simultaneously in soil and water. One such instance is inorganic ions containing Na^+ present in drainage water, limiting plant growth and yields. However, appreciable amount of trace elements, such as chromium (Cr), cadmium (Cd), selenium (Se) and nickel (Ni), may occur due to geochemical and artificial activities in soil (Deverel and Fujii 1990). Another instance is organic fertilizers such as biosolids and phosphorus fertilizers, extensively applied in saline soils to ameliorate soil quality. These fertilizers are known to contain considerable amount of chromium (Bini et al. 2000). Moreover chromium is used on large scale in industries, such as paints and pigments, wood preservation, tanneries, paper and pulp production, leading to increased Cr level in soil and water (Zayed and Terry 2003). Therefore, interaction of chromium and salinity in its influence on crop growth and yield formation should be taken into consideration and studied.

Salinity causes the growth inhibition by decreasing chlorophyll contents, disturbing net photosynthesis rate, stomatal conductance and nutrient imbalance (Qin et al. 2010). Activity of antioxidant enzymes, such as SOD and POD activity and lipid peroxidation (MDA) content distinctly increased in plants subjected to moderate salinity stress for barley (Liang 1999, Huang et al. 2006). The plants exposed to chromium stress were shown to limit growth and yield of plants by decreasing chlorophyll contents, photosynthesis rate and disturbing stomatal conductance (Ali et al. 2010a). It was demonstrated that chromium induced SOD, POD, and MDA in plants at higher concentration, such as in barley (Ali et al. 2010b).

Most researchers focus on response of plants to single stress but in nature plants often face multiple stresses, the interaction of which may be far from additive (Chapin et al. 1987). There are very few reports on the concurrent behavior of salinity and trace elements. It was reported that cadmium and salinity interaction affected antioxidant enzymes of barley plants (Huang et al. 2006). However, interaction of salinity and Cr stress on the growth, photosynthesis and antioxi-

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dant enzymes in crops has rarely been reported. Thus, it is of significant importance to determine the combined effects of salinity and Cr stress on the growth, photosynthesis and antioxidant enzymes of barley. In this study, a hydroponic experiment was conducted aiming at investigating single and combined effects of salinity and Cr on the growth, photosynthesis and antioxidant enzymes in two barley genotypes differing in salinity tolerance.

MATERIALS AND METHODS

Plant material, growth and treatment conditions. The experiment was conducted in a greenhouse of Huajiachi Campus, Zhejiang University (Hangzhou, China; 31°16/N, 120°12/E). Uniform seeds of two barley genotypes differing in salt tolerance (tolerant, CM 72 and sensitive, Gairdner) (Chen et al. 2007) were surface sterilized in a 3% H_2O_2 for 20 min, rinsed with distilled water 10 times. Then seeds were soaked in deionized water in the dark for 12 h and sown into moist quartz sand in a controlled chamber with photoperiod of 16 h light/8 h dark and light intensity of 225 ± 25 μmol/m^2/s. The light/dark temperatures were set at 22°C/18°C, and relative humidity was kept at 85%. When seedlings grew the second leaf (10 days old), they were selected for uniformity and transplanted into 5 L pots containing 4.5 L nutrient solution. The pot was covered with a polystyrol-plate with seven evenly spaced holes and placed in a greenhouse; in each hole two seedlings were located. The composition of the basic nutrient solution was used as described by Ali et al. (2010b). Half strength nutrient solution was applied for the first 4 days and then changed to full strength nutrient solution for 8 days. Thereafter, different amounts of $K_2Cr_2O_7$ were added to the nutrient solutions to form 3 Cr levels (0, 10, and 50 μmol) and NaCl to form 2 NaCl levels (0 and 150 mmol). Thus, combination of Cr and NaCl levels resulted in six treatments viz. NaCl 0 mmol-Cr 0 μmol, NaCl 0 mmol-Cr 10 μmol, NaCl 0 mmol-Cr 50 μmol, NaCl 150 mmol-Cr 0 μmol, NaCl 150 mmol-Cr 10 μmol, NaCl 150 mmol-Cr 50 μmol. The experiment was arranged as a split-split plot design with barley cultivars as main plot, salinity levels as subplot, and Cr levels as sub-subplot; each treatment had six replicates. The nutrient solution in the pots was continuously aerated with pumps and renewed every four days. The solution pH was adjusted to 6.5 with 1 mol/L NaOH or HCl, as required.

Plant harvest. After 25 days of treatment (37 days after transplanting), twenty-four barley plants in each treatment with three replications were harvested, washed thoroughly with distilled water and then separated into roots and shoots, dried in an oven at 80°C and weighed.

Determination of photosynthetic characters. The measurements were carried out on the topmost secondary fully expanded leaves. Chlorophyll concentration, expressed as SPAD value, was measured with a chlorophyll meter (Minolta Co. Ltd., Nobi, Japan). Photosynthetic parameters, including net photosynthetic rate (P_n) and stomatal conductance (G_s) were determined using a photosynthesis system (ADC Bio-scientific Ltd., Furlong Way, UK).

Extract preparation. The second fully expanded leaves and roots of the plants were sampled for enzymatic analysis at 7, 14, and 25 days after start of the treatment. Samples were homogenized in 0.05 mol/L phosphate buffer (pH 7.8) by grinding with a mortar and pestle under chilled condition with liquid nitrogen. The homogenate was filtered through four layers of muslin cloth and centrifuged at 12 000 × g for 10 min at 4°C, and the supernatants were used for measurements of SOD, POD activities and MDA concentration according to Zhang (1992).

Statistical analysis. All values reported in this study are mean of at least three replicates. The data were analyzed using a statistical package, SPSS version 16.0 (SPSS, Chicago, IL). A two-way variance analysis (ANOVA) was carried out, followed by the Duncan's multiple range test to determine the significant difference between means of treatments.

RESULTS

Plant height, biomass and tillers per plant. Plant height, biomass and tillers per plant of two barley cultivars, Gairdner and CM72 are presented in Table 1. NaCl addition inhibited growth of the two barley genotypes, leading to a significant reduction in all examined plant growth parameters. However, the reduction was more pronounced in Gairdner in than CM72. Cr at low level (10 μmol) did not affect or slightly increased plant height, biomass and tillers per plant, but at high level (50 μmol) significantly decreased these parameters as compared to the control. On combining with NaCl, Cr at low level (10 μmol) stimulated growth and alleviated NaCl stress, being more pronounced for Gairdner than for CM72, but at 50 μmol level caused further inhibi-

Table 1. The effect of salinity and chromium stresses on plant height, biomass and tillers per plant of different treatments

Treatment		Plant height (cm)	Dry root weight (g/plant)	Dry shoot weight (g/plant)	Tillers/plant
Genotype	CM72	35.22[a]	0.16[a]	0.39[a]	2.95[b]
	Gairdner	25.08[b]	0.11[b]	0.31[b]	3.80[a]
Salinity level (mmol)	0	36.06[a]	0.20[a]	0.49[a]	4.12[a]
	150	26.07[b]	0.08[b]	0.25[b]	2.90[b]
Cr level (μmol)	0	31.80[a]	0.15[a]	0.43[a]	3.75[a]
	10	33.98[a]	0.16[a]	0.48[a]	4.10[a]
	50	26.35[b]	0.12[b]	0.22[b]	2.68[b]
Interaction					
Genotype and salinity		**	**	ns	**
Genotype and Cr		**	ns	*	**
Cr and salinity		**	ns	**	**

The same letters after the data between genotypes or salinity levels and among Cr levels within a column mean no significant difference at 95% probability level. $P < 0.05$; $^{**}P < 0.01$; ns – non significant

tion of these growth parameters as compared to the salinity stress alone.

Chlorophyll concentration (SPAD value). Exposure of plants to NaCl resulted in a significant decrease of SPAD value in Gairdner but no significant difference was observed in CM72 (Table 2). Cr addition in the growth medium, at low level (10 μmol) had no significant effect on SPAD value but at high level (50 μmol) a significant reduction of SPAD value could be detected. Combined NaCl and Cr stresses resulted in a slight increase in SPAD value at low Cr level (10 μmol), while at 50 μmol a higher reduction was noted as compared to NaCl or Cr 50 μmol alone.

Photosynthetic rate (P_n) and stomatal conductance (G_s). Photosynthetic rate and stomatal conductance of two barley cultivars are presented in Table 3. P_n and G_s decreased significantly when plants were exposed to salinity stress for the two genotypes. CM72 had consistently higher P_n and

Table 2. The effect of salinity and chromium stresses on SPAD values of different treatments

Treatment		SPAD value		
		7 days	14 days	25 days
Genotype	CM72	36.2[a]	37.0[a]	34.7[a]
	Gairdner	34.8[a]	37.5[a]	34.9[a]
Salinity level (mmol)	0	36.6[a]	38.6[a]	36.8[a]
	150	34.4[a]	36.0[a]	32.9[b]
Cr level (μmol)	0	36.9[a]	39.0[a]	38.8[a]
	10	36.6[a]	39.1[a]	39.1[a]
	50	33.1[b]	33.7[b]	26.6[b]
Interaction				
Genotype and salinity		ns	ns	*
Genotype and Cr		ns	ns	ns
Cr and salinity		ns	ns	ns

The same letters after the data between genotypes or salinity levels and among Cr levels within a column mean no significant difference at 95% probability level. $^{*}P < 0.05$; $^{**}P < 0.01$; ns – non significant

Table 3. The effect of salinity and chromium stresses on net photosynthetic rate (P_n) and stomatal conductance (G_s) of the different treatments

Treatment		G_s [mol/(m²s)]			P_n [μmol CO_2/(m²s)]		
		7 days	14 days	25 days	7 days	14 days	25 days
Genotype	CM72	0.13[a]	0.15[a]	0.13[a]	4.51[a]	6.24[a]	9.93[a]
	Gairdner	0.13[a]	0.12[b]	0.11[a]	3.65[a]	5.78[a]	7.67[b]
Salinity level (mmol)	0	0.20[a]	0.20[a]	0.18[a]	5.95[a]	7.97[a]	11.90[a]
	150	0.06[b]	0.08[b]	0.05[b]	2.22[b]	4.05[b]	5.70[b]
Cr level (μmol)	0	0.14[a]	0.16[a]	0.13[a]	4.50[a]	6.91[a]	10.02[a]
	10	0.16[a]	0.16[a]	0.14[a]	4.80[a]	6.79[a]	10.39[a]
	50	0.09[b]	0.09[b]	0.09[b]	2.94[b]	4.34[b]	5.99[b]
Interaction							
Genotype and salinity		ns	*	ns	ns	ns	**
Genotype and Cr		ns	ns	ns	ns	ns	ns
Cr and salinity		*	ns	*	ns	*	*

The same letters after the data between genotypes or salinity levels and among Cr levels within a column mean no significant difference at 95% probability level. *$P < 0.05$; **$P < 0.01$; ns – non significant

G_s than Gairdner, irrespective of duration of stress. Under low Cr level, a slight increase was found, but at high Cr level, a significant decrease was noted for both genotypes. Moreover, the effect of Cr treatment on P_n and G_s of barley plants under NaCl stress varied with genotype, time and Cr level. At low Cr level, the values of the two parameters increased slightly relative to the NaCl stress alone, but at high Cr level, both P_n and G_s decreased significantly as compared to NaCl and 50 μmol Cr stress alone. Overall less reduction was observed in CM72 than in Gairdner.

Enzyme activity. As shown in Table 4, a significant increase in SOD activity under NaCl stress was observed as compared to the control, irrespective of plant organs and genotype. Comparatively,

Table 4. The effect of salinity and chromium stresses on SOD (U/g FW) activity of the leaves and roots of the different treatments

Treatment		Leaves			Roots		
		7 days	14 days	25 days	7 days	14 days	25 days
Genotype	CM72	201.6[a]	208.9[a]	228.5[a]	185.5[a]	191.4[a]	208.0[a]
	Gairdner	184.4[a]	192.9[a]	208.1[a]	173.8[a]	180.6[a]	190.1[b]
Salinity level (mmol)	0	176.0[b]	184.4[b]	196.3[b]	165.5[b]	170.4[b]	178.5[b]
	150	209.9[a]	217.3[a]	240.4[a]	193.8[a]	201.6[a]	219.5[a]
Cr level (μmol)	0	172.6[b]	181.8[b]	195.1[b]	165.0[b]	170.9[b]	178.4[b]
	10	166.0[b]	172.8[b]	186.7[b]	162.1[b]	166.3[b]	172.8[b]
	50	240.4[a]	248.0[a]	273.1[a]	211.8[a]	220.9[a]	245.9[a]
Interaction							
Genotype and salinity		ns	**	**	**	ns	**
Genotype and Cr		ns	ns	**	**	**	**
Cr and salinity		ns	**	**	ns	*	*

The same letters after the data between genotypes or salinity levels and among Cr levels within a column mean no significant difference at 95% probability level. *$P < 0.05$; **$P < 0.01$; ns – non significant

Table 5. The effect of salinity and chromium stresses on POD (nmol/g FW/min) activity of the leaves and roots of the different treatments

Treatment		Leaves			Roots		
		7 days	14 days	25 days	7 days	14 days	25 days
Genotype	CM72	112.8[a]	121.6[a]	133.6[a]	570.4[a]	646.5[a]	679.0[a]
	Gairdner	99.6[a]	111.0[a]	118.9[b]	526.2[a]	573.0[b]	590.9[b]
Salinity level (mmol)	0	93.2[b]	102.9[b]	110.9[b]	466.7[b]	508.4[b]	506.6[b]
	150	119.2[a]	129.7[a]	141.6[a]	629.9[a]	711.0[a]	763.3[a]
Cr level (μmol)	0	95.3[b]	105.5[b]	114.2[b]	503.7[b]	568.5[b]	597.8[b]
	10	90.0[b]	101.1[b]	107.1[b]	476.5[b]	546.4[b]	561.8[b]
	50	133.3[a]	142.2[a]	157.5[a]	664.7[a]	714.2[a]	745.3[a]
Interaction							
Genotype and salinity		**	*	**	**	**	**
Genotype and Cr		ns	ns	**	ns	ns	ns
Cr and salinity		**	ns	**	ns	ns	**

The same letters after the data between genotypes or salinity levels and among Cr levels within a column mean no significant difference at 95% probability level. *$P < 0.05$; **$P < 0.01$; ns – non significant

CM72 showed higher and earlier increase than Gairdner when exposed to NaCl stress. A slight decrease of SOD activity was observed in plants subjected to 10 μmol Cr when compared with the control. However, the plants grown in high Cr level showed a significant increase in either plant parts of the two genotypes. Low Cr level under NaCl stress resulted in a decrease of SOD activities, irrespective of plant parts and genotype. However, the combined treatment of high Cr level and NaCl stress caused a significant increase of SOD activity in both roots and leaves of the two genotypes relative to NaCl or 50 μmol Cr alone, with CM72 having a higher increase than Gairdner.

Similarly, NaCl stress caused a significant increase in POD activities of both plant parts for the two genotypes; it was higher with expanding exposure time (Table 5). CM72 showed a more rapid increase and higher values than Gairdner. Low Cr level had no significant effect on POD activity. However, high Cr level caused a marked increase of POD activities, irrespective of plant part, exposure time and genotype. Low Cr level under NaCl stress led to a slight decrease of POD activity in CM72 at 7, 14, and 25 days. But in case of Gairdner at 25 days a significant decrease was found. The combined treatment of high Cr level and NaCl caused a significant increase of POD activity in CM72 as compared to NaCl or 50 μmol Cr stresses alone. But in case of Gairdner there was no significant difference between the combined stress and 50 μmol Cr alone, although the difference was significant between the combined stress and NaCl stress alone.

MDA content. MDA contents in the two barley cultivars are presented in Table 6. Exposure of barley plants to NaCl resulted in a significant increase of MDA content in either plant parts, with Gairdner increasing more than CM72. At low Cr level, MDA content in both roots and shoots was slightly reduced, while at high Cr level it was markedly increased. Low Cr level under NaCl stress slightly reduced MDA content in roots of either genotypes and leaves of CM72 relative to NaCl alone. In case of Gairdner, MDA content in leaves was significantly reduced at 14 and 25 days. The combined stresses of high Cr level and NaCl caused a marked increase of MDA content relative to the treatment of Cr stress alone, with Gairdner having a higher increase than CM72.

DISCUSSION

Salinity exposure can lead to various biochemical and physiological changes in plants. In the present study, salinity reduced plant height and biomass, stomatal conductance and photosynthetic rate in barley plants. Moreover the reduction varied with the genotype, and salt-sensitive genotype Gairdner showed a higher reduction, confirming the findings of the previous reports (Chen et al.

Table 6. The effect of salinity and chromium stresses on MDA (nmol/g FW) concentration of the leaves and roots of the different treatments

Treatment		Leaves			Roots		
		7 days	14 days	25 days	7 days	14 days	25 days
Genotype	CM72	15.5^{b}	17.8^{b}	21.7^{a}	16.3^{a}	19.2^{a}	22.7^{b}
	Gairdner	21.0^{a}	21.2^{a}	24.8^{a}	18.2^{a}	21.0^{a}	26.4^{a}
Salinity level (mmol)	0	14.3^{b}	13.5^{b}	16.5^{b}	14.7^{b}	15.8^{b}	18.0^{b}
	150	22.3^{a}	25.5^{a}	30.1^{a}	19.8^{a}	24.4^{a}	31.1^{a}
Cr level (μmol)	0	16.8^{b}	18.2^{b}	20.7^{b}	16.2^{b}	18.5^{b}	22.1^{b}
	10	15.8^{b}	16.3^{b}	19.2^{b}	15.6^{b}	17.6^{b}	20.7^{b}
	50	22.2^{a}	24.0^{a}	30.0^{a}	20.1^{a}	24.2^{a}	30.8^{a}
Interaction							
Genotype and salinity		*	**	**	**	ns	*
Genotype and Cr		ns	*	*	ns	ns	ns
Cr and salinity		ns	**	*	ns	ns	*

The same letters after the data between genotypes or salinity levels and among Cr levels within a column mean no significant difference at 95% probability level. *$P < 0.05$; **$P < 0.01$; ns – non significant

2007). In this study, the plants exposed to 10 μmol Cr showed slight increases in the most examined physiological and growth parameters, including plant height and biomass, stomatal conductance and photosynthetic rate. Similarly, the beneficial effect of low Cr level on plant growth and nutrient accumulation were reported (Zeng et al. 2010). But at higher level (50 μmol); phytotoxicity of Cr was observed (Tables 1–3). Similar results were found in different plants by Pandey et al. (2005).

To our best knowledge, it was the first study on interaction of NaCl and Cr on plant growth and physiological traits. It was found that the inhibiting effect of salinity stress on plant growth could be alleviated under low Cr level, with salt-sensitive genotype being more prominent. Wei et al. (2007) found synergistic interaction of Cd and NaCl on growth and photosynthesis in barley plants. On the other hand, we found that the combined stress of high Cr level and NaCl caused further reduction of the measured parameters as compared to the stress alone. Moreover the reduction was more pronounced in salt-sensitive genotype. Similarly, Sepehr and Ghorbanli (2006) found that interaction between Cd and NaCl at higher doses decreased plant biomass, growth and chlorophyll in maize as compared to Cd or NaCl stress alone.

Generally, MDA content is used to measure the degree of cell membrane lipid peroxidation, which will be enhanced when plants are exposed to different abiotic stresses, including salinity and Cr toxicity (Liang 1999, Gallego et al. 2002). In current study salinity increased SOD and POD activities, and MDA contents in barley plants. Moreover it was found that SOD and POD activities were much higher in the CM72, a salt-tolerant genotype than in Gairdner, a salt-sensitive genotype, While MDA content was just opposite for the two genotypes. It was reported that salt-tolerant genotypes might develop a tolerance response by increasing antioxidant enzymes and decreasing MDA (Liang 1999, Huang et al. 2006).

Low Cr level could alleviate the oxidative stress caused by salinity, which was reflected by lower SOD and POD activities, and MDA content in the treatment combined 10 μmol Cr and salinity than in the salinity alone. Thus, it may be assumed that there might be a beneficial effect of low Cr level on the plant growth, but it needs to be tested. But at high Cr level (50 μmol), oxidative stress was further enhanced relative to salinity alone. It was reported that that Cr toxicity increased MDA content due to oxidative damage to cell membrane (Pandey et al. 2005, Ali et al. 2010b). To our knowledge, it was the first study about the interaction of Cr and NaCl on oxidative stress. The current results showed that the effect of Cr on oxidative stress of barley plants exposed to NaCl varied with Cr level. Similarly, Huang et al. (2006) found that interaction between Cd and NaCl increased oxidative damage in barley plants.

Moreover, the extent of enhanced oxidative stress under high Cr level differed in the two genotypes, with salt-sensitive genotype Gairdner being more affected than salt-tolerant genotype CM72.

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