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Variation in antioxidant enzyme activities, growth and some physiological parameters of bitter melon (*Momordica charantia*) under salinity and chromium stress

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Abstract

In general, salinity and heavy metals interfere with several physiological processes and reduce plant growth. In order to evaluate of three levels of salinity (0, 4 and 8 ds m^{-1}) and three concentration of chromium (0, 10 and 20 mg kg^{-1} soil) in bitter melon (*Momordica charantia*), a plot experiment was conducted in greenhouse at university of Shahrood, Iran. The results revealed that chromium treatment had no significant affect on fresh and dry weight, but salinity caused reduction of fresh and dry weight in growth parameter. Salinity and chromium enhanced antioxidant enzymes activities like catalase (CAT), guaiacol peroxidase (GPX) and sodium content in leaves. However salinity and chromium treatments had no effect on potassium, phosphorus in leaves, soluble carbohydrate concentration in leaves and root, but decreased the carotenoid content in leaves. On increasing salinity from control to 8 ds m^{-1} chlorophyll a, b and anthocyanin content decreased by 41.6%, 61.1% and 26.5% respectively but chromium treatments had no significant effect on these photosynthetic pigments.

Key words

Antioxidant enzymes, Bitter melon, Heavy metal, Ion content, Salinity

Introduction

Momordica charantia a member of cucumber family-cucurbitaceae is commonly known as bitter melon. It is a tropical plant currently distributed across the globe but mainly concentrated in East Africa, Asia and throughout South America. The plant is adapted to wide variation of climates although production is best in hot areas (Binder *et al.*, 1989). Concerning with condition requirements of cultivation, the plant is similar to cucurbit crops especially in irrigation, fertilization and weather. Bitter melon is an annual, slender, climbing plant with long-stalked leaves. All plant parts, including fruit, tastes bitter. Although the seeds, leaves and vines of bitter melon have all been used as food, but the fruit is the safest and most prevalent part of the plant used (Yen and Hwang, 1985).

Agricultural soil in many parts of the world are slightly to moderately contaminated by heavy metals like Cd, Cu, Ni, Co, Cr, Pb and As. Chromium (Cr) is highly toxic to human health. Its presence in agricultural soils can be attributed to use of organic wastes such as fertilizer and waste water for irrigation (Chatterjee and Chatterjee, 2000). Chromium compounds are highly toxic to plants and are detrimental to their growth and development. Although some crops are not affected by low Cr concentration (3.8×10^{-4} µ M) (Huffman and Allaway, 1973), however, 100 µ Mkg^{-1} d. wt. Cr is highly toxic (Davies *et al.,* 2002). At low concentration, Cr has been found to promote plant growth, (Hu *et al.*, 2010).

The first prerequisite for higher yield in plants is an increase in biomass production in terms of dry matter. Carbon compounds account for 80-90% of total dry matter produced by plants. Higher source size and increased photosynthetic process has found to be the basis for building up of organic substances and dry matter production under heavy metal stress in general and Cr in particular (Bishnol et al., 1993). In a study conducted on *Vallisneria spiralis* to evaluate the Cr accumulation and toxicity in relation to biomass production, it was found that dry matter production was severely affected by Cr (VI) concentrations above 2.5 µg ml^{-1} in nutrient medium (Vajpayee *et al*., 2001). Zeid (2001) observed that Cr at highest concentration (10^{-2} M) decreased photosynthesis drastically in pea plant. However, plants have developed potential mechanism to combat such adverse heavy metal toxicity. Plants produce low molecular weight thiols that show high affinity for toxic metals. Another mechanism, induction and activation of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorate perxidase (APX) and guaiacol peroxidase (GPX) (Shanker *et al*., 2003). Gwozdz *et al.* (1997) found that low heavy metal concentrations, activity of antioxidant enzymes increased, whereas at higher concentrations, SOD activity did not increase further and catalase activity decreased.

Bioavailability of metals in soil is strongly influenced by soil pH and complexation with ligands. Cr available for plant uptake depends on the oxidation state of Cr, pH, presence of colloidal binding sites and Cr-organic complexes in soil (Losi *et al*., 1994). Zurayk *et al*. (2001) reported that salinity and Cr interaction caused a significant decrease in the dry biomass accumulation in *Portulaca oleracea*.

Salinity is one of the most significant abiotic factors limiting crop productivity. Photosynthesis is the most important process affected by plants, growing under saline conditions. Reduced photosynthesis under salinity is not only attributed to stomata closure leading to a reduction of intercellular CO_2 concentration, but also to non-stomata factors. There is a strong evidence that salt affects photosynthetic enzymes, chlorophylls and carotenoids (Stepien and Klobus, 2006). High salt concentrations, usually sodium chloride, cause osmotic stress by decreasing water potential within the cells, and ionic stress due to specific inhibition of metabolic processes (Bohnert *et al*., 1995).

Salinity stress effected in plant metabolism is mediated by enhanced production of reactive oxygen species (ROS), such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), singlet oxygen and hydroxyl radical (Foyer and Noctor, 2003). These ROS are extremely cytotoxic and can seriously disrupt normal metabolism through oxidative damage to lipids (Alscher *et al.*, 2002), nucleic acids and proteins. In order to avoid damage caused by ROS compounds, plants have evolved molecular defence systems that limits formation of ROS and promotes its removal (Alscher *et al.*, 2002). Plant enzymatic defences include antioxidant enzymes such as phenol peroxidase (POX), ascorbate peroxidase (APX), glutathione peroxidase (GPX), superoxide dismutase (SOD) and catalase (CAT), which together with other enzymes of ascorbate–glutathione cycle promote scavenging of ROS (Cavalcanti *et al,* 2004).

Most studies with Cr toxicity have focused on the effects of Cr solution on plant tolerance, growth and survival with only a few studies. The purpose of this study was to provide additional information on antioxidant activity enzymes, photosynthesis pigments, nutrient elements and soluble carbohydrate in bitter melon (*Momordica charantia*) under different concentrations of salinity and chromium supply.

Materials and Methods

Plant culture: A plot experiment was conducted in greenhouse at Agricultural University of Shahrood, Iran during 2013 to study the effects of different salinity and chromium levels on bitter melon (*Momordica charantia*). The experiment was laid out in a completely randomized 3×3 factorial design with three replicates. Each plot (26×20 cm) was filled with sandy loam soil (Ec= 1.1 ds/m and pH= 7.56). Eight seeds of bitter melon (*Momordica charantia*) were sown at uniform depth (2 cm) and after completion of emergence, thinning was done and three plants were maintained in each plot. Recommended dose of commercial fertilizer @ of 75 and 50 kg ha^{-1} N and P was supplied to each plot. Air temperature in greenhouse was controlled between 25° and 35°C during day and 20 and 25°C during night. Relative humidity ranged from 50 to 80%. Light averaged 1200 µmol m^{-2} S^{-2}, with a minimum of 344 and a maximum of 1500 µmol m^{-2} S^{-2} at noon.

In this study, three chromium levels of Cr C_1=0, C_2=10 and C_3=20 mg kg^{-1} soil was used as chromium chloride ($CrCl_3$. $6H_2O$) source and applied in plot before sowing of seeds at the 0-90 cm depth of soil. Bitter melon plants were subjected to different salinity levels including of S_1= 0, S_2= 4 and S_3= 8 ds m^{-1} through irrigation water by addition of salt (NaCl) in increments after thinning.

Thirty days after salt treatment, the plants were harvested. At the harvest time, data collected included plant height and biomass or dry weight of a plant (g/plant). Biomass was measured after drying plants at 70 °C for 48 h in an air oven (Schuurman and Goedewaagen).

Chlorophyll, cartotenoid, anthocyanin, soluble carbohydrate, sodium and potassium : Thirty days after the initiation of the salinity treatments, one of plant in plot was

harvested, and the organic and inorganic solutes extracted from the mature leaf blades. Soluble carbohydrates were determined according Horwitz's method (1975). Chlorophyll 'a' & 'b' and carotenoid in the leaves were extracted with 80% acetone and determined according Arnon's method (1967), wherein the chlorophyll spectrum absorptions were measured at 645 and 663 nm, respectively, and the carotenoid calculated at 440 nm. Ion contents of Na^+ and K^+ in leaves were determined by using a Jemway PFP7 Flam photometer.

Enzyme assays : Thirty days after salinity treatment antioxidant enzyme activities in leaves tissues were assayed. Leaf samples were collected in a ice bucket and brought to laboratory. Leaves were then washed with distilled water and surface moisture was wiped out. Leaf samples (0.5 g) were homogenized in ice cold 0.1 M phosphate buffer (pH 7.5) containing 0.5 mM EDTA with pre-chilled pestle and mortar. The homogenate was transferred to centrifuge tubes and was centrifuged at 48^0C in Beckman refrigerated centrifuge for 15 min at 15 000 /g. The supernatant was transferred to 30 ml tubes and referred to enzyme extract.

Total APX activity was determined by the method of Nakno and Asada (1981). Ascorbate oxidation was monitored by reading the absorbance at 290 nm at the moment of H_2O_2 addition and 1 min later. Difference in absorbance was divided by ascorbate molar extinction coefficient (2.8 $mM^{-1}.cm^{-1}$) and enzyme activity expressed as μmol of H_2O_2 $min^{-1}.mg^{-1}$ protein, taking into consideration that 1.0 mol of ascorbate is reqired for the reduction of 1.0 mol of H_2O_2 (McKersie and Leshem, 1994).

Total GPX activity was determined as described by Urbanek et al. (1991) in a reaction mixture (2.0 mL) containing 100mM phosphate buffer (pH 7.0), 0.1 μM EDTA, 5.0mM guaiacol, 15.0mM H_2O_2 and 50 μL enzyme extract. The addition of enzyme extract started the reaction and the increase in absorbance was recorded at 470 nm for 1 min. Enzyme activity was quantified by the amount of tetraguaiacol formed using its molar extinction coefficient (26.6mM^{-1} cm^{-1}). The results were expressed as μmol H_2O_2 min^{-1} g^{-1} d.wt. taking into consideration that 4 mol H_2O_2 reduced to produce 1 mol tetraguaiacol (Plewa *et al.*, 1991).

Statistical analyses: All data were analyzed with SAS Institute Inc. 9.2 software. All data were first analyzed by ANOVA to determine significant (P=0.05) treatment effects. Significant differences between individual means were determined using Duncan multiple range test. Data points in the figures represent the means ± SE of three replicates.

Results and Discussion

Application of Cr in the rooting medium had no significantly effect on fresh and dry weight, but on increasing salinity level from 0 to 8 ds m^{-1}, dry and fresh weight of bitter melon plants significantly reduced. Fresh and dry weight of plants growing in 8 ds m^{-1} salinity decreased by 41.2% and 15.2% as compared to control plants (Table 1).

Analysis of variance for chlorophyll a, chlorophyll b and anthocyanin revealed that salinity showed significant difference on all these photosynthetic pigments. When bitter melon plants were exposed to 8 ds m^{-1} salinity, the amount of chlorophyll a, chlorophyll b and anthocyanin decreased by 41.6%, 61.1% and 26.5% respectively, as compared to the control plants (Table 1).

Chromium addition in growing medium, from 0 to 20 mg kg^{-1} soil, had significant effect only on chlorophyll a while no effect on chlorophyll b and anthocyanin content (Table 1) was noted. With a increasing salinity level in rooting medium from 0 to 8 ds m^{-1} and chromium concentration from control to 20 mg kg^{-1} soil, the carotenoid content decreased in bitter melon plants. Maximum carotenoid content was observed at A_1C_3 treatment (Fig. 1).

Salt stress (From 0 to 8 ds m^{-1}) accelerates formation of active oxygen species. The lifetime of active oxygen species within cellular environment is determined by antioxidant system, which provides crucial protection against oxidative damage. Bitter melon plants grown at various salinity levels and chromium concentration showed changes in activities of antioxidants enzymesin leaf tissues, more activity was observed at 8 ds m^{-1} salinity levels and 20 mg kg^{-1} chromium treatment.

CAT activity at different salinity and chromium level, showed that at 8 ds m^{-1} level and 20 mg kg^{-1}, the bitter melon plants had the highest activity in leaf tissue (Fig. 2).

GPX activity increased remarkably in leaf tissue as a result of salt stress and increasing chromium concentration. GPX activity was high at 8 ds m^{-1} level and 20 mg kg^{-1} in leaf tissue of plants (Fig 3). There was a slight increase in GPX activity of bitter melon plants in response to increasing NaCl and chromium concentration and its activity was 60% higher in the presence of 8 ds m^{-1} and 20 mg kg^{-1} soil chromium as compared to control leaf tissue (Fig 3).

Table 1, shows, that application of chromium and salinity in growing medium, except for sodium, had no significantly effect on carbohydrate content in root and leaves; potassium and phosphorus content in leaves of bitter melon plants (Table 1).

Salinity and chromium treatment increased sodium content in leaves. As chromium concentration increased from control to 20 mg kg^{-1}, and salinity from 0 to 8 ds m^{-1}, sodium content in leaves increased by 37.3% & 48.1% respectively. (Table 1).

Salinity and chromium problem can arise simultaneously in soil and water. One such instance is inorganic ions containing Na^+ present in drainage water,

Table 1.: The effect of salinity and chromium treatments on plant growth, some physiological parameters and nutrient elements in bitter melon plants.

Treatments	Fresh weight (g)	Dry weight (g)	Chlorophyll a (mg g^{-1}.f.wt.)	Chlorophyll b	Anthocyanin
Salinity levels (ds/m)					
S_1=0	29.2a	18.4a	4.8a	4.8a	1.47ab
S_2=4	29.4a	19.4a	4.2ab	2.7ab	1.55a
S_3=8	17.2b	15.6b	2.8b	1.4b	1.08b
Chromium levels (mg/kg Soil)					
C_1=0	26.1a	17.3a	5.2a	3.3a	1.5a
C_2=10	24.2a	18.6a	3.6ab	2.22a	1.3a
C_3=20	25.6a	17.3a	3.1b	2.2a	1.22a
Salinity	***	*	*	*	*
Chromium interaction	ns	ns	*	ns	ns
Salinity and chromium	ns	ns	ns	ns	ns

	Antioxidant enzyme activities		Nutrient elements				Soluble carbohydrate	
	Cartoenoid (µmol H_2O_2 min^{-1} mg^{-1} protein)	CAT	GPX	Sodium (mg g^{-1}. d.wt.)	Potassium	Phosphorus	Leaves (µmol glucose g f.wt)	Root
Salinity levels (ds/m)								
S_1=0	0.59	0.14ab	0.03b	81.2b	561.9a	0.07a	18.4a	19.6a
S_2=4	0.47a	0.08b	0.05b	101.7b	516a	0.07a	19.2a	20.17a
S_3=8	0.46a	0.21a	0.103a	156.2a	517a	0.072a	19.8a	20.96a
Chromium levels (mg/kg Soil)								
C_1=0	0.64a	0.138a	0.06a	86.2b	567.1a	0.06a	20.7a	19.6a
C_2=10	0.44b	0.16a	0.05a	115.2a	526.3a	0.071a	18.1a	20.3a
C_3=20	0.43b	0.137a	0.067a	137.6a	502.5a	0.072a	18.9a	20.8a
Salinity	Ns	**	***	***	ns	ns	ns	ns
Chromium interaction		ns	ns	**	ns	ns	ns	ns
Salinity and chromium	*	*	**	ns	ns	ns	ns	ns

limiting plant growth and yields. However, appreciable amount of trace elements such as chromium, cadmium, selenium and nickel, may occur due to geochemical and artificial activities in soil (Deverel and Fujii, 1990).

Salinity stress is an intricate phenomenon which includes osmotic stress, specific ion effect and nutrient deficiency thereby affecting various physiological and biochemical mechanisms associated with plant growth and development (Sairam *et al*., 2002). In the present study, the results revealed that fresh and dry weight of bitter melon plants, was affected by salinity stress. Increased salt levels from control to 8 ds m^{-1} caused decline by 41.2% and 15.2% fresh and dry weight, respectively (Table 1).

The negative effect of salinity on plant growth might be due to defective metabolism in plant cells. Salinity restricted plant cells to uptake water and some mineral nutrients dissolved in the culture medium (Cicek and Cakirlar, 2002). Reduction of plant growth under salinity stress might be related to reduction in photosynthesis. Salinity reduces the chlorophyll content in salt susceptible plants and increases in salt tolerant plants. Salinity reduced growth and photosynthesis pigments in basil (Heidari , 2012) and radish. At high salinity level, reduction in growth could be attributed to reduction of leaf chlorophyll content and leaf area expansion and low light interception (Marcelis and Hooijdonk, 1999).

In the present study, bitter melon plants exposed to 8 ds m^{-1} salinity showed decreased amount of chlorophyll a, b and anthocyanin content by 41.6%, 61.1% and 26.5% as compared to control plant (Table 1).

Heavy metals interfere with several physiological processes reducing plant growth, photosynthesis and consequently biomass (Jamal *et al*., 2006). Chlorophyll is an important pigment and plays an important role in photosynthesis (Liu *et al*., 2001). Decrease in total chlorophyll, chlorophyll a and b and cartotenoid content has been well documented in Cr stressed plants (Panda and Choudhury, 2005).

In the present study, chromium in growing medium, from 0 to 20 mg kg^{-1} soil, had no significant effect on fresh and dry weight. Among the photosynthetic pigments, interaction between salinity and chromium had significant effect only on carotenoid content in leaves. On increasing salinity and chromium level, the amount of carotenoid decreased was decline. Maximum carotenoid content was observed at 0 ds m^{-1} salinity and 20 mg kg^{-1} chromium chloride hydrate treatment (Fig. 1).

Salinity affects growth and yield of most crops; high salinity is known to cause hyperionic and hyper osmotic effects in plants, leading to membrane disorganization, increase in reactive oxygen species production and metabolic toxicity (Hasegawa *et al*., 2000; Gosset *et al*., 1994). Various protective mechanisms limit ROS production and scavenger ROS. Enzymatic antioxidant systems are located in various cell compartments (Ashraf, 2009). In the present study, salinity stress accelerated enzymatic antioxidants in bitter melon plants. On increasing salinity level from control to 8 ds m^{-1}, CAT and GPX enzyme activity increased. In addition to salinity, heavy metals enter frond chloroplast and cause oxidative stress (Romero-puertas *et al*., 2004). CAT and GPX activity increased remarkably in leaf tissue as a result of increased chromium concentration. CAT and GPX activity was higher in leaf tissue (Fig. 2 and 3). SOD scavenge free radicals (O_2^-) while POD and CAT oxygen radicals (H_2O_2). These enzymes maintain the stability of cell membranes and protect plant cell from reactive oxygen (Luo *et al*., 2004). Huang *et al*. (2006) found that interaction between Cd and NaCl increased oxidative damage in barley plants.

Salinity stress in plant cells is primarily caused by combined effect of osmotic and ionic stress in soil (Hasegawa *et al*., 2000). Metabolic acclimation via accumulation of compatible solutes is often regarded as basic strategy for the protection and survival of plants under abiotic stress (Shabala and Cuin, 2006). Many plant species accumulate significant amounts of glycine betaine, proline and carbohydrate in response to high salinity (Di Martino *et al.,* 2003).

Application of salinity and heavy metal (Cr) in the growing medium had no significant effect on soluble carbohydrate concentration in the leaves and roots of bitter melon plants (Table 1).

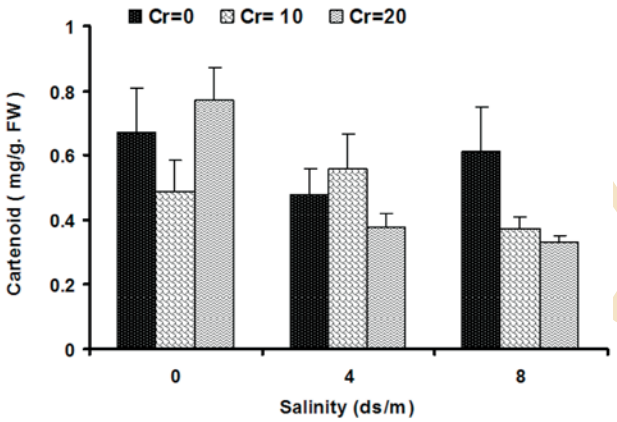

Fig. 1: Effect of salinity and chromium on cartoenoid content in bitter melon

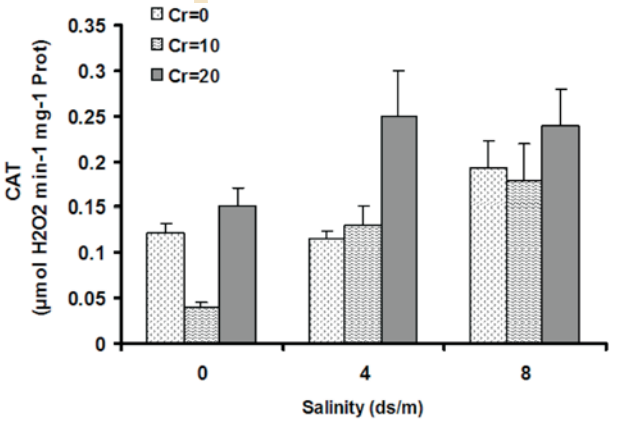

Fig. 2: Effect of salinity and chromium on CAT activity in leaves of bitter melon.

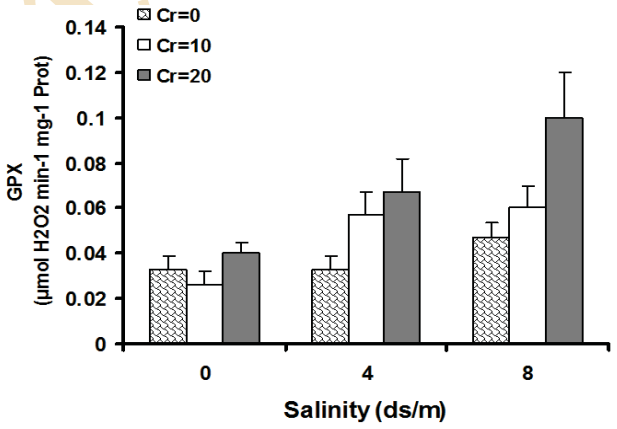

Fig. 3: Effect of salinity and chromium on GPX activity in leaves of bitter melon

Among the nutrient elements, salinity and chromium only accelerated sodium content in leaves while no significant effect on potassium and phosphorus content was noted. De Lacerda *et al*. (2003) reported that Na^+

increased and K^+ decreased in salinity stressed plants. They concluded that elevated concentration of Na^+ inhibited K^+ ions in sunflower plants resulting in an increase in the Na^+/K^+ ratio.

In conclusion, our data demonstrate that salinity and chromium had different effect on bitter melon plants (*Momordica charantia*). Data revised that addition of 20 mg of $CrCl_3 \cdot 6H_2O$ per kg of soil was sufficient to impart a significant improvement in salinity tolerance to bitter melon plants when the plants were interactively heated with 8 dS/m of NaCl. Consequently, when plants were subjected to salinity and chromium, They accelerated enzymatic antioxidant systems such as GPX and CAT activity enzymes. Antioxidant enzymes activity acts as the main defense mechanism in plants. Bitter melon plants did not use soluble carbohydrates as the osmotic adjustment during stress. Salinity and chromium treatments increased sodium content and reduced carotenoid in leaves of the plants.

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