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Research article/Araştırma makalesi

A role of catalase (CAT) in detoxification of reactive oxygen species (ROS) in tomato (*Lycopersicum esculentum*) contaminated with manganese (Mn^{2+})

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Abstract

In the current study, tomato (*Lycopersicum esculentum* L.) plants were exposed to various concentrations of Mn^{2+} (80, 160, 320, 640 and 1280 µM) cation. In the first part, in order to obtain evidence that plants were in stress, following the Mn^{2+} treatments, lipid peroxidation were determined. In the second part, to gain an idea about regulation of the CAT gene product, enzyme activities were also recorded in Mn^{2+} treated tomato plants. The maximum lipid peroxidation level was determined in plants which were exposed to 1280 µM concentration of Mn^{2+} contamination. Changes in lipid peroxidation and CAT enzyme activities in tomato plants exposed to different concentration of Mn^{2+} contamination revealed no positive correlation. The current study revealed that, ROS induced lipid peroxidation level has depended on concentration of Mn^{2+} contamination. Also, antioxidant responses to Mn^{2+} contamination could be reflected as changes in CAT enzyme activity in tomato plants.

Key words: tomato (*Lycopersicum esculentum*), lipid peroxidation, catalase (CAT).

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Mangan (Mn^{2+}) ile kontamine olmuş domates (*Lycopersicum esculentum*) bitkisinde reaktif oksijen türlerinin (ROS) detoksifikasyonunda katalazın (CAT) rolü

Özet

Bu çalışmada, domates bitkileri (*Lycopersicum esculentum* L.) çeşitli konsantrasyonlardaki (80, 160, 320, 640 ve 1280 µM) Mn^{2+} katyonuna maruz bırakılmıştır. Çalışmanın ilk bölümünde Mn^{2+} stresini takiben bitkilerin stres etkisine gitmiş olduklarını göstermek için lipit peroksidasyonu düzeyleri belirlenmiştir. İkinci bölümde CAT gen ürününün regülasyonu değerlendirmek için Mn^{2+} stresli domates bitkilerinde enzim aktivitesi ölçülmüştür. En yüksek lipit peroksidasyonu 1280 µM konsantrasyonda Mn^{2+} stresine maruz kalan bitkilerde gözlenmiştir. Farklı konsantrasyonlardaki Mn^{2+} stresi altındaki domates bitkisinde lipit peroksidasyonu ve CAT enzim aktivitesi düzeyleri arasında pozitif bir korelasyona rastlanmamıştır. Bu çalışma ile ROS ile tetiklenen lipit peroksidasyonu seviyesinin Mn^{2+} stresinin konsantrasyonuna bağlı olduğu belirlenmiştir. Aynı zamanda Mn^{2+} stresine karşı oluşan antioksidan cevapların domates bitkisinde CAT enzim aktivitesinin değişimi olarak yansıyabildiği gözlenmiştir.

Anahtar kelimeler: domates (*Lycopersicum esculentum*), lipitper oksidasyonu, katalaz (CAT).

1. Introduction

Heavy metals are mainly considered as beneficial for continuation of normal life in plants but above certain threshold levels could be dangerous. They have been generally used by referring to a group of metals such as Cd, Co,

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Ni, Zn, As, Cu and Mn which contamination often cause to toxicity and environmental problems. As a result of anthropogenic and industrial effluents, heavy metals might accumulate in environment (air, water and soil) and lead to alterations in physiological process such as stunted plant growth, root damage, chlorosisor biochemical parameters which effect life quality of plants and ultimately human health (Kumar et al., 2010; Çolak et al., 2011; Cansaran-Duman et al., 2012; Aras et al., 2012; Soydam-Aydın et al., 2013).

Manganese (Mn)is an important micronutrient for plant nutrition which can be found in largest amounts in the soil and present in three stable forms: Mn^{2+}, Mn^{3+} and Mn^{+4}. Mn^{2+} participates in different enzyme systems include oxidoreductases, transferases, hydrolases, lyases, isomerases, ligases, lectins and intregrins and structure of photosynthetic proteins (Millaleo et al., 2010). It functions in the uptake of carbon dioxide and in the transport of electrons in photosynthesis, participates in synthesis of chlorophyll and in assimilation of nitrate and activates lipid forming enzymes in plants (Fecht-Christoffers, 2003).The cellular concentration of Mn^{2+}, which is a transition element comprised in redox signaling, needs to be strongly adjusted in plants. Any alteration above or under threshold level can lead to toxic effect in plants (Elstner et al., 1987). Severalresearchers have indicated that the growth of tomato plants may be restricted by either too high or too low Mn^{2+} concentration (Reuter and Robinson, 1986; Shenker et al., 2004). In hazardous concentrations,Mn^{2+}can affect photosynthesis and transpiration mechanism of plants due to generation ofreactive oxygen species (ROS) such as superoxide radical (O_2^-), hydroxyl radical (OH^-), hydrogen peroxide (H_2O_2)(Mukhopadhyay and Sharma, 1991).ROS are highly reactive and toxic for plants in exceeded concentrations.Under steady state conditions, there is anequilibrium betweenproduction and scavenging of ROS molecules by various antioxidative defense mechanisms in plants (Rao et al., 2006). Changes in this equilibrium can damage cell structure and integrity (Mittler et al., 2002; Chahid et al., 2013).Mn^{2+}toxicity can perturb this equilibrium and lead to increased accumulation of ROS and breakdown in cells membrane lipid (Rellan-Alvarez et al., 2006).Therefore, determination of oxidative lipid injury in response to stress conditions is important topic in plants. Small hydrocarbon fragments such as ketones, malondialdehydes (MDAs) etc. and compounds related to them used as an indicator of lipid peroxidation or membrane damages, which are considered as the first evidence of stress in plants (Lyons, 1973;Hodges et al., 1999).There are lots of studies reported increased lipid peroxidation levelsin plants due to heavy metal contaminations(Krupa and Baszynski, 1985; Krupa and Baszynski, 1989; Malik et al.,1992; Gaur and Gupta, 1994; Quariti et al., 1997; Vassilev et al., 2004; Ben Ammar et al., 2005;Nouairi et al., 2006; Cansaran-Duman et al., 2013).

In recentyears, screening antioxidant defense system has been another considerable approach to understanding the response mechanisms of plants under stress conditions (Alscher et al., 1997; Cansaran-Duman et al. 2011; Cansaran-Duman, 2011). ROS-interacting antioxidant defense enzymes such as peroxidases, monodehydroscorbate (MDHAR), glutathione reductase (GR), superoxide dismutases (SOD) and catalase (CAT) play role for scavenging of the ROS and prevent plants from cell damages and death (Gout et al., 2001; Verma and Dubey, 2003; Nocito et al., 2007; DalCorso et al., 2008).CATdismutasehighly reactive H_2O_2into non-toxic H_2O and $O_2$and by this way it prevents hazardous effects of ROS during stress conditions in plants. One molecule of CAT can convert almost six million molecules of $H_2O_2$to non-toxic components per minute and thus it is known as one of the highest turnover rates for all enzymes (Polidoros and Scandolios, 1999).

The current study was designed to understand the effect of Mn^{+2}(ranging from 80,160,320, 640 and1280 μM) on lipid peroxidation and CAT enzyme activity in tomato plants (*Lycopersicumesculentum*L.).

2. Materials and methods

2.1. *Plant material, growth conditions and stress treatment*

Tomato (*Lycopersicumesculentum* L. 'Falcon') seeds were germinated and grown hydroponically in pots containing 0.2 L of modified 1/10 Hoagland's solution. Hoagland solution includes macronutrients (K_2SO_4, KH_2PO_4, $MgSO_4.7H_2O$, Ca $(NO_3)_2$. $4H_2O$ and KCl) and micronutrients (H_3BO_3, $MnSO_4$, $CuSO_4.5H_2O$, NH_4Mo, $ZnSO_4.7H_2O$) with a final concentration of ions as 2mM Ca, 10^{-6} M Mn, 4mM NO_3, 2.10^{-7} M Cu, 1mM Mg, 10^{-8} M NH_4, 2mM K, 10^{-6} M Zn, 0.2 mM P, 10^{-4} M Fe ve10^{-6} M B.Six plants were grown in each pot in a controlled environmental growth chamber in the light with 250 mmol m^{-2} s^{-1} photosynthetic photon flux at 25 ºC, 70 % relative humidity. Twenty-five-days-old plants grown in controlled media were used for stress treatments. For application of heavy metal stress,$MnSO_4H_2O$ were addedto the hydroponic solution for 24h at concentrations; 0(control), 80,160,320, 640 and1280 μM of Mn^{2+}.

2.2. *Estimation of Lipid Peroxidation*

Malondialdehyde (MDA) is a marker of oxidative lipid injury which changes in response to environmental factors that lead to stress in plants. TBA-MDA content was determined as described by Hodges et al.(1999). Plant tissue samples were homogenized with liquid nitrogene and homogenized with 80:20 (v:v) ethanol:water, followed by centrifugation at 3000g for 10 min. A 1-ml aliquot ofappropriately diluted sample was added to a test tube with 1 ml of

either (i) TBA solution comprised of 20.0% (w/v) trichloroacetic acid and 0.01% butylatedhydroxytoluene, or (ii) +TBA solution containing the above plus 0.65% TBA. Samples were then mixed vigorously, heated at 95 °C in a hot plate (neoBlock1, 2-2503) for 25 min, cooled, and centrifuged at 3000 g for 10 min. Absorbance values were measured at 440 nm, 532 nm, and 600 nm by ELISA microplate reader (SpectraMax M2). The equivalents of malondialdehyde were calculated by the following equations;

1) $[(Abs\ 532_{+TBA})-(Abs\ 600_{+TBA})-(Abs\ 532_{-TBA}-Abs\ 600_{-TBA})] = A$

2) $[(Abs\ 440_{+TBA}-Abs\ 600_{+TBA})\ 0.0571] = B$

3) MDA equivalents $(nmol.ml^{-1}) = (A-B/157\ 000)\ 10^6$

2.3. Catalase Enzyme Activity Assay

Frozen plant tissues were ground in liquid nitrogen and the powder was suspended in extraction buffer, containing 50 mM potassium phosphate (pH 7.0) and 0.1 mM EDTA. The homogenates were centrifuged at 15000g for 20 min, and the supernatant fraction was used for the assays of enzyme activity (Jovanovic, 2006). All steps were carried out at 4°C. Catalase activity assay was performed with the method reported by Aebi et al. (1988) based on 240 nm absorbance values. Enzyme activity was evaluated using the extinction coefficient of 0,0396 $cm^2\ \mu mol^{1-}$ for H_2O_2..

3. Results

3.1. Effects of Mn^{2+} on lipid peroxidation

Oxidative damage in membrane lipids due to environmental stress could be estimated by MDA content. In this study, the levels of lipid peroxidation in tomato plants exposed to different concentration of Mn^{+2}wereanalyzed in terms of thiobarbituric (TBA)-reactive substancesand total MDA values were calculated (Table 1).

Table 1.Absorbancesand MDA equivalent levels (nmol $ml^{1-}g\ FW^{1-}$) of various plant materials assayed immediately after processing. MDA equivalents are given as the means of at least n = 4 samples

Plant	Assayed without TBA (-TBA)		Assayed with TBA (+TBA)			A	B	Total MDA
material	532	600	440	532	600			equivalents
Control	0,1475	0,133	0,094	0,118	0,102	0,0015	-0,00046	0,012464
80 µM	0,15	0,1335	0,148	0,1515	0,137	-0,002	0,000628	-0,01674
160 µM	0,1385	0,1215	0,1465	0,1425	0,1265	-0,001	0,001142	-0,01364
320 µM	0,127	0,116	0,1175	0,1055	0,0935	0,001	0,00137	-0,00236
640 µM	0,1665	0,1555	0,185	0,164	0,142	0,011	0,002455	0,054425
1280 µM	0,2025	0,204	0,25	0,2305	0,1895	0,0425	0,003455	0,248697

MDA level which reflects lipid peroxidationwere slightly increased in tomato samples exposed to 80 and 160 µM concentrations of Mn^{2+}treatment. Additionally, this level was less than the control level in tomato plants exposed to 320 µM concentrations of Mn^{2+}. Upon application of 640 µM concentration of Mn^{2+} contamination, MDA level was suddenly increased and reached fivefold of controllevel. 1280 µM concentration of Mn^{2+} contamination had the maximum effect on lipid peroxidation in tomato plants, it was generated approximately twenty fivefold of control level (Figure 1).

3.2. Effects of Mn^{2+} on the activity of catalase enzyme

All concentrations of Mn^{2+}treatment(80, 160, 320, 640 and 1280 µM)altered the capacity of H_2O_2quenching enzyme catalase (CAT). CAT activity was slightly increased in tomato plantsexposed to 80 µM concentration of Mn^2.After this initial increase, 160 µMconcentration of Mn^{2+} treatment led to significant increase in CAT enzyme activity and this was the maximum level compared to control and all concentrations of Mn^{2+}treatment. 320µM concentration of Mn^{2+} treatment was the only application which led to lower CAT enzyme activity thanthe control (Figure 2). After this concentration,CAT enzyme activity was again increased and decreased in tomato samples exposed to 640 and 1280µM concentration of Mn^{2+}treatment, respectively.

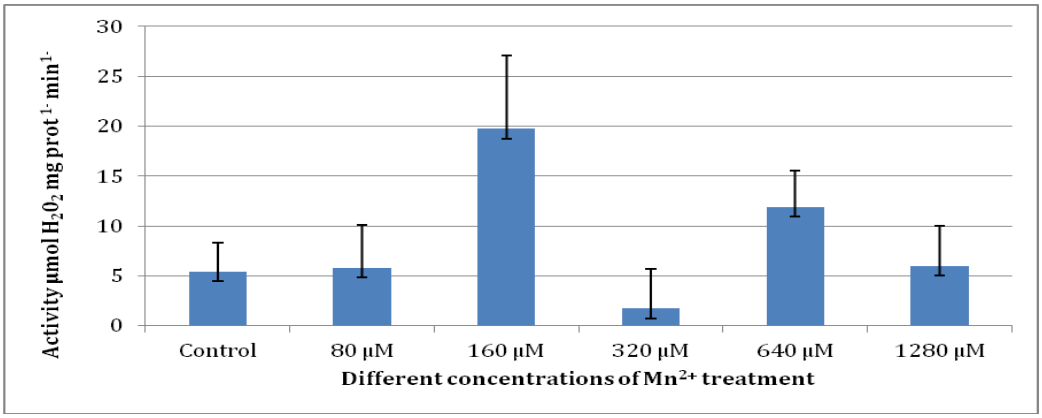

Figure 1. Lipid peroxidation (malondialdehyde=MDA content) in the *Lycopersicum esculentum* L.plant exposed to different concentrations (80, 160, 320, 640 and 1280 µM) of Mn^{2+}

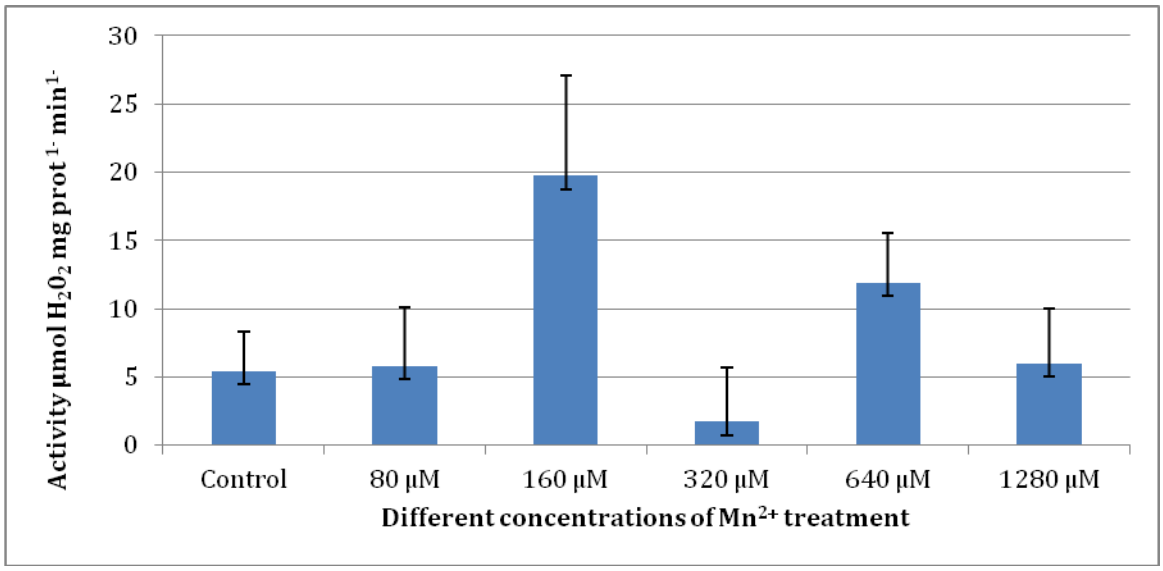

Figure 2. Catalase enzyme profile in the *Lycopersicum esculentum* L.plant exposed to different concentrations (80, 160, 320, 640 and 1280 µM) of Mn^{2+}

4. Conclusions

Manganese toxicity in plants have been correlated with function deficiency, growth inhibition, lower respiration rates, decrease in chlorophyll content, iron deficiency, structure damage,increased activity of indoleacetic acid oxidase, peroxidase and phenol oxidase; lower activities of catalase,ascorbic acid oxidase and glutathione oxidase; lower ATP content depends on many factors such as pH, plant species etc. (Morgan et al., 1976; Cheng, 2003; Sinha et al., 2006; Liu et al., 2010).In spite of these physiological and biochemical knowledge about Mn^{2+}toxicity in plants, little interest has been focused on tomato plants (Millaleo et al., 2010).

Lipid peroxidation was also correlated with variety of heavy metals induced overproduction of H_2O_2 (Quariti et al., 1997; Ben Ammar et al., 2005;Vassilev et al., 2004; Gaur and Gupta, 1994; Nouairi et al., 2006; Jouili and Ferjani, 2003).In recent researches, Li et al. (2007)reported that 10-200 µM Mn^{2+} toxicity did not lead to lipid peroxidation in microalga *Pavlovaviridis*.Bueno and Piguares 2002 reported increased accumulation of lipid peroxidation products in 10-100 mM Mn^{2+}treated tobacco cell.Boojar and Goodarzi(2008) evaluated that MDA is asensitive indicator of ROS productionand reported significantly increased MDA content in three plant species (*Daturastramonium, AlhagicamelthornandChenopodiumambrosioides*)which were cultivated in center of Mn mine(Boojar and Goodarzi, 2008).In the current study; compatible with all these researches,Mn^{2+} toxicity did notled to important alterationsin MDA content in tomato plants exposed to low concentration of Mn^{2+} contamination (80µM, 160µM, 320µM). 640 µM concentration of Mn^{2+} contamination slightly increased lipid peroxidation in tomato cells. 1280 µM concentration of Mn^{2+} contamination had significant increase on lipid peroxidation(Figure 1).Results of the current study is compatible

with the idea that the threshold of Mn^{2+} injury is substantially depend onconcentration of metal contamination and also plant species, cultivars or genotypes withina species(Foy et al., 1988; Horst, 1988).

The lipid peroxidation degree under stress conditionsalso depends on the rate of ROS formation and on the impact and capacity of the detoxification and repair mechanisms of plants (Stadtman and Oliver, 1991). Plants respond to increased ROS accumulation during stress conditions by activating their antioxidant defense systems. CATwhich is one of the components of antioxidant defense systemdismutases highly reactive H_2O_2 into non-toxic H_2O and O_2 and isknown as one of the highest turnover rates for all antioxidant enzymes (Mittler et al., 2004; Polidoros and Scandolios, 1999; Behera et al., 2009).

In astudy performed by Liu et al. (2010), CAT activity was increased in tomato leaves contaminated with low (from 10 to 200 μMol L^{-1}) concentrations of Mn^{2+} treatment. But above 200 μMconcentration of Mn^{2+} treatments(400 and 600 μM), CAT activity reduced with the increasing concentrations of Mn^{2+} treatment.As shown in Figure 2, results of the current study are compatible with Liu et al.(2010) findings and suggest that low concentrations of Mn^{2+}contamination (80μM and 160 μM)stimulates activation of CATin tomato plant.There is a gradual increase in CAT activity up to 160 μM concentration of Mn^{2+}treatment and a sudden decrease at 320 μM concentration ofMn^{2+}treatment.Above 320 μM concentration ofMn^{2+}treatment,CAT activity was again increased in tomato plants exposed to 640 μM concentration of Mn^{2+} and slightly decreased in samples exposed to 1280 μM Mn^{2+} treatment compared to 640 μM but still above control level (Figure 2). When overall MDA contents and CAT enzyme activities of tomato plantsexposed to 160 μM and 320 μMMn^{+2} concentrations were evaluated, it was considered that lipid peroxidation levels may have effect on modulatingCAT enzyme activity at these concentrations.These results indicate a balanced equilibrium between production of ROS which cause lipid peroxidation and cell death and CAT enzyme activity as a component of scavenging and defense mechanisms of ROS. Alsowhen the MDA levels and CAT enzyme activities in 640μM and 1280μMMn^{+2} treated tomato plants were evaluated; it was suggested that high levels of Mn^{+2} contamination may be concluded with a lower CAT activityor these concentrations could be such high levels which perturbedthe equilibrium between ROS production and scavenging-defense mechanisms(Mittler, 2002).

The current study highlights some of the points in CAT enzyme activity under Mn^{2+} toxicity but further analysis are necessary to explain the complex connection between Mn^{2+} toxicity and scavenging mechanism. Because it is still unclear that whether the activation of CAT enzyme belongs to protection against to Mn^{2+} toxicity or this defense reaction occursnon-dedicatedly due to enhanced production of ROS under Mn^{2+} contamination.

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