American Journal of Plant Sciences, **2010, 1, 24-31**
doi:10.4236/ajps.2010.11004 Published Online September 2010 (http://www.SciRP.org/journal/ajps)

Effects of Hypoxia Stress and Different Level of Mn^{2+} on Antioxidant Enzyme of Tomato Seedlings

Airong Liu[1], Shuangchen Chen[1*], Yinfa Mi[1], Zhou Zhou[1], Golam Jalal Ahammed[2]

[1]College of Forestry, Henan University of Science and Technology, Luoyang, P.R. China; [2]College of Horticulture, Bangladesh Agricultural niversity, Mymensingh , Bangladesh.
Email: [*]lamont@126.com

Received June 2nd, 2010; August 2nd, 2010; September 13th, 2010

ABSTRACT

The changes of antioxidant enzyme activities and related genes expression of tomato seedlings were evaluated under hypoxia stress with different levels of Mn^{2+}. Activities of superoxide dismutase (*SOD*), *peroxidase* (*POD*), *ascorbate peroxide* (*APX*), *glutathione reductase* (*GR*), *catalase* (*CAT*), *the contents of H_2O_2, ascorbic* (*AsA*) *and malondialdehyde* (*MDA*) *were studied to investigate how active oxygen damaged the membrane lipid under hypoxia stress. With* 10-200 *$\mu mol \cdot L^{-1}$ Mn^{2+}, the activities of SOD, POD, APX, GR and the contents of H_2O_2, AsA, MDA of leaves and roots increased significantly, which indicated that low Mn^{2+} could eliminate the active oxygen and protect the membrane lipid from hurt. But the activities of catalase* (*CAT*) *decreased evidently in the root. When the concentration of Mn^{2+} reached* 400-600 *$\mu mol \cdot L^{-1}$ under hypoxia stress, the activities of SOD, POD, APX, GR and ASA content decreased remarkably. However, the contents of H_2O_2 and MDA increased contrarily. A series of resistance genes level achieved peak value with* 10 *$\mu mol \cdot L^{-1}$ Mn^{2+}. The expression level of SOD, CAT, APX, POD, GR were* 6.28, 2.19, 5.66, 5.21 *and* 6.79 *times compared to control respectively. These results illustrated appropriate amount of Mn^{2+} could reduce the damage of active oxygen under hypoxia stress, but reversely, high level of Mn^{2+} just aggravated the already serious damage to the tomato seedlings.*

***Keywords*:** *Tomato*, *Hypoxia Stress*, *Mn^{2+}*, *Antioxidant Enzyme*

1. Introduction

Manganese (Mn) is an essential trace element for plant systems. It is involved in photosynthesis, respiration and activation of several enzymes including superoxide dismutase, NADPH-specific decarboxylating malate dehydrogenase and nitrate reductase [1]. The availability of manganese (Mn) to plants is governed by redox processes, which depend on soil Mn reserve, pH and the availability of electrons [2].

However, the presence of Mn at higher concentrations retards plant growth and development by interfering with metabolic processes [3,4], and consequently it represents an important factor in environmental contamination with various phytotoxic effects. But during plant cultivation, lack of oxygen or anoxia is a common environmental challenge which plants have to face throughout their life. Winter ice encasement, seed imbibition, spring floods and excess of rainfall are examples of natural conditions leading to root hypoxia or anoxia. Hypoxia occurs when the decomposition gives rise to water oxygen concentrations less than 2 $ml \cdot L^{-1}$ [5].

Due to lack of oxygen, the reduction potential and pH will become much lower around the root-zoon of plants. On the one hand, under this reducing conditions, Mn^{4+} will be deoxygenized to Mn^{2+}, which is the most form of manganese absorbed by plants in the soil. If the anoxic soil keep this low reducing and pH conditions for a long time, Mn^{2+} will be accumulated to a high concentration, which lead to serious acidification of soil and result in increasing available Mn concentration [6] and Mn toxicity to plants maybe occur. It has been reported that excess Mn disturbs the metabolism of plants and inhibit the plant growth [7-9]. Mn in excess inhibit respiration, affect negatively nitrogen and protein metabolism, cause a reduction of chlorophyll contents and inhibit some photosynthetic functions in leaves [10]. As Mn is essential micronutrients imported actively in the chloroplasts, participating in the structure of different photosynthetic proteins and enzymes, the excess of Mn seems to be particularly damaging to chloroplasts. Some studies have indicated that excess Mn causes deficiency of Fe, Mg

and Ca [11,12] and induces inhibition of chlorophyll biosynthesis and a decline in the photosynthetic rate [13,14].

Research indicates that the degree of cell damage under heavy metal stress depends on the rate of ROS formation and on the efficiency and capacity of detoxification and repair mechanisms. ROS were partially reduced forms of atmospheric oxygen and under normal conditions their production in cells was low and tightly controlled [15]. Stress that disrupts the cellular homeostasis, including heavy metal toxicity, can enhance the production of ROS and increase the steady-state level of H_2O_2 up to 30-fold [16]. The degree of cell damage under heavy metal stress depends on the rate of ROS formation and on the efficiency and capacity of detoxification and repair mechanisms. Many other proteins require metal ions for their catalytic activities and contain metal binding sites making these enzymes highly susceptible to site specific metal-catalyzed oxidation. Any system capable of producing H_2O_2 and of reducing Fe^{3+} or Cu^{2+} can provoke this type of oxidative modification and convert some amino acid residues to carbonyl derivates [17]. This mechanism of oxidative damage could be relevant with Cu and Mn toxicity. Metal-catalyzed oxidation of proteins has been implicated in the marking of proteins for subsequent proteolytic degradation [18].

Tomato (*Solanum lycopersium* Mill.) plants are very sensitive to water flooding or excess of rainfall, which can easily produce root-zoon hypoxia stress and reducing conditions. So Mn toxicity is incidental to the tomato plants during growing season. Previous researches had mainly focused on plant growth and chlorophyll fluorescence in plants [12]. However, little work has been done to determine the antioxidant enzyme activity and related genes expression.

The objectives of this study were to identify changes in activities of SOD, POD, CAT, APX, GR and their gene expressions, along with the contents of ASA, H_2O_2 and MDA in tomato seedlings leaves and roots under hypoxia stress with different level of Mn^{2+}.

2. Material and Methods

2.1. Plant Materials and Treatment Conditions

The experiments were carried out in a greenhouse of Henan University of Science and Technology. Tomato seeds (*S. lycopersium* Mill. cv. Zhongza 9) were sown in growth medium containing a mixture of peat and vermiculite (7:3, v:v) in trays. When the first true leaf fully expanded, groups of eight seedlings were transplanted into a container (40 cm × 25 cm × 15 cm) filled with Hoagland nutrient solution. When the seedlings reached 5 leaves, they were transferred to 10 L plastic pots containing aerated full nutrient solution, six seedlings per pot, pH was maintained close to 6.5 by adding diluted H_2SO_4 or KOH.

After 7 days of pre-culture, the plants were treated with 10 μM Mn^{2+} concentration (normal Mn^{2+}) and 200 μM, 400 μM, 600 μM Mn^{2+} concentration (excess Mn^{2+}) (supplied with $MnSO_4$) and two level of dissolved oxygen (DO) with about 8.0-8.5 $mg \cdot L^{-1}$ at a normal level and about 0.9-1.1 $mg \cdot L^{-1}$ at a low level.

The experiments seedlings were subjected to hypoxia by flushing nutrient solution with N_2 gas for 10 days. The nutrient solution of control plants was continuously flushed with air with an air pump. Oxygen concentration in the vessels was monitored with an oxygen meter.

The normal DO (8.0-8.5 $mg \cdot L^{-1}$) was an optimum intensity and the low DO (0.9-1.1 $mg \cdot L^{-1}$) was suboptimum intensity for plant growth. The experiment had eight treatments: Mn^{2+} 10 μM + normal DO; Mn^{2+} 200 μM + normal DO; Mn^{2+} 400 μM + normal DO; Mn^{2+} 600 μM + normal DO; Mn^{2+} 10 μM + low DO; Mn^{2+} 200 μM + low DO; Mn^{2+} 400 μM + low DO; Mn^{2+} 600 μM + low DO. The experiment was arranged as a randomized, complete block design with four replicates, giving a total 64 pots. The growth conditions were as follows: photoperiod of 14/10 h (day/night), temperature of 25/17˚C (day/night), and photosynthetic photon flux density (PPFD) of 600 $\mu mol\ m^{-2}\ s^{-1}$.

The youngest fully developed leaves and tender roots were rinsed carefully in water, dried with filter paper and used either immediately or frozen in liquid nitrogen for assays of antioxidant enzymes. ASA, H_2O_2 and MDA contents were measured as soon as symptoms were extremely evident.

2.2. Determination of MDA and H_2O_2 Contents in Leaves

The thiobarbituric acid (TBA) test, which determines malonaldehyde (MDA) as an end-product of lipid peroxidation in the leaves, was used to measure MDA. Leaves were homogenized and centrifuged in a potassium phosphate buffer (pH 7.8) for 20 min at 12,000 g, with 1 ml of the supernatant incubated in boiling water for 30 min. The tubes were placed in an ice bath to stop the reaction, after which the samples were centrifuged at 1500 g for 10 min and the absorption was read at 532 nm. The value for nonspecific absorption at 600 nm was measured simultaneously and then subtracted from OD_{532}. The extinction coefficient of 155 mM cm^{-1} was used to calculate the amount of the MDA-TBA complex.

The concentration of H_2O_2 in leaves was measured by monitoring the absorbance of the titanium-peroxide complex at 415 nm, using the method of Brennan and Frenkel [19]. Absorbance values were quantified using a standard curve generated from known concentrations of H_2O_2.

2.3. Enzyme Extraction and Activity Assay

For the enzyme assays, 0.3 g of leaf were ground with 3 ml ice-cold 25 mM HEPES buffer (pH 7.8) containing 0.2 mM EDTA, 2 mM AsA and 2% PVP. The homogenates were centrifuged at 4˚C for 20 min at 12,000 g and the resulting supernatants were used for the determination of enzymatic activity. A photochemical method published by Giannopolitis and Ries [20] was used to determine superoxide dismutase (SOD). One unit of SOD activity was defined as the amount of enzyme required to cause a 50% inhibition in the rate of p-nitro blue tetrazolium chloride reduction at 560 nm. Catalase (CAT) was measured in a reaction mixture containing 25 mM phosphate buffer (pH 7.0), 10 mM H_2O_2, and the enzyme. The decomposition of H_2O_2 was determined at 240 nm (E = 39.4 mM cm^{-1}) [21].

APX activities were measured by a decrease in absorbance at 290 nm and an increase in absorbance at 265 nm according to Nakano and Asada [22]. Glutathione reducetase (GR) activity was measured according to Foyer and Halliwell [23], which depends on the rate of decrease in the absorbance of NADPH at 340 nm.

All data presented were the mean values of four replicates and were analyzed by Duncan's multiple new range tests using Origin Pro 7.5 and SAS software.

2.4. RNA Extraction and RT-PCR for Gene Expression Analysis

Total RNA was isolated from eggplant leaves with 0.3% using TRIZOL reagent (Invitrogen) according to the manufacturer's instruction. Genomic DNA was removed with RNeasy Mini Kit (Qiagen).

The cDNA used as template for RT-PCR was synthesized using a RevertAid^{TM} first strand cDNA Synthesis Kit (Fermentas) from 2 μg purified RNA. On the basis of mRNA or EST sequences, the gene-specific primers were shown in **Table 1** and used for amplification.

Quantitative real time PCR was performed using the iCycler iQ^{TM} Real-time PCR Detection System (Bio-Rad, Hercules, CA, USA). PCRs were performed using the SYBR Green PCR Master Mix (Applied Biosystems). The PCR conditions consisted of denaturation at 95˚C for 3 min, followed by 40 cycles of denaturation at 95˚C for 30 s, annealing at 58˚C for 30 s and extension at 72˚C for 30 s. A dissociation curve was generated at the end of each PCR cycle to verify that a single product was amplified using software provided with the iCycler iQ^{TM} Real-time PCR Detection System. The identity of the PCR products was verified by single strand sequencing using MegaBACE 1000 DNA analysis system (Amersham Biosciences, USA). To minimize sample variations, mRNA expression of the target gene was normalized relative to the expression of the housekeeping gene actin. All experiments were repeated three times for cDNA prepared for two samples of eggplants leaves. The quantification of mRNA levels is based on the method of Livak and Schmittgen [24]. The threshold cycle (Ct) value of actin was subtracted from that of the gene of interest to obtain a ΔCt value. The Ct value of untreated control sample was subtracted from the ΔCt value to obtain a ΔΔCt value. The fold changes in expression level relative to the control were expressed as $2^{-\Delta\Delta Ct}$.

Table 1. Primers used for real-time RT-PCR assays.

Gene	Encoding protein	Accession no.	Primer pairs
SOD	Superoxide dismutase	AY262025.1	F: GGCTTGCATACAAACCTGAA R: CTGACTGCTTCCCATGACAC
CAT	catalase	M93719.1	F: GTCGATTGGTGTTGAACAGG R: AGGACGACAAGGATCAAACC
cAPX	cytosolic ascorbate peroxidase	DQ099420.1	F: GACTCTTGGAGCCCATTAGG R: AGGGTGAAAGGGAACATCAG
POD	peroxidase	DQ099421.1	F: TTAGGGAGCAGTTTCCCACT R: AGGGTGAAAGGGAACATCAG
GR	glutathione reductase	AW033378	F: TTGGTGGAACGTGTGTTCTT R: TCTCATTCACTTCCCATCCA
actin		AB199316	F: TGGTCGGAATGGGACAGAAG R: CTCAGTCAGGAGAACAGGGT

F: forward primer; R: reverse primer.

3. Results

3.1. Effects of Excess Mn under Hypoxia Stress on Activities of SOD, POD, CAT, APX and GR

According to **Figure 1**, SOD activity under hypoxia conditions with 10-400 μmol·L^{-1} Mn^{2+} greatly increased in the leaves and roots than aeration condition (**Figure 1**). Under hypoxia, SOD activity of tomato seedlings treated by 200 μmol·L^{-1} Mn^{2+} reached maximum, which increased averagely by 194.5%, 206.9% in leaves and roots respectively.

Compared with aeration, SOD activity under hypoxia reduced greatly by 600 μmol·L^{-1} excess Mn^{2+}, in the leaves and roots depressed by 27.3%, 43.13% (P<0.05) respectively. It showed that SOD activity could be greatly induced by 10-400 μmol·L^{-1} excess Mn^{2+}, which could reduce the damage of $O_2^{\cdot-}$ under hypoxia stress.

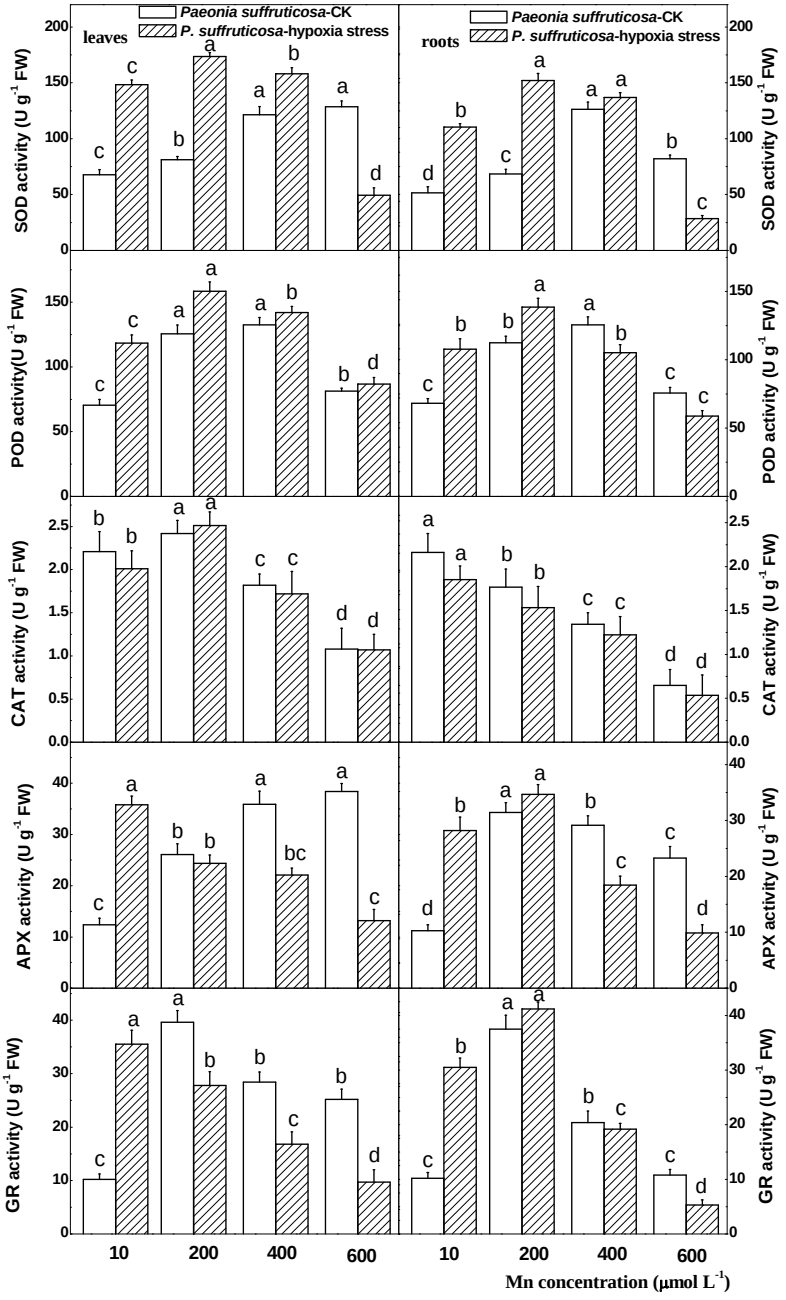

Figure 1. Effects of excess Mn under hypoxia stress on activities of SOD, POD, CAT, APX and GR in tomato seedlings. Different letter indicates significant difference between treatments (P < 0.05). The same is as below.

POD activity in the leaves and roots were increased evidently under hypoxia conditions with 10-200 μmol·L^{-1} Mn^{2+} (**Figure 1**). However, POD activity were gradually decreasing with 200 to 600 μmol·L^{-1} Mn^{2+} under hypoxia stress. POD activity in the leaves and roots researched the highest point with 400 μmol·L^{-1} Mn^{2+} under aeration conditions. POD activity under hypoxia in the roots was gradually lower than those of aeration with the increasing concentration of Mn^{2+}. But in the leaves, POD activity under hypoxia was kept nearly in the same level as those of aeration. Thus, it indicated that 200 μmol·L^{-1} Mn^{2+} could eliminate the contents of O_2^- and H_2O_2 produced by hypoxia stress, which could avoid O_2^- and H_2O_2 reacting to form another serious kind of free radical .OH and reduced their harmfulness.

According to **Figure 1**, CAT activity in the leaves increased with the increasing Mn^{2+} concentration from 10 to 200 μmol·L^{-1}. CAT activities enhanced averagely by 11.9%, 11.3% under aeration and hypoxia stress respectively. When Mn^{2+} was higher than 200 μmol·L^{-1}, CAT activity in the leaves and roots both reduced with the increasing Mn^{2+} concentration. Moreover, with the same Mn^{2+} concentration, CAT activity in leaves and roots had no significant under aeration and hypoxia.

Varying tendency of APX activities in leaves had much difference from those in roots (**Figure 1**). Under aeration conditions, APX activities in the leaves increased obviously with the increasing of Mn^{2+}. But under hypoxia conditions, APX activities in the leaves reduced obviously with the increasing of Mn^{2+}. APX activity in the leaves under hypoxia were distinctly lower than those of aeration when Mn^{2+} was higher than 400 μmol·L^{-1}.

APX activity in the roots had peak value when Mn^{2+} was 200 μmol·L^{-1}. When Mn^{2+} was above 200 μmol·L^{-1}, APX activities in the roots reduced rapidly with the increasing of Mn^{2+} under hypoxia conditions. But under normal aeration conditions, the reducing tendency of APX activity was much gently.

When Mn^{2+} was less than 200 μmol·L^{-1}, APX activities of hypoxia in the roots were higher than those of aeration. At the concentration of 200 μmol·L^{-1} Mn^{2+}, APX activities enhanced averagely by 237.9%, 208.4% in the roots under hypoxia and aeration respectively compared with the control respectively. When Mn^{2+} was higher than 400μmol·L^{-1}, APX activities of hypoxia in the roots were evidently lower than those of aeration.

GR activity in the leaves enhanced obviously with the increase of Mn^{2+} under aeration conditions when Mn^{2+} was less than 200 μmol·L^{-1}, but it reduced obviously with the increasing of Mn^{2+} (**Figure 1**). When Mn^{2+} was 10 μmol·L^{-1}, GR activities in the leaves of hypoxia increased averagely by 243.9%, which were much higher than those of aeration. When Mn^{2+} was less than 200 μmol·L^{-1}, GR activity under aeration or hypoxia conditions increased obviously with the increase of Mn^{2+} in the roots. When Mn^{2+} was higher than 200 μmol·L^{-1}, GR activity under aeration or hypoxia conditions reduced obviously with the increase of Mn^{2+}. It could come to the conclusion that 200 μmol·L^{-1} Mn^{2+} could effectively eliminate the harmfulness to the seedlings of free radical.

3.2. Effects of Excess Mn under Hypoxia Stress on H_2O_2, MDA and ASA Content

AsA activity in the leaves and roots increased obviously with the increase of Mn under aeration or hypoxia conditions when Mn^{2+} was less than 200 μmol·L^{-1}, and AsA activity in the leaves and roots of hypoxia were much higher than those of aeration (**Figure 2**). The AsA activity in the leaves of hypoxia and aeration enhanced 262.1%, 226.9% respectively. AsA activity in the roots of hypoxia and aeration enhanced 312.1%, 261.2% respectively. However, when Mn^{2+} was higher than 400 μmol·L^{-1}, AsA activity in the leaves and roots of hypoxia were obviously lower than those of aeration. AsA activity in the leaves of hypoxia and aeration were reduced averagely by 10.2%, 57.5% respectively compared with the control and AsA activity in the roots of hypoxia and aeration were reduced averagely by 121.8%, 81.7% respectively compared with the control. Therefore, low level of Mn^{2+} could relieve the harmfulness from hypoxia stress, on the contrary, high level of Mn^{2+} could only make more serious.

As shown in **Figure 2**, contents of H_2O_2 in the leaves and roots reduced when Mn^{2+} was less than 200 μmol·L^{-1} and then increased obviously when Mn^{2+} was higher than 200 μmol·L^{-1} under hypoxia conditions. However, contents of H_2O_2 in the roots raised all the way under aeration condition.

Under hypoxia, contents of MDA in the leaves and roots reduced at the critical point of 200 μmol·L^{-1} Mn^{2+} and then increased (**Figure 2**). Under the conditions of other things being equal, the hypoxia contents of MDA in leaves and roots were much higher than those of aeration. Contents of MDA in the hypoxia leaves and roots, aeration leaves and roots increased by 246.1%, 157.7%, 396.7%, and 320.2% respectively. In term of hypoxia stress, 200-400 μmol·L^{-1} Mn^{2+} could reduce the contents of MDA evidently and the contents of MDA in hypoxia leaves and roots were lowered by 57.9%, 52.2% than those of control. It indicated appropriate amount of Mn^{2+} could effectively alleviate the over-oxidation to the membrane lipid of cell under hypoxia.

3.3. Changes in Gene Expressions in Response to Mn^{2+} Levels under Hypoxia Stress

To analyze the underlying molecular mechanisms for

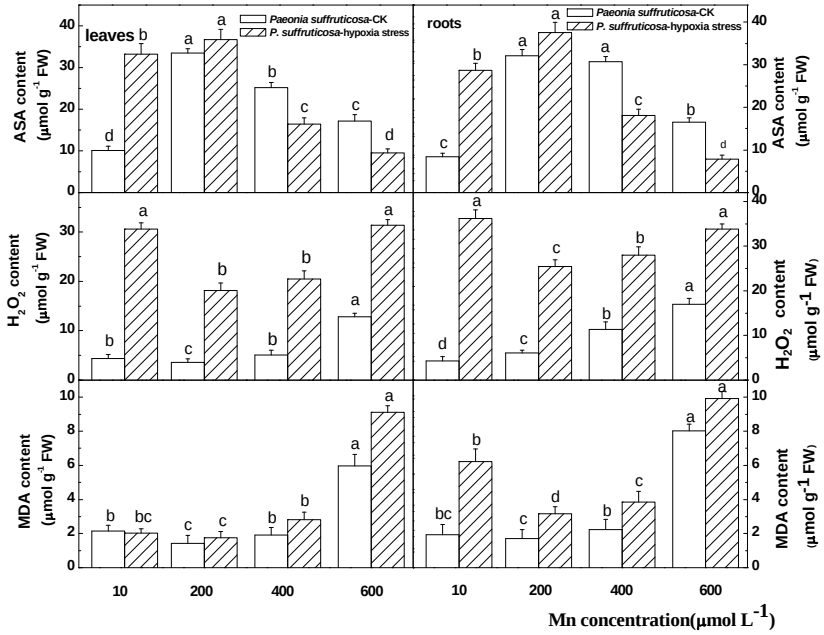

Figure 2. Effects of excess Mn under hypoxia stress on content of ASA, H_2O_2 and MDA in tomato seedlings.

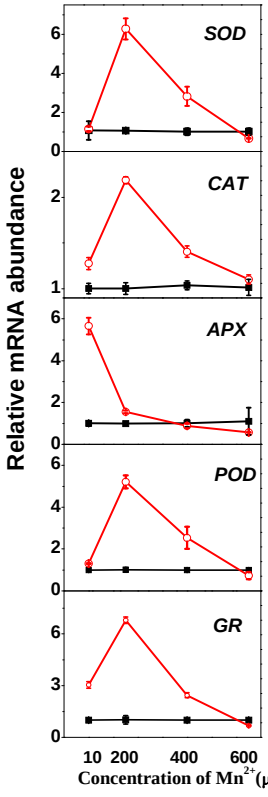

Figure 3. Changes in stress responsive genes expressions in response to Mn^{2+} levels under hypoxia stress. Related genes were analyzed by quantitative real time PCR using the gene-specific primer pairs shown in Table 1. The data were obtained from three independent experiments. Each value in the graph shows mean with ± SD of three experiments.

hypoxia stress tolerance, we examined the effects of different Mn^{2+} concentrations on stress responsive genes expression.

As shown in **Figure 3**, the gene expression was inspired on the low level of Mn^{2+}, and a series of resistance genes level achieved peak value with 10 μmol·L^{-1} Mn^{2+}. The expression level of *SOD*, *CAT*, *APX*, *POD*, *GR* were 6.28, 2.19, 5.66, 5.21 and 6.79 times compared to control respectively. And the expression level decreased in resistance genes with the increasing level of Mn^{2+}. The gene expression levels with excessive Mn^{2+} under hypoxia stress was less than the low level Mn^{2+}.

4. Discussion

Mn toxicity is one of important abiotic stresses in acidic soil [21], and affects some physiological and biochemical processes associated with plant growth and development [3,25]. Results of experiment in cucumber have shown that excess Mn inhibited plant growth [12]. It has been reported that the occurrence of Mn toxicity was closely related with Mg in muskmelon and Fe concentrations in lichen tissues [3,26]. Excess Mn significantly reduced the contents of Mg and Fe in cucumber leaves. The decrease in the contents of Mg and Fe may increase the SOD activity to hypoxia stress [21,27]. The antioxidant enzymes were important enzymes in preventing the oxidative stress in plants as is based on the fact that the activity of one or more of these enzymes in generally increased in plants when exposed to stressful conditions and this enhanced activity is related to increased stress tolerance [28,30].

The oxidative stress is a key component of environmental stress [31]. Various environmental stresses cause accumulation of H_2O_2 in different tissue segments and the regulation of H_2O_2 levels is of utmost importance in plant cell metabolism [32]. The enhancement of antioxidant enzymes activities was correlated with increased protection from damage associated with oxidative stress [33]. In the present experiment, excess Mn increased the activities of SOD, APX and GR, particularly under hypoxia stress. It may be concluded that appropriate amount of Mn^{2+}could reduce the damage of active oxygen under hypoxia stress, but reversely, excessive level of Mn^{2+} were just to aggravate the already serious damage to the tomato seedlings.

REFERENCES

[1] M. J. Mukaopadhyay and A. Sharma, "Manganese in Cell Metabolism of Higher Plants," *Botanical Review*, Vol. 51, No. 2, April 1991, pp.117-149.

[2] C. Negra, D. S. Ross and A. Lanzirotti, "Oxidizing Behavior of Soil Manganese: Interactions among Abundance, Oxidation State, and pH," *Soil Science Society of America Journal*, Vol. 69, No. 1, January 2005, pp. 87-95.

[3] M. Hauck, A. Paul, S. Gross and M. Raubuch, "Manganese Toxicity in Epiphytic Lichens: Chlorophyll Degradation and Interaction with Iron and Phosphorus," *Environmental and Experimental Botany*, Vol. 49, No. 2, April 2003, pp. 181-191.

[4] C. O. Dimkpa, D. Merten, A. Svatoš, G. Büchel and E. Kothe, "Metal-Induced Oxidative Stress Impacting Plant Growth in Contaminated Soil is Alleviated by Microbial Siderophores," *Soil Biology & Biochemistry*, Vol. 41, No. 1, January 2009, pp. 154-162.

[5] R. V. Tyson and T. H. Pearson, "Modern and Ancient Continental Shelf Anoxia: An Overview," In: R. V. Tyson, T. H. Pearson, Ed., *Modern and ancient continental shelf anoxia*, London, Geological Society Special Publication, 1991, pp. 1-24.

[6] L. Blake and K. W. T. Goulding, "Effects of Atmospheric Deposition, Soil pH and Acidification on Heavy Metal Contents in Soils and Vegetation of Semi-Natural Ecosystems at Rothamsted Experimental Station, UK," *Plant Soil*, Vol. 240, No. 2, March 2002, pp. 235-251.

[7] F. C. Vlidon and M. G. Teixeira, "Oxygen Radical Production and Control in the Chloroplast of Mn-Treated Rice," *Plant Science*, Vol. 152, No. 1, January 2000, pp. 7-15.

[8] H. Markus, P. Alexander, G. Shana and R. Markus, "Manganese Toxicity in Epiphytic Lichens: Chlorophyll Degradation and Interaction with Iron and Phosphorus," *Environmental and Experimental Botany*, Vol. 49, No. 2, April 2003, pp. 181-191.

[9] Q. H. Shi, Z. Y. Bao, Z. J. Zhu, Q. S. Ying and Q. Q. Qian, "Effects of Different Treatments of Salicylic Acid on Heat Tolerance, Chlorophyll Fluorescence, and Antioxidant Enzyme Activity in Seedlings of *Cucumis Sativa* L.," *Plant Growth Regulation*, Vol. 48, No. 2, February 2006, pp. 127-135.

[10] T. El-Jaoual and D. A. Cox, "Manganese Toxicity in Plants," *Journal of Plant Nutrition*, Vol. 24, No. 2, February 1998, pp. 353-386.

[11] J. Le Bot, E. A. Kirkby and M. L. Van Beusichem, "Manganese Toxicity in Tomato Plants: Effects on Cation Uptake and Distribution," *Journal of Plant Nutrition*, Vol. 13, No. 5, May 1990, pp. 513- 525.

[12] Q. H. Shi, Z. J. Zhu, Y. He, Q. Q. Qian and J. Q. Yu, "Silicon-Mediated Alleviation of Mn Toxicity in *Cucumis Sativus* in Relation to Activities of Superoxide Dismutase and Ascorbate Peroxidase," *Phytochemistry*, Vol. 66, No. 13, July 2005, pp. 1551-1559.

[13] S. M. Macfie and G. J. Taylor, "The Effects of Excess Manganese on Photosynthetic Rate and Concentration of Chlorophyll in *Triticum Aestivum* Grown in Solution Culture," *Physiologia Plantarum*, Vol. 85, No. 3, June 2008, pp. 467-475.

[14] M. Hauck, A. Paul, C. Mulack, E. Fritz and M. Runge, "Effects of Manganese on the Viability of Vegetative Diaspores of the Epiphytic Lichen *Hypogymnia Physodes*," *Environmental and Experimental Botany*, Vol. 47, No. 2, March 2002, pp. 127-142.

[15] S. S. Sharma and K. J. Dietz, "The Relationship between Metal Toxicity and Cellular Redox Imbalance," *Trends in Plant Science*, Vol. 14, No. 1, January 2009, pp. 43-50.

[16] R. Mittler, "Oxidative Stress, Antioxidants and Stress Tolerance," *Trends in Plant Science*, Vol. 7, No. 9, September 2002, pp. 405-410.

[17] E. R. Stadtman and C. N. Oliver, "Metal-Catalyzed Oxidation of Proteins. Physiological Consequences," *Journal of Biological Chemistry*, Vol. 266, No. 4, February 1991, pp. 2005-2008.

[18] J. A. Harrigan, J. Piotrowski, L. D. Noto, R. L. Levine and V. A. Bohr, "Metal-Catalyzed Oxidation of the Werner Syndrome Protein Causes Loss of Catalytic Activities and Impaired Protein-Protein Interactions," *Journal of Biological Chemistry*, Vol. 282, No. 50, December 2007, pp. 36403-36411.

[19] T. Brennan and C. Frenkel, "Involvement of Hydrogen Peroxide in the Regulation of Senescence in Pear," *Plant Physiology*, Vol. 59, No. 3, March 1977, pp. 411-416.

[20] C. N. Giannopolitis and S. K. Ries, "Superoxide Dismutases I. Occurrence in Higher Plants," *Plant Physiology*, Vol. 59, No. 2, 1977, pp. 309-314.

[21] I. Cakmak and H. Marschner, "Magnesium Deficiency and High Light Intensity Enhance Activities of Superoxide Dismutase, Ascorbate Peroxidase and Glutathione Reductase in Bean Leaves," *Plant Physiology*, Vol. 98, No. 4, April 1992, pp. 1222-1227.

[22] Y. Nakano and K. Asada, "Purification of Ascorbate Peroxidase in Spinach Chloroplasts; its Inactivation in Ascorbate-Depleted Medium and Reactivation by Monodehydroascorbate Radical," *Plant and Cell Physiology*, Vol. 28, No. 1, January 1987, pp. 131-140.

[23] C. H. Foyer and B. Halliwell, "The Presence of Glutathione and Glutathione Reductase in Chloroplasts: A Proposed Role in Ascorbic Acid Metabolism," *Planta*, Vol. 133, No. 1, January 1976, pp. 21-25.

[24] K. J. Livak and T. D. Schmittgen, "Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the $2^{-\Delta\Delta CT}$ Method," *Methods*, Vol. 25, No. 4, December 2001, pp. 402-408.

[25] A. Paul, M. Hauck and E. Fritz, "Effects of Manganese on Element Distribution and Structure in Thalli of the Epiphytic Lichens *Hypogymnia Physodes* and *Lecanora Conizaeoides*," *Environmental and Experimental Botany*, Vol. 50, No. 2, October 2003, pp. 113-124.

[26] O. M. Elamin and G. E. Wilcox, "Effects of Magnesium and Manganese Nutrition on Muskmelon Growth and Manganese Toxicity," *Journal of the American Society for Horticultural Science*, Vol. 111, No. 4, April 1986, pp. 582-587.

[27] I. Cakmak, B. Erenoglu, K. Y. Gulut, R. Derici and V. Romheld, "Light-Mediated Release of Phytosiderophores in Wheat and Barley under Iron or Zinc Deficiency," *Plant Soil*, Vol. 202, No. 2, May 1998, pp. 309-315.

[28] G. Santandrea, T. Pandolfini and A. Bennici, "A Physiological Characterization of Mn-Tolerant Tobacco Plants Selected by *In Vitro* Culture," *Plant Science*, Vol. 150, No. 2, January 2000, pp. 163-177.

[29] M. M. Fecht-Christoffers, P. Maier and W. J. Horst, "Apoplastic Peroxidases and Ascorbate are Involved in Manganese Toxicity and Tolerance of *Vigna Unguiculata*," *Physiologia Plantarum*, Vol. 117, No. 2, March 2003, pp. 237-244.

[30] A. Hameed, S. Naseer, T. Iqbal, H. Syed and M. A. Haq, "Effects of NaCl Salinity on Seedling Growth, Senescence, Catalase and Protease Activities in Two Wheat Genotypes Differing in Salt Tolerance," *Pakistan Journal of Botany*, Vol. 40, No. 3, March 2008, pp. 1043-1051.

[31] A. C. F. de Vasconcelos, X. Z. Zhang, E. H. Ervin and K. J. de Castro, "Enzymatic Antioxidant Responses to Biostimulants in Maize and Soybean Subjected to Drought," *Scientia Agricola*, Vol. 66, No. 3, May 2009, pp. 395-402.

[32] T. S. Gechev and J. Hille, "Hydrogen Peroxide as a Signal Controlling Plant Programmed Cell Death," *Journal of Cell Biology*, Vol. 168, No. 1, January 2005, pp. 17-20.

[33] H. H. Abd El-Baky, F. K. El Baz and G. S. El-Baroty, "Enhancement of Antioxidant Production in *Spirulina Platensis* under Oxidative Stress," *Acta Physiologiae Plantarum*, Vol. 31, No. 3, May 2009, pp. 623-631.

