

# Effect of Zinc Heavy Metal on Stress-Related Genes in Tomato (*Solanum lycopersicum* L.) Plants

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**Abstract**— Heavy metal pollution is a major environmental problem all over the world. It is known that high concentration of heavy metals in soils and waters cause genotoxicity and damage to most of the functional biomolecules. The aim of this study was to determine the molecular changes in tomato (*Solanum lycopersicum* L.) genome under heavy metal of zinc stress. Zinc is a microelement which should be taken in very less amounts by plants, animals and humans. In plants, zinc in low concentration is essential for root and stem elongation, RNA levels, the cell's ribosome content and protein formation mechanism. But at high concentrations, it is toxic for plants like cadmium, lead and copper. In this current study, the molecular response of tomato (*Solanum lycopersicum* L.) plants to zinc stress was examined by transcript accumulation analysis of two stress-related genes: (i) *MT2* (metallothionein) gene, coding for a metal-binding protein and (ii) *GR1* (glutathionreductase) gene, a marker of enzymatic ROS scavenging mechanism. A quantitative Real-Time PCR experiment was performed with *MT2* and *GR1* genes using RNA isolated from tomato roots or shoots treated for 24h with zinc at concentrations ranging from 20 to 1280ppm. Results showed that the genes were over-expressed in zinc-stressed tomato. The highest relative fold change value was measured on *GR1* for both root and shoot indicating the activation of the oxidative stress enzyme to tolerate zinc stress.

**Keywords**— Zinc Heavy Metal, Tomato Plants, *MT2*, *GR1*.

## I. INTRODUCTION

Crop plants such as tomato are frequently exposed to a variety of abiotic stresses including drought, salinity and heavy metal pollutions. Several industrial activities, urban waste, spraying and fertilization held in agriculture and uses of heavy metal-containing pesticides are some of the sources of heavy metal pollution. These pollutants cause a decrease in the quality of agricultural products [1-3]. Heavy metal toxicity affects biological molecules, for example, when metals bind to S group, blocks the active site of enzyme, and may cause conformational changes in enzymes, disrupts the cellular homeostasis and cause oxidative damage by generating reactive oxygen species (ROS) such as singlet oxygen, hydrogen peroxide, hydroxyl radical which cause lipid peroxidation, membrane defects and instability of enzymes in higher plants [4-7]. Zinc (Zn) is an indispensable metal for normal growth. Zinc is a microelement which should be taken in very less amounts by plants, animals and humans. In plants, zinc in low concentration is essential for root and stem elongation, RNA levels, the cell's ribosome content

and protein formation mechanism. But at high concentrations, it is toxic for plants like cadmium, lead and copper [8, 9]. The toxic effect of zinc causes damages to the cell division and it especially gives damages to the cell nucleus of meristematic stem cells and cell division. Also genetic variations in sensitivity to Zn toxicity have been mapped in plants [10, 12]. Zinc (Zn) is a potential environmental toxicant for plants under excessive conditions. In this study, to understand the molecular response of tomato (*Solanum lycopersicum* L.) plants to high zinc stress was examined by transcript accumulation analysis of two stress-related genes: (i) *MT2* (metallothionein) gene, coding for a metal-binding protein and (ii) *GR1* (glutathionreductase) gene, a marker of enzymatic ROS scavenging mechanism [13, 14].

So, in our study we demonstrated the activation of these genes under Zn treatment in nutrient solution. In addition, this study reports that the activation of these genes could serve as a possible additional Zn-tolerance mechanism to cope with toxic levels of Zn in tomatoes.

## II. MATERIAL AND METHODS

### Plant growth and Zinc treatment

Tomato plants were grown in seedling trays were filled with sterilized perlite. Tomato seeds were germinated and grown hydroponically in pots containing 0.2 L of modified 1/10 Hoagland's solution. Tomato plants with three biological replicates, each consisting of one pot with ten plants were grown in a controlled environmental growth chamber with light of 250  $\mu\text{mol m}^{-2} \text{s}^{-1}$  photosynthetic photon flux at 23-26 °C, and with 50-60% relative humidity.

After 21 days of growth with hoagland solution containing macronutrients and micronutrients, seedlings were exposed to 20, 40, 80, 160, 320, 640, 1280ppm zinc solution ( $\text{ZnSO}_4 \cdot 6\text{H}_2\text{O}$ ) for 24h. Roots and shoots were harvested and stored at -80°C until RNA isolation.

### RNA isolation and first strand cDNA synthesis

Total RNA of all root and shoot samples were extracted by using Trizol RNA extraction protocol followed by RNeasy mini kit (Qiagen, Cat no: 74104) to cleanup [15]. The quantity and quality of RNA was determined by NanoDrop Lite Spectrophotometer and also confirmed by gel electrophoresis which contains 1.5% agarose gel and formaldehyde. Extracted RNAs were stored at -80 °C.

### First strand cDNA synthesis

A two-step procedure was used for real-time reverse transcriptase-polymerase chain reaction (RT-PCR). Reverse transcription reactions were performed with 2  $\mu\text{g}$  of RNA, 2.5  $\mu\text{M}$  Anchoredoligo(dT)18, 1X Transcriptor High Fidelity Reverse Transcriptase Reaction Buffer, 20 U Protector Rnase Inhibitor, 1 mM deoxynucleotide mix and 10 U Transcriptor highfidelity reverse transcriptase using the high fidelity cDNA synthesis kit (Roche). The quantity and quality of cDNA was determined by NanoDrop Lite Spectrophotometer.

### qRT-PCR analysis of *GR1* and *MT2* genes

Real-time PCR was performed using Light Cycler® Nano System (Roche). Gene-specific qRT-PCR primers (Table 1) for *GR1* and *MT2* genes and actin (*ACT*) were designed using Primer 3 software [16] based on the sequence information of tomato genes available in the databank (<http://www.ncbi.nlm.nih.gov/>). Amplifications of the PCR product were monitored via SYBR Green I dye.

PCR conditions consisted of a 95 °C for 10 min, 40 cycles of 95 °C for 7s, 58-62 °C for 15s, 72 °C for 10s and a melting analysis of 52 to 95 °C with an increasing temperature 0.5 °C  $\text{min}^{-1}$ . The qRT-PCR analysis

contained three biological replicates, consisting of three technical replicates.

Table 1. Sequences, melting temperatures of primers and accession numbers of genes used in qRT-PCR

Gene		Sequence (5'-3')	Tm (°C)	Accession no.
<b>MT-2</b>	Forward	GCTGTGGATCTAGCTGCAAGTGCG	60	EU884310
	Reverse	AAGGGTTGCACTTGCAGTCAGATC		
<b>GR-1</b>	Forward	CGTGCTGTGATACTTGGTGG	60	FJ265823
	Reverse	TCGTGCAAGGATGCATAGTG		
<b>ACT</b>	Forward	GGGATGGAGAAGTTTGGTGGTGG	60	EU884309
	Reverse	CTTCGACCAAGGGATGGTGTAGC		

### Statistical analysis

The abundance of target genetranscripts was normalized to *ACT* and set relative to the  $2^{-\Delta\Delta\text{CT}}$  method [17]. Changes in relative expression levels (REL) of the gene were checked for statistical significance according to one way ANOVA.  $P < 0.05$  was considered to be statistically significant.

## III. RESULTS AND DISCUSSION

One of the effects of toxins is to prevent the root and body growth. The accumulation of heavy metals in plant causes negative effects on roots, stems and germination of seeds; when it is exposed to the increasing concentrations of heavy metals [18]. Similarly, as expected in tomato plants, the findings of this study on the length of tomato seedlings' roots and stems are similar with the related literature.

MTs protect plants from metal stresses. High transcription rate of MTs was particularly shown in metallophyte tolerant plant varieties [7, 14]. They have the ability to bind both physiological (such as zinc, copper, and selenium) and xenobiotic (such as cadmium, mercury, silver, and arsenic) heavy metals through their cysteine residue thiol groups. Also, MTs were proposed to function in both metal chaperoning and scavenging of ROS [19]. Studies showed that Arsenic and cadmium stress induces metalloprotein expression and accumulation [20].

GR is a potential enzyme of the ASH-GSH cycle and plays an essential role in the defense system against ROS by sustaining the reduced status of GSH [21]. It plays a crucial role in determining the tolerance of a plant under various types of stress [22]. It has recently been observed that the GR activity increases in the presence of Cd in various plants [21].

In this study, the tomato seedlings were subjected to Zinc for 24 hour. By referring to the several studies it was

decided that 24 h is enough period to trigger to the early stress response.

The expression levels of the *MT2*, *GR1* and *ACT* gene at mRNA level were analyzed in, tomato samples by Real-time PCR (Light CyclerNano, Roche) and the results were summarized in Figure-1 and Figure-2. *MT2* and *GR1* gene transcript levels were calculated in tomato samples

exposed to various concentration of zinc solution. To avoid error, real-time RT-PCR is typically normalized with *ACT* as an housekeeping and internal control gene and also with the control treatment samples. To evaluate the stability of the results, *MT2*, *GR1* and *ACT* transcript levels of all samples were measured three times for each time period.

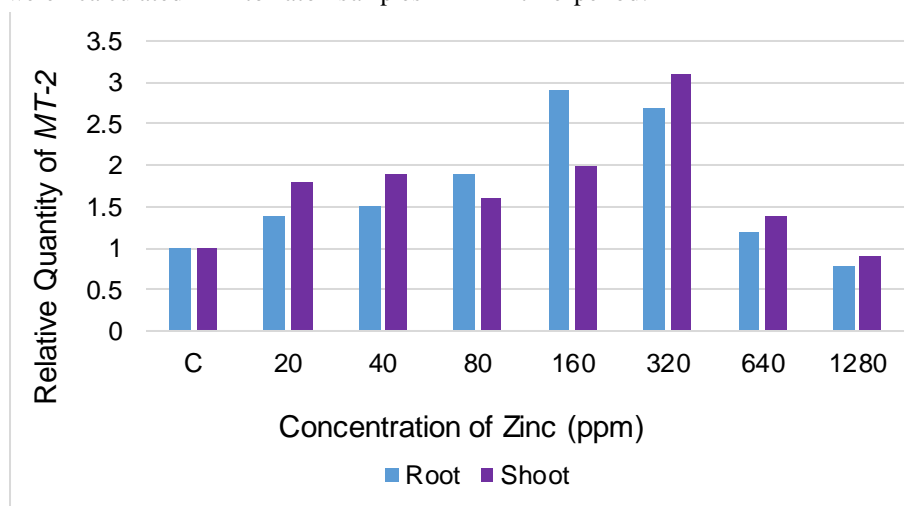


Fig.1: Metallothionein (*MT*) gene expression profile of root and shoot in the tomato samples exposed to different concentration of zinc solution.

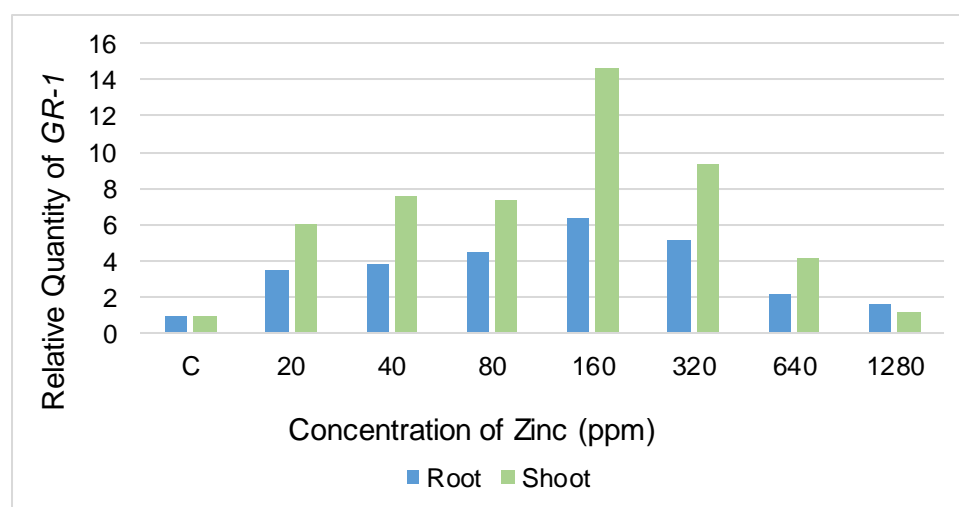


Fig.2: Glutathion reductase (*GR*) gene expression profile of root and shoot in the tomato samples exposed to different concentration of zinc solution.

In this study, the *GR1* accumulation was significantly observable under zinc stress for both roots and shoots ( $P < 0.01$ ). The highest relative fold change value was measured on *GR1* for both root and shoot. In roots, the abundance of transcript level reached to 6.4 and 5.2-fold increase at concentrations 160 and 320ppm, respectively [Figure-3, ( $P < 0.01$ )].

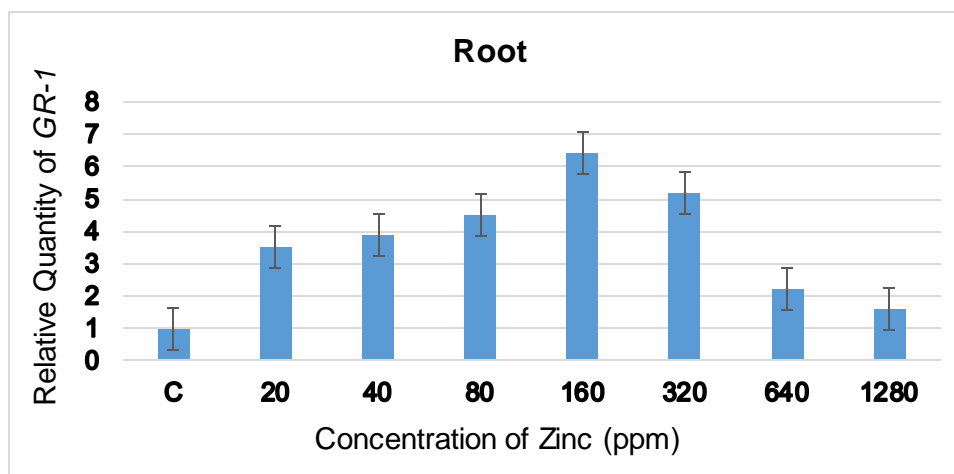


Fig.3: Glutathion reductase (GR) gene expression profile of root in the tomato samples exposed to different concentration of zinc solution.

In shoots the abundance of transcript level reached to 14.6-fold increase at 160ppm concentration and 9.4-fold increase at concentration of 320ppm [Figure-4, ( $P < 0.01$ )].

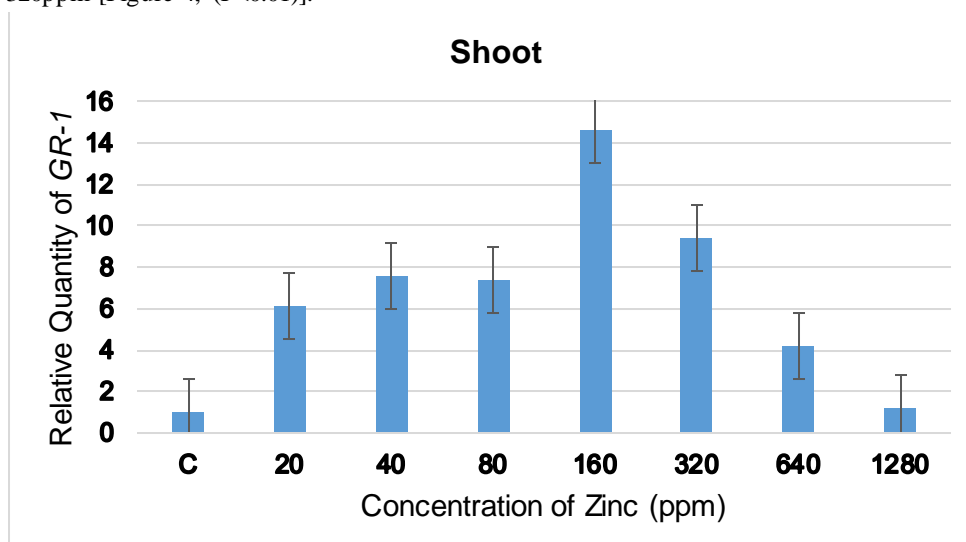


Fig.4: Glutathion reductase (GR) gene expression profile of shoot in the tomato samples exposed to different concentration of zinc solution.

After the concentration of 160 ppm, the abundance of *GR1* dramatically decreases nearly down to the same as in the control group for both root and shoot, indicating that the resistance mechanism does not allow any protection for the amelioration of the zinc stress by using the GR pathway, due to possible cellular damage. Similar results were observed in zinc-treated tomato root and shoots that

of *MT2* accumulations was apparent. *MT2* accumulation was significantly observable under zinc stress for both roots and shoots ( $P < 0.01$ ).

In roots, the abundance of transcript level reached to 2.9 and 2.7-fold increase at concentrations 160 and 320 ppm, respectively [Figure-5, ( $P < 0.05$ )].

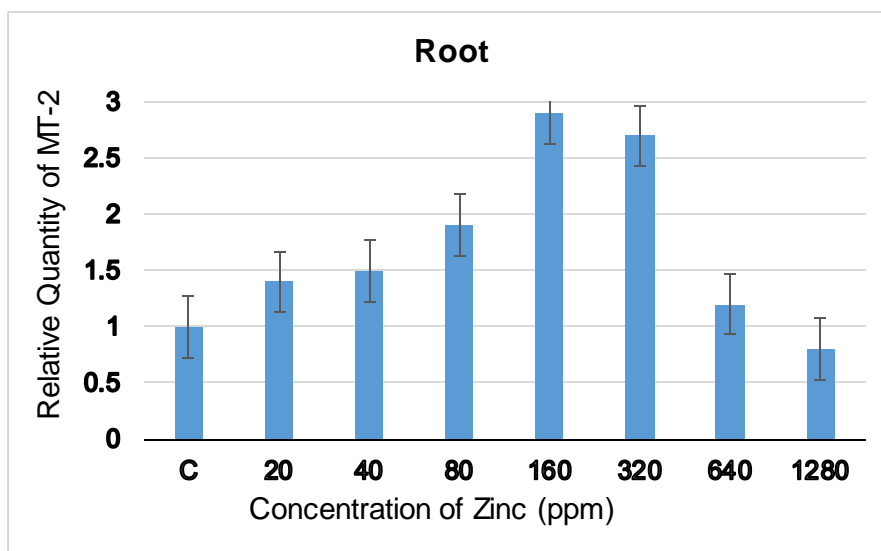


Fig.5: Metallothionein (MT) gene expression profile of root in the tomato samples exposed to different concentration of zinc solution.

In shoots the abundance of transcript level reached to 2 and 3.1-fold increase at concentrations 160 and 320 ppm, respectively. These results indicated that metallothionein binding protein-MT2 transcript accumulation may induce the zinc-toxicity tolerance in tomato plant [Figure-6, ( $P < 0.05$ )].

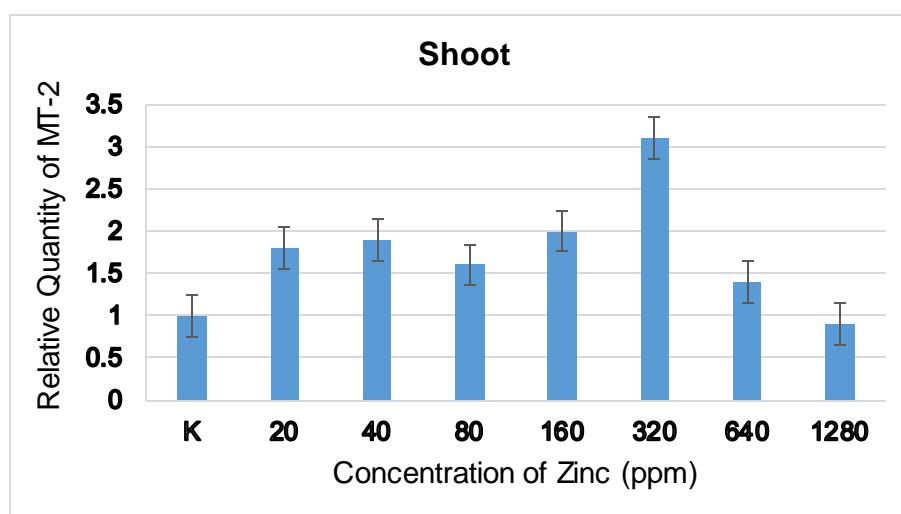


Fig.6: Metallothionein (MT) gene expression profile of shoot in the tomato samples exposed to different concentration of zinc solution.

**In conclusion**, it was shown here that the activation of MT2 and GR1-like protein transcripts under zinc stress. The accumulation of these genes increases at first and then, the curve reflects a descending profile, revealing the disruption of mechanisms which regulates the cellular homeostasis under high zinc levels. The activation of these genes could be a protection mechanism to zinc stress and play important role in the detoxification or tolerance mechanism.

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