Changes in the Photosynthetic Responses and Biochemical Constituents of Tomato (*Lycopersicon esculentum* Mill.) Under Zinc Stress

**P. Vijayarengan** and **G. Mahalakshmi**
Department of Botany, Annamalai university, Annamalainagar 608 002, Tamilnadu, India

**Abstract:** Tomato (cultivar PKM-1) plants were raised in pots containing the soil amended with various levels of zinc (control, 50, 100, 150, 200 and 250 mg kg$^{-1}$ soil). Photosynthetic responses (Photosynthesis and stomatal conductance) and biochemical constituents (chlorophyll-a, chlorophyll-b, total chlorophyll, carotenoid, sugars, amino acids and protein) were analyzed on 45th day. Zinc treatment at all levels tested (except 50 and 100 mg kg$^{-1}$) decreased the photosynthetic responses (photosynthesis and stomatal conductance) and biochemical constituents (chlorophyll and carotene content of leaves; sugars, amino acids and protein contents of root and shoots) of tomato plants. However the 50 and 100 mg kg$^{-1}$ zinc level in the soil showed a positive effect on the photosynthetic responses and biochemical constituents of tomato plants.

**Key words:** Zinc • Tomato • Photosynthesis • Stomatal conductance • Pigments • Sugars • Amino acids • Protein

**INTRODUCTION**

High heavy metal availability induces an ion stress in plants clearly distinct from salt stress. Heavy metals do not affect plant growth by a significant decrease of the osmotic potential of the substrate but by their own toxicity, acting at relatively low concentrations (10$^{-7}$ M or lower) [1]. The primary toxicity mechanisms of the different metal ions may be as different as their chemical properties, especially valence, ion radius and capacity to form organic complexes. Nevertheless, an excess of these metal ions or of soluble metal chelates may induce a series of biochemical and physiological alterations in plants which present some common characteristics. Membrane damage, alteration of enzyme activities and the inhibition of root growth are considered characteristic features of heavy metal stress [2]. These early events lead to a large range of secondary effects, such as disturbance of hormone balance [3], deficiency of essential nutrients [4], inhibition of photosynthesis [5], alteration of water relation [6], etc., which further enhance the metal-induced growth reduction.

Zinc is one of the essential nutrients of plants for normal growth and development. So, zinc is classified as a micro-nutrient. Zinc is very essential for plant nutrition at low level because it is vitally involved in a number of metalo-enzymes to provide stability to cytoplasm and ribosomes. It also catalyses the process of oxidation which is concerned with the synthesis of auxin-indole acetic acid and transformation of carbohydrates [7]. There are 59 kinds of zinc containing enzymes representing almost all enzymes group in plants. Zinc also participates in glycolate pathway, TCA, pentose phosphate pathway and also in nitrogen and nucleic acid metabolisms. But in excess levels zinc causes significant reduction in growth and yield of plants [8-9]. Keeping these points in view the present investigation has been made to study the impact of zinc on photosynthetic responses and biochemical constituents of tomato (*Lycopersicon esculentum* Mill.) cultivar PKM-1.

**MATERIALS AND METHODS**

**Seed Materials:** The experimental plant, the tomato belongs to the family Solanaceae, cultivar PKM-1 is one of the important vegetables of the world. Certified seeds were obtained from the market. Seeds with uniform size and weight were chosen for experimental purpose.

**Pot Culture Experiments:** Tomato (*Lycopersicon esculentum* Mill.) cultivar PKM-1 plants were grown in pots in untreated soil (control) and in soil to which zinc...
had been applied (50, 100, 150, 200 and 250 mg kg\(^{-1}\) soil). The inner surfaces of pots were lined with a polythene sheet. Each pot contained 6 kg of air dried soil. The zinc as finely powdered (ZnSO\(_4\) \(7H_2O\)) was applied to the surface soil and thoroughly mixed with the soil. Fifteen seeds were sown in each pot. All pots were watered to field capacity daily. Plants were thinned to a maximum of seven per pot, after a week of germination. Each treatment including the control was replicated seven times.

**Sample Collection:** The photosynthetic parameters (photosynthesis and stomatal conductance) and biochemicals constituents (pigments, sugars amino acids and protein) of the plants were estimated on 45\(^{th}\) day. Seven plants from each replicate of a pot was analyzed for its various parameters and the average was calculated. These mean values were used for statistical analysis.

**Photosynthetic Parameters:** Photosynthesis and stomatal conductance were measured using a Li-Cor 6200 portable infrared gas analyzer (Li-cor Inc., USA). All the measurements were made during the noon period when the stomata were fully open. Precautions were taken to avoid any water vapor on the leaf surface during the measurements. Always a 1 liter, leaf chamber was used. Readings were taken at 5 sec intervals and 10 readings were computed in each measurement. Five to six such measurements were analyzed, only natural light was used during these measurements.

**Biochemical Analysis:** Chlorophyll-a, Chlorophyll-b, total chlorophyll, carotene, total sugars, amino acids and protein contents in plant samples were estimated by the following methods.

**Estimation of Chlorophyll [10]:** Hundred milligram of fresh leaf was ground in a mortar and pestle with 20 ml of 80% acetone. The homogenate was centrifuged at 3000 rpm for 15 minutes. The supernatant was saved. The pellet was reextracted with 5ml of 80% acetone each time, until it become colourless. All the supernatants were pooled and utilized for chlorophyll determination. Absorbance was read at 645nm and 663nm in spectrophotometer. The chlorophyll content was measured by using the formula given by Arnon (1949).

**Estimation of Carotene [11]:** The same chlorophyll extract was measured at 480nm, in spectrophotometer to estimate the carotene.

**Estimation of Total Sugars [12]:** Plant samples were treated with 80 per cent boiling ethanol for taking extractions (5ml extract representing 1g of tissue). Five readings for each sample were taken.

One ml of ethanol extract taken in the test tubes was evaporated in a waterbath. To the residue, 1 ml of distilled water and 1ml of 1N sulphuric acid were added and incubated at 49°C for 3 minutes. The solution was neutralised with 1N sodium hydroxide using methyl red indicator. One ml of Nelson’s reagent was added to each test tube prepared by mixing reagent A and reagent B in 25: 1 ratio (Reagent A: 25g sodium carbonate, 25g sodium potassium tartarate, 20g sodium bicarbonate and 200g anhydrous sodium sulphate in 1000 ml; Reagent B: 15g cupric sulphate in 100ml of distilled water with 2 drops of concentrated sulphuric acid). The test tubes were heated for 20 minutes in a boiling water bath, cooled and 1ml of arsenomolybdate reagent (25g ammonium molybdate, 21ml concentrated sulphuric acid, 5g sodium arsenate dissolved in 475ml of distilled water and incubated at 37°C in a waterbath for 48 hours) was added. The solution was thoroughly mixed and diluted to 25ml and measured at 495nm in a spectrophotometer. The reducing sugar contents of unknown samples were calculated from glucose standard.

**Estimation of Amino Acids [13]:** One ml ethanol extract was taken in 25 ml test tubes and neutralized with 0.1N sodium hydroxide using methyl red indicator. One ml of ninhydrin reagent was added (800mg stannous chloride in 500ml citrate buffer, pH 5.0, 20g ninhydrin in 500ml methyl cellosolve; both solutions were mixed). The contents were boiled in a waterbath for 20 minutes, 5 ml of diluent solution (distilled water and n-propanol mixed in equal volume) was added, cooled and diluted to 25 ml with distilled water. The absorbance was measured at 570nm in a spectrophotometer. The standard graph was prepared using leucine.

**Estimation of Crude Protein [14]:** The crude protein content of the plant material was arrived by multiplying the nitrogen value with 6.25.

**RESULTS**

**Photosynthetic Responses**

**Photosynthetic Rate (M\(\mu\)m\(^{2}\)S\(^{-1}\)):** The effect of zinc on the photosynthetic rate of tomato plants was represented in Table 1. The photosynthetic rate of tomato leaves in of zinc level was found to be 27.2, 29.2, 28.4, 25.2, 22.3 and 20.1 respectively.
Table 1: Photosynthetic responses of *Lycopersicon esculentum* Mill. plants grown at various concentrations of zinc (45th day)

<table>
<thead>
<tr>
<th>Zinc added in the soil (mg kg(^{-1}))</th>
<th>Photosynthesis (or) CO(_2) uptake (µ M m(^{-2})S(^{-1}))</th>
<th>Stomatal conductance (mM m(^{-2})S(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>27.2</td>
<td>0.80</td>
</tr>
<tr>
<td>50</td>
<td>29.2 (+ 7.35)</td>
<td>0.85 (+ 6.25)</td>
</tr>
<tr>
<td>100</td>
<td>28.4 (+ 4.41)</td>
<td>0.83 (+ 3.75)</td>
</tr>
<tr>
<td>150</td>
<td>25.2 (-7.35)</td>
<td>0.75 (-6.25)</td>
</tr>
<tr>
<td>200</td>
<td>22.3 (-18.01)</td>
<td>0.73 (-8.75)</td>
</tr>
<tr>
<td>250</td>
<td>20.1 (-26.10)</td>
<td>0.68 (-15.0)</td>
</tr>
</tbody>
</table>

Per cent over control values are given in parentheses

Table 2: Effect of zinc on pigment content (mg g\(^{-1}\) fresh wt.) of *Lycopersicon esculentum* Mill. (45th day)

<table>
<thead>
<tr>
<th>Zinc added in the soil (mg kg(^{-1}))</th>
<th>Chlorophyll-a</th>
<th>Chlorophyll-b</th>
<th>Total chlorophyll</th>
<th>Carotenoid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.456</td>
<td>0.842</td>
<td>1.298</td>
<td>0.021</td>
</tr>
<tr>
<td>50</td>
<td>0.545 (+ 19.51)</td>
<td>0.993 (+ 17.93)</td>
<td>1.563 (+ 20.41)</td>
<td>0.025 (+ 19.04)</td>
</tr>
<tr>
<td>100</td>
<td>0.532 (+ 16.66)</td>
<td>0.986 (+ 17.10)</td>
<td>1.519 (+ 17.02)</td>
<td>0.023 (+ 9.52)</td>
</tr>
<tr>
<td>150</td>
<td>0.450 (-1.31)</td>
<td>0.838 (-0.47)</td>
<td>1.287 (-0.84)</td>
<td>0.15 (-28.57)</td>
</tr>
<tr>
<td>200</td>
<td>0.431 (-5.48)</td>
<td>0.808 (-4.03)</td>
<td>1.240 (-4.46)</td>
<td>0.013 (-38.09)</td>
</tr>
<tr>
<td>250</td>
<td>0.410 (-10.08)</td>
<td>0.772 (-8.31)</td>
<td>1.182 (-8.93)</td>
<td>0.012 (-42.85)</td>
</tr>
</tbody>
</table>

Per cent over control values are given in parentheses.

Table 3: Effect of zinc on total sugar, amino acid (mg g\(^{-1}\) fresh wt.) and protein (mg g\(^{-1}\) dry wt.) content of *Lycopersicon esculentum* Mill (45th day)

<table>
<thead>
<tr>
<th>Zinc added in the soil (mg kg(^{-1}))</th>
<th>Total sugar</th>
<th>Amino acid</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Root</td>
<td>Shoot</td>
<td>Root</td>
</tr>
<tr>
<td>50</td>
<td>12.768 (+35.52)</td>
<td>16.087 (+25.90)</td>
<td>14.734 (+8.67)</td>
</tr>
<tr>
<td>100</td>
<td>10.828 (+14.08)</td>
<td>11.821 (-7.48)</td>
<td>13.737 (+1.32)</td>
</tr>
<tr>
<td>150</td>
<td>8.697 (-35.52)</td>
<td>9.320 (-27.05)</td>
<td>11.991 (-11.55)</td>
</tr>
<tr>
<td>200</td>
<td>7.302 (-23.06)</td>
<td>7.221 (-43.48)</td>
<td>10.972 (-19.07)</td>
</tr>
<tr>
<td>250</td>
<td>5.317 (-43.97)</td>
<td>6.328 (-50.47)</td>
<td>9.640 (-28.89)</td>
</tr>
</tbody>
</table>

Per cent over control values are given in parentheses.

**Stomatal Conductance (mM m\(^{-2}\)S\(^{-1}\))**: Stomatal conductance of leaves of tomato plants under zinc stress is represented in Table 1. Stomatal conductance of leaves of tomato plants was maximum at 50 mg kg\(^{-1}\) (0.85) and decreased further with an increase in zinc level in the soil. Minimum stomatal conductance of tomato leaves (0.68) was observed at 250 mg kg\(^{-1}\) zinc level in the soil.

**Biochemical Constituents (mg g\(^{-1}\) fresh wt.)**

**Pigments**: Effects of zinc on the pigment contents of tomato were represented in Table 2. Pigments, such as chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoid content of tomato leaves increased at lower concentration (50-100 mg kg\(^{-1}\)) and decreased further with an increase in the zinc level (150-250 mg kg\(^{-1}\)). Total chlorophyll content of the control is 1.298 and increased at 50 mg kg\(^{-1}\) (1.563) and 100 mg kg\(^{-1}\) (1.519) and decreased 8.93% at 250 mg kg\(^{-1}\). The carotenoid content of tomato leaves in the control 50, 100, 150, 200 and 250 was found to be 0.021, 0.025, 0.23, 0.015, 0.013 and 0.012 respectively.

**Total Sugars**: Total sugar content of root and shoot of tomato plants under zinc stress is represented in Table 3. Sugar content of root (12.768) and shoot (16.087) were maximum at 50 mg kg\(^{-1}\) soil level. Minimum sugar content of tomato root (5.317) and shoot (6.328) were recorded at 250 mg kg\(^{-1}\) soil level.

**Amino Acid**: Amino acid content of root and shoot of tomato plants are presented in Table 3. Amino acid content of root (14.734) and shoot (16.891) of tomato plants increased at 50 mg kg\(^{-1}\) soil level and decreased further with an increase in zinc level in the soil. Minimum amino acid content of tomato root (9.640) and shoot (8.812) were observed at 250 mg kg\(^{-1}\) soil level.
Protein (mg g\textsuperscript{-1} dry wt.): Protein content in the root (153.125) and shoot (185.625) of tomato were found to be the highest at 50 mg kg\textsuperscript{-1} soil level (Table 3). Protein content of tomato plants root (93.125) and shoot (98.75) were the lowest at 250 mg kg\textsuperscript{-1} zinc level in the soil.

**DISCUSSION**

**Photosynthetic Parameters**

*Photosynthesis and Stomatal Conductance:* The photosynthetic parameters such as photosynthesis and stomatal conductance of tomato leaves were analyzed on 45\textsuperscript{th} day. Photosynthesis and stomatal conductance of tomato leaves showed a gradual decline with the increase in zinc levels in the soil. Photosynthesis and stomatal conductance of tomato leaves and found to be higher at 50 and 100 mg kg\textsuperscript{-1}. Similar reduction in photosynthesis and stomatal conductance due to the metal treatments were registered by Schlegel et al. [15], Krupa et al. [16] and Mehindirata et al. [17]. Reduction in photosynthesis may be an indirect effect of heavy metals through a decrease in chlorophyll content and stomatal conductance [18] or due to the lower CO\textsubscript{2} assimilation rate, compared to control [19]. Metals may inhibit photosynthesis by increasing stomatal resistance [20] or through such process as chlorophyll degradation and impairment of PS II activity [21].

**Biochemical Constituents**

*Pigments:* The photosynthetic pigments such as chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoid contents of tomato decreased with increasing zinc level in the soil. Similar changes in the content by various metal treatments were recorded by Stobart et al. [22] with cadmium, Prasad and Prasad [23] with mercury and Schlegel et al. [15] with cadmium, zinc and mercury. The chlorophyll content of leaves varied greatly with the zinc level in the soil indicating the pronounced effect on chlorophyll biosynthesis. The increased chlorophyll content at lower level of zinc was obviously due to better growth.

The excess zinc treatment brought about by a marked depression in photosynthetic pigments in tomato plants. It might be due to excess supply of zinc resulting in interference with the synthesis of chlorophyll. The formation of chlorophyll pigments depends on the adequate supply of iron [24]. Granick [25] has suggested protoporphyrin is a precursor for chlorophyll synthesis. The excess supply of zinc seems to prevent the incorporation of iron in protoporphyrin molecule resulting in the reduction of chlorophyll pigment. This was strengthened by the fact that excessive amounts of a range of heavy metals such as copper [26], cobalt [27] and zinc [28] induced chlorosis in plants which were usually similar to the chlorosis of iron deficiency. Impaired chlorophyll development by heavy metals may be due to interference of protein, the structural component of chloroplast [29]. Zinc treatment presumably blocked the synthesis and activities of enzyme proteins responsible for chlorophyll biosynthesis.

*Sugars:* Total sugar content of tomato plants showed a decreasing trend with progressive increase in zinc level in the soil. However 50 and 100 mg kg\textsuperscript{-1} zinc level produced positive effect on the total sugar contents which is in consonance with the findings of Mahadeswaraswamy et al. [30] in *Phaseolus mungo*. The accumulation of total sugar decreased with increase in zinc level. The response is similar to that reported by Samarakoon and Rauser [31] in bush bean and Greger and Lindberg[32] in sugar beets.

*Amino Acids and Proteins:* Zinc level above 100 mg kg\textsuperscript{-1} significantly reduced the amino acid and protein contents in root and shoot of tomato plants. Zinc at 50 and 100 mg kg\textsuperscript{-1} soil level increased the amino acid and protein contents of tomato plants. Nag et al. [29] observed similar trends due to heavy metals like copper, zinc, mercury, lead and cadmium in rice. Further increase in zinc level decreased the amino acid and protein content. This was strengthened by the findings of Stiborova et al. [33] (copper and cadmium), Kastori et al. [34] (lead, cadmium, copper and zinc) and Bhattacharjee and Mukherjee [3] (cadmium and lead). Nitrogen is a precursor for the synthesis of amino acids [35]. Since the nitrogen content of the metal treated plants was found reduced, ultimately amino acids and protein contents of plants were also reduced, because there was only limited availability of nitrogen for the synthesis of amino acid [36].

**CONCLUSION**

From the present investigation it was concluded that the 50 and 100 mg kg\textsuperscript{-1} level of zinc in the soil was beneficial for the photosynthetic responses and biochemical constituents of tomato plants. The level of zinc in the soil above 150 mg kg\textsuperscript{-1} proved to be toxic. The results indicated that the zinc levels 50 to 100 mg kg\textsuperscript{-1} can be applied for increasing the photosynthetic responses and biochemical constituents of tomato plants.
REFERENCES