Response of Iodine on Antioxidant Levels of *Glycine max* L. Grown under Cd\(^{2+}\) Stress

Neha Gupta, Monika Shukla Bajpai, Rita Singh Majumdar and Pankaj K Mishra

Department of Biotechnology, School of Engineering & Technology, Plot No 32-34, Knowledge Park III, Sharda University, Greater Noida- 201306, India

Department of Biochemistry, Pandit Jawahar Lal Nehru Medical College, Raipur, Chattisgarh, India

**Abstract:** Heavy metal (HM) toxicity is one of the major abiotic stresses leading to hazardous effects in plants. A common consequence of HM toxicity is the excessive accumulation of reactive oxygen species (ROS) that cause lipids peroxidation, oxidation of protein, inactivation of enzymes, DNA damage and/or interactions with other vital constituents of plant cells. The purpose of this study was to determine whether the application of IO\(_3^-\) can improve the levels of antioxidants to severe cadmium stress in soybean seeds (*Glycine max* L).

The activity of antioxidant enzymes such as Superoxide dismutase, Ascorbate peroxidase and Glutathione reductase was boosted up when IO\(_3^-\) was applied in different concentrations of 20, 40, 80µM. SDS PAGE analysis of total protein was carried out to evaluate the seed proteins response of the *Glycine max* L. to 100mM CdCl\(_2\) and 100mM CdCl\(_2\) with IO\(_3^-\) in dosages of 20, 40, 80µM. SDS PAGE analysis of protein reveals that the protein expression decreased on the application of 100mM CdCl\(_2\) but supplementing with iodine in low concentrations (20 µM) enhanced the expression of seed proteins of *Glycine max* L. Our study recommends that IO\(_3^-\) could be considered a possible beneficial element to counteract the harmful effects of cadmium chloride stress.

**Key words:** *Glycine max* L-Oxidative Stress-HeavyMetalStress-SDS-PAGE Iodate

**INTRODUCTION**

Currently, it is estimated that 250–300 thousand ha of agricultural land are lost every year to heavy metal contamination. Heavy metal concentration has increased in soil, surface water and posed potential threat to terrestrial and aquatic biota [1]. Cadmium (Cd\(^{2+}\)) is a strongly phytotoxic heavy metal that inhibits plant growth and leads to plant death as studied in a number of plants. The antioxidative response due to oxidative stress induced by Cd\(^{2+}\) varies in different plants and is dependent on the concentration of Cd\(^{2+}\). Interestingly Cd\(^{2+}\) hyperaccumulator plants show strong tolerance to oxidative stress [2]. Plants employ various strategies to cope with the toxic effects of metals or metalloids. The plant’s molecular response to HM stress is characterized by the synthesis of stress-related amino acids, protein, genes and signaling molecules which are released continually as metabolic by-products in different metabolic pathways such as photosynthesis, photorespiration, mitochondrial electron transport chain and protein oxidation [3, 4]. Cd\(^{2+}\) being a redox-inactive HM can only generate ROS indirectly by enzyme inactivation and by inducing the expression of lipoxygenase in plant tissues and therefore causing oxidation of polyunsaturated fatty acids [5, 6]. The over expression of ROS can damage other biomolecules essential for growth and development. HM that bind to the cell nucleus cause promutagenic damage including DNA base modification, inter- and intramolecular crosslinking of DNA and proteins, DNA strand breaks, rearrangement and depurination [7].

The oxidative stress caused due to HM stress induces antioxidative defense systems, which in turn develop free radical scavenging molecules such as ascorbic acid and glutathione and the related enzymes that are involved in their biosynthesis and reduction [8]. It is one of the major antioxidant and redox buffers in
plants found abundantly in all cell compartments. GSH takes part in the control of H$_2$O$_2$ levels through the AsA-GSH cycle. It can also function directly as a free radical scavenger by reacting with O$_2^-$, O$_2^*$ and •OH [9].

Iodine is vital to human health and iodine biofortification programs help improve the human intake through plant consumption. Iodine is applied in two forms, one as potassium iodide (KI) and other as potassium iodate (KIO$_3$). Research has shown the successful use of KI as a chemical desiccant first for the screening of drought tolerant upland rice varieties at reproductive stage [10] and second in simulating the effect of terminal drought by chemical desiccants during grain filling [11].

The application of iodine in the form of iodate influences the antioxidant capacity of a number of plants like lettuce and tomato [12, 13]. Our principle goal was to study the effect of application of iodine in the form of potassium iodate on the antioxidant system of Glycine max L. seeds.

**MATERIALS AND METHODS**

**Plant Materials and Growth Conditions:** The experiment was conducted in the rainy season at the experimental field of Sharda University, Department of Biotechnology, Greater Noida, India, under controlled environmental conditions where (Minimum/maximum temperature and relative humidity were: 22.4/37.6°C and 76 to 81%, respectively. The plants were grown under the average photoperiod of 12 h of light. Seeds of Glycine max L. were procured from Indian Agriculture Research Institute, Pusa, New Delhi. Seeds were sown in pots carrying soil and cow dung manure at the ratio of 3:1. Ten days old seedlings having four-five true leaves were administered with three treatments of 100 mM of CdCl$_2$, three of which were applied in increasing doses of IO$_3^-$ (20, 40 and 80 µM as KIO$_3$) [14]. Two controls were kept. One was supplied with 100 mM CdCl$_2$ and the other was administered only Hogleland Nutrient solution. The CdCl$_2$ and iodine were supplied in Hogleland Nutrient Solution for one month (Alternate days). The young plants were harvested after 15 days of the last treatment. The seeds were collected and wrapped in an aluminium foil and stored at -20°C to see the following effects.

**Seed Growth and Germination:** Seeds after harvesting were measured for their size and mass.

Standard blotter technique was followed for seed germination percentage of Glycine max. For germination percentage, pure soaked seeds of each sample were spread over a wet filter paper in a Petri plate. The entire setup was placed in dark for 72 h. Seeds were observed for germination at an interval of 4 h till 3 days.

**Rate of H$_2$O$_2$ Production:** H$_2$O$_2$ content was determined by the method of Patterson [16]. Harvested seeds preserved in freezer were grounded in 6 ml of ice-cold acetone. The homogenate was centrifuged at 8,000g at 4°C for 30 min. 0.5 ml of supernatant was mixed with a 1.5 ml mixture of CHCl$_3$ and CCl$_4$ (1:3, v/v). The mixture was developed by adding the following reagents: a mixture of CHCl$_3$ and CCl$_4$ (1:3, v/v), 0.5 ml of supernatant, 1.5 ml of phosphate buffer (pH 7.8), 0.05% nitroblue tetrazolium (NBT) and 10 mM NaN$_3$. After 5 minutes incubation in the dark, 2 ml of the solution was taken up from the tubes and heated at 85°C for 15 minutes. Then the samples were cooled and optical density was measured at a wavelength of 580 nm [15] and the O$_2^*$ content was expressed as A$_{580}$ g$^{-1}$ fresh weight (FW).

**Rate of Superoxide anion production (O$_2^-$):** 0.1gm seeds were placed in a test tube and poured over with a solution containing 0.05 M PBS (pH 7.8), 0.05% nitroblue tetrazolium (NBT) and 10 mM NaN$_3$. After 5 minutes incubation in the dark, 2 ml of the solution was taken up from the tubes and heated at 85°C for 15 minutes. Then the samples were cooled and optical density was measured at a wavelength of 580 nm [15] and the O$_2^*$ content was expressed as A$_{580}$ g$^{-1}$ fresh weight (FW).

**Malondialdehyde Production:** For the MDA production, 0.1gm seeds were homogenized with 5ml of 80% cold ethanol. Homogenates were centrifuged at 4°C for 30 min. The 0.5 ml of supernatant was mixed with a 1.5 ml mixture of CHCl$_3$ and CCl$_4$ (1:3, v/v). The mixture was centrifuged at 1000rpm for 1 min and the water phase was collected for H$_2$O$_2$ determination. 1ml of phosphate buffer (pH 7.8) and 4-2-pyridylazol of 200mM were added to the supernatant and incubated at 45°C for 20 min. The absorbance was taken at 508 nm and concentration was expressed as µmol g$^{-1}$ FW.

**Antioxidant Enzymatic Assays:** Superoxide dismutase (SOD, EC 1.15.1.1) activity was determined by following the method of Bauchamp and Fridovich [18]. Enzyme extract was prepared by homogenizing 1g of sample with 1.5ml of phosphate buffer (pH 7.8). The extract was centrifuged at 3000 rpm for 20 min at 4°C. The reaction mixture was developed by adding the following reagents: 100µl of enzyme extract supernatant, 0.2 ml of 200mM methionine, 100µl of NBT, 100µl of 3mM Ethylene diamine...
tetra acetic acid, 1.5ml of PBS, 100 µl of 1.5 M Na₂CO₃ and 100µl of 60µM riboflavin. Reaction mixtures were illuminated for 15min at light intensity of 5000 lux. The absorbance of solution was measured at 560 nm.

Catalase (CAT, EC 1.11.1.6) activity was measured by following the method Cakmak and Marschner [19]. The catalase was determined by following the consumption of H₂O₂ at 240nm for 3 min. The reaction mixture contained 0.1ml of 50 mM potassium phosphate buffer (pH 7.0), 0.1 ml of 0.1 mM EDTA, 0.1 ml of 100 mM H₂O₂ and 0.7 ml of enzyme aliquot. The degradation of H₂O₂ was monitored at 240 nm.

Ascorbate peroxidase (APX, EC 1.11.1.11) activity measurement was done by homogenizing 0.1g of sample in 50mM sodium phosphate buffer (pH 7.0) and centrifuged at 1000rpm for 15min. The reaction mixture was prepared by adding 0.5mM ascorbic acid, 0.1mM H₂O₂ and 200µl of enzyme extract supernatant. The decrease in absorbance at 290nm was read. Activity was calculated using the extinction coefficient (2.8mM⁻¹ cm⁻¹) [20].

Glutathione reductase (GR) activity was determined according to Jablonski and Anderson [21]. Enzyme extract was prepared by homogenizing 100mg of sample with .067M of phosphate buffer (pH 7.6). The reaction mixture consisted of 10 mM Glutathione disulfide and 1mM EDTA. The supernatant was pre incubated at 25 °C for 5 min. The reaction was initiated by an addition of 1mM NADPH and the rate of oxidation of NADPH was monitored at 340 nm.

Non Enzymatic Antioxidants Assay: Ascorbic acid content was measured [22] 0.1g seed homogenized in 10ml of 0.4% oxalic acid and then centrifuged at 8000rpm at 4°C for 15min. 500µl of supernatant was taken in a tube and 7ml of 2,6- Dichlororindophenol dye solution was added to the same tube. The absorbance was taken at 518nm in spectrophotometer. The results were expressed as mmol total AsA g⁻¹ fresh weight (FW).

Proline content was determined based on the method of Bates et al. [23] 0.1g seed was homogenized with 2ml of 3% aqueous sulfosalicylic acid and centrifuged at 10,000 rpm for 10 min, 1ml of supernatant were mixed with 1ml of glacial acetic acid and 1ml of acid ninhydrin for 1 h at 100°C. The developed colour was extracted in 2ml toluene and measured at 520nm.

Total glutathione was determined by the glutathione recycling method of Anderson [24]. Fresh seed (0.1g) were homogenized in 2ml of 5% sulphosalicylic acid at 4°C. The homogenate was centrifuged at 10,000rpm for 10 min. To a 0.5ml of supernatant, 0.6ml of reaction buffer (0.1 M Na-phosphate, pH 7, 1mM EDTA) and 50µl of 3mM 5, 5-dithiobis-2-nitrobenzoic acid (DTNB) were added and read at 412 nm after 5 min.

Protein Profiling: Control and potassium iodate treated seeds were collected and washed with the doubled distilled water. Total soluble proteins from the seeds were isolated. One gram fresh seeds were homogenized with 10 ml phosphate buffer (1mM, pH 7.0). The homogenated samples were centrifuged at 8000 rpm for 30 minutes. After centrifugation the supernatant was collected and protein concentration in each samples were measured [25]. Protein samples (20µg) were separated on 12% SDS-PAGE as described by Laemmli et al. [26]. The amount of protein was calculated by comparison with standard curve of BSA drawn under identical experimental conditions.

Protein Extraction and Sodium Dodecyl Sulphate - Polyacrylamide Gel Electrophoresis (SDS-PAGE): Total proteins (200mg) of whole mature seeds of Soybean (Glycine max L. Merr.) were extracted with alkaline lysis buffer of 1N NaOH, containing 10 ml of 10% sodium dodecyl sulfate (SDS) and 70 ml of deionised water. Samples after mixing with loading buffer were boiled for 5 min and then loaded in the gels. SDS-PAGE was performed with the GeNei vertical unit, according Laemmli [26] using (12.5% polyacrylamide in resolving gel, 5% polyacrylamide in stacking gel, 180× 160 ×1.5 mm in size) gels. The run was carried out at 70 mA per gel at the beginning and then 85 mA per gel until the end of electrophoresis. Gels were stained with 0.25% Coomassie Brilliant Blue R dissolved in methanol: water: acetic acid (5:5:1 v/v) and destained in the same solvent. The protein standard used was Bovine Serum Albumin.

Statistical Analysis: All the experiments were performed in triplicate. Values in the tables indicate mean values ± SD. Differences among treatments were analyzed by Two Way ANOVA with multiple observations, taking p < 0.05 as significant according to Fisher’s multiple range test.

RESULTS

The major challenge imposed by heavy metal stress on plants is reduction in growth and therefore major loss in yield. Biomass reduction is one of the major parameters of agricultural indices for defining heavy metal stress tolerance. Here, in our experiment the maximum tolerance of Glycine max seeds was studied in the pots containing Hogland Nutrient Solution with 100 mM of CdCl₂ treated with 80 µM of IO₃⁻ (Table 1).
Table 1: The effect of iodate supplementation on O$_2^*$, H$_2$O$_2$ and MDA concentration in CdCl$_2$ stress Glycine max-L.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>IO$_3^-$ (µM)</th>
<th>CdCl$_2$ (mM)</th>
<th>O$<em>2^*$ (A$</em>{550}$ g$^{-1}$ FW)</th>
<th>H$_2$O$_2$ (µmol g$^{-1}$ FW)</th>
<th>MDA (A$_{532}$Abs g$^{-1}$ FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>12.263±3.34432</td>
<td>17.023±0.84310</td>
<td>16.855±0.744698</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>100</td>
<td>16.34±0.38974</td>
<td>21.66±0.86927</td>
<td>20.46±0.921954</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>100</td>
<td>13.303±0.44117</td>
<td>18.04±0.11789</td>
<td>18.02±0.072194</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>100</td>
<td>15.176±0.75434</td>
<td>19.63±0.53351</td>
<td>18.88±0.77033</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>100</td>
<td>17.743±0.58389</td>
<td>17.42±0.62115</td>
<td>18.05±0.055302</td>
<td></td>
</tr>
<tr>
<td>Mean±SE</td>
<td></td>
<td>0.570±0.632</td>
<td>0.513±0.831</td>
<td>0.566±0.080</td>
<td></td>
</tr>
<tr>
<td>CD</td>
<td></td>
<td>3.471±0.057</td>
<td>3.124±0.056</td>
<td>3.446±0.061</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: The effect of iodate supplementation on SOD, APX, CAT and GR concentration in CdCl$_2$-stressed Glycine max-L.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>IO$_3^-$ (µM)</th>
<th>CdCl$_2$ (mM)</th>
<th>SOD (A$_{550}$ min$^{-1}$.mg$^{-1}$.protein)</th>
<th>APX (A$_{290}$ min$^{-1}$.mg$^{-1}$.protein)</th>
<th>CAT (A$_{340}$ min$^{-1}$.mg$^{-1}$.protein)</th>
<th>GR (A$_{340}$ min$^{-1}$.mg$^{-1}$.protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>1482.3±69.0386</td>
<td>10.44±0.047634</td>
<td>17.32±0.42332</td>
<td>16.61±1.391079</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>100</td>
<td>1203.6±71.5913</td>
<td>10.98±0.464258</td>
<td>20.56±0.493288</td>
<td>15.79±1.09</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>100</td>
<td>1417.6±58.7054</td>
<td>12.47±0.030315</td>
<td>18.30±0.387341</td>
<td>21.30±1.315611</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>100</td>
<td>1639.6±67.2261</td>
<td>12.53±0.061809</td>
<td>19.30±0.519393</td>
<td>21.33±1.267294</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>100</td>
<td>1482.7±59.0028</td>
<td>11.13±0.552037</td>
<td>17.98±0.92433</td>
<td>18.31±0.53966</td>
<td></td>
</tr>
<tr>
<td>Mean±SE</td>
<td></td>
<td>78.9±36.55</td>
<td>0.36±0.36</td>
<td>0.65±0.05</td>
<td>1.31±0.15</td>
<td></td>
</tr>
<tr>
<td>CD</td>
<td></td>
<td>479.9±22.85</td>
<td>3.99±0.33</td>
<td>7.99±0.70</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3: The effect of iodate supplementation on AsA, GSH and Proline concentration in CdCl$_2$-stressed Glycine max-L.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>IO$_3^-$ (µM)</th>
<th>CdCl$_2$ (mM)</th>
<th>AsA (mmol g$^{-1}$ FW)</th>
<th>GSH (mmol g$^{-1}$ FW)</th>
<th>Proline (µg g$^{-1}$ FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>13.80±0.43712</td>
<td>13.64±0.55387</td>
<td>16.06±0.802268</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>100</td>
<td>15.39±0.19891</td>
<td>15.91±0.241777</td>
<td>18.56±0.066583</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>100</td>
<td>17.51±0.39513</td>
<td>19.29±0.202724</td>
<td>16.85±0.460145</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>100</td>
<td>18.23±0.17967</td>
<td>18.18±0.150766</td>
<td>15.33±0.589604</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>100</td>
<td>19.42±0.09430</td>
<td>17.14±0.110014</td>
<td>14.92±0.688398</td>
<td></td>
</tr>
<tr>
<td>Mean±SE</td>
<td>0.33±0.01</td>
<td>0.36±0.58</td>
<td>0.59±0.04</td>
<td>0.68±0.8888</td>
<td></td>
</tr>
<tr>
<td>CD</td>
<td>2.01±0.08</td>
<td>2.12±0.06</td>
<td>3.59±0.65</td>
<td>4.12±0.75</td>
<td></td>
</tr>
</tbody>
</table>

Seed Yield, Size, Growth and Germination: The number of seeds yield per pod were found to be 3 in all the controls and treated plants and Size of the seeds increased from 0.5 cm in both the controls to 0.7 cm in treated plants.

There was a variation in the mass of the seeds, in control the mass was found to be 0.15 g. Where as in plants treated with CdCl$_2$ and increasing conc of iodate, the mass was found to be 0.22 g. However the mass of the seed in control only treated with CdCl$_2$ was same as the control supplied only with Hoagland nutrient solution. Colour of all the seeds was found to be greenish yellow. All seeds showed hundred percent germination.

Superoxide radical generation in control plants was less than in the treatment with 100mM of CdCl$_2$ however on treatment with IO$_3^-$ along with 100mM concentration of CdCl$_2$, the superoxide radical generation increases considerably, 80 µM showing the highest levels of generation (Figure 1). In the case of H$_2$O$_2$ production, the effect of exogenous iodine was not prominent. The production of H$_2$O$_2$ increased under the 100mM CdCl$_2$ stress, however under IO$_3^-$ application no remarkable change was observed (Figure 1). Our results indicate that MDA levels in the treatment of 100mM CdCl$_2$ increased with respect to the MDA content in control plants however the MDA content was reduced in the IO$_3$-CdCl$_2$ treatments (Figure 1).

In order to combat the production of oxidative stress, plants have an extensive network of antioxidant enzymes. From these enzymes SOD is the first line of defence. Our results show that the SOD activity increased remarkably on the application of IO$_3^-$, the 40 µM IO$_3$-CdCl$_2$ treatments showing the maximum activity of SOD (Figure 2). APX activity showed very little fluctuation however on treatment with IO$_3^-$ along with 100mM showing an increase of only 5% in 100mM CdCl$_2$ against control (Figure 3). The APX activity reached the highest values at the dosages of 20µM and 40µM IO$_3$-CAT.
Fig. 1: Effect of I$_2$-application on (A) Superoxide anion (B) Hydrogen peroxide and (C) Lipid peroxidation (MDA) concentration in seeds of Glycine max L plants under heavy metal stress. Data represent mean value ± SD (n=5) for one control and three treatment each of three replicate (CD = Coefficient of Determination).

Fig. 2: Effect of IO$_3$-application on Superoxide dismutase (SOD) activities in seeds of Glycine max L plants under heavy metal stress. Data represent mean value ± SD (n=5) for one control and three treatment. (CD = Coefficient of Determination)

Fig. 3: Effect of IO$_3$-application on (A) Ascorbate Peroxidase (APX) (B) Catalase (CAT) and (C) Glutathione Reductase (GR) activities in seeds of Glycine max L plants under heavy metal stress. Data represent mean value ± SD (n=5) for one control and three treatment each of three replicate. (CD = Coefficient of Determination)
Fig. 4: Effect of IO<sub>3</sub>- application on (A) Glutathione (GSH) (B) Ascorbic acid (AsA) and (C) Proline activities in seeds of Glycine max L plants under heavy metal stress. Data represent mean value (n=5) for one control and three treatment each of three replicate. (CD = Coefficient of Determination)

Fig. 5: SDS-PAGE of soybean seeds proteins extracted with tris-buffer, pH 6.8. (A) BSA; (B) control; (C) 20µM /100mM; (D) 40µM/100mM; (E) 80µM/100mM; (F)100mM

showed an increase in its activity in all the dosages, reaching its maximum value in 100mM CdCl<sub>2</sub> treatment against control. It showed an increase of some 18% in 100mM CdCl<sub>2</sub> treatment.

CAT, showed an increase in its activity under 100mM CdCl<sub>2</sub> conc. (Figure 3). CAT showed an increase in its activity in all the dosages, reaching its maximum value in 100mM CdCl<sub>2</sub> treatment against control. Our results show that all the combination dosages of IO<sub>3</sub>- and CdCl<sub>2</sub> boosted the activity of GR with respect to control treatment, 40µM IO<sub>3</sub>- showing the highest activity. The activity of GR decreased in the 100 mM CdCl<sub>2</sub> treatment (Figure3).

Non Enzymatic antioxidants such as Glutathione, ascorbic acid, proline and non protein thiol constitute an important plant defense system against environmental stresses [27].Our findings show that ascorbic acid content of the seeds of Glycine max L. increased with the treatment of 100 mM of CdCl<sub>2</sub> and joint application of both 100 mM CdCl<sub>2</sub> and increasing dosage of IO<sub>3</sub>- (Figure 4). Similar pattern was observed in the case of GSH against control however the 20 µM IO<sub>3</sub>- 100 mM CdCl<sub>2</sub> showed the maximum activity among all treatments (Figure 3). In the case of Proline, a 3 fold increase in concentration was observed in 100mM CdCl<sub>2</sub> treatment against control. More than 2 fold increase was observed in the joint application of both 100 mM CdCl<sub>2</sub> and increasing dosage of IO<sub>3</sub>- (Figure 4).

SDS PAGE analysis showed plants treated with 100mM CdCl<sub>2</sub> treatment two protein bands. Bands in the control plant are light and more than observed in 100mM CdCl<sub>2</sub>. The treatments supplied with increasing dosage of potassium iodate shows high expression of proteins as compared to the controls. Among the CdCl<sub>2</sub>-IO<sub>3</sub>- combination concentrations, 20µM showed the maximum band width of the obtained proteins. However, the number of bands obtained in all the CdCl<sub>2</sub>-IO<sub>3</sub>- combinations remains 6 (Figure 5).

**DISCUSSION**

Although there are no direct results on the essentiality of iodine to plant growth, low iodine
concentrations in the growth environment is beneficial to a number of plant species [28, 29] and its high concentrations are detrimental to plant growth [30, 31]. In the present study, the effect of CdCl$_2$ and combination of CdCl$_2$ with different (20, 40, 80 µM) dosage of IO$_3^-$ was observed on the seeds of *Glycine max* L.

The major results of exposure of heavy metal stress to plants are reduction in biomass and hence yield. Under stress conditions, transfer of electrons leads to the free radical generation. These electrons damage DNA, lipids and proteins. Chloroplast is the site of maximum production of superoxide radical through the Mehler reaction and H$_2$O$_2$. Cd has been found to inhibit the electron flow on the reducing side of photo system I [32]. In our experiment, 33% of increase in superoxide radical generation was observed in 100mM CdCl$_2$ against control. However, with the application of IO$_3^-$, its levels were reduced at 20 µM and 40 µM concentrations. Highest concentration of IO$_3^-$ showed an increase in the superoxide radical generation, similar to 100mM CdCl$_2$ treatment.

In our work, production of H$_2$O$_2$ showed an increase of 27% for 100mM CdCl$_2$ treatment against control. The application of IO$_3^-$ reduced the production of H$_2$O$_2$ in all the three dosages. 80 µM of IO$_3^-$ along with 100mM CdCl$_2$ showed similar results as that of control.

In our work, lipid peroxidation was determined by measuring the concentration of MDA in cells. MDA is formed through auto oxidation and enzymic degradation of polyunsaturated fatty acids in cells. Our findings show that there was an increase of 21% in the MDA content of seeds of *Glycine max* in 100 mM CdCl$_2$ treatment. However, the combination dosages of CdCl$_2$ and IO$_3^-$ reduced the MDA content against 100mM CdCl$_2$ treatment.

Plants have developed an extensive network of antioxidant enzymes to combat the effect of heavy metal stress. Metalloenzyme SOD is the most effective intracellular enzymatic antioxidant against the toxic effects of elevated levels of ROS. SOD reduces O$_2^-$ to H$_2$O$_2$ and hence decrease the chances of production of OH$^-$ via Haber-Weiss reaction [33]. Our results indicate that there was a considerable decrease in the concentration of SOD in 100mM CdCl$_2$ treatment. However, application of exogenous iodine triggered the activity of SOD. The SOD activity was found maximum in the dosage of 40 µM IO$_3^-$.

APX activity showed very little fluctuation showing an increase of only 5% in 100mM CdCl$_2$ against control. The APX activity reached the highest values at the dosages of 20µM and 40µM IO$_3^-$, CAT showed an increase in its activity in all the dosages, reaching its maximum value in 100mM CdCl$_2$ treatment against control. It showed an increase of some 18% in 100mM CdCl$_2$ treatment. Foyer and Noctor [34] has shown that GR is a key player of Halliwell Asada pathway along with the enzyme DHAR. GR along with DHAR regenerates AsA from DHA using reduced glutathione as reducing agent. Our results show that all the combination dosages of IO$_3^-$ and CdCl$_2$ boosted the activity of GR, 40µM IO$_3^-$ showing the highest activity.

Non Enzymatic antioxidants such as Glutathione, ascorbic acid and proline constitute an important plant defense system against environmental stresses [35]. They are found abundantly in all cell compartments. AsA and GSH are the substrates of Halliwell Asada pathway and work to keep the levels of H$_2$O$_2$ under control. Our present findings show an increase in the activity of both AsA and GSH. The maximum concentration of AsA was reached in 80 µM of IO$_3^-$ perhaps to balance the O$_2$ accumulation with this dosage. GSH reached its peak value at 20 µM IO$_3^-$ exhibiting an increase of 21% with respect to 100mM treatment of CdCl$_2$. Non protein thiol showed an increase of 26% in 100mM CdCl$_2$ treatment against control. Its activity showed an increase except in 80µM IO$_3^-$ conc where it showed a slight decrease as compared to control. Proline has been shown to play an important role in recovering from environmental stresses in plants and its accumulation might be induced as a result of Reactive Oxygen Species (ROS). The mechanism by which Proline reduces oxidative damage include physical quenching of singlet oxygen and chemical reaction with hydroxyl radicals [36]. Proline, an important osmolyte, was also measured and it showed an increase of 16% in 100mM CdCl$_2$ treatment against control. Among the combination dosages it reached its peak value in 20 µM IO$_3^-$ CdCl$_2$ treatment. Our findings are in agreement of the findings of Leyva *et al.* [30] where they showed the beneficial effects of low conc. of iodine (>40 µM) in lettuce plant under 100 mM NaCl stress.

Dai *et al.*, have shown that iodine when supplied in the form of IO$_3^-$ has positive effects on the biomass of plants used for their edible leaves. Our results are almost in agreement with Dai’s findings showing that supply of iodine in the form of IO$_3^-$ increased the antioxidant response of *Glycine max* seed proteins. It was also proved by the SDS-PAGE electrophoresis results of seed proteins of *Glycine max* in our work.

Seed protein SDS-PAGE analysis showed that in control the number of bands obtained were 6 in number. Under 100mM CdCl$_2$ concentration the expression of proteins declined in *Glycine max* L. seeds showing only two bands with one of the band showing a different
banding pattern. Similar results were reported by Rout and Sahoo [37] that, a group of polypeptides were completely disappeared in 100 and 200 µM treatment. The disappearance and reappearance of some proteins and de novo synthesis of others in response to Fe exposure indicated a direct relationship of metal stress induced proteomics.

Our results prove that the application of iodine in the form of iodate enhances the level of proteins in seeds of Glycine max L. under CdCl₂ stress. Among the plants treated with combinations of CdCl₂–IO₃⁻, 20 µM conc of IO₃⁻ showed the maximum band width of the bands however, the number of bands remained the same in all the combination dosages. The result shows that iodine in low concentrations (20 µM) can accelerate the enzymatic antioxidant system of Glycine max L. under CdCl₂ stress.

CONCLUSION

The present study suggests that supplementing soil with lower concentrations of iodine (20 µM) improves the response of Glycine max L. plants under 100mM CdCl₂ stress. The application of IO₃⁻ increased the conc of O₂⁻, H₂O₂ and MDA in the seeds of Glycine max L. It also enhanced the activities of CAT, APX and GR which were kept the levels of ROS low. IO₃⁻ also played a significant role in increasing the conc. of AsA and GSH. Our study strongly recommends that use of low conc. of IO₃⁻ (20 µM) improves the response of Glycine max L. under 100mM CdCl₂ stress. Further research is needed in this respect to prove the beneficial effects of exogenous application of iodine in eliminating or suppressing the harmful effects of other stresses.

ACKNOWLEDGEMENT

We would like to extend our thanks to Sharda University for providing the necessary infrastructure for carrying out this research work.

REFERENCES


