Abstract
Nickel toxicity has been recognized as a primary growth-limiting factor in plants that results in decrease in plant growth and production. In this experiment, we studied the effect of 200µM and 400µM nickel treatments on H$_2$O$_2$ content, lipid peroxides, proline content and protein thiolation as well as the activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX) and glutathione reductase (GR) in the roots and leaves of barley plants (cv HB 393) after 12 days of growth period. Exposure of the barley plants to nickel led to reduction of length and fresh weight of the roots and leaves. As early as 6 days after treatment, visible symptoms of nickel toxicity: leaf chlorosis and necrosis were observed. Treatment with nickel resulted in the increase in H$_2$O$_2$ contents in both roots and the leaves. There was no change in CAT activity in roots while a significant increase in CAT activity was observed only under 200µM nickel treatment in leaves. We observed significant increase in GPX, APX, SOD and GR activities in leaves and roots under both 200µM and 400µM nickel treatments. Significant change in lipid peroxide content was observed only under 400µM Ni treated leaves. There was significant decline in protein thiol in leaves but no change was observed in roots. Proline accumulation was observed to be significant only in roots treated with nickel. Our results indicate that despite increase in H$_2$O$_2$ levels, oxidative damage, measured as the level of lipid peroxidation, did not occur in the roots of nickel treated barley but at higher concentration state of oxidative damage occurred in leaves.

Key words: Antioxidants, oxidative stress, nickel, H$_2$O$_2$, lipid peroxide, proline

Introduction
Nickel (Ni) is considered to be an essential micronutrient for plants (Eskew et al., 1983) and in small amount is known to improve plant yield and quality (Brown et al., 1987; Gerendas et al., 1999). However, like other micronutrients; when present at high concentrations in the soil environment, Ni becomes phytotoxic (Parida et al., 2003; Gajewska and Sklodowska, 2005). In plant tissues, the concentrations of Ni may vary from 0.1 to 5.0 ppm (dry wt. basis) with a threshold range of toxicity of 40-246 ppm, depending on plant species (Gonnelli et al., 2001). The most common symptoms of nickel toxicity in plants are inhibition of growth, chlorosis, necrosis and wilting (Madhava Rao and Sresty, 2000, Pandey and Sharma, 2002, Nakazawa et al., 2004). Toxicity of this metal has been attributed to its negative effect on photosynthesis (Tripathy et al., 1981), mineral nutrition (Parida et al., 2003), sugar transport (Samarakoon and Rouser, 1979), water relations (Pandey and Sharma, 2002) and induction of oxidative stress (Gajeweska and Scelodowska, 2007, 2008). Overproduction of reactive oxygen species (ROS) seems to be common response of plants to different stress conditions. To maintain normal metabolic functions under the stress conditions, the balance between generation and degradation of ROS is essential, otherwise oxidative injuries may occur. The ROS level in plant tissues is controlled by antioxidant system consisting of antioxidative enzymes and nonenzymatic low molecular mass antioxidants. Superoxide dismutase (SOD, EC 1.15.1.1) is a key antioxidative enzyme that catalyzes disproportionation of superoxide anion (O$_2$–) to H$_2$O$_2$ and O$_2$. Catalase (CAT, EC 1.11.1.6) scavenges H$_2$O$_2$ by converting it to H$_2$O and O$_2$. Peroxidase (POD, EC 1.11.1.7) reduces H$_2$O$_2$ using several reductants such as phenolic compounds. Induction of POD activity has been observed in response of plants to different stress factors, including heavy metals (Diaz et al.,...
2001). It has been suggested that Ni-induced phytotoxicity is associated with the generation of oxidative stress in plants (Baccouch et al., 2001; Boominathan and Doran, 2002). In relation to this, enzymes related to antioxidant defense systems have been shown to be stimulated in response to Ni-induced oxidative stress (Gajewska and Sklodowska, 2005, 2008; Dubey and Pandey, 2011; Sharma et al., 2010). Although, nickel is considered a redox-inactive metal, it cannot directly generate ROS. Membrane peroxidation, however, has been observed in various plants subjected to Ni toxicity (Boominathan and Doran, 2002; Gajewska and Sklodowska, 2005; Dubey and Pandey, 2011), suggesting role of ROS in Ni induced oxidative stress. Moreover, results concerning effect of Ni toxicity on the antioxidant enzymes are contradictory and both increase and decrease in their activities have been observed. This warrants the further study of Ni induced antioxidant system in other plants. In addition, the increase in activities of APX and GPX has been shown in barley seedlings by Jocsak et al. (2008) but this study did not consider the effect of Ni toxicity on other antioxidant enzymes. The purpose of the present work was therefore to contribute to a better understanding on the possible ability of Ni to the generation of oxidative stress and on the activities of antioxidant enzymes in barley roots and leaves.

Materials and Methods

Growth and collection of plant material

Seeds of grain barley (cv HB 393) were soaked overnight in minimum distilled water and surface sterilized next day in 0.1% HgCl$_2$. The sterilized seeds were germinated on moistened filter paper lined in plastic petriplates. During growth, seedlings were treated with various regimes of nickel chloride i.e. 0, 50, 100, 200, 400,800 and 1000µM for preliminary screening of the tolerance range of plant in seed germinator incubator at 25°C. The pre-soaked and sterilized seeds were then regerminated in petriplates in the nutrient solution containing 8 mM KNO$_3$, 2 mM Ca(NO$_3$)$_2$, 1 mM KH$_2$PO$_4$, 1 mM MgSO$_4$ and micronutrients: 30 µM H$_2$BO$_3$, 5 µM MnSO$_4$, 1 µM CuSO$_4$, 1 µM (NH$_4$)$_6$MoO$_4$ and 1 µM ZnSO$_4$. The solution with conc. of 200µM or more produced visible morphological symptoms of toxicity in barley and hence 200µM and 400µM solutions of NiCl$_2$ were used to irrigate two sets of plants (3rd day onwards) besides a third set of plants without nickel as control.

Measurement of growth

During growth, the root and shoot samples were taken for fresh weight determination and compared with those of control. The root and shoot length assessment was done at both levels of nickel treatments at 3 days interval upto 12 days using ten random samples in triplicate.

Preparation of crude extract

After growth period, fresh plant tissue was homogenized (1:5, w/v) in ice-cold 0.1M potassium phosphate buffer of pH 7.0 containing 2% PVP in prechilled mortar and pestle. The homogenate was centrifuged at 4°C for 15 min at 12000 x g and supernatant was used for enzyme assays. The protein content of the supernatant was determined by the method of Bradford (1976) using bovine serum albumin.

Determination of hydrogen peroxide content

The H$_2$O$_2$ content of both control and nickel treated barley roots was determined according to Sagisaka (1976). One gram of root tissue was homogenized in 5% trichloroacetic acid (TCA) and the homogenate was centrifuged at 16,000 x g at 4°C for 10 min. The reaction mixture contained 1.6 ml supernatant of root extract, 0.4 ml TCA (50 %), 0.4 ml ferrous ammonium sulfate and 0.2 ml potassium thiocyanate. The absorbance was recorded at 480 nm.

Antioxidant enzymes assays

Catalase activity was determined by consumption of H$_2$O$_2$ in absorbance at 240 nm by the method of Vitoria et al. (2001). The assay mixture consisted of 0.1ml extract and 25 mM potassium phosphate buffer (pH 7.0) containing 10 mM H$_2$O$_2$. The decreases in absorption were recorded at 240 nm and quantified from the extinction coefficient of 0.036 mM$^{-1}$ cm$^{-1}$ and activity expressed as µmol H$_2$O$_2$ oxidized min$^{-1}$ mg$^{-1}$ protein.

Guaiacol peroxidase was estimated with guaiacol as substrate according to the method of Vitoria et al. (2001). The assay mixture contained 0.1 ml extract in 25 mM potassium phosphate buffer (pH 7.0) containing 10 mM H$_2$O$_2$ and 9 mM guaiacol. The formation of tetraguaiacol was monitored by noting increase in absorbance at 470 nm and quantified using the extinction coefficient 26.6 µM$^{-1}$ cm$^{-1}$ and activity expressed as µmol guaiacol oxidized min$^{-1}$ mg$^{-1}$ protein.

Ascorbate peroxidase activity was estimated by the method of Nakano and Asada (1981) with modification by monitoring the rate of ascorbate oxidation (extinction coefficient=2.8 mM$^{-1}$ cm$^{-1}$). The assay mixture was 0.1ml extract added to 50 mM potassium phosphate buffer (pH 7.0) containing 0.1mM H$_2$O$_2$, 0.5 mM ascorbate and 0.1 mM EDTA. The change in absorbance was monitored at 290 nm and activity expressed as µmol ascorbate oxidized/min/mg protein.

Total SOD activity was assayed by monitoring inhibition of photochemical reduction of nitroblue tetrazolium (NBT) as described by Beauchamp and Fridovich (1971). The 3 ml reaction mixture consisted of 2.9 ml 50 mM potassium phosphate buffer (pH 7.8) containing 10 mM methionine, 168 µM NBT, 0.025 % Triton X-100, 1.17 µM riboflavin, and 0.1 ml enzyme. The assay was carried out by placing the test-tubes below a 20W fluorescent lamp for 30 min. The formation of methionine-mediated formazan formed was calculated using its extinction coefficient of 7 mM$^{-1}$ cm$^{-1}$ and compared with amount of formazan formed in the absence of enzyme. The reaction mixture with no enzyme developed maximum color because of maximum rate of reduction of NBT. Non-irradiated reaction mixture was used as the control as it did not develop color. One unit of SOD was defined as the enzyme causing 50 % inhibition of formazan formation. 

GR activity was determined by monitoring the glutathione-dependant oxidation of NADPH at 340 nm as described by Schaedle and Bassham (1977) with minor modifications. The reaction mixture was 0.2 ml enzyme sample added to 1.8 ml 50 mM potassium phosphate buffer (pH 7.6) containing 0.12 mM NADPH, 0.5 mM GSSG and 0.1 mM EDTA at 25°C. Activity was calculated by using extinction
coefficient 6.22 mM⁻¹ cm⁻¹, and expressed in enzyme units/mg protein. One unit of enzyme is the amount necessary to decompose 1 µmol of NADPH per min at 25°C.

**Lipid peroxidation and proline content**

The level of lipid peroxidation in barley roots and leaves was determined as the amount of 2-thiobarbituric acid-reactive substances (TBARS) mainly malondialdehyde (MDA) content formed as described by Dhindsa et al. (1981). 1g of root tissue was homogenized in 5 ml 0.1% TCA and centrifuged at 10,000 rpm for 15 min. To 2 ml supernatant, 2 ml of 20 % TCA containing 0.67 % TBA was added. The mixture was heated at 90°C for 30 min for formation of pink-colored 1:2 adduct between MDA and TBA and then quickly cooled on ice. After centrifugation at 10,000 rpm for 10 min, the absorbance of the supernatant at 532 nm was read, and the value for the non specific absorption at 600 nm was subtracted. The concentration of MDA was calculated using an extinction coefficient of 155 mM⁻¹ cm⁻¹ and expressed as n mol/g fresh weight (FW).

Proline concentration in barley roots and leaves was determined following the method of Bates et al. (1973). 0.5 g sample was homogenized with 5 ml of sulfosalicylic acid (3 %) using mortar and pestle and filtered through Whatman No. 1 filter paper. The volume of filtrate was made up to 10 ml with sulfosalicylic acid and 2.0 ml of filtrate was incubated with 2.0 ml glacial acetic acid and 2.0 ml ninhydrin reagent and boiled in a water bath at 100°C for 30 min. After cooling the reaction mixture, 6.0 ml of toluene was added and after cyclomixing it, absorbance was read at 570 nm. The proline content was determined using a standard curve prepared by 10-100µg proline.

**Thiol contents**

The contents of protein thiols (PT) and non-protein thiols (NPT) were estimated as described by Romero-Puertas et al. (2007). First total thiols were estimated by reaction with DTNB [5,5’-dithio-bis(2-nitrobenzoic acid)] which gets reduced to TNB (2-nitro-5-thiobenzoic acid) having absorption maximum at 412 nm. 500 mg sample was homogenized in 5 ml of 50 mM Tris-HCl buffer (pH 7.5) containing 1 mM EDTA and 0.2 % Triton X-100. After centrifugation at 15000 rpm for 15 min, 0.5 ml supernatant was taken and mixed with 0.95 ml methanol and 0.05 ml of 0.1 M DTNB. The absorbance was taken at 412 nm against 1 ml of methanol containing 0.05 ml 0.1 M DTNB.

For non protein thiol content, proteins were removed by 12 % (w/v) TCA precipitation and after centrifuging at 12000 rpm for 10 min thiol group were assayed by reaction with DTNB as described above. The content of protein thiol was obtained by deduction of NPT content from total thiol. The amount of thiol was calculated using extinction coefficient 14.35 mM⁻¹ cm⁻¹ and thiols were reported as n mol/g FW.

**Statistical analysis**

All the assays and estimations were done in triplicates. The mean and standard deviations were calculated and the significance of differences between control and treatment mean values was determined by Student’s t-test. Differences at p ≤ 0.05 were considered significant.

**Results**

**Growth parameters**

Treatment of barley with 200 and 400µM Ni for 12 days resulted in changes in morphology of plant. Biomass production and elongation of the barley leaves and roots were substantially reduced in response to Ni application. As early as 6 days after treatment, the leaf chlorosis and necrosis was observed at both the doses of Ni (not shown). During growth period, the effect on growth as well as fresh weight of both roots and shoots were observed (Table 1).

**Effect of Ni on H₂O₂ generation**

Ni treatment to barley for 12 days enhanced the production of H₂O₂ in barley roots and leaves. The increase in H₂O₂ content in barley roots treated with 200 and 400µM of Ni 52% and 65% (p≤0.01) respectively compared to controls, while this increase was about 65% and 80% (p≤0.01) respectively in barley leaves treated with 200 and 400µM of Ni compared to controls (Fig 1).

**Antioxidant enzyme assays**

Activity of CAT did not show any significant change in barley roots treated with 200µM and 400µM Ni after 12 days of growth period (Fig 2). Similarly, no change in CAT activity was observed in leaves treated with 400µM Ni. In contrary to this, a significant increase of about 93% (p≤0.05) in CAT activity was observed in the leaves treated with 200µM Ni for 12 days (Fig 2). A profound dose dependent increase in GPX activity was observed in 200 or 400 µM Ni treated roots and leaves. The activity of GPX was higher in roots compared to leaves. There was about 170% and 220% increase in GPX activity in the roots exposed to 200 and 400 µM Ni for 12 days compared to the controls (Fig 3), while an increase of about 110% and 180% was observed in GPX activity in the leaves exposed to 200 and 400 µM Ni concentrations respectively (Fig 3). The increase in GPX activity was significant at both 200 µM (p≤0.01) and 400 µM (p≤0.001) Ni treatments. The activity of APX showed about 80% (p≤0.05) increase in roots treated with 200µM Ni while this increase was about 100% (p≤0.05) under 400µM Ni treatment. In leaves, APX activity increased about 55% (p≤0.01) and 70% (p≤0.01) respectively under 200µM and 400µM Ni treatments for 12 days (Fig 4).

The SOD activity showed an increase of about 90% (p≤0.01) and 110% (p≤0.01) above the control level was observed in roots exposed to 200µM and 400µM Ni for 12 days while an increase of about 140% (p≤0.001) above the control level in SOD activity was observed in barley leaves treated with 200µM Ni for 12 days. Further increase in Ni treatment caused decline in SOD activity. There was only 80% increase in SOD activity in leaves under 400µM Ni treatment (Fig 5). The level of GR activity was higher in Ni treated roots and leaves compared to controls. 12 day-old roots treated with 200 and 400 µM Ni showed significant increase of about 70% (p≤0.05) and 115% (p≤0.01) in GR activity compared to controls. In leaves also, there was about 55% (p≤0.01) and 70% (p≤0.01) increase in enzyme activity respectively under both 200 µM and 400 µM Ni treatments compared to controls (Fig 6).
Table 1: Effect of nickel treatment on root length, shoot length and fresh masses of roots and shoot of barley plant.

<table>
<thead>
<tr>
<th>Time</th>
<th>Treatment</th>
<th>Root length (cm)</th>
<th>Shoot length (cm)</th>
<th>Fresh mass of Root (mg)</th>
<th>Fresh mass of Shoot (mg)</th>
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<tbody>
<tr>
<td>3rd day</td>
<td>Control</td>
<td>2.0</td>
<td>3.4</td>
<td>9.5</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>200µM</td>
<td>2.2 NS</td>
<td>3.2 NS</td>
<td>9.6 NS</td>
<td>12 NS</td>
</tr>
<tr>
<td></td>
<td>400 µM</td>
<td>2.1 NS</td>
<td>3.2 NS</td>
<td>9.4 NS</td>
<td>14 NS</td>
</tr>
<tr>
<td>6th day</td>
<td>Control</td>
<td>3.2</td>
<td>5.0</td>
<td>16.2</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>200µM</td>
<td>3.0 NS</td>
<td>4.5 NS</td>
<td>14.8 NS</td>
<td>22 NS</td>
</tr>
<tr>
<td></td>
<td>400 µM</td>
<td>2.9*</td>
<td>4.4*</td>
<td>14.5*</td>
<td>20 NS</td>
</tr>
<tr>
<td>9th day</td>
<td>Control</td>
<td>4.2</td>
<td>7.0</td>
<td>28</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>200µM</td>
<td>4.0*</td>
<td>5.8 NS</td>
<td>23**</td>
<td>32 NS</td>
</tr>
<tr>
<td></td>
<td>400 µM</td>
<td>3.8*</td>
<td>5.6*</td>
<td>22*</td>
<td>28*</td>
</tr>
<tr>
<td>12th day</td>
<td>Control</td>
<td>6.0</td>
<td>11.0</td>
<td>36</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>200µM</td>
<td>5.0**</td>
<td>10.0*</td>
<td>30**</td>
<td>46*</td>
</tr>
<tr>
<td></td>
<td>400 µM</td>
<td>5.0**</td>
<td>9.6**</td>
<td>27**</td>
<td>45**</td>
</tr>
</tbody>
</table>

The values are mean of ten random samples in triplicate. *p<0.05 significantly different from control. **p<0.01 significantly different from control. NS-not significant.

Fig 1: H$_2$O$_2$ content in barley roots and leaves treated with 200 and 400µM nickel for 12 days. Values are mean ± S.D. (N=3). **p<0.01 significantly different from control.

Fig 2: Catalase activity in barley roots and leaves treated with 200 and 400µM nickel for 12 days. Values are mean ± S.D. (N=3). *p<0.05 significantly different from control; NS - not significant.

Fig 3: Guaiacol peroxidase activity in barley roots and leaves treated with 200 and 400µM nickel for 12 days. Values are mean ± S.D. (N=3). ***p<0.001 significantly different from control; **p<0.01 significantly different from control

Fig 4: Ascorbate peroxidase activity in barley roots and leaves treated with 200 and 400µM nickel for 12 days. Values are mean ± S.D. (N=3). **p<0.01 significantly different from control; *p<0.05 significantly different from control.
Effect of Ni treatment on lipid peroxidation and proline content

Lipid peroxidation is an effective indicator of cellular oxidative damage (Verma and Dubey, 2003). In our study, lipid peroxidation, measured as MDA levels, did not show any significant change in roots treated with 200 µM and 400 µM Ni after 12 days growth period. In leaves also, there was no significant change in lipid peroxidation under 200 µM Ni treatment; however, a significant increase (p≤0.05) in MDA content in 400µM Ni treated barley leaves was observed (Fig 7). The proline content was found to be accumulated with an increase in Ni concentration in the roots. A significant increase of about 80% (p≤0.01) proline was observed in 200µM Ni treated roots while it was increased by 120% (p≤0.001) following 400µM Ni treatment. In contrast to the roots, there was no significant change in proline level in barley leaves under both treatments (Fig 10).

Effect of Ni on protein thiolation

Several heavy metals including Cd, Pb and Hg have been shown to cause the depletion of protein bound thiol groups (Stohs and Bagchi, 1995). The oxidation of thiol groups of amino acid residues (a major target of attack of ROS) may reduces the protein thiol content in Ni-treated barley plants. A non-significant decline in protein thiol level in 200 and 400µM Ni treated barley roots was observed (Fig 8). Contrary to this, a significant decline of about 35% (p≤0.05) and 70% respectively was observed in 200µM and 400µM Ni treated leaves (Fig 8). The content of non-protein thiol was however, found to be higher in 200µM and 400µM Ni treated roots and leaves. The level of non-protein thiol was found to be increased significantly by 45% and 88% (p≤0.01) respectively in Ni-treated roots and by 44% and 93% (p≤0.01) respectively in 200µM and 400µM Ni-treated leaves compared to controls (Fig 9).

Discussion

Ni is considered to be an essential micronutrient for plants (Eskew et al., 1983), however at higher concentrations this metal becomes toxic for the plant species. Effect of Ni treatments on plant growth inhibition has been reported by many authors (Ewais 1997, Parida et al., 2003, Vinterhalter and Vintherhalter, 2005). In the present study, we observed reduction in growth parameters as well as visible symptoms of toxicity at 200µM and 400µM Ni treatments in barley. The roots showed brownish colour appearance in nickel treated plants. We also observed decrease of fresh mass of roots and shoots under Ni treatment. Decrease in fresh weight may be partly due to the metal-induced decline in tissue water content, as demonstrated earlier (Gajewska et al., 2006). As early as 6th day following Ni treatment, leaf necrosis was observed at both the treatments (not shown). Chlorosis is the common symptom of toxicity of heavy metals, including Ni (Pandey and Sharma, 2002).

Our results show that Ni toxicity in barley leaves and roots is strongly correlated with increased production of H$_2$O$_2$. Ni induced increase in this ROS content has been reported for roots of wheat (Hao et al., 2006), hairy roots of Alyssum bertolonii and Nicotiana tabacum (Boominathan and Doran, 2002) and wheat leaves (Gajewska and Sklodowska, 2007). The concentration of H$_2$O$_2$ in plant
tissues is controlled by H$_2$O$_2$-scavenging enzymes as well as by non-enzymatic antioxidants. APX is considered to be the most important H$_2$O$_2$ scavenger operating both in the cytosol and chloroplasts. Ni treatments led to a marked increase in APX activity in barley roots and leaves. Elevated APX activity in plants exposed to toxic levels of Ni has also been reported earlier in wheat and rice (Gajewska and Sklodowska, 2008; Maheshwari and Dubey, 2009), barley seedlings (Jocsak et al., 2008) and in maize shoots (Baccouch et al., 1998). In contrast, inhibition of this enzyme activity has been shown for Ni treated hairy roots of Alyssum bertoloni and Nicotiana tabacum (Boominathan and Doran, 2002). The increased activity of APX in the present study, suggests that APX might play an important role in the removal of this ROS from cells in the roots and leaves of Ni-stressed barley. It has been speculated that under abiotic stress, H$_2$O$_2$ acts as a systemic intracellular signal for the induction of APX (Hernandez et al., 2004). Similar to APX, a several-fold induction of GPX activity was found in both barley roots and leaves in response to Ni exposure. The increase in GPX activity has also been reported in Silene paradoxa (Gonnelli et al., 2001), barley seedlings (Jocsak et al., 2008) and wheat shoots (Gajewska et al., 2006), indicating a potential defensive role of GPX against Ni-induced oxidative stress. Our findings are consistent with reports on elevated root GPX and APX activities in several plant species grown under toxic levels of metals (Radotic et al., 2000; Madhav Rao and Sresty, 2000; Gajewska and Sklodowska, 2007). Contrary to APX and GPX activity, CAT activity in the barley roots did not show any significant change in response to Ni treatment, however, a significant increase in CAT activity in the leaves in response to 200µM Ni treatment was observed. It may be speculated that either up-regulation of CAT does not seem to be a requirement for the removal of H$_2$O$_2$ in roots and in 400 µM treated leaves or this decline in CAT activity may result from enzyme deactivation (Dat et al., 2000). Although, enhancement of CAT activity have been shown in many plants subjected to salinity and toxicity of certain heavy metals (Hsu and Kao, 2004; Kim et al., 2005), other findings suggest decrease in CAT activity in the leaves in response to 200µM Ni treatment was observed. It may be speculated that either up regulation of CAT does not seem to be a requirement for the removal of H$_2$O$_2$ in roots and in 400 µM treated leaves or this decline in CAT activity may result from enzyme deactivation (Dat et al., 2000). Although, enhancement of CAT activity have been shown in many plants subjected to salinity and toxicity of certain heavy metals (Hsu and Kao, 2004; Kim et al., 2005), other findings suggest decrease in CAT activity in the leaves in response to 200µM Ni treatment was observed. It may be speculated that either up regulation of CAT does not seem to be a requirement for the removal of H$_2$O$_2$ in roots and in 400 µM treated leaves or this decline in CAT activity may result from enzyme deactivation (Dat et al., 2000). Although, enhancement of CAT activity have been shown in many plants subjected to salinity and toxicity of certain heavy metals (Hsu and Kao, 2004; Kim et al., 2005), other findings suggest decrease in CAT activity in the leaves in response to 200µM Ni treatment was observed. It may be speculated that either up regulation of CAT does not seem to be a requirement for the removal of H$_2$O$_2$ in roots and in 400 µM treated leaves or this decline in CAT activity may result from enzyme deactivation (Dat et al., 2000).
Sresty, 2000), Silene paradoxa (Gonnelli et al., 2001) and rice seedlings (Maheshwari and Dubey, 2009). However, the increase in GR activity was not sufficient for combating the reduction of protein thiol in leaves as observed by decline in protein thiol but protection to protein thiol might have been provided by increased GR or other antioxidant enzymes in roots. The increase in GR activity and decline in protein thiol has also been shown by Maheshwari and Dubey (2009) in rice seedling under Ni treatments.

Excessive production of ROS has been shown to cause lipid peroxidation, enzyme inactivation and oxidation of protein thiol groups and nucleic acids (Dat et al., 2000). Lipid peroxidation is an effective indicator of cellular oxidative damage (Verma and Dubey, 2003). To check the possibility of oxidative damage, we determined the level of lipid peroxidation in the barley roots and leaves. The results showed that lipid peroxide content in roots and 200µM Ni treated leaves remained unaltered. However, significant increase in lipid peroxidation was observed in 400µM Ni treated leaves, suggesting a state of oxidative damage in leaves at higher concentration. The increase in lipid peroxidation has also been observed recently in the findings for black gram (Dubey and Pandey, 2011), Zea mays (Sharma et al., 2010) and Lemma gibbi (Yilmaz and Parlık, 2011). The state of no significant change in lipid peroxidation in roots and 200µM Ni treated leaves in our study, is in agreement to the induction of GPX, APX and SOD activities that may be involved in the protection of membranes from oxidative damage by ROS, but at 400 µM Ni treatment the induction of antioxidant enzymes was not sufficient to protect barley leaves from oxidative damage as also indicated by decrease in SOD activity at this concentration. Proline occurs widely in plants and normally accumulates in large quantities in response to environmental stresses (Rhodes et al., 1999; Hsu et al., 2003; Kavi Kishore et al., 2005). Proline contributes to stabilizing sub-cellular structures (membranes and proteins) and scavenging free radicals. The proline content was found to be accumulated with an increase in Ni concentration in the barley roots but was unaffected in leaves suggesting protective mechanism of proline accumulation from ROS under Ni induced oxidative stress in barley roots. In conclusion, our results suggest that Ni toxicity induces oxidative stress in barley roots and leaves resulting in increased generation of H₂O₂ and activation of antioxidant enzymes. The increase in APX, SOD and GPX activity suggests their role in scavenging ROS in barley roots as further evidenced by no change in lipid peroxidation. However, activated antioxidant enzymes were not sufficient to combat Ni induced oxidative stress in leaves as suggested by lipid peroxidation and protein oxidation.

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References


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