EFFECTS OF CADMIUM-INDUCED OXIDATIVE STRESS ON GROWTH AND NITROGEN ASSIMILATION IN BLACKGRAM [Vigna mungo (L.) Hepper]

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Abstract: Cadmium (Cd) accumulation, oxidative damage, and nitrogen metabolism were studied in roots and leaves of 30-d-old blackgram plants [Vigna mungo (L.) Hepper], grown in a mixture of soil and compost (3:1) with different Cd concentrations. Significant reductions in both root and shoot dry weight were noted. The concentration of Cd in roots and leaves increased with increasing Cd levels. The level of lipid peroxidation elevated with a consequent increase in H₂O₂ content under Cd stress in both plant organs. The activity of enzymes mediating the nitrogen assimilation in roots and leaves was greatly reduced in the presence of Cd, except glutamate dehydrogenase (GDH) which showed a significant increase.

Key words: cadmium toxicity, blackgram, nitrogen assimilation, nitrate reductase, glutamate dehydrogenase, oxidative stress, lipid peroxidation.

Introduction

Several studies revealed that the toxicity of Cd results from oxidative stress induced by the generation of activated oxygen species (AOS) or by the inhibition/stimulation of the enzymatic and non-enzymatic antioxidants (Mobin and Khan, 2007; Romero-Puertas et al., 2007; Gallego et al., 2012). The assimilation of nitrogen is especially sensitive to oxygen and reactive oxygen species (Hernandez et al., 1997; Boussama et al., 1999). The exposure to Cd severely depresses the activities of the enzymes of nitrogen metabolism (Wahid et al., 2007; Wang et al., 2008).

Blackgram [Vigna mungo (L.) Hepper] is an important pulse crop grown in an area of about 3 million hectares in India. It is an early maturing crop and is suitable for cultivation throughout the country with the average yield of 1.0-1.2 t/ha.

The detoxification of Cd could be achieved by metal binding by low-molecular-weight polypeptides (phytochelatins, PC) (Sanita di Toppi and Gabbielli, 1999). PCs are a class of cysteine-rich polypeptides with general

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structure ($\gamma$-Glu-Cys)$_n$-Gly (Rauser, 1995). The structural components of PCs imply the significance of glutamate and cysteine along with GSH in the tolerance mechanisms. Therefore, the present study is undertaken to find out the effects of Cd exposure on oxidative damage and regulatory enzymes of nitrogen assimilation (which furnishes the glutamate and cysteine) in blackgram.

**Material and Methods**

An experiment was conducted in the naturally illuminated greenhouse of the Department of Botany, Aligarh Muslim University, Aligarh, India. A mixture of soil and compost (3:1; pH 7.1) was used for the study. The chemical properties of the soil were organic carbon 0.38%; cation exchange capacity (CEC) 78 meq 100 g$^{-1}$ soil; nitrogen 88.4 mg kg$^{-1}$ soil; phosphorus 8.4 mg kg$^{-1}$ soil; potassium 110.6 mg kg$^{-1}$ soil and total cadmium 0.31 mg kg$^{-1}$ soil. Soil was mixed with an appropriate amount of CdCl$_2$ to achieve 0, 25 and 50 mg Cd kg$^{-1}$ soil. The seeds of blackgram [Vigna mungo (L.) Hepper] cultivar T9 were sown in 23 cm diameter clay pots. After germination, two plants per pot were maintained and watered with deionized water when required. All treatments were replicated five times. Thirty days after sowing, cadmium concentration, activities of enzymes of nitrogen assimilation, nitrate reductase (NR), nitrite reductase (NiR), glutamine synthetase (GS) and glutamate dehydrogenase (GDH) and formation of thiobarbituric acid reactive substances (TBARS) and H$_2$O$_2$ were determined in roots and leaves.

**Determination of cadmium**

Leaves were washed with deionized water, while roots were immersed in an ice-cold 5 mM CaCl$_2$ solution for 10 min to displace extracellular Cd (Rauser, 1987). The root/leaf samples were dried for 48 h at 80ºC, weighed and ground to fine powder, and digested with concentrated HNO$_3$-HClO$_4$ (3:1, v/v). Cadmium concentration was determined by atomic absorption spectrophotometer (GBG, 932 plus, Australia).

**Determination of TBARS and H$_2$O$_2$ content**

The level of lipid peroxidation products in the roots/leaves was determined by thiobarbituric acid reactive substances (TBARS) as described by Cakmak and Horst (1991). The TBARS content was calculated using the extinction coefficient (155 mM$^{-1}$ cm$^{-1}$). H$_2$O$_2$ level was colorimetrically measured as described by Okuda et al. (1991) and calculated using the extinction coefficient (0.28 µmol$^{-1}$ cm$^{-1}$).
Determination of enzymes of nitrogen assimilation pathway

Leaf or root sample (0.5 g) was extracted according to Gouia et al. (2003). The activities of nitrate reductase (NR, E.C.1.6.6.1), nitrite reductase (NiR, E.C.1.7.7.1), glutamine synthetase (GS, E.C.6.3.1.2) and glutamate dehydrogenase (GDH, E.C.1.4.1.2) were determined according to Dey and Harbone (1990).

The protein content in the samples was determined using bovine serum albumin (BSA, Sigma) as standard (Bradford, 1976).

Statistical analysis

The results are presented as means ± standard deviations. Data were subjected to ANOVA test (SPSS ver. 11, Chicago, USA) and means were compared using Duncan’s multiple range test, taking P = 0.05 as the significant level.

Results and Discussion

The accumulation of Cd in the roots and leaves increased with increasing Cd concentrations. The Cd content of roots was 3.2 and 5.8 times higher than that of shoots in 25 and 50 mg Cd kg\(^{-1}\) soil treatments (Figure 1A).

Data revealed that treatment with Cd increased the endogenous content of H\(_2\)O\(_2\) in both the root and leaf (Figure 1B). Maximum level (164.6%) of H\(_2\)O\(_2\) in roots was attained at 25 mg Cd kg\(^{-1}\) soil while in leaf the highest level (80.8%) was observed at 50 mg Cd kg\(^{-1}\) soil. The levels of TBARS increased with increasing Cd concentrations. Roots showed higher contents of TBARS than the leaves. In roots, the enhancement in TBARS content was 62.9% and 114.6%, while in leaves it was increased by 13.6% and 38.0% at 25 and 50 mg Cd kg\(^{-1}\) soil respectively in comparison to the control plants (Figure 1C).

The activity of enzymes mediating nitrogen assimilation in roots and leaves was significantly affected by Cd stress (Table 1). The activity of both reductases (NR and NiR) in root as well as in leaf was remarkably reduced when Cd concentration increased in the soil. Significant reductions were noted in NR activity in root (64.8% and 81.8%) and leaf (84.6% and 98.3%) at 25 and 50 mg Cd kg\(^{-1}\) soil respectively in comparison to the control plants. The activity of NiR showed reductions of 64.9% and 81.9% in root and 83.3% and 88.9% in leaf at 25 and 50 mg Cd kg\(^{-1}\) soil respective to the control. GS activity was severely depressed in roots with increasing levels of Cd in the soil where the reductions were 89.3% and 92.3% at 25 and 50 mg Cd kg\(^{-1}\) soil respectively, compared to the control. However, in leaf, a different trend was noted for GS activity where at lower concentration of Cd it was marginally increased (13.3%) but at higher Cd concentrations a reduction of 58.1% was noted. Treatment with Cd resulted in the
enhancement of GDH activity in root and leaf. In the roots, an increase in GDH activity was 25.1% and 55.6%, whereas in leaf, the enhancement was 11.6% and 130.6% at 25 and 50 mg Cd kg⁻¹ soil, respectively.

Figure 1. Cadmium content (A), H₂O₂ content (B), and TBARS content (C) in roots and leaves of blackgram plants exposed to 0, 25 and 50 mg Cd kg⁻¹ soil for 30 days. Data are expressed as means ± SD (n > 5).
The accumulation of heavy metals in roots and shoots relies on binding to extracellular matrix (Horst, 1995), chelating inside the cell (Cobbett et al., 1998) and on the translocation efficiency (Marchiol et al., 1996). Cd accumulation in root and leaf increased with increasing Cd level. Moreover, Cd concentration was lower in shoots than in roots, indicating that a higher proportion of Cd taken up by plants remained in the roots. Our results are in agreement with the findings of Wu and Zhong (2002).

As shown in Figure 1 (B-C), a constant induction of H$_2$O$_2$ and TBARS concentrations in blackgram roots and leaf with increasing Cd concentration was noted which signals the oxidative stress and the peroxidative damage of membrane lipids. Similar increases of TBARS and H$_2$O$_2$ content by Cd treatment have been observed in *Phaseolus vulgaris* (Chaoui et al., 1997) and *Helianthus annuus* (Gallego et al., 1996).

Table 1. Effect of cadmium treatments on enzymes of nitrogen assimilation pathway (activities expressed as nmols min$^{-1}$ mg protein$^{-1}$) in *Vigna mungo*.

<table>
<thead>
<tr>
<th>Plant organ/Enzyme</th>
<th>Cadmium concentration (mg kg$^{-1}$ soil)</th>
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<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Root Nitrate reductase</td>
<td>4.28 ± 0.68a</td>
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<tr>
<td>Nitrite reductase</td>
<td>5.07 ± 0.78a</td>
</tr>
<tr>
<td>Glutamine synthetase</td>
<td>21.47 ± 3.21a</td>
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<tr>
<td>Glutamate dehydrogenase</td>
<td>14.92 ± 1.79a</td>
</tr>
<tr>
<td>Leaf Nitrate reductase</td>
<td>2.87 ± 0.35a</td>
</tr>
<tr>
<td>Nitrite reductase</td>
<td>3.89 ± 0.71a</td>
</tr>
<tr>
<td>Glutamine synthetase</td>
<td>16.22 ± 2.18a</td>
</tr>
<tr>
<td>Glutamate dehydrogenase</td>
<td>11.89 ± 2.09a</td>
</tr>
</tbody>
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The values are means ± SD of five replications. Means in a row followed by different letters are significantly different according to Duncan’s multiple range test (P = 0.05).

Nitrogen metabolism is important for the response of plants to Cd toxicity. Upon exposure to Cd, plants often synthesize a set of N-containing metabolites through N metabolism, such as proline, GSH and PCs, which play a significant role in Cd tolerance of plants (Sharma and Dietz, 2006). Accordingly, plants might exhibit a higher Cd tolerance by the maintenance of normal N metabolism levels under Cd stress (Gussarsson et al., 1996). An in-depth analysis of activities of principal enzymes of nitrogen metabolism revealed that exposure to Cd differently affected all the enzymes of nitrogen assimilation pathway (Table 1). Significant reductions were noted in the two inorganic nitrogen reducing enzymes, nitrate reductase and nitrite reductase. The level in both the root and leaf declined up to an imperceptible amount. As we know, an established pathway for nitrogen
assimilation is expected to originate from nitrate and is accomplished through the two reductases and the glutamine synthetase-glutamate synthase cycle by the incorporation into glutamate. As our results demonstrated, under Cd exposure, the ability of plant to utilize nitrate is severely repressed or even abolished. Several investigators have also reported the diminutive response of nitrogen assimilation to Cd stress (Chugh et al., 1992; Singh et al., 1994).

Under physiological conditions, GS plays a prominent role in nitrogen metabolism, catalyzing the conversion of glutamate to glycine (Gln), an essential component of proteins and also a donor for the synthesis of most of the nitrogenous compounds in the organism (Lea and Miflin, 2004; Suzuki and Knaff, 2005). As shown in Table 2, the activity of GS was strongly inhibited under Cd stress. Interestingly, our results indicate that GDH activity was induced in the presence of Cd in root and leaf of blackgram. Earlier investigations revealed that higher concentration of ammonium either supplied exogenously (Lea and Ireland, 1999) or by hydrolysis of protein (Masclaux et al., 2000) increased the GDH activity. According to Dominguez et al. (2003), an induced accumulation of ammonium under Cd stress should be accompanied by perpetuation of NR activity. Nonetheless, under these conditions, the aminating GDH activity should contribute to the synthesis of the constitutive glutamate and subsequent induction of PC synthesis. Contrarily, this does not help the plant to tolerate the impact of toxic Cd level. Furthermore, GS catalyzes the elimination of ammonium from the cells and allows recycling of carbon skeletons (pyruvate or α-ketoglutarate) generated in the photosynthetic pathway into the Calvin cycle (Sauer et al., 1987). Thus, inactivation of GS inexorably arrests growth and, eventually, provokes cell death.

Conclusion

It can be concluded that the accumulation of cadmium in leaves and roots of blackgram plants grown under increasing concentrations of cadmium strongly alters the nitrogen metabolism with the consequent increase in TBARS and H$_2$O$_2$ contents.

References

Effects of Cd-induced oxidative stress on growth and N assimilation in blackgram


Received: June 6, 2013
Accepted: July 22, 2013
UTICAJ OKSIDATIVNOG STRESA INDIKOVANOG KADMIJUMOM NA RAST I ASIMILACIJU AZOTA KOD CRNE VIGNE [Vigna mungo (L.) Hepper]

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Rezime

Akumulacija kadmiijuma (Cd), oksidativno oštećenje i metabolizam azota su proučavani u korenovima i listovima 30 dana starih biljaka crne vigne [Vigna mungo (L.) Hepper] uzgajane na mešavini zemljišta i komposta (3:1) sa različitim koncentracijama Cd. Zabeležena su značajna smanjenja suve mase korena i izdanka. Koncentracija Cd u korenovima i listovima se povećavala sa povećanjem nivoa Cd. Nivo peroksidacije lipida se povećavao sa povećanjem sadržaja H₂O₂ u uslovima stresa indukovanog Cd kod oba biljna organa. Aktivnost enzima koji posreduju u asimilaciji azota u korenovima i listovima je bila veoma smanjena u prisustvu Cd, osim glutamat dehidrogenaze (GDH) koja je pokazala značajno povećanje.

Ključne reči: toksičnost kadmiijuma, crna vigna, asimilacija azota, nitrat reduktaza, glutamat dehidrogenaza, oksidativni stres, peroksidacija lipida.


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