Effects of Cadmium on The Nitrate and Potassium Uptake in Guar

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The NET uptake, distribution and assimilation of $\text{NO}_3^-$ were studied in guar plants subjected to either long-term continuous Cd treatment for 252hr (9 or 45 $\mu M$ Cd) or short-term treatment for (84hr) with 45 $\mu M$ Cd. In the latter treatment, the effects of transferring the plants to a Cd- free nutrient solution for “recovery period” of 108hr were also studied. All these treatments were compared with “controls” plants which received no Cd. In both experiments, the reduction in fresh weight was associated with a decrease in the content (%) of shoot and root water and in transpiration rates as Cd concentration increased. The concentration of $\text{NO}_3^-$ in the shoots and sap decreased dramatically and net $\text{NO}_3^-$ uptake was severely inhibited, effects associated with a loss of shoot nitrate reductase (NR) activity. In the short-term Cd treatment, net $\text{NO}_3^-$ uptake was almost completely inhibited after 36hr, but recovered after the transfer of plants to a Cd- free nutrient solution. Similarly, a dramatic decrease in the shoot NR activity was observed.

The uptake, distribution and tissue partitioning of K was also studied, which is considered to be the major counterion of $\text{NO}_3^-$. Potassium uptake was similarly affected by Cd, as inferred from the ratio $\text{NO}_3^-$/uptake, which was ca. 9. The ratio $K/\text{NO}_3^-$ tissue content increased in the shoot concomitantly to Cd in both long-term and short-term metal supply. These parameters showed a tendency of K similar to that observed for $\text{NO}_3^-$, although its relative tissue distribution was not affected by Cd.

Keywords: Cadmium, Nitrate, Guar, Uptake, Distribution, Assimilation.

Abbreviations: LDS$_{0.05}$ = Least significant differences with 0.05 of probability. NR=nitrate reductase, g Fw-fresh weight.
Many different phytotoxic effects have been observed induced by the exposure of higher plants to Cd (Van Assche & Clijsters, 1990 and El-Kassas, 1999). Alterations to fundamental physiological processes of plant metabolism have been attributed to Cd including photosynthesis (Krupa et al., 1993), cell respiration (Greger et al., 1991) and plant water relationships (Barcelo and Poschenrieder, 1990).

Several authors found that NO\textsubscript{3} assimilation was affected by the presence of Cd in the growing medium of higher plants; in particular, NO\textsubscript{3} content in tissues was reduced in broad bean (Khater et al., 1991 and El Sayed, 1999a), cucumber (Burzynski, 1988 and El-Sayed, 1998a) and sugar beet (Petrovic et al., 1990 and El-Sayed, 1998b). Cadmium accumulation in plant tissues resulted also in the loss of NR activity (Burzynski, 1989, Nussbaum et al., 1988; Petrovic et al., 1990 and El-Shebiny, 1998). In maize plants, NR activity decreased when Cd was added to the nutrient solution, which was related to a decrease in NO\textsubscript{3} content in tissues. However, in maize scutella, the presence of Cd (upto 45\textmu M) had no effect on in vitro NR activity nor on the de novo synthesis of the enzyme (Ghaly and El Sayed, 1997). Mathys (1975) observed that the presence of Cd (>5 \textmu M) and other-heavy metals in the incubation medium, inhibited NR activity of Silene cucubalus plants. However, Chugh et al. (1992) showed that Pisum sativum NR activity was not affected by exposure to concentration of Cd below 45 \textmu M and Burzynski (1988) found that NR activity of cucumber plants was only inhibited above 100\textmu M Cd. Therefore, depending on the stability of the NR extracted from different plants, Cd might alter NR activity in vitro. Nevertheless, there is little information available about the putative mechanisms involved in the alteration of NO\textsubscript{3} absorption and assimilation under Cd exposure in vivo.

In the present work, guar plants were supplied with varying amounts of Cd supplied in a long-and short-term experiments (maximum exposition for 25hr and 84hr, respectively), to evaluate its effect on the uptake and distribution of NO\textsubscript{3}. I have also studied the uptake and distribution of K, which is considered to be the major counterion in the processes of NO\textsubscript{3} uptake and tissue distribution (Rufty et al., 1986, Tremblay et al., 1988 and El-Sayed, 1999b).

**Material and Methods**

**Plant material**

Guar seeds (Cyamopsis tetragonoloba L. Taub) variety Giza 1 were germinated on moistened paper for 4 days. Seedlings were cultivated on plastic
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Grids suspended in nutrient solution (macronutrients (mM): 2.0 Ca (NO₃)₂, 1.5 KNO₃, 1.0 Mg (NO₃)₂, 1.0 KH₂PO₄, 0.5 Mg SO₄, 0.1 NaCl, and micronutrients [μM element]: 45.0 Fe (EDDHA), 18.0 MnSO₄, 3.0 CuSO₄, 6.0 ZnSO₄, 2.0 Mo₇O₂₄(NH₄)₆, 4H₂O, 23.5 H₃BO₃, pH 6.0) in greenhouse.

Long-term exposure to Cd

Fifteen selected seedlings suspended in plastic grids were treated with absence (control, 0.0), 9 or 45 μM Cd (CdSO₄·H₂O), for 7 days in plastic pots containing 1.9L of nutrient solution in plants were then transferred to glass jars containing 320 mL of the same nutrient solution and sealed to avoid evaporation losses. After 0, 36, 60 and 84hr of treatment the volume of solution last through transpiration was replaced, to avoid any concentration effects, and an aliquot of the nutrient solution (15 mL) was taken to determine the concentration of the remaining NO₃⁻ and K. In NO₃ vitro NR activity was measured in one plant per glass jar at each harvest. After 84 hr (accounting for a total Cd-exposure of 252hr), plants were harvested to determine: fresh weight of plant (shoot, root and seed), shoot and root. The content of NO₃⁻ and K in the plant tissues and NO₃ concentration in sap.

Short-term-addition of Cd

In the short term treatment, 15 selected guar seedlings were grown in control nutrient solution for 7 days in the plastic pots described above. Then, cultivated in identical conditions as described above, plants were transferred to control or 45 μM nutrient solutions. In vitro NR activity, and K-and NO₃⁻ -uptake were measured after 0, 36, 60 and 84hr of treatment. Plants were sampled after 84hr exposure to Cd to determine (Van Erp and Van Lune, 1989), the same parameters as indicated above. Another batch of plants was transferred to control nutrient solution in the recovery period and the same parameters measured after 36, 60, 84 and 108hr following transfer.

Determination of in vitro NR activity

The in vitro NR activity assay was done according to Ramon et al. (1989). Fresh tissue (0.5g) was quickly frozen in liquid N₂ and homogenized in extraction buffer (25 mM KH₂PO₄ / K₂ HPO₄, 5mM EDTA, 1% W/V polyvinylpyrrolidone and 1 mM cysteine, pH 7.7). The extract was centrifuged at 10000 xg for 15 min at 4°C. To avoid the putative hydrolysis of the solubilized
NR by proteases, the supernatant was kept at ice-cold temperature to slow down the protein degradation and its activity was immediately determined. In previous experiments the extraction buffer was supplemented with 2% (W/V) casein, as unspecific substrate for proteases, and activity measurements demonstrated that under the described assay conditions no significant difference in activity was detected. NR was assayed by adding 0.1 mL of 100 mM KNO₃, 0.5 mL of reaction buffer (100 mM KH₂PO₄/K₂HPO₄, 1 mM EDTA, pH 7.5) and 0.1 mL of 1 mg/mL NADH as reductant substrate. The NR reaction was started by adding 0.1 mL of NR extract and incubated for 15 min at 20°C. NO₂⁻ was analyzed using freshly prepared colorimetric reagent comprising sulphanilamide (1% W/V) in 3 M HCL and 0.02% (W/V) N-(1-naphthyl) ethylenediamide dihydrochloride, in a 1:1 ratio. The strongly acidic colorimetric reagent denaturated all proteins present in the reactions medium and, before the absorbance was measured, the tubes were centrifuged at 1000 xg for 15 min to obtain a clear supernatant. The absorbance was read at 540 nm.

**NO₃⁻ determination in plant tissues**

The NO₃⁻ content of plant tissues was determined essentially as described by Gojon et al. (1991). Dried sample (100 mg) was digested with 100 mM HCL (5 mL) for 30 min at 70°C, the extract filtered and made to 25 mL with water. NO₃⁻ was analyzed by using a Tecnicon Acta 11 colorimetric autoanalyzer, with a Cd/Cu reducing column. The sample was diluted in reduction buffer (10 g/L NH₄Cl, pH 8.5), reacted with the colorimetric reagent (1% W/V sulphanilamide, 14 M H₃PO₄, 0.05% W/V N-(1-naphthyl) ethylenediamide dihydrochloride and 0.5 mg/L Brij 35) and the absorbance read at 520 nm.

The NO₃⁻ content of sap was determined by extracting stems sap according to Cottenie et al. (1982); Page et al. (1982); Evenhuis and De Waard (1978). FAO (1978), with minor modifications. Stems were chopped, placed in plastic flask, immersed in diethyl ether and stored at -25°C. Sap was extracted under pressure and the aqueous phase isolated using a separating funnel. An aliquot (0.1 mL) was diluted in 1 mL of 2.5 mg/mL sulphasalicylic acid, centrifuged at 15000 xg and NO₃⁻ analyzed in the protein-free supernatant as described above.

The net uptake of NO₃⁻ was measured from the amount of NO₃⁻ that was lost from the nutrient solution, after its volume was corrected with deionised H₂O to take into account the losses due to plant transpiration.

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*K determination*

Plant tissues were dried at 70°C to constant weight. After homogenization, samples were digested with a mixture of acids; HNO₃; H₂SO₄; HClO₄ (5:1:2) at low temperature (< 200°C) to avoid evaporation losses and formation of foam. Samples was diluted with deionized water, thoroughly vortexed and after filtration. The volume was made to 25 mL. Potassium was analyzed by atomic emission spectrophotometry (Perkin-Elmer 4000), with acetylene-air flame as described (Garte et al., 1993). Negligible losses of metals by filtering were detected by using internal standards, and accuracy and precision of atomic spectrophotometry methods were checked with high-pure commercial available standards (Merck and Carlo-Erba) diluted to appropriated concentrations in the acidic matrix used. The concentration K in the nutrient solution was determined in an aliquot of 5ml diluted with 1mL of mixture of acids, and analyzed as described above. Potassium net uptake was determined as described above for NO₃⁻.

**Data analysis**

Data are the average of at least three independent experiments. Significance of differences was determined by using the test of Duncan for analysis of variance. In the tables, data significantly different to the control at 0.05 and 0.02 probability are indicated with the corresponding asterisks. In figures, the least significant difference at 0.05 of probability (LSD₀.₀₅) is given; so if the difference between two values is larger than the LSD₀.₀₅ both are significantly different.

**Results**

The fresh weight and size of the whole plant, shoots and roots declined as the concentration of Cd in the nutrient solution increased following 252hr of total Cd-exposure (Table 1), in agreement with previous results of guar plants treated with Cd (Leita et al., 1993; El-Sayed, 1999 C). In the short-term experiment after 84hr of treatment with 45 µM Cd, only the weight of root was affected but during the recovery period, the fresh weight of shoots was also reduced (Table 1). Those results might indicate that the roots were affected first by the presence of Cd, and that those phytotoxic effects were irreversible.
TABLE 1. Fresh weight of the whole plant, shoot and root (g/plant) and the shoot (SWC) and root (RWC) water content (%) of guar plants, treated with control, 9 or 45 uM Cd in the long-term experiment, and treated with control or 45 uM Cd in the shoot-term experiment. Plants were sampled at the end of the Cd exposure (84 hr) and at the end of the recovery period (108 hr) after transfer to a Cd-free nutrient solution. (Supply Cd, n=9, Cd-recovery period n = 6).

<table>
<thead>
<tr>
<th></th>
<th>Plant</th>
<th>Root</th>
<th>Root</th>
<th>SWC(%)</th>
<th>RWC(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Long-term</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.86</td>
<td>0.28</td>
<td>0.30</td>
<td>88.51</td>
<td>92.76</td>
</tr>
<tr>
<td>9 uM Cd</td>
<td>0.82**</td>
<td>0.17**</td>
<td>0.20**</td>
<td>86.78**</td>
<td>91.32**</td>
</tr>
<tr>
<td>45 uM Cd</td>
<td>0.36**</td>
<td>0.05**</td>
<td>0.05**</td>
<td>81.66**</td>
<td>88.37**</td>
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<td><strong>Short-term addition of Cd</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.98</td>
<td>0.31</td>
<td>0.38</td>
<td>88.89</td>
<td>93.91</td>
</tr>
<tr>
<td>45 uM Cd</td>
<td>0.89**</td>
<td>0.28</td>
<td>0.30**</td>
<td>88.40</td>
<td>92.79**</td>
</tr>
<tr>
<td><strong>Recovery</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.89</td>
<td>0.70</td>
<td>0.72</td>
<td>89.05</td>
<td>94.15</td>
</tr>
<tr>
<td>45 uM Cd</td>
<td>1.19**</td>
<td>0.31**</td>
<td>0.31**</td>
<td>85.65**</td>
<td>9158**</td>
</tr>
</tbody>
</table>

* P < 0.05 (treatment vs. control).
** p < 0.02 (treatment vs. control).

In both experiments, Cd-treated plants suffered a reduction in their relative water contents (Table 1). This reduction was concomitant with a decrease in transpiration rate, measured by the water consumption of the nutrient solution (Fig. 1). As daily, less volume of water was needed to restore the original volume of the nutrient medium of Cd-treated plants. Also, the total consumption of water decreased consistently as the concentration of Cd increased. Thus, Cd treated plants exhibited the characteristics of water-stress, as observed with bean plants (Poschenrieder et al., 1989 and Darwish & Ahmad, 1997).

The net absorption of NO\textsubscript{3}\textsuperscript{-} from the nutrient solution was reduced dramatically in the long-term presence of Cd (Fig. 2A). After 60 hr, NO\textsubscript{3}\textsuperscript{-} uptake of plants treated with 9 uM Cd was less than a third of that found in control plants and this loss was even greater in plants treated with 45 uM Cd. The concentration of NO\textsubscript{3}\textsuperscript{-} in all tissues also decreased (Table 2). In the shoot, NO\textsubscript{3}\textsuperscript{-} declined sharply in plants treated with 45 uM Cd, whereas in the root there was only a decrease of ca. 50% the concentration of the control. In addition, the concentration of NO\textsubscript{3}\textsuperscript{-} found in sap was reduced as the concentration of Cd increased, the values...

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observed being of the same order of magnitude as those obtained in the shoot (Table 2). Similarly, net K uptake completely inhibited in plants treated with 45\mu M Cd and plants supplied with 9 \mu M Cd showed an identical trend than that observed for net NO_3 uptake (Fig. 2B). The concentration of K in plant tissue decreased when Cd accumulated in plants. Both shoot and root tissue were equally affected (Table 2).

![Graph showing transpiration of guar plants](image)

**Fig. 1.** Transpiration of guar plants (ml H_2O g FW / root/day) after 36, 60 and 84 from the beginning of the long-term experiment (A. LSD_0.05 :0.39) and of the short-term experiment (B, LSD_0.05 : 1.03). Control (\Diamond), (\diamond) or 45 \mu M Cd (\blacktriangle). The accumulated volume of H_2O transpired (ml H_2O g FW / root) is represented in the right - hand Y axis. Control (?), 9 (\square) or 45 \mu M Cd (?). Supply Cd, n=9; Cd-recovery period n=6.

In the short term experiment, the net uptake of NO\textsuperscript{3} from the nutrient solution of treated plants declined sharply after only 36 hr from the addition of Cd, and only resumed when plants were transferred to Cd-free nutrient solution (Fig. 3A). During Cd supply, less NO\textsuperscript{3} accumulated in the shoots, whereas the concentration of NO\textsuperscript{3} in the roots and the sap was not significantly different from that in the control plants (Table 2). In the recovery period, Cd-treated plants only accumulated ca. 33\% of the NO\textsuperscript{3} found in the control, whereas in the roots the concentration of NO\textsuperscript{3} in treated plants was significantly greater than that observed in control plants (Table 2). Also, a significant decrease (ca. 45\% of the control) was found in the sap NO\textsuperscript{3} content (Table 2). In parallel, K net uptake was completely inhibited after 36 hr of short-term treatment with 45 \textmu M Cd. During the recovery period following Cd removal from the nutrient solution, the absorption of K increased although values were ca. 5 times less than those

observed for control plants (Fig. 3B). Consecutively, shoot and root K concentration declined after Cd supply, whereas no significant differences were observed following Cd removal (Table 3). Potassium uptake was similarly inhibited in birch plants under Cd stress with a decrease of ca. 45% when plants were exposed to 5 μM Cd (Asp et al., 1994; El-Gendi et al., 1999).

In a previous work, I have observed that in vitro NR activity correlates ($r^2 > 0.90$) with the endogenous content of NO$_3^-$ in plant tissues (Hernandez et al., 1995 and El-Sayed, 1999d). However, little or no in vitro activity was detected in roots by using the procedure described above. It is possible that guar root NR was more labile than that of shoots under the conditions used for the in vitro NR assay. Nevertheless, the data presented of shoot NR activity were valid and were used as an index for the shoot NO$_3^-$ nutritional status of plants treated with Cd. Thus, the reduction of shoot NO$_3^-$ content observed in Cd-treated plants was associated with the loss of NR activity (Table 3). Similarly, in the short-term experiments, the decrease in shoot NO$_3^-$ concentration was accompanied by a sharp decrease in the in vitro NR activity of Cd-treated plants (Table 3). After transferring the plants to Cd-free nutrient solution, there was no significant recovery of NR activity (Table 3).

Although K and NO$_3^-$ absorption were similarly affected by the presence of Cd in the long- and short-term treatments, their partitioning between root and shoot was different. Thus, while NO$_3^-$ accumulated mainly in root of Cd-treated plants, K distributed equally in root and shoot under the long-term exposure to Cd or was not affected after the supply of 45 μM Cd in the short-term experiment (Table 2). Therefore, the ratio K/NO$_3^-$ of root content remained constantly at ca. 2 in both experiments (Table 2). However, this ratio increased almost 10-fold in the shoot of 45 μM Cd long-term treated plants. Similarly, the ratio K/NO$_3^-$ increased in plants subjected to the short-term Cd exposure (ca. 6-fold after the recovery period). This might indicate that NO$_3^-$ content was dramatically reduced in shoot in the presence of Cd, due to its assimilation and/or reduced transport from the root, whereas K shoot content was less affected.

Cd addition

Recovery

Fig. 3. Short-term experiment. Concentration of NO$_3^-$ (A, LSD$_{0.05}$ : 8.6) and K (B, LSD 0.05 : 11.7) absorbed from the nutrient solution after 0.36, 60 and 84hr from the beginning of the short-term addition of Cd, and after recovery at 36, 60, 84 and 108hr from the transfer of plants to a Cd-free nutrient solution (umol nutrient g FW/root). Control (•) or 45 µM Cd (○). Supply Cd, n=9; Cd-recovery period, n=6.

Table 4 shows an estimated balance of K and NO$_3^-$ uptake. There was higher requirement of NO$_3^-$ than of K, and the ratio NO$_3^-$ /K uptake fluctuate in all samples around 10. Thus, regardless to Cd treatments, plants withdraw similar relative amounts of both nutrients from the growing solution, indicating that the effects of Cd on their uptake was similar. On the other hand, the ratio of total plant content of nutrient vs. their uptake revealed different behavior between

NO$_3^-$ (NCNU) and K (KCKU; Table 4). In the long-term Cd exposure experiment, UCNU increased only slightly in Cd-treated plants (from 2 to 4), whereas KCKU was ca. 60 times higher in 45 $\mu$M Cd treated plants. Similar pattern was observed in the short-term Cd supply. Therefore, in Cd-treated plants, much more K was accumulated in the tissue than absorbed from the nutrient solution. It is possible that NO$_3^-$ tissue content was more dependent on its uptake, because it is assimilated into organic N, and a continuous extracellular replenishment is required (Hoff et al., 1992; Tischner et al., 1993; Eissa and El-Kassas, 1999) This is demonstrated in the recovery period, following the 108hr transfer to Cd-free nutrient solution, where NCNU was below 1, indicating that more NO$_3^-$ was absorbed than accumulated, probably due to its assimilation.

**TABLE 2. NO$_3^-$ and K concentration (Umol/gFW) of shoot, root and sap, and ratio between K and NO$_3^-$ tissue content (RKN) of guar plants treated with control, 9 or 45$\mu$M Cd in the long-term experiment, and of guar plants with control or 45 $\mu$M Cd in the short-term experiment. Plants were sampled at the end of the Cd exposure (84hr) and at the end of the recovery period. 108hr after transfer to a Cd-free nutrient solution. (Supply Cd, n=9, Cd-recovery period n=6).**

<table>
<thead>
<tr>
<th></th>
<th>NO$_3^-$ concentration</th>
<th>K Concentration</th>
<th>RKN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot</td>
<td>Root</td>
<td>Sap</td>
</tr>
<tr>
<td>Long-term</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>37.33</td>
<td>44.51</td>
<td>38.53</td>
</tr>
<tr>
<td>9 $\mu$M Cd</td>
<td>18.16</td>
<td>45.77</td>
<td>20.91</td>
</tr>
<tr>
<td>45 $\mu$M Cd</td>
<td>1.83</td>
<td>22.49</td>
<td>1.29</td>
</tr>
<tr>
<td>Short-term addition of Cd</td>
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<td></td>
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<tr>
<td>Control</td>
<td>40.19</td>
<td>42.96</td>
<td>37.11</td>
</tr>
<tr>
<td>45 $\mu$M Cd</td>
<td>18.76</td>
<td>42.19</td>
<td>31.91</td>
</tr>
<tr>
<td>Recovery</td>
<td></td>
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<tr>
<td>Control</td>
<td>30.30</td>
<td>29.41</td>
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<tr>
<td>45 $\mu$M Cd</td>
<td>9.04</td>
<td>31.67</td>
<td>11.00</td>
</tr>
</tbody>
</table>

* P< 0.05 (treatment vs. control).
** P < 0.02 (treatment vs. control)

**Discussion**

To my knowledge, these results are the first to describe the effects of Cd on the NO$_3^-$ and K uptake and tissue distribution in guar plants. The long-term experiments reveal that plants suffered a mild-stress and strong-stress due to their exposure to 9 and 45 $\mu$M Cd respectively, according to the reduction observed in fresh weight (Table 1).

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Nitrate absorption was affected by the exposure of plants to Cd (Fig. 2A and 3A). This was in agreement with the lower tissue concentration of NO$_3^-$ observed in sugar beet (Petrovic et al., 1990), cucumber (Burzynski, 1988) and maize (El-Sayed, 1995) treated with Cd. The short-term exposure to 45 uM Cd reflected a quick response of guar plants to the presence of a high concentration of Cd. This was demonstrated by the severe decrease in NO$_3^-$ uptake, even after 36 hr of Cd supply (Fig. 3A), which was associated with a little response of the fresh weight (Table 1). Therefore, the reduction in NO$_3^-$ depletion from the nutrient solution in the presence of Cd is not exclusively due to a decrease in plant size. Nitrate uptake is mediated by transporters in the plasma membrane of root cells and the synthesis of these transporters is activated by the presence of in NO$_3^-$ in the growing medium (Agüera et al., 1990; Henriksen & Spanswick, 1993 and El-Sayed, 1995). It is possible that the inhabitation of NO$_3^-$ uptake could be explained by an alteration in transporter function arising from a decrease in the trans-plasma membrane potential, responsible for the facilitated uptake, of NO$_3^-$ (Aidid and Okamoto, 1992; Meharg & Blatt, 1995 and Tahoun et al., 1999). The transmembrane potential is mainly due to plasma membrane-bound Mg$^{2+}$-ATP as activity, which is the presence of Cd (100 uM) is inhibited in wheat and sunflower root (Fodor et al., 1995; El-Sayed 1998b), and in guar root after 36 hr of exposure to 45 uM Cd. The decline in NO$_3^-$ uptake of plants subjected to the Cd short-term treatment (Fig. 3A) could be explained in this context.
TABLE 4. Balance of NO$_3^-$ and K uptake and tissue accumulation in the long- and short treatments with Cd. The ratios between NO$_3^-$ and K-uptake is shown in the brackets. The ratios (NUNC) and (KUKC) between NO$_3^-$ and K uptake and their respective total plant concentration are also shown.

<table>
<thead>
<tr>
<th>Total nutrient uptake (umole/plant) VS. hours</th>
<th>Ratios a</th>
<th></th>
</tr>
</thead>
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<tr>
<td></td>
<td>36</td>
<td>60</td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long - term experiment</td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>0.808(8.8)</td>
<td>4.529(11.1)</td>
</tr>
<tr>
<td>9 uM Cd</td>
<td>0.374(12.8)</td>
<td>0.965(8.3)</td>
</tr>
<tr>
<td>45 uM Cd</td>
<td>0.041(10.4)</td>
<td>0.110(6.8)</td>
</tr>
<tr>
<td>Sort - term experiment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.801(6.2)</td>
<td>3.431(8.9)</td>
</tr>
<tr>
<td>45 uM Cd</td>
<td>0.333(5.1)</td>
<td>0.743(7.1)</td>
</tr>
<tr>
<td>K</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long - term experiment</td>
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<td>Control</td>
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<tr>
<td>9 uM Cd</td>
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<td>0.015</td>
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<tr>
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<td>0.377</td>
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<tr>
<td>45 uM Cd</td>
<td>0.063</td>
<td>0.102</td>
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</table>

$^a$ NUNC - Ratio of NO$_3^-$ total plant content vs. NO$_3^-$ uptake when plants were collected.
$^b$ KCNU - ratio of K total plant content vs. K uptake when plants were collected.
$^c$ Ratio calculated for the recovery period.

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Similar decrease in NO$_3^-$ uptake due to a modification of the plasma membrane NO$_3^-$ permeability was observed in the presence of Al (Cakmak and Horst, 1991; Durieux et al., 1993; Ghaly and El-Sayed, 1997). In this sense, Pandolfini et al. (1992) observed that in wheat roots exposed to Ni a leakage of K occurred, which was associated to the peroxidation of membrane lipids. This finding is in agreement with my results of K uptake (Fig. 2B and 3B), where a severe loss of K uptake was observed, indicating that a general alteration of absorption processes in root cells may occur in the presence of Cd. This would reflect a general loss of plant plasma membranes integrity (Meharg, 1993; Eissa and El-Kassas, 1999).

In the shoots, the increase in Cd nutrient solution concentration was accompanied by a decrease in NR activity, which was inhibited dramatically by the greatest concentration of Cd supplied in the long-term experiment (Table 3). This loss of NR activity in shoot was associated with a similar reduction in NO$_3^-$ content (Table 2), symptoms also observed in maize plants under similar concentrations of Cd (El-Sayed, 1999c). Likewise, several authors reported a decrease in NR activity of different plant species when Cd was supplied to the nutrient solution (Burzynski, 1988; Chugh et al., 1992; Nussbaum et al., 1988; Petrovic et al., 1990; Darwish and Ahmad, 1997). After 36hr exposure to Cd in the short-term treatment, the NR shoot activity was ca. 45% of that in the control shoot and was almost totally inhibited after 84hr (Table 3). This loss of NR activity was concomitant to a severe restriction in net NO$_3^-$ uptake, and its extreme accumulation of NO$_3^-$ in roots.

The largest proportion of Cd was found in the soluble fraction (consisting mainly of vacuolar and cytosolic contents), according to the subcellular distribution of Cd in guar plants. However, Cd was associated to proteins or polypeptides up to 85% of the total Cd concentration in this fraction (El-Gendi et al., 1999). It is possible that Cd is released from cellular storage pools by the tissue homogenization needed for the solubilization of the NR. This might provoke its inhibition due to the interaction of Cd with the enzyme. However, as indicated, many other proteins are expected to form complexes with Cd, in particular phytochelatins (Gupta and Goldsborough, 1991), which suggests that Cd does not interact alone with NR. Therefore, it is unlikely that the in vitro NR inhibition is primarily due to an artifact. Moreover, shoot in vivo NR activity (determined) as described by Hernandez et al. (1995) of sunflower plants was
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severely inhibited (from 1.14±0.16 to 0.08±0.02 umol NO$_3^-$ /g FW shoot/hr) when treated with 15 $\mu$M Cd for 7 days, results that support those presented here (Table 3).

The accumulation of NO$_3^-$ in the roots might indicate that the xylem transport of NO$_3^-$ was limited in the presence of Cd in the growing medium. The long-term exposure to Cd caused a severe reduction in the shoot and sap NO$_3^-$ content, but in the roots the decrease was observed only in plants treated with 45 $\mu$M Cd (Table 2). The results of the short-term treatment also support this hypothesis, where after 84hr the shoot concentration of NO$_3^-$ declined to ca. 50% of the control values, whereas in the roots the NO$_3^-$ content was not affected and increased after the recovery (Table 2). Nitrate long distance transport to the shoot via xylemis mainly controlled by the transpiration (Barthes et al., 1996; El-Sayed 1998a; El-Kassas, 1999) The exposure of soybean plants to Cd provoked a reduction in their sap flow, transpiration and water uptake (Leita et al., 1995). Therefore, the loss of transpiration observed in the presence of Cd (Fig. 1), an effect also described in bean (Poschenrieder et al., 1989) and sugar beet (Greger and Johansson, 1992), might explain partially the decrease in NO$_3^-$ transport.

De novo synthesis of NR is induced by the concentration of NO$_3^-$ in the cytosol (Hoff et al., 1992; Tischner et al., 1993 and El-Sayed, 1999a) which represents a minor fraction compared to the storage pool of NO$_3^-$ . This major proportion is located in the vacuole (Martinoia et al., 1981, Zhen et al., 1991; El-Sayed, 1999b), and is available to NR only under conditions of external NO$_3^-$ deprivation (Grandstedt and Huffaker, 1982; Hernandez et al., 1995; El-Shebiny, 1998). The concentration of cytosolic NO$_3^-$ in aerial parts is mainly controlled by the xylem flux, and by the concentration of incoming NO$_3^-$ from the xylem (Mcknown et al., 1983, Soussana et al., 1989 and El-Sayed, 1999d). Therefore, the loss in uptake and transport of NO$_3^-$ due to exposure of guar plants to Cd would lead to a decrease in the cytosolic NO$_3^-$ concentration. This effect could partially explain the inhibition of NR activity observed in those plants by a decrease in NO$_3^-$ availability. This hypothesis is supported by the complete inhibition of net NO$_3^-$ uptake and transport to assimilating tissues observed after 36hr of short-term Cd treatment, effect also observed onwards (Fig. 3A, Table2). Furthermore, the almost complete inhibition of water transpiration (Fig. 1B) which would probably affect the transport of NO$_3^-$ via xylem (Barthes et al., 1996; Tahoun et al., 1999), confirms that hypothesis.

Conclusion

It is possible that the inhibition of NO\textsuperscript{3} and K uptake and the loss of water transpiration reflect a general Cd phytotoxic effect on plasma membranes integrity. As a result, NO\textsuperscript{3} content and assimilation is severely reduction in shoot.

References


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تأثير الكادميوم على استئصاص كل من النباتات والبوتاسيوم في الجوار

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تم دراسة محصلة الاستئصاص وتوزيع وتمثيل النباتات في نباتات الجواف لفترة طويلة، حيث تم إضافة الكادميوم لمدة 42 ساعة بتركيزات 50 ميكرومول، ثم إضافة الكادميوم لفترة قصيرة بتركيز 5 ميكرومول لمدة 8 ساعات. وفي المقابلة الأخيرة تم دراسة انتقال الكادميوم الحرج في الطول المغربي إلى النباتات وسببات هذه الفترات بفترة استعداد الكادميوم. وتم دراستها عنده 108 ساعة. يتم مقارنة جميع هذه الحصائل مع الكنبوزول الذي يمثل النباتات التي لم يضاف فيها عنصر الكادميوم لوحظ في كل من التجريبيتين أن هناك نقصا في الوزن الطازج للنباتات مماجرد نقص في النسبة المئوية تحتوي الماء، في كل من سيقان وجدور نباتات الجواف وكذلك في معدل النثأ عند زيادة تركيز الكادميوم.

وأوضح النتائج حدوث نقص مافي في تركيز النباتات في كل من سيقان ومضارورة نباتات الجواف، حيث حدث تضييق كبير لاستئصاص النباتات وصاحب هذا التأثير فقد للفترة بواسطة نشاط إنزيم Reductase. إضافة ذلك أوضح النتائج أن النباتات لفترة القصيرة من معاملة الكادميوم كانت المحصلة النهائية لاستئصاص النباتات وهو حدوث نثأ لا بعد 26 ساعة. كذلك حدث نقص مافي في نشاط إنزيم Reductase.

تم دراسة استئصاص البوتاسيوم وتوزيعه في أنجسية نباتات الجواف حيث حول حدوت تضاؤ البوتاسيوم في وجود النباتات كذلك تأثير استئصاص البوتاسيوم لوجود الكادميوم وذلك من معرفة K/NO_3 أوضحت النتائج أن نسبة K/NO_3 في أنجسية نباتات الجواف تزداد في السيقان مع زيادة الكادميوم المضاد في كل من الفترة الطويلة والقصيرة. أوضح هذه الدراسة أن سلاك البوتاسيوم بشابة سلاك النباتات بالرغم من عدم تأثر التوزيع النسبى للكادميوم في أنجسية النباتات.