Differential Toxicological Response to Cadmium Stress of Bean Seedlings Grown With NO\textsubscript{3}\textsuperscript{−} or NH\textsubscript{4}\textsuperscript{+} as Nitrogen Source

Gouia Houda, Chaffei Chiraz, Debouba Mohamed and Ghorbel Mohamed Habib
Département de Biologie, Faculté des Sciences de Tunis,
Campus Universitaire El Manar I, 2092,
Unité de Recherche: Nutrition et Métabolisme Azotés et Protéines de Stress (99UR/09-20), Tunis, Tunisie

Abstract: We examined the effects of cadmium (Cd) in bean seedlings (Phaseolus vulgaris L. cv. Morgan) grown with nitrate (4 mM) or ammonium (4 mM) as the nitrogen source. Depending on the N source supplied, there were significant differences in the sensitivity of plants to Cd. The Cd treatment had a greater depressive effect on shoot and root dry weight production in plants fed with NO\textsubscript{3}\textsuperscript{−}. Accumulation of Cd in roots exceeds by far that of shoots, with the greatest Cd accumulation occurring in plants supplied with NO\textsubscript{3}\textsuperscript{−}. Increasing Cd supply resulted in a dramatic decrease of CO\textsubscript{2} assimilation and transpiration rate in NO\textsubscript{3}\textsuperscript{−}-fed plants compared to NH\textsubscript{4}\textsuperscript{+}-fed plants. Concurrently, cadmium treatment enhanced the phosphoenolpyruvate carboxylase (PEPC) and glutamate dehydrogenase (GHD) activities in ammonium-fed plants. PEPC maximal activity significantly increased in Cd-treated leaves. Immunohistochemical analysis by determination of the equivalence point indicated that Cd treatment increased the amount of PEPC protein in the leaves at all Cd concentrations examined. These results suggest that ammonium assimilating capacity by GHD in bean plants-fed with NH\textsubscript{4}\textsuperscript{+} compared to NO\textsubscript{3}\textsuperscript{−}-fed plants, plays a crucial role in Cd tolerance. In addition, our results suggest that the detrimental effects of cadmium can be reduced by partial substitution of NO\textsubscript{3}\textsuperscript{−} with NH\textsubscript{4}\textsuperscript{+} and that this is due to the lower energy cost of N assimilation with NH\textsubscript{4}\textsuperscript{+} as opposed to NO\textsubscript{3}\textsuperscript{−} nutrition.

Key words: Phaseolus vulgaris, cadmium, ammonium, nitrate, glutamate dehydrogenase, phosphoenolpyruvate carboxylase

INTRODUCTION

Cd is one of the important heavy metal pollutants and its toxic effects on plants are well documented. However, increased industrial and mining activities, mineral and organic fertilizers, pesticides and the disposal of urban and industrial water have led to the input of large amounts of Cd in the environment (Alloway, 1990). During prolonged periods of Cd stress, the decrease in water availability for transport associated processes leads to change in the concentrations of many metabolites, followed by disturbance in amino acid and carbohydrate metabolism (Boussama et al., 1999; Liao et al., 2005; Chaffei et al., 2004). Several investigations have demonstrated a marked reduction in the overall rate of photosynthesis by cadmium in different plants species (Chught and Sawhney, 1999; Sandalio et al., 2001; Chaffei et al., 2006a). This effect of Cd may, however, be modified by the nitrogen source supplied depending on plant species and experimental conditions. The growth of many plant species is clearly affected by the form of nitrogen nutrition (Lewis and Chadwick, 1983). If ammonium (NH\textsubscript{4}\textsuperscript{+}) is the sole N source, growth of many plants is impaired (Clausen and Lenz, 1999; Walsh-Lu et al., 2000). The smaller biomass of many plants grown on NH\textsubscript{4}\textsuperscript{+} compared to NO\textsubscript{3}\textsuperscript{−} nutrition was associated with the reduced carbon assimilation (Guo et al., 2002). Furthermore, NH\textsubscript{4}\textsuperscript{+} dose not only influence production of dry matter but also water uptake. The water uptake rate was higher in NO\textsubscript{3}\textsuperscript{−} than in NH\textsubscript{4}\textsuperscript{+} grown plants (Adler et al., 1996). This effect was explained by differences in stomatal conductance. NH\textsubscript{4}\textsuperscript{+} is assimilated for the greatest part within the root and NO\textsubscript{3}\textsuperscript{−} for major part in the shoot (Cramer and Lewis., 1993). Some studies support the involvement of GHD in the assimilation of ammonium produced in stress conditions like heavy metallic stress such that induced by Cd (Maselaux-Daubresse et al., 2006; Chaffei et al., 2003). Generally, those conditions bring about disturbances on the activities of enzymes involved in the ammonium
assimilation such as an inhibition of GS (Kamachi et al., 1991) and GOGAT (Singh and Srivastava, 1986; Gouia et al., 2000). The catabolic activity of PEPC (Guo et al., 2002) in primary leaves of bean plants was higher in NO$_3^\text{-}$ than in NH$_4^\text{+}$ grown plants. Similarly, Arnizis et al. (1988) found that the activity of PEPC was little affected by the nitrogen source in the leaves of wheat and maize. However, with addition of Cd, a marked increase in the PEPC was found.

Many studies of the toxic effects of Cd were mainly concerned with growth rate, detoxification processes and nitrogen metabolism (Ouari et al., 1997; Jing et al., 2005; Romero-Puertas et al., 2002; Papazoglou et al., 2005; Ohraya et al., 2005). Only a few and recent studies of the effects of Cd on plants under ammonium or nitrate supply are available (Singh, 1987; Bolanos et al., 1992). The aim of this study was to investigate the effects of Cd stress on bean seedlings grown under NO$_3^\text{-}$ or NH$_4^\text{+}$ nutrition.

MATERIALS AND METHODS

Growth conditions: Seeds of bean Phaseolus vulgaris L. cv. Morgan were surface sterilised in 10% H$_2$O$_2$ for 20 min, washed in distilled water and germinated between wet paper towels at 25°C in the dark for 3 days. Subsequently plants were cultivated hydroponically in a growth chamber at a light intensity of 150 μmol m$^{-2}$ s$^{-1}$ (16 h light/8 h dark). The air temperature was set at 22°C during the day and 18°C during the night, the relative humidity was set at 65%. The nutrient solution contained, 4 mM N as Ca(NO$_3^\text{-}$)$_2$, or SO$_4$(NH$_4^\text{+}$)$_2$, 1 mM KH$_2$PO$_4$, 1 mM MgSO$_4$, 30 μM H$_2$BO$_3$, 50 μM Fe-EDTA, 10 μM MnSO$_4$, 1 μM CaSO$_4$, and 0.03 μM (NH$_4^\text{+}$)$_2$Mo$_7$O$_{24}$ (pH 5.7) in the NH$_4^\text{+}$ containing nutrient solution, Ca$^\text{2+}$ was supplied as Ca(SO$_4$)$_2$, 2 mM.

Leaf exchange measurements: Rate of CO$_2$ assimilation (μmol CO$_2$ m$^{-2}$ s$^{-1}$) and transpiration rate (mmol H$_2$O m$^{-2}$ s$^{-1}$) were measured by IRGA techniques (model LCA4, Analytical Development Co, Hodeston, UK). During analyses, plants were maintained in the corresponding culture conditions.

Analyses of Cd: Cd content in leaves and roots was analysed by digestion of dried plant material in HNO$_3$/HClO, mixture (3/2, v/v) and determined by atomic absorption spectrophotometry (Perkin Elmer Analyst 300).

Measurement of enzyme activities: The reaction of ODH activity was measured at 30°C in 100 mM Tris-HCl (pH8.2), containing 100 mM NH$_4$Cl, 10 mM 2-oxoglutarate, 160 μM NADH and 4 mM CaCl$_2$. The 2-oxoglutarate dependent oxidation of NADH was followed at 340 nm.

Phosphoenolpyruvate carboxylase (PEPC) activity was assayed spectrophotometrically, at 340 nm, in a final volume of 1 mL containing 100 mM HEPES-HCl, 10 mM MgCl$_2$, 5 mM NaHCO$_3$, 0.2 mM NADH at the optimal pH (8.0) and at 2 mM PEP. Assays were initiated by addition of the plant extracts. PEPC sensitivity to the inhibitor L-malate was measured as described by Echevarria et al. (1994) and expressed as IC_{50} values (L-malate concentration producing a 50% decrease in the initial activity of the enzyme) at pH 7.3. PEPC protein amounts in control and Cd-treated plants were measured by immunochromatographic techniques. Equivalence points for bean leaf PEPC contained in 2 mg dry material were obtained using a polyclonal antibody built against the Sorghum C$_4$ type PEPC.

RESULTS

Cd content: The data on Cd content in leaves and roots of treated-plants is given in Fig. 1. The Cd amount increased almost linearly with concentration of Cd in medium. It may however, be noted that the Cd content in leaves and roots were relatively higher in NO$_3^\text{-}$-fed plant (Fig. 1A) than in NH$_4^\text{+}$ medium (Fig. 1B). Thus in plants receiving 20 μM Cd for 7 day in NH$_4^\text{+}$ and NO$_3^\text{-}$ medium, accumulated 642 and 3335 μg of the metal in 1 g of dry matter, respectively. As shown in Fig. 1, most of the Cd absorbed by the plants was retained in roots. In both NO$_3^\text{-}$ and NH$_4^\text{+}$-fed plants, Cd accumulation was more important in the roots than in the shoots. The Cd content in the roots was linearly correlated to externally applied Cd concentrations (NH$_4^\text{+}$: r$^2$ = 0.997; NO$_3^\text{-}$: r$^2$ = 0.974).

Growth and rate of photosynthesis: Present results have shown that with absence of Cd dry matter production in both organs of plants grown on nutrient solution was lower with NH$_4^\text{+}$ (Fig. 2B) than NO$_3^\text{-}$ nutrition (Fig. 2A).

The higher shoot: root ratio of the NH$_4^\text{+}$-fed plants indicates the retarding effect of ammonium nutrition on root growth (Table 1). Exposure of seedlings to Cd showed no reduction in dry mass of ammonium-fed plants and only a slight reduction in dry mass at 20 μM Cd level (Fig. 2). The effect of Cd on the dry mass production of nitrate-fed plants was very marked even at a concentration of 2 μM and became increasingly so with increasing Cd concentration. Inspection of the shoot: root ratios recorded in Table 1 showed that regardless of the nitrogen source, the Cd effect on growth was more noticeable in the roots than in leaves. Fresh and dry weights of nitrate-fed plants (Fig. 3A) and ammonium-fed plants (Fig. 3B) varied in parallel, indicating that plant moisture content was not significantly modified by the nitrogen source (Table 1).
Fig. 1: Changes in cadmium (Cd) contents in the shoots (●) and roots (□) of bean seedlings, grown with NO$_3^-$ (A) or NH$_4^+$ (B) as N source after 7 days of exposure to different concentrations of Cd in nutrient solution. Values are means±SE of five individual plants. Standard errors are not shown when they are smaller than the symbol.

Fig. 2: Changes in dry weight of shoots (●) and roots (□) of bean plants grown with NO$_3^-$ (A) or NH$_4^+$ (B) as N source after 7 days of exposure to increasing concentration of Cd (0, 5, 10 and 20 μM). DW values are expressed as% of the control, with NO$_3^-$, 1309±97 mg DW of shoots and 238±16 mg DW of roots, with NH$_4^+$: 575±25 mg DW of shoots and 71.5±6.5 mg DW of roots. Values are means of six replicates expressed as percents of controls±SE of five individual plants. Standard errors are not shown when they are smaller than the symbol.

Table 1: Effect of nitrogen (N) form on the shoot to root Dry Weight (DW) and Fresh Weight (FW) ratios and moisture contents of shoots and roots of bean seedlings grown in solutions containing different concentrations of cadmium (0, 5, 10 and 20 μM).

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<th>CdCl$_2$ (μM)</th>
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Moisture contents are given as percent. Each value represents the mean of three experiments.

Net photosynthetic rate showed no significant response to Cd stress of ammonium-fed plants except at 20 μM Cd treatment (Fig. 4A). Whereas, nitrate-fed plants showed that in Cd treatments, the rate of photosynthesis diminished progressively with increasing concentrations of Cd. Thus in plants receiving 20 μM Cd, the rate of photosynthesis per plant was lowered by 95% after 7 day of treatment (Fig. 4A). A remarkable feature of the non-Cd-stressed plants in this experiment was that the photosynthetic rate was significantly lower in NH$_4^+$ compared to NO$_3^-$-fed plants.

As shown in Fig. 4B, changes in CO$_2$ assimilation induced by the addition of CdCl$_2$ in the culture medium was accompanied by a partial stomatal closure as
Fig. 3: Changes in fresh weight (FW) in the shoots (■) and roots (□) of bean seedlings grown with NO$_3^-$ (A) or NH$_4^+$ (B) as N source after 7 days of exposure to increasing concentration of Cd (0, 5, 10 and 20 μM). FW values are expressed as % of the control: with NO$_3^-$: 8006±797 mg FW of shoots and 2044±166 mg DW of roots, with NH$_4^+$: 5004±425 mg FW of shoots and 1004.5±66.5 mg FW of roots. Values are means of six replicates expressed as percents of controls±SE of five individual plants. Standard errors are not shown when they are smaller than the symbol.

Fig. 4: Changes in the rate of net photosynthetic CO$_2$ assimilation (A) and the rate of transpiration (B) in the leaves of bean seedlings grown with NO$_3^-$ (A) or NH$_4^+$ (B) as N source after 7 days of exposure to increasing concentration of Cd (0, 5, 10 and 20 μM).

Enzyme activities: The extractable activity of PEPC based on fresh weight from the first trifoliate leaves of NH$_4^+$ grown plants was higher than that of NO$_3^-$ grown plants (Fig. 5A). After 7 days of treatment with Cd, the activity of PEPC isolated from leaves of plants supplied with NH$_4^+$ remained constant in all the concentration of Cd. Whereas, in nitrate-fed plants, the PEPC activity increased by Cd stress; it remained at a same value for all Cd treatments (about 150% of the control, Fig. 5A). PEPC activity in extracts from control plants were again negatively affected by Cd concentrations in the incubation medium exceeding 0.1 Mm Cd (Fig. 5B).

To compare the protein PEPC content from Cd-stress bean leaves and controls of NO$_3^-$ grown plants, the maximal extractable protein PEPC were precipitated with (NH$_4$)$_2$SO$_4$ and were resuspended in buffer. Following this procedure, the net increase in protein PEPC content was still observed (Fig. 6A). The higher protein content in the leaves of Cd-stressed plants were found independent of Cd concentration (about 60%). This indicates that the observed differences in PEPC activities were not due to the presence of any activating substances.

To compare the malate sensitivity of PEPC from Cd-stressed leaves with controls, L-malate was added into the assay medium. The corresponding IC$_{50}$ values were estimated from the concentration of L-malate that determines a 50% decrease in the initial catalytic activity (Fig. 6B). The effect of malate concentration on the
Fig. 5: Effect of increasing concentrations of cadmium (0, 5, 10 and 20 \( \mu \text{M} \)) on the activity of phosphoenolpyruvate carboxylase \textit{in vivo} (A) from the shoots of bean seedlings supplied with \( \text{NO}_3^- \) or \( \text{NH}_4^+ \) treatment and \textit{in vitro} (B) from the shoots of bean seedlings supplied with \( \text{NO}_3^- \) treatment. Values shown are means of 3 replicates. Activity values are expressed as% of the control: with \( \text{NO}_3^- \), 6.08\( \pm \)1.25 \( \mu \text{mol min}^{-1} \text{g}^{-1} \text{DW} \) in shoots and 18.04\( \pm \)4.90 \( \mu \text{mol min}^{-1} \text{g}^{-1} \text{DW} \) roots, with \( \text{NH}_4^+ \): 3.12\( \pm \)0.25 \( \mu \text{mol min}^{-1} \text{g}^{-1} \text{DW} \) in shoots.

Fig. 6: Changes in protein amount and enzymatic properties of PEPC extracted from leaves of bean seedlings grown with \( \text{NO}_3^- \) (A–D) as N source, after a 7-day treatment by different \( \text{CdCl}_2 \) concentrations (0, 5, 10, and 20 \( \mu \text{M} \)). Amount of IgG (polyclonal antibodies directed against Sorghum PEPC) necessary to precipitate all PEPC contained in extract aliquots corresponding to 2 mg leaf dry weight (A). PEPC sensitivity to malate expressed as \( IC_{50} \) (L-malate concentration (mM) determining a 50% decrease in the initial activity to the enzyme, at pH 7.3) (B). PEPC responses to pH are expressed as the activity ratio pH 8/pH 7.3 (C) and pH 8/pH 7.1 (D).

Inhibition of PEPC activity in the leaves of Cd-stressed compared to controls plants is described in the Fig. 6. It is notable that increasing malate concentration increased the degree of malate inhibition of both PEPC. However, in Cd-stressed plants, the enzyme became less sensitive to the inhibition than the enzyme extracted from control plants.

Thus Cd stress at 5 \( \mu \text{M} \) can lead to a predominant change in the \( IC_{50} \) (approximately by 10 fold higher) (Fig. 6B). In addition, the extent of the \textit{in vivo} phosphorylation of PEPC was estimated by measuring \( IC_{50} \) and pH dependence activity. Cd treatments induced the decrease of PEPC sensitivity to the inhibitor malate in Cd-treated
In the absence of Cd, NADH-GDH activity was higher in roots and leaves of seedlings growing on ammonium medium than those in seedlings growing on nitrate medium (Table 2). After 7 days of treatment with 5 μM CdCl₂, GDH activity both in roots and leaves of plants supplied with different N form was significantly increased. Under NH₄⁺ supply, Cd stress stimulated the GDH activity (Fig. 7). NH₄⁺ content was more important to evaluate for the plant grown in NO₃⁻ medium. All external Cd concentrations caused an increase in the content of ammonium. Concomitant to these changes, an enhancement of protease activity was recorded in Cd-treated plants (Table 3).

**DISCUSSION**

Present results showed that dry and fresh weight productions were reduced by ammonium nutrition (Fig. 2 and 3). Furthermore, the partitioning of fixed carbon between shoot and root is affected by the N form: NH₄⁺ nutrition resulted in higher shoot: root ratio (Table 1). The roots are particularly sensitive to the form of nitrogen nutrition resulting in difference in the shoot: root ratio. The increase of the shoot: root ratio under NH₄⁺ supply in our experiments does illustrate the negative effects of NH₄⁺ on root growth. Many plant species showed growth depression when NH₄⁺ was the sole N form (Raab and Terry, 1994; Guo et al., 2002; Graaf et al., 2001; Paulissen et al., 2001). It is thought that the difference in the response of plant growth to NO₃⁻ assimilation is primarily foliar while nutrient NH₄⁺ assimilation is root based (Lasa et al., 2002). The energetic costs of NH₄⁺ absorption and assimilation in the root are lower (Bloom et al., 1996). However, detoxification of NH₄⁺ may diminish this energetic advantage, if NO₃⁻ is transiently stored and predominantly reduced in the shoot (Gerendas et al., 1997). Some authors such as Protocor (2000) and Limpens and Berendse (2003) have concluded that pH rather than ammonium ion was responsible for the effects observed during ammonium nutrition. It is well established that the uptake of nitrate ions is coupled to efflux of hydroxyl or bicarbonate ions, resulting in a higher pH to the medium surrounding the roots. Uptake of ammonium ions is also on active process, coupled to the efflux of protons that lowers the pH of the medium.

The response to elevated Cd in the rooting medium depends on the nitrogen form supply. The Cd concentrations in the different parts of the plants under various forms of N supply are given in Fig. 1. Present results showed that most of the Cd that has been taken up by plants remained accumulated in roots tissues. Roots thus function as a barrier than restricts the transport of plants. Cd-treatment also led to a shift of the pH-response curve of the leaf PEPC (activity ratio between optimal and sub-optimal pH 8.0/7.1) to a phosphorylated form. These properties were shown to be typical for acquisition of the phosphorylated state of the enzyme (Fig. 6C and 6D).
We found that plants grown in NH₄⁺ absorbed about five times less Cd than those supplied with NO₃⁻. In addition to severely reduced total Cd uptake, proportionally to the Cd absorbed, the roots of the plants with NH₄⁺ treatment retain more Cd than those with NO₃⁻ treatment. The Cd translocated to the shoot was more limited when NH₄⁺ was present in the nutrient medium than when the plants were grown with NO₃⁻ as N source. Nitrate-fed plants show a considerably greater sensitivity to Cd than to ammonium-fed plants (Fig. 2 and 3). Present results have shown that total dry matter and production in nitrate treated plants were reduced considerably by Cd, which obtained from NH₄⁺ treated plants which were only slightly affected by increasing concentration of Cd. The cause of this effect is not immediately apparent. Some studies have demonstrated that biototoxicity of Cd is determined by free ion concentration of the metal (Barcelo et al., 1998). Thus, we found that in the presence of NO₃⁻ there is an increase in Cd content and its toxic effect. The low metal concentration in NH₄⁺ cultures was a result from interactions between Cd²⁺ and NH₄⁺ or with protons excreted during active NH₄⁺ uptake for the cellular binding sites. Haynes (1980) indicated that high concentrations of H⁺ ions in the free space of roots could inhibit involvement of other cations within the free space. We can conclude that could explain the low effect of Cd on the biomass production of both roots and shoots of NH₄⁺ grown plants compared with NO₃⁻ nutrition. In addition, we found the more of Cd was accumulated in plant grown with NO₃⁻ than grown with NH₄⁺ which resulted in a decrease of growth and photosynthetic activities in plants grown with NO₃⁻ than grown with NH₄⁺.

The assimilation of the bulk of nutrient nitrogen in the roots of ammonium-fed plants necessitates the diversion of large quantities of carbon to the root to provide the carbon skeletons of the products of nitrogen assimilation. It is possible that this carbon metabolism is inhibited by the presence of high concentration of Cd ions. In order to identify the process affected by Cd, effects of this metal on CO₂ assimilation were examined. The results in Fig. 4A show that the lower photosynthetic capacity of bean plants under NH₄⁺ supply may promote the effects of competition between root extension and root based NH₄⁺ assimilation by providing sufficient carbohydrate to support both of these processes. The influence of Cd on CO₂ assimilation in the leaves indicate significant differences with the form of nitrogen. Rate of CO₂ assimilation was significantly affected by the Cd in leaf tissue of NO₃⁻ plants compared to NH₄⁺ plants (Fig. 4A). We did not find any modifications in CO₂ assimilation and transpiration rate under NH₄⁺ supply (Fig. 4A and B). We explain this effect by differences in stomatal closure (Costa et al., 1994) and subsequent decrease in transpiration water loss and inhibition of CO₂ fixation (Gouia et al., 2000). Earlier investigations have demonstrated a marked reduction in the overall rate of photosynthesis by Cd in different plant species (Chugh et al., 1999, Krupa et al., 1993). The deleterious effect on the rate of photosynthesis per unit leaf area and per g FW of plant under NO₃⁻ medium could be a consequence of an overall reduction in growth with concomitant decrease in total area. The effect of this metal on various facets of photosynthesis, have been also reported, such as biosynthesis of chlorophyll (Papazoglou et al., 2005), functioning of photochemical reactions (Chugh et al., 1999) and metabolic disturbances in photosystem I and II (Sandalio et al., 2001).

Most fast-growing plant reduce nitrate in their leaves where the main part of the reducing power arises directly from light via ferredoxin (Bevers and Hageman, 1980). Nitrate is reduced to NH₄⁺ by the plant enzymes nitrate reductase and nitrite reductase. Since NH₄⁺ is toxic (Britto et al., 2002), it must be rapidly assimilated into non toxic metabolites. It is converted to glutamine and glutamate by the enzymes glutamine synthetase (GS) and glutamate synthase (GOGAT) (Ireland and Lea, 1999). Even though the GS/GOGAT pathway is the major route in higher plants, the glutamate dehydrogenase (GDH), can also catalyse the reversible amination of 2-oxoglutarate to yield glutamate. In our study, GDH activity was higher in ammonium-fed plants compared to nitrate-fed plants in both organs (Fig. 7). The metabolic pathways of nitrogen and carbon are linked since nitrogen assimilation requires the provision of C skeletons by tricarboxylic acid cycle. Arozitis et al. (1988) and Chaffee et al. (2004) strongly supports a function of PEP carboxylase in providing carbon sources for nitrogen assimilation, our results show that PEPC is more efficient for providing carbon skeletons under ammonium nutrition than in nitrate nutrition (Fig. 5A). The increased PEPC activity might be attributed to a number of factors. First, a rise in cytoplasmic pH under Cd exposure may stimulate the PEPC activity (Sagi et al., 1998). Secondary, increased PEPC activity in presence of Cd may reflect the need of the plant for increased production of carbon skeletons due to high decrease of the photosynthetic activity, in order to regulate the cell ionic neutrality by synthesis of organic acids (Cramer and Lewis, 1993). However, when Cd is introduced directly in the incubating medium of the enzyme extracted from control plants, it has a depressive effect on PEPC activity (Fig. 5B).

Studies of the effects of Cd on N assimilation have frequently constitutes the rate-limiting step of N
assimilation catalysed by nitrate reductase (Gouia et al., 2000). In general, heavy metal toxicity is attributed to binding of heavy metal to enzymes, resulting in alteration and inhibition of metabolism Van Asse and Clijsters (1990). Enzymes of nitrogen metabolism have generally been shown to lose their activity to different extents during Cd stress (Chaffei et al., 2006b). The stimulation of GDH in response to Cd observed in bean plant grown with the two types of N source, has been noted equally by Boussama et al. (1999) Van Asse and Clijsters (1990) and Papazoglou et al. (2005). This stimulation of GDH activity under Cd stress resulted to the increase of GDH protein content and in induction of the transcription of GDH gene accompanied by an increase of ARNm content (Chaffei et al., 2006a). Thus the adjustment of N metabolism to Cd stress, occurred though a high increase in glutamate dehydrogenase, confirming the central role of this enzyme in the responses to changes in environmental conditions: a detoxification role for the recycling of the high ammonium content. The present study has shown that in Cd-stressed plants under NO$_3^-$ medium where was recorded an inhibition in both NR and Nitr activities (Singh, 1987; Gouia et al., 2000) strongly suggests that accumulated ammonium (Fig. 7) is produced through proteolysis, as was evidenced by the increase in protease activity (Table 3). NH$_4^+$ has to be assimilated at its site of production/uptake to prevent toxicity and there is little export of NH$_4^+$ from roots to shoots (Majerowicz et al., 2000).

Despite the fact that the effect of Cd on the activity of GDH shows a substantial rise in the roots of the NO$_3^-$-fed plants in comparison with those of NH$_4^+$ treatment, GDH is predominant in ammonium-fed plants. This suggested a possible relation-ship between GDH activity and Cd tolerance. PEPC activity was followed in order to assess that the Cd-induced changes contribute to provide C skeletal for effective functioning of GDH, which plays an important role in ammonium assimilation under stress conditions. Ammonium has been shown to induce expression of gene encoding the 2-oxoglutarate of GDH (Loulakakis and Roubelakis-Angelakis, 1992; Turano et al., 1997) and this induction occurred also at the level of GDH protein (Turano et al., 1997; Tercé-Lafargue et al., 2004; Chaffei et al., 2006b). The increase in PEPC activity in presence of Cd, proceeds not only from de novo synthesis of protein but also from changes in enzymatic properties related to phosphorylation process (Fig. 6). PEPC might provide 2-oxaloacetate to replace a lower availability of oxoglutarate whose requirement in respiratory cycle to produce ATP for transpiration increases under Cd stress, or to provide 2-oxoglutarate for the TCA cycle.

In conclusion, the present study shows that differences found in Cd stress effects between ammonium-fed plants and nitrate-fed ones seem to be related to differences in the capacity of the plant to assimilate ammonium. These results suggest that in plants under ammonium nutrition, the GDH activity contributes efficaciously to the ammonium detoxification. We suggest that the ammonium assimilating capacity of different plant organs plays a crucial role in Cd tolerance.

REFERENCES


