Journal of Experimental Botany, Vol. 53, No. 371, pp. 1177–1185, May 2002

Cadmium accumulation and buffering of cadmium-induced stress by arbuscular mycorrhiza in three *Pisum sativum* L. genotypes

Facundo Rivera-Becerril[1,5], Catherine Calantzis[2], Katarzyna Turnau[3], Jean-Pierre Caussanel[1], Andrei A. Belimov[4], Silvio Gianinazzi[1], Reto J. Strasser[2] and Vivienne Gianinazzi-Pearson[1,6]

[1] *UMR 1088 INRA/Université de Bourgogne, BBCE-IPM, INRA-CMSE, BP 86510 21065 Dijon Cédex, France*

[2] *Laboratoire de Bioénergétique, Université de Genève, 1254 Jussy-Lullier, Genève, Switzerland*

[3] *Institute of Botany, Jagiellonian University, ul. Lubicz 46, 31–512 Kraków, Poland*

[4] *All Russia Research Institute for Agricultural Microbiology, Podbelsky Shossee 3, St.-Petersburg-Pushkin 8, 189620 Russia*

[5] *Departamento El Hombre y su Ambiente, CBS, Universidad Autónoma Metropolitana-Xochimilco, Calz. del Hueso 1100, Col. Villa Quietud, 04960 México DF, México*

Received 10 September 2001; Accepted 19 December 2001

Abstract

The role of arbuscular mycorrhiza in reducing Cd stress was investigated in three genotypes of *Pisum sativum* L. (cv. Frisson, VIR4788, VIR7128), grown in soil/sand pot cultures in the presence and absence of 2–3 mg kg^{-1} bioavailable Cd, and inoculated or not with the arbuscular mycorrhizal fungus *Glomus intraradices*. Shoot, root and pod biomass were decreased by Cd in non-mycorrhizal plants. The presence of mycorrhiza attenuated the negative effect of Cd so that shoot biomass and activity of photosystem II, based on chlorophyll *a* fluorescence, were not significantly different between mycorrhizal plants growing in the presence or absence of the heavy metal (HM). Total P concentrations were not significantly different between mycorrhizal and non-mycorrhizal plants treated with Cd. From 20–50-fold more Cd accumulated in roots than in shoots of Cd-treated plants, and overall levels were comparable to other metal-accumulating plants. Genetic variability in Cd accumulation existed between the pea genotypes. Concentration of the HM was lowest in roots of VIR4788 and in pods of VIR4788 and VIR7128. *G. intraradices* inoculation decreased Cd accumulation in roots and pods of cv. Frisson, whilst high concentrations were maintained in roots and pods of mycorrhizal VIR7128. Shoot concentrations of Cd increased in mycorrhizal cv. Frisson and VIR4788. Sequestration of Cd in root cell walls and/or cytoplasm, measured by EDS/SEM, was comparable between non-mycorrhizal pea genotypes but considerably decreased in mycorrhizal cv. Frisson and VIR7128. Possible mechanisms for mycorrhiza buffering of Cd-induced stress in the pea genotypes are discussed.

Key words: Cadmium, *Glomus intraradices*, pea genotypes, phytoremediation.

Introduction

Cadmium (Cd), which is termed a heavy metal (HM) or a metal trace element, is dispersed in natural and agricultural environments principally through human activities such as mining, refining, municipal waste incinerators, and fossil fuel combustion sources (Wagner, 1993), as well as natural rock mineralization processes (Sanità di Toppi

[6] To whom correspondence should be addressed. Fax: +33 3 80 69 37 53. E-mail: gianina@epoisses.inra.fr

Abbreviations: AM, arbuscular mycorrhiza; ANOVA, analysis of variance; Chl, chlorophyll; DF, driving force; EDS, energy dispersion spectrometer; HM, heavy metal; PI, performance index; PDW, pod dry weight; RDW, root dry weight; SDW, shoot dry weight; SEM, scanning electron microscope.

© Society for Experimental Biology 2002

and Gabrielli, 1999). Major inputs of Cd into agricultural soils are due to the application of phosphatic fertilizers (Williams and David, 1976; McLaughlin *et al.*, 2000), soil amendments with municipal sewage sludges and atmospheric deposition (Wagner, 1993; Weissenhorn and Leyval, 1995).

Cd is relatively mobile in plants where it can influence mineral nutrition (Siedlecka *et al.*, 1997), and symptoms of toxicity are leaf chlorosis, leaf and root necrosis, and a general decrease in growth and tissue-size (Hernandez and Cooke, 1997). The main basis of Cd toxicity in biological systems lies in its strong affinity for SH-containing ligands, particularly polythiols, and it is considered principally to target zinc metalloenzymes, membrane phospholipids and oxidative phosphorylation (Wagner, 1993), so causing impairment of cell respiration, inhibition of enzyme activities, protein denaturation (Das *et al.*, 1997; Hernandez and Cooke, 1997), or disruption of cell transport processes (Williams *et al.*, 2000). In plants, Cd is particularly damaging to the photosynthetic apparatus. Although inhibition of Rubisco activity in the Calvin cycle is considered as a primary plant response to Cd stress (Siedlecka *et al.*, 1997), photosystems I and II are also affected (Krupa *et al.*, 1993; Siedlecka and Baszynsky, 1993; Siedlecka and Krupa, 1996), and levels of total chlorophyll (Chl) and carotenoid can be lowered. In addition, Cd has been demonstrated to increase non-photochemical quenching in *Brassica napus* (Larsson *et al.*, 1998).

Tolerance of plants to HM is genetically determined (Sanità di Toppi and Gabrielli, 1999) so that identification of plant genotypes differing in resistance to HM is a promising approach, not only for studying mechanisms protecting plants against toxic metals but also for the selection of those adapted to production in HM-contaminated soils. Pea (*Pisum sativum* L.), one of the most important legume crops in Europe, is an ideal candidate for restoring and maintaining soil fertility in HM-polluted agroecosystems. This crop plant shows genetic diversity *vis-à-vis* HM tolerance (Belimov *et al.*, 1999) and it establishes a symbiotic root association with P-scavenging arbuscular mycorrhizal (AM) fungi which has been reported to improve resistance to HM in other plants. Suggested mechanisms for the latter include enhanced nutrient supply or decreased water stress by the AM fungi (Meharg and Cairney, 2000), metal sequestration through the production of binding substances or bioaccumulation in microbial cells (Leyval *et al.*, 1997).

The aim of the present work was to evaluate how root colonization by the AM fungus *Glomus intraradices* modifies the effect of Cd-induced stress on three genotypes of pea of different geographical origin. Plant growth, photosynthesis, and P and Cd accumulation were measured in order to evaluate possible mechanisms of Cd effects.

Materials and methods

Plants and growth conditions

Seeds of *Pisum sativum* L. cv. Frisson (provided by G Duc, Laboratoire des Legumineuses, INRA, Dijon), and the genotypes VIR4788 (from Mongolia) and VIR7128 (from Daghestan) (World Collection, Vavilov Institute of Plant Industry, St Petersburg) were surface-disinfected (10 min 3.5% Ca hypochlorite, 10 min 96° ethanol). Prior to disinfection, VIR4788 and VIR7128 seeds were also treated for 15 min with 98% H_2SO_4 to scarify the seed coat. Seeds were incubated overnight at room temperature in sterile water, in the dark, and then germinated in sterile vermiculite (16 h photoperiod, 20–24 °C). Five-day-old seedlings were individually transplanted into 400 g of a 1 : 1 (v/v) soil : sand mix as growth substrate. Soil (clay loam, pH 8.1 (H_2O), 16.6 g C kg^{-1}, 1.8 g N kg^{-1}, 26 mg Olsen P kg^{-1}, 0.9 µg Cd kg^{-1}) was γ-irradiated (10 kGy) and heat sterilized for 4 h at 180 °C. Sand (Special Aquarium, Quartz, Nr. 3, Zolux, France) was washed and autoclaved three times for 1 h at 121 °C.

The experimental treatments (five replicates each) consisted of (i) added Cd, (ii) inoculation with *Glomus intraradices* Schenck & Smith (BEG 141), (iii) *G. intraradices*+Cd, and (iv) control plants (no inoculation with *G. intraradices*, no added Cd). Ground $CdCl_2 \cdot 2.5H_2O$ was added to the soil : sand substrate to obtain 100 mg Cd kg^{-1} and the mix was humidified for 12 h before planting. Because of the high soil pH, the amount of bioavailable Cd after mixing was measured using a $CaCl_2$ extraction method (analysis performed by INRA-Arras, France). This gave a final concentration of 2–3 mg bioavailable Cd kg^{-1}, which is comparable to levels of the HM reported in polluted soils (Baker, 1987; Haag-Kerwer *et al.*, 1999). In the case of plants inoculated with *G. intraradices*, all the soil in the substrate mix was replaced by inoculum (10-week-old mycorrhizal onion roots, spores and hyphae in the same soil). Non-inoculated plants received filtered (Whatman No. 2) washings of the inoculum to reconstitute the associated soil microflora without *G. intraradices*. Pots were placed in a random design in a growth chamber (20–24 °C night/day, 16 h photoperiod, 330 µmol m^{-2} s^{-1}, 70% relative humidity). Plants received 20 ml of a modified Long Ashton solution (no phosphate, 2-fold nitrate to compensate for the lack of *Rhizobium*) (Dumas-Gaudot *et al.*, 1994) three times a week and Milli-Q water on other days.

In a first experiment, plants were harvested after 6 weeks' growth and root systems were thoroughly washed in running tap water, then deionized water. Root samples were taken for mycorrhiza and scanning electron microscopy analyses. Shoots and roots were weighed fresh and after drying at 80 °C for 72 h. Dried shoots and roots were ground and analysed for P content (Duval, 1963), and for Cd content by a $CaCl_2$ extraction method and electrothermal atomic absorption spectrometry (Lebourg *et al.*, 1996) by INRA-Arras. A root sample was stained with trypan blue for the determination of root colonization by *G. intraradices*, following the method described previously (Trouvelot *et al.*, 1986). The parameters considered were frequency of colonized root fragments (F%), extent of cortex colonization in the whole root system (M%), and abundance of arbuscules (A%), and vesicles (V%) in the whole root system.

In a second experiment, plants were grown to maturity (18 weeks' growth). Pods were harvested, dried at 80 °C for 72 h, weighed then ground and analysed for Cd-content as described above.

Chlorophyll *a* fluorescence measurements

Chlorophyll *a* fluorescence induction kinetics were measured at growth room temperature in 6-week-old plants from the first experiment by a fast and non-destructive screening method. An intact pea leaflet was directly attached to the head of a Plant Efficiency Analyser (King's Lynn, Norfolk, UK) with the help of a leaf clip and plants were dark-adapted for 1 h before measurement. Light was then provided by an array of six light-emitted diodes (peak 650 nm) focused on the sample surface to provide direct illumination over the exposed area (about 5 mm in diameter). The light intensity was 600 W m^{-2}. Chl *a* fluorescence signals were detected using a PIN (positive-intrinsic-negative) photocell after passing through a long-pass filter (50% transmission at 720 nm). Chl *a* fluorescence signals were recorded up to 1 s, with an acquisition rate of 10 μs for the first 2 ms and of 1 ms thereafter, and with a 12 bits resolution (Strasser *et al.*, 1995). The first reliable point, measured 50 μs after the onset of illumination, was taken as F_o. Time zero was within 5% of this value. Ten to 15 measurements were made per plant. The activity of photosystem II was evaluated using the JIP-test based on the Chl *a* Polyphasic Fluorescence Transient O-J-I-P (Strasser *et al.*, 1995). A performance index (PI) based on the JIP-test was calculated as described earlier (Strasser *et al.*, 2000). The PI combines three parameters favourable to photosynthetic activity: (1) the density of reaction centres (expressed on an absorption basis), (2) the quantum yield of primary photochemistry, and (3) the ability to feed electrons into the electron chain between photosystem II and photosystem I (Strasser *et al.*, 1999).

Ultrastructural analyses of Cd accumulation

Root samples taken at 6 weeks from each treatment and pea genotype were frozen in isopentane and lyophilized. Cross-sections of 0.5 mm were hand cut, mounted on carbon stubs and covered with a carbon layer as described previously (Turnau, 1998). Because tissues were lyophilized it was not possible to discriminate between cell walls and cytoplasm in the root material. Cd concentration was therefore analysed in cell wall/cytoplasm regions of the exodermis, cortex and vascular tissue using a scanning electron microscope (SEM) (Analiser Noran Instruments, JEOL-JSM-5410) coupled to an energy dispersion spectrometer (EDS) at 20 kV, 100 s, with a silicon/lithium detector (Noran). Net counts obtained for Cd were transformed to μmol Cd g^{-1} root dry weight using an EDS standard prepared by dissolving 0.066 g of $Cd(NO_3)_2$ in 5 ml double-distilled water, and different dilutions (1 : 2, 1 : 4, 1 : 8) of this solution were combined with gelatin (Sigma, G-1890) to a final concentration of 20%, giving a Cd concentration in the undiluted sample equivalent to 469 mmol Cd kg^{-1} dry weight. The different dilutions were frozen in isopentane, lyophilized, coated with carbon and analysed. A calibration curve was plotted of average net counts from SEM/EDS against Cd concentration.

Statistical analyses

Data were subjected to three-way ANOVA with Cd treatment, mycorrhizal status and genotype as independent factors. The Neuman–Keuls test ($P \leq 0.05$) was used to evaluate differences between treatments and interactions means, using the SAS statistical software package (SAS Institute, 1986). Percentage data and Cd concentration data were arcsin and square root transformed, respectively, prior to analysis.

Results

Mycorrhiza development

Neither mycorrhiza nor nodules were observed in root systems of non-inoculated pea plants. Roots of *G. intraradices*-inoculated pea genotypes were colonized by the AM fungus but not nodulated. All stages of arbuscular mycorrhiza (appressoria, intercellular hyphae, arbuscules, and vesicles) were present at 6 weeks after inoculation. The intensity of root cortex colonization was not significantly different between cv. Frisson ($M\%=50$), VIR4788 ($M\%=39$) and VIR7128 ($M\%=39$) in the absence of Cd amendment. The addition of Cd to the growth substrate had no significant effect on root cortex colonization, arbuscule or vesicle formation in cv. Frisson ($M\%=41$, $A\%=29$, $V\%=31$), VIR4788 ($M\%=30$, $A\%=23$, $V\%=24$) and VIR7128 ($M\%=52$, $A\%=38$, $V\%=39$).

Analysis of variance (ANOVA)

Genotype, Cd and mycorrhiza effects were significant ($P \leq 0.01$) for shoot (SDW), root (RDW) and pod dry weight (PDW) (Table A of the Supplementary data, which can be found at JXB online) indicating an intrinsic genetic variability between the three pea genotypes as well as variable responses to Cd-induced stress and mycorrhiza. Genotype×Cd interactions were detected for RDW and PDW confirming different responses of the pea genotypes to Cd-induced stress. Genotype×mycorrhiza interactions were observed for SDW and PDW and Cd×mycorrhiza interactions existed for all the measured growth parameters.

Genotype effects were also significant for Cd concentrations in roots and pods, and Cd effects were significant for shoots, roots and pods ($P \leq 0.01$). Genotype×Cd interactions existed for Cd concentrations in shoots, roots and pods, again indicating different responses of the pea genotypes *vis-à-vis* Cd-induced stress. Significant ($P \leq 0.01$) genotype×mycorrhiza, Cd×mycorrhiza, and genotype×Cd×mycorrhiza interactions were only detected for shoot Cd concentrations.

Plant growth

As seen in the ANOVA analysis, growth of the three pea genotypes varied somewhat, and flowers and pods began to develop after 6 weeks in cv. Frisson and VIR7128, but later in VIR4788. Plants treated with Cd showed root necrosis and some chlorosis but anthocyanin production, a typical stress response in Cd-sensitive plants, was only observed on leaflets of non-mycorrhizal VIR4788.

Shoot and root growth was significantly decreased by the addition of Cd in all non-mycorrhizal pea plants (Table 1). Reduction in SDW was greatest in non-mycorrhizal VIR7128 (−54.8%) and least in cv. Frisson (−32.7%) whilst RDW was most reduced in non-mycorrhizal VIR4788 (−52.4%) and least in cv. Frisson (−44.3%). By contrast, Cd stress did not significantly affect SDW of any of the *G. intraradices*-colonized pea genotypes compared to mycorrhizal plants growing without Cd (Table 1). The SDW of VIR7128 was significantly enhanced in mycorrhizal plants growing in the Cd-amended substrate as compared to non-mycorrhizal ones. RDW was not significantly reduced by the HM treatment in mycorrhizal cv. Frisson, and in VIR4788 it remained higher in mycorrhizal than in non-mycorrhizal Cd-stressed plants (Table 1). Roots became necrotic and fragile in all cases. The presence of mycorrhiza attenuated the overall negative effect of Cd, so that shoot and root growth losses in the three pea genotypes were significantly ($P \leq 0.05$) less in mycorrhizal than in non-mycorrhizal plants (Fig. A of the Supplementary data, which can be found at JXB online).

Pod yield

The cv. Frisson genotype had consistently higher pod yields when inoculated with *G. intraradices*, whether plants were grown in the presence or the absence of added Cd (Table 1). This mycorrhizal effect on pod yield was also significant for VIR7128 in the absence of added Cd, but no differences were observed between Cd-stressed mycorrhizal and non-mycorrhizal plants. Inoculation with *G. intraradices* had little influence on the pod yield of VIR4788. Seed production in the absence of added Cd was significantly ($P \leq 0.05$) increased by 85% (2.4 g plant^{-1}) and 46% (1.9 g plant^{-1}) in mycorrhizal cv. Frisson and VIR7128, respectively, as compared to non-mycorrhizal plants, but neither genotype produced seeds under the Cd-stress conditions. VIR4788 seed production was unaffected by *G. intraradices* inoculation and decreased from 0.9 to 0.2 g per plant in the presence of Cd.

Table 1. *Biomass (g dry weight) of pea genotypes inoculated (M) or not (NM) with* Glomus intraradices *and grown in the absence or presence (Cd) of 100 mg kg^{-1} cadmium*

Data are the means of five repetitions. Means with the same letter are not significantly different ($P \leq 0.05$) for shoot, root or pod data.

Genotype	M	NM	M+Cd	NM+Cd
Shoots (6-week-old plants)				
cv. Frisson	0.97 abc	1.01 abc	0.83 bcd	0.68 de
VIR4788	0.74 cd	0.78 bcd	0.57 ed	0.47 e
VIR7128	1.17 a	1.04 ab	0.94 abc	0.47 e
Roots (6-week-old plants)				
cv. Frisson	0.57 b	0.61 b	0.46 bc	0.34 cd
VIR4788	0.88 a	0.82 a	0.55 b	0.39 cd
VIR7128	0.57 b	0.55 b	0.37 cd	0.29 d
Pods (18-week-old plants)				
cv. Frisson	3.1 a	1.9 c	1.4 d	0.8 f
VIR4788	1.3 de	1.1 e	0.7 f	0.5 f
VIR7128	2.4 b	1.8 c	0.8 f	0.7 f

Photosynthetic activity

In the absence of added Cd, PI values for photosystem II were significantly higher in mycorrhizal than non-mycorrhizal plants of cv. Frisson (Table 2), showing that the AM symbiosis can promote higher photosynthetic activity. Treatment with Cd decreased PI values to a greater extent in non-mycorrhizal (−62.1 to −76.6%) than in the mycorrhizal plants (−15.8 to −31.9%) of the three genotypes (Fig. A of the Supplementary Information). PI values were not significantly affected by Cd stress in plants colonized by *G. intraradices*, illustrating again the effect of AM in attenuating Cd-induced stress in *P. sativum* (Table 2).

P accumulation in pea tissues

P concentrations and total P uptake were significantly higher in mycorrhizal than in non-mycorrhizal plants growing in the absence of added Cd (Table 3), showing a clear positive mycorrhizal effect on P accumulation. Such an effect was less evident in the presence of Cd where only total P uptake was greater in mycorrhizal than

Table 2. *Performance index (PI) values for photosynthesis in 6-week-old pea genotypes inoculated (M) or not (NM) with* Glomus intraradices, *and grown in the absence or presence (Cd) of 100 mg kg^{-1}cadmium*

Data are the means of five repetitions. Means with the same letter are not significantly different ($P \leq 0.05$).

Genotype	M	NM	M+Cd	NM+Cd
cv. Frisson	57.6 a	45.3 b	48.8 ab	10.6 de
VIR4788	31.9 c	22.5 cd	22.3 cd	5.8 e
VIR7128	23.2 cd	18.2 de	15.8 de	6.9 e

Table 3. *Phosphorus concentrations (µg g^{-1}dry weight) and total phosphorus uptake (µg plant^{-1}) by 6-week-old pea genotypes after inoculation (M) or not (NM) with* Glomus intraradices *and grown in the absence or presence (Cd) of 100 mg kg^{-1} cadmium*

Data for concentrations and total uptake are the means of three repetitions. Means for concentrations or total uptake values with the same letter are not significantly different ($P \leq 0.05$).

Genotype	M	NM	M+Cd	NM+Cd
Phosphorus concentrations				
cv. Frisson	0.36 a	0.18 cd	0.26 b	0.23 bc
VIR4788	0.30 b	0.17 cd	0.24 bc	0.19 cd
VIR7128	0.24 bc	0.16 d	0.24 bc	0.17 cd
Total phosphorus uptake				
cv. Frisson	0.80 a	0.43 b	0.49 b	0.25 c
VIR4788	0.71 a	0.44 b	0.46 b	0.23 c
VIR7128	0.77 a	0.41 b	0.40 b	0.27 c

in non-mycorrhizal plants, due to the higher biomass of the former.

Cd accumulation in pea tissues

Very low levels of Cd accumulated in roots and shoots of plants growing in the absence of added Cd, and tissue concentrations of the metal tended to decrease in mycorrhizal plants (Table 4). Addition of Cd to the growth substrate greatly increased uptake of the HM by the three pea genotypes, and root concentrations were from 20–50 times more than in shoot tissues (Table 4). VIR7128 had the highest concentration of Cd in roots, with more than 1100 μg g^{-1} RDW, and VIR4788 the lowest concentrations with below 700 μg g^{-1} RDW. Under Cd-stressed conditions, the presence of mycorrhiza significantly increased the concentration of Cd in shoots of cv. Frisson and VIR4788, and decreased root concentrations in cv. Frisson.

Root colonization by *G. intraradices* significantly increased total Cd uptake into shoots of all three pea genotypes (Table 5). In the absence of a Cd amendment, transfer to shoots of the HM was significantly reduced by *G. intraradices* root colonization in cv. Frisson. When plants grew in the Cd-amended substrate, the large majority of absorbed Cd (94.2–96.7%) was retained in the root systems. Under these conditions, mycorrhiza development had little effect on Cd transfer to the shoots.

Very little Cd was detected in pods of pea plants which had grown in soil without the addition of the HM (Tables 4, 5). Cd accumulation increased greatly in pods in the presence of Cd, but to different extents in the pea genotypes. Levels were significantly higher in pods of cv. Frisson. Cd concentrations decreased considerably in pods of mycorrhizal plants of both cv. Frisson and VIR4788 (Table 4).

Table 4. *Cadmium concentrations (μg g^{-1} dry weight) in tissues of pea genotypes after inoculation (M) or not (NM) with* Glomus intraradices *and grown in the absence or presence (Cd) of 100 mg kg^{-1} cadmium*

Data for shoots and roots are the means of five repetitions, and for pods three. Means for shoot, root or pod values with the same letter are not significantly different ($P \leq 0.05$).

Genotype	M	NM	M + Cd	NM + Cd
Shoots (6-week-old plants)				
cv. Frisson	0.21 f	3.54 d	26.88 b	20.04 c
VIR4788	0.16 f	0.69 e	32.56 a	23.18 b
VIR7128	0.20 f	0.59 ef	26.8 b	24.36 b
Roots (6-week-old plants)				
cv. Frisson	1.14 d	3.58 d	870.2 b	1027.2 a
VIR4788	0.81 d	2.37 d	695.6 c	646.6 c
VIR7128	1.23 d	4.28 d	1112.6 a	1153.6 a
Pods (18-week-old plants)				
cv. Frisson	0.03 d	0.05 d	30.65 b	58.10 a
VIR4788	0.04 d	0.13 d	9.79 c	18.67 bc
VIR7128	0.03 d	0.14 d	22.01 bc	21.21 bc

Cd concentrations measured by SEM/EDS in cell wall/cytoplasm regions did not differ significantly between the cell types considered, and values were grouped together for all root tissues. Analyses confirmed the significantly greater accumulation of the HM in the Cd-treated pea genotypes (12.2–27.0 μmol g^{-1} RDW) as compared to non-treated plants (0.6–2.8 μmol g^{-1} RDW). In Cd-treated plants, Cd concentrations were comparable in the root cell wall/cytoplasm regions of non-mycorrhizal plants of cv. Frisson, VIR4788 and VIR7128 (19.2–22.1 μmol g^{-1} RDW) and according to ANOVA, mycorrhiza development did not significantly affect this localized Cd accumulation in the pea root systems. However, when data for Cd-treated mycorrhizal and non-mycorrhizal plants were compared separately for each genotype, there was a significant ($P \leq 0.05$) decrease in Cd concentration in the cell wall/cytoplasm regions for mycorrhizal roots (13.2 μmol g^{-1} RDW) of cv. Frisson as compared to non-mycorrhizal roots (22.1 μmol g^{-1} RDW).

Discussion

Mycorrhiza development

The fact that the intraradical development of *G. intraradices* BEG141 was not particularly affected by Cd, even though this isolate had not previously been exposed to the HM, confirms the previously observed HM tolerance of this fungus (Jacquot *et al.*, 2000). This contrasts with reports that AM fungi isolated from

Table 5. *Total cadmium uptake (μg $plant^{-1}$) by pea genotypes after inoculation (M) or not (NM) with* Glomus intraradices, *and grown in the absence or presence (Cd) of 100 mg kg^{-1} cadmium*

Data for shoots, roots, or transfer to shoots are the means of five repetitions, and for pods three. Means for shoot, root, transfer to shoot or pod values with the same letter are not significantly different ($P \leq 0.05$).

Genotype	M	NM	M + Cd	NM + Cd
Shoots (6-week-old plants)				
cv. Frisson	0.20 e	3.55 d	22.24 ab	13.67 c
VIR4788	0.11 e	0.54 e	18.50 b	11.03 c
VIR7128	0.23 e	0.63 e	25.40 a	12.43 c
Roots (6-week-old plants)				
cv. Frisson	0.65 c	2.18 c	396.1 a	355.8 a
VIR4788	0.71 c	1.93 c	387.6 a	256.2 b
VIR7128	0.70 c	2.30 c	415.7 a	344.1 a
Transfer to shoots (%) (6-week-old plants)				
cv. Frisson	23.7 b	59.1 a	5.3 d	3.7 d
VIR4788	14.4 c	21.7 bc	4.6 d	4.2 d
VIR7128	24.4 b	21.3 bc	5.8 d	3.3 d
Pods (18-week-old plants)				
cv. Frisson	0.09 c	0.11 c	47.22 a	32.39 a
VIR4788	0.05 c	0.13 c	7.76 bc	8.57 bc
VIR7128	0.08 c	0.24 c	18.43 b	14.41 b

non-polluted soils show reduced root colonization levels in the presence of HM, as compared to isolates from polluted soils (Gildon and Tinker, 1983; Leyval *et al.*, 1997).

Plant growth and photosynthetic activity

When the non-mycorrhizal pea genotypes cv. Frisson, VIR4788 and VIR7128 were treated with Cd, they showed stunted growth and chlorosis which are general symptoms of Cd toxicity (Das *et al.*, 1997; Chugh and Sawhney, 1999). The Cd-induced anthocyanin pigmentation on leaflets of VIR4788 has also been reported in pea plants subjected to low temperature stress (Christie *et al.*, 1994; Hasegawa *et al.*, 2001) and it suggests a greater sensitivity of this pea genotype to the HM. Another symptom of Cd stress in the treated pea plants was root necrosis, which is similar to that associated with Cd accumulation in maize seedlings (Khan *et al.*, 1984). The negative impact of Cd on shoot and root development was clearly attenuated by the presence of mycorrhiza in all three pea genotypes, and this buffering effect of AM *vis-à-vis* Cd-induced stress was also seen in the PI of photosystem II of leaves which was less affected by Cd treatment in mycorrhizal plants as compared to non-mycorrhizal ones (Fig. A of the Supplementary Information). In addition, the presence of mycorrhiza had consistently positive effects on plant growth and PI when the three pea genotypes were treated with Cd (Fig. B of the Supplementary data, which can be found at JXB online).

Another parameter of photosynthetic activity, called the driving force (DF), can be defined as $DF = \log PI$ in analogy to the Nernst-equation for redox reactions. The DF represents the extent to which absorbed light energy is conserved in photosynthetic electron transport. The DF showed a clear sigmoidal relation to RDW (Fig. 1). In a soil without Cd stress, there is virtually no effect of mycorrhiza on the RDW/DF relationship (Fig. 1C). By contrast, RDW and DF decrease under Cd stress in non-mycorrhizal plants (Fig. 1A), but in mycorrhizal plants this negative effect is attenuated (Fig. 1B), and there is a strong positive mycorrhizal effect on the relationship between the two parameters in the presence of Cd (Fig. 1D).

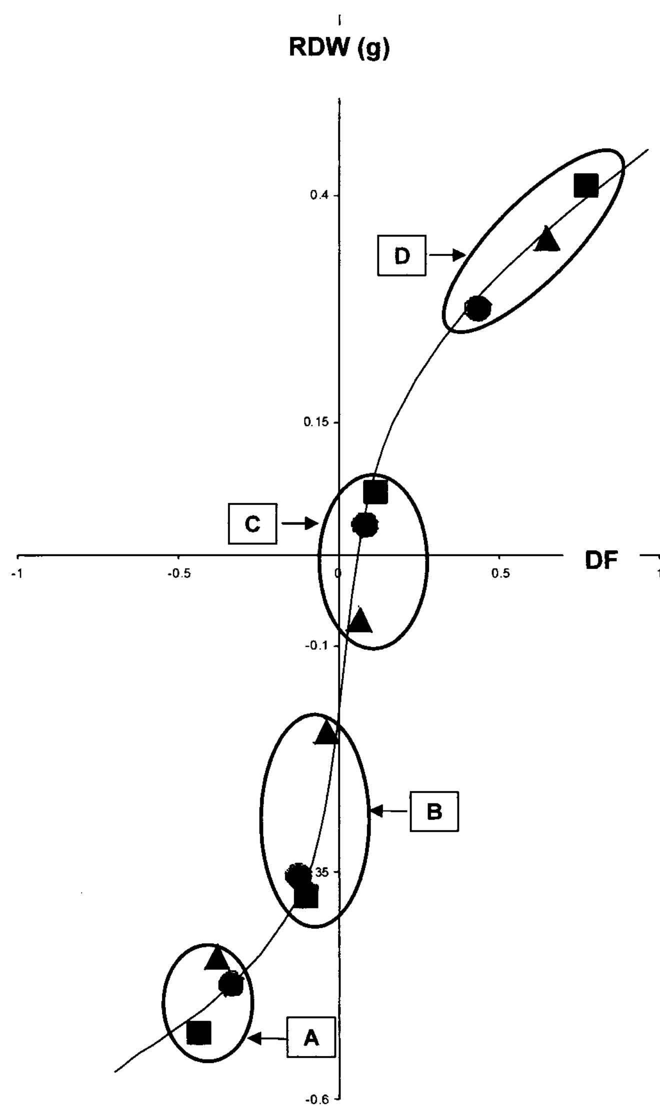

Fig. 1. (A, B) Effect of Cd on the relationship between the fluorescence signals of leaflets, expressed as the driving force (DF, *x*-axis), and root dry weight (RDW, *y*-axis) in non-mycorrhizal (A) and mycorrhizal (B) 6-week-old pea genotypes treated with 100 mg kg^{-1} cadmium, expressed as values of non-treated plants. (C, D) Effect of mycorrhiza on the relationship between DF and RDW in 6-week-old pea genotypes grown in the absence (C) and presence (D) of 100 mg kg^{-1} cadmium, expressed as values of non-mycorrhizal plants. Pea genotypes: cv. Frisson (▲); VIR4788 (■); VIR7128 (●).

Cd has been reported to have a direct effect on the structure, composition and functioning of photosystem II domains in the thylakoid membranes of other plants (Becerril *et al.*, 1988), and it has been suggested that photosystem II is more sensitive to Cd than photosystem I in pea (Chugh and Sawhney, 1999). In studies on another legume, *Phaseolus vulgaris*, it was postulated that Cd toxicity provokes a reduced demand for ATP and NADPH within the Calvin cycle, the inhibition of which causes a down-regulation of photosystem II and decreased linear electron transport (Krupa *et al.*, 1993). Since AM frequently enhance P acquisition by plant roots (Smith and Read, 1997), one mechanism for the compensatory effect on photosynthetic capacities in the mycorrhizal pea plants growing under Cd-stress may be through an improved P nutrition. This, however, does not appear to be a main cause of the attenuated effect of Cd in mycorrhizal plants since P concentrations in tissues were comparable to those of non-mycorrhizal plants growing under the same Cd-stress conditions.

Cd accumulation in pea tissues

The present observations of Cd accumulation in Cd-treated plants indicate that levels can attain 20–32 μg g^{-1} dry weight in shoots of pea, which are in the range of those reported to accumulate in tobacco shoots (Wagner, 1993). Cd accumulation in pods of the Cd-treated pea genotypes reached similar or higher levels than those previously reported earlier (Paul *et al.*, 1991) for seeds of pea growing in a soil with a lower Cd content than that of the substrate used in the present work. The values for Cd in shoots and pods, together with the high amounts of Cd in the roots of all three pea genotypes, make *P. sativum* a relatively high metal-accumulating plant within the classification proposed previously (Reeves and Baker, 2000) and, consequently, a possible candidate for vegetation and phytoextraction of contaminated soils.

Roots of several plant species have been shown to act as a barrier in restricting the transport of Cd to shoots, a behaviour which is considered to be one of several strategies of tolerance to the pollutant (Lozano-Rodríguez *et al.*, 1997). The high quantities of Cd retained in the roots of the three genotypes of *P. sativum* agree with results obtained previously in pea (Leita *et al.*, 1992, 1993; Lozano-Rodríguez *et al.*, 1997), bean plants (Weigel and Jäger, 1980) and maize seedlings (Rauser, 2000). The higher accumulation of Cd in roots and/or pods of cv. Frisson and VIR7128 together with the lack of anthocyanin toxicity symptoms in these two genotypes, as compared to VIR4788, suggest that cv. Frisson and VIR7128 are more tolerant to the HM than VIR4788, confirming the existence of genetic variability in Cd tolerance in pea (Belimov *et al.*, 1999).

Conclusions

This is the first report that AM development attenuates the toxic effect of Cd in pea, and that genetic variability can exist within one plant species in the extent of the protective effect. The role of AM in Cd uptake can differ between plant species, depending on the level of the HM present in soils. Contrary to results from the present study on pea, enhanced foliar concentrations of Cd have been reported in AM clover or soybean grown in soils low in Cd, whilst AM decreased or did not affect foliar concentrations of plants in soils which contain high amounts of the HM (Gildon and Tinker, 1983; Heggo *et al.*, 1990; Tonin *et al.*, 2001). Different mechanisms have been proposed to explain the protective effect of AM *vis-à-vis* HM stress (Leyval *et al.*, 1997). As mentioned above, enhanced nutrient supply by the symbiotic fungi to the host plant may lessen the effect of such stress on plant physiology (Meharg and Cairney, 2000). Alternatively, production of metal-binding molecules by the fungi may lead to HM sequestration within the microbial cells (Joner *et al.*, 2000) so that there is reduced transfer to root cells. It has been shown that extraradical hyphae can transport Cd from soil to roots, but that transfer of the HM from fungal to plant tissues is limited (Joner and Leyval, 1997). It seems unlikely that this mechanism was active in the pea genotypes colonized by *G. intraradices* since Cd uptake into tissues was similar or greater in mycorrhizal than non-mycorrhizal plants.

Metal-binding to cell walls is one proposed strategy whereby plants may reduce HM concentrations to physiologically compatible levels in tissues. Cd was reported to be bound to the cell wall fraction in pea, although a far greater proportion (>70%) was localized in the cytoplasmic fraction (Weigel and Jäger, 1980). In the present study, the pea genotypes did sequester high amounts of Cd in cell wall/adjacent cytoplasm regions of their roots. However, quantities were not increased by mycorrhiza formation and values even decreased in tissues of cv. Frisson and VIR7128. Another mechanism of HM tolerance in plants is based on detoxification and sequestration into the cell vacuole by molecules like metallothioneins and phytochelatins (Cobbett, 2000; Cobbett and Goldsbrough, 2000). Ongoing research is presently aimed at evaluating the role of this molecular pathway of HM sequestration in the alleviation or buffering of Cd stress by arbuscular mycorrhiza and in Cd tolerance of pea genotypes.

Acknowledgements

We would like to thank G Duc (Unité de Génétique et d'Amélioration des Plantes, INRA-Dijon, France) for providing seeds of cv. Frisson, and to V Monfort for inoculum production of *G. intraradices* (BEG 141). The SEM and EDS equipment used was donated by the Foundation for Polish Science (FNP) REGLE 25/97 and SUBIN 2000. F Rivera-Becerril was supported by a fellowship from the CONACyT-SFERE (Mexico–France) programme. Research was performed within the framework of an EU-RTD INCO-Copernicus project (IC15-CT 98-0116), the France/Poland Polonium programme (Project No. 99072), COST Action 838, the Swiss Project OFES C990075 and a Swiss National Foundation project (No. 3100-057046.99).

References

Baker AJM. 1987. Metal tolerance. *New Phytologist* **106,** Supplement, 93–111.

Becerril JM, Muñoz-Rueda A, Aparicio-Tejo P, Gonzalez-Murua C. 1988. The effects of cadmium and lead on photosynthetic electron transport in clover and lucerne. *Plant Physiology and Biochemistry* **26,** 357–363.

Belimov AA, Safronova VI, Tsyganov VE, Borisov AY, Tikhonovich IA, Soboleva VN, Kvokova NA, Zaitseva LN, Poddubnykh ON, Dolgaya LN, Sukhanov PA. 1999. Screening of garden pea varieties by their resistance to cadmium and accumulation of heavy metals. *Pisum Genetics* **31,** 39–40.

Christie PJ, Alfenito MR, Walbot V. 1994. Impact of low-temperature stress on general phenylpropanoid and anthocyanin pathways: enhancement of transcript abundance and anthocyanin pigmentation in maize seedlings. *Planta* **194,** 541–549.

Chugh LK, Sawhney SK. 1999. Photosynthetic activities of *Pisum sativum* seedlings grown in the presence of cadmium. *Plant Physiology and Biochemistry* **37,** 297–303.

Cobbett CS. 2000. Phytochelatins and their roles in heavy metal detoxification. *Plant Physiology* **123,** 825–832.

Cobbett CS, Goldsbrough PB. 2000. Mechanisms of metal resistance: phytochelatins and metallothioneins. In: Raskin I, Ensley BD, eds. *Phytoremediation of toxic metals: using plants to clean up the environment*. London: John Wiley & Sons, 247–269.

Das P, Samantaray S, Rout GR. 1997. Studies on cadmium toxicity in plants: a review. *Environmental Pollution* **98,** 29–36.

Dumas-Gaudot E, Guillaume P, Tahiri-Alaoui A, Gianinazzi-Pearson V, Gianinazzi S. 1994. Changes in polypeptide patterns in tobacco roots colonized by two *Glomus* species. *Mycorrhiza* **4,** 215–221.

Duval L. 1963. Etude des conditions de validité du dosage céruléomolybdique de l'acide phosphorique. Conséquences pratiques. *Chimie Analytique* **45,** 237–250.

Gildon A, Tinker PB. 1983. Interactions of vesicular–arbuscular mycorrhizal infection and heavy metals in plants. I. The effects of heavy metals on the development of vesicular–arbuscular mycorrhizas. *New Phytologist* **95,** 247–261.

Haag-Kerwer A, Schäfer HJ, Heiss S, Walter C, Rausch T. 1999. Cadmium exposure in *Brassica juncea* causes a decline in transpiration rate and leaf expansion without effect on photosynthesis. *Journal of Experimental Botany* **50,** 1827–1835.

Hasegawa H, Fukasawa-Akada T, Okuno T, Niizeki M, Suzuki M. 2001. Anthocyanin accumulation and related gene expression in Japanese parsley (*Oenanthe stolonifera*, DC.) induced by low temperature. *Journal of Plant Physiology* **158,** 71–78.

Heggo A, Angle JS, Chaney RL. 1990. Effects of vesicular–arbuscular mycorrhizal fungi on heavy metal uptake by soybeans. *Soil Biology and Biochemistry* **22,** 865–869.

Hernandez LE, Cooke DT. 1997. Modifications of the root plasma membrane lipid composition of cadmium-treated *Pisum sativum*. *Journal of Experimental Botany* **48,** 1375–1381.

Jacquot E, van Tuinen D, Gianinazzi S, Gianinazzi-Pearson V. 2000. Monitoring species of arbuscular mycorrhizal fungi *in planta* and in soil by nested PCR: application to the study of the impact of sewage sludges. *Plant and Soil* **226,** 179–188.

Joner EJ, Briones R, Leyval C. 2000. Metal-binding capacity of arbuscular mycorrhizal mycelium. *Plant and Soil* **226,** 227–234.

Joner EJ, Leyval C. 1997. Uptake of ^{109}Cd by roots and hyphae of a *Glomus mosseae*/*Trifolium subterraneum* mycorrhiza from soil amended with high and low concentrations of cadmium. *New Phytologist* **135,** 353–360.

Khan DH, Duckett JG, Frankland B, Kirkham JB. 1984. An X-ray microanalytical study of the distribution of cadmium in roots of *Zea mays* L. *Journal of Plant Physiology* **115,** 19–28.

Krupa Z, Öquist G, Huner NPA. 1993. The effects of cadmium on photosynthesis of *Phaseolus vulgaris*—a fluorescence analysis. *Physiologia Plantarum* **88,** 626–630.

Larsson EH, Bornman JF, Asp H. 1998. Influence of UV-B radiation and Cd^{2+} on chlorophyll fluorescence, growth and nutrient content in *Brassica napus*. *Journal of Experimental Botany* **49,** 1031–1039.

Lebourg A, Sterckeman T, Ciesielski H, Proix N, Gomez A. 1996. Estimation of soil trace metal bioavailability using unbuffered salt solutions: degree of saturation of polluted soil extracts. *Environmental Technology* **19,** 243–252.

Leita L, Baca García MT, Maggioni A. 1992. Cadmium uptake by *Pisum sativum*: accumulation and defense mechanism. *Agrochimica* **XXXVI,** 253–259.

Leita L, De Nobili M, Mondini C, Baca García MT. 1993. Response of Leguminosae to cadmium exposure. *Journal of Plant Nutrition* **16,** 2001–2012.

Leyval C, Turnau K, Haselwandter K. 1997. Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. *Mycorrhiza* **7,** 139–153.

Lozano-Rodriguez E, Hernandez LE, Bonay P, Carpena-Ruiz RO. 1997. Distribution of cadmium in shoot and root tissues of maize and pea plants: physiological disturbances. *Journal of Experimental Botany* **48,** 123–128.

McLaughlin MJ, Bell MJ, Wright GC, Cozens GD. 2000. Uptake and partitioning of cadmium by cultivars of peanut (*Arachis hypogaea* L.). *Plant and Soil* **222,** 51–58.

Meharg AA, Cairney JWG. 2000. Co-evolution of mycorrhizal symbionts and their hosts to metal-contaminated environments. *Advances on Ecological Research* **30,** 69–112.

Paul R, Rahmoune C, Delcarte E. 1991. Bioaccumulation du cadmium par les végétaux. *Annales de Gembloux* **97,** 133–147.

Rauser WE. 2000. Roots of maize seedlings retain most of their cadmium through two complexes. *Journal of Plant Physiology* **156,** 545–551.

Reeves RD, Baker AJM. 2000. Metal-accumulating plants. In: Raskin I, Ensley BD, eds. *Phytoremediation of toxic metals: using plants to clean up the environment*. London: John Wiley & Sons, 193–229.

Sanità di Toppi L, Gabrielli R. 1999. Response to cadmium in higher plants. *Environmental and Experimental Botany* **41,** 105–130.

SAS Institute Inc. 1986. *Introductory guide for personal computers*. Cary: SAS Institute Inc.

Siedlecka A, Baszynsky T. 1993. Inhibition of electron flow around photosystem I in chloroplasts of cadmium-treated maize plants is due to cadmium-induced iron deficiency. *Physiologia Plantarum* **87,** 199–202.

Siedlecka A, Krupa Z. 1996. Interaction between cadmium and iron and its effects on photosynthetic capacity of primary leaves of *Phaseolus vulgaris*. *Plant Physiology and Biochemistry* **34,** 833–841.

Siedlecka A, Krupa Z, Samuelsson G, Öquist G, Gardeström P. 1997. Primary carbon metabolism in *Phaseolus vulgaris* plants under Cd/Fe interaction. *Plant Physiology and Biochemistry* **35,** 951–957.

Smith SE, Read DJ. 1997. *Mycorrhizal symbiosis*. London: Academic Press.

Strasser RJ, Srivastava A, Govindjee. 1995. Polyphasic chlorophyll *a* fluorescence transient in plants and cyanobacteria. *Photochemistry and Photobiology* **61,** 32–42.

Strasser RJ, Srivastava A, Tsimilli-Michael M. 1999. Screening the vitality and photosynthetic activity of plants by fluorescence transient. In: Behl RK, Punia MS, Lather BPS, eds. *Crop improvement for food security*. Hisar: SSARM, 79–126.

Strasser RJ, Srivastava A, Tsimilli-Michael M. 2000. The fluorescence transient as a tool to characterize and screen

photosynthetic samples. In: Yunus M, Pathre U, Mohanty P, eds. *Probing photosynthesis: mechanisms, regulation and adaptation*. London: Taylor & Francis.

Tonin C, Vandenkoornhuyse P, Joner EJ, Straczek J, Leyval C. 2001. Assessment of arbuscular mycorrhizal fungi diversity in the rhizosphere of *Viola calaminaria* and effect of these fungi on heavy metal uptake by clover. *Mycorrhiza* **10,** 161–168.

Trouvelot A, Kough JL, Gianinazzi-Pearson V. 1986. Mesure du taux de mycorhization VA d'un système radiculaire ayant une signification fonctionnelle. In: Gianinazzi-Pearson V, Gianinazzi S, eds. *Physiological and genetical aspects of mycorrhizae*. Paris: INRA Presse, 217–221.

Turnau K. 1998. Heavy metal content and localization in mycorrhizal *Euphorbia cyparissias* from zinc wastes in Southern Poland. *Acta Societatis Botanicorum Poloniae* **67,** 105–113.

Wagner GJ. 1993. Accumulation of cadmium in crop plants and its consequences to human health. *Advances in Agronomy* **51,** 173–212.

Weigel HJ, Jäger HJ. 1980. Subcellular distribution and chemical form of cadmium in bean plants. *Plant Physiology* **65,** 480–482.

Weissenhorn I, Leyval C. 1995. Root colonization of maize by a Cd-sensitive and a Cd-tolerant *Glomus mosseae* and cadmium uptake in sand culture. *Plant and Soil* **175,** 233–238.

Williams CH, David DJ. 1976. The accumulation in soil of cadmium residues from phosphate fertilizers and their effect on the cadmium content of plants. *Soil Science* **121,** 86–93.

Williams LE, Pittman JK, Hall JL. 2000. Emerging mechanisms for heavy metal transport in plants. *Biochimica et Biophysica Acta* **1465,** 104–126.