Cd affects the translocation of some metals either Fe-like or Ca-like way in poplar

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Abstract

In plants, Cd causes perturbation of root metal uptake and is known to interfere with the metal translocation to the shoot. The most significant effect is the strongly reduced transport of Fe. Fe accumulation in roots under Cd stress revealed that it is not the Fe acquisition but the Fe loading to xylem elements that is blocked by Cd, which can be a result of competition between Fe and Cd for the transporters. However, in animal cells as well as in plant stomata guard cells, Cd was shown to move through Ca channels.

To clarify whether the perturbation of metal translocation/xylem loading caused by Cd show any regularity, translocation ability was tested by the determination of the metal content in leaves of hydroponically cultured (¼ Hoagland nutrient solution, Fe source: 10 μM $Fe^{(III)}$-citrate) poplar plants grown for three weeks with or without 10 μM $Cd(NO_3)_2$ treatment. Metals could be classified into two groups according to the behavior of their translocation under Cd treatment: alkaline earth metals (except Mg), Zn and Mn were influenced similarly to Ca, but other transition metals (together with alkali metals and Al) behaved like the Fe. Based on the translocation pattern, Cd seems to inhibit the transport of Ca-like metals competitively, but a different type of inhibition is exerted on the transition metal transport, with which Cd can share a common translocation system. The strongly decreased translocation of chelator-dependent transition metals may indicate Cd related disturbances in signalling pathways and gene expression of xylem transporters or chelators.

Keywords: cadmium, translocation, transition metals, alkaline earth metals

1. Introduction

1.1 Metal translocation of plants

The metal content of shoot tissues depends on the uptake and translocation ability of root and vascular tissues. The transport mechanisms for essential alkali and alkaline earth metals (sodium, potassium, magnesium and calcium) has the ability to discriminate between divalent or monovalent, larger or smaller cations, which is essential to translocate them differentially. Calcium transport routes, however, can hardly separate strontium and calcium [1] pointing out that this finely balanced system is only evolved to take discrimination among essential cations. Some of the metals, as potassium are mobile in the phloem, but most of them, like calcium, seem to be only mobile in the xylem. Essential transition metals also have dedicated transporters and specialized transport routes in which not only the uptake, but the xylem loading, and the assembly of metal-complexes is strongly controlled [2, 3]. Some of the non-essential metal ions are also taken up by the transporters of essential transition metals, and translocated as organic complexes [2, 4]. Furthermore, it is well known that changes in the availability of nutrient ions cause perturbations in the accumulation of others. Particularly, essential transition metals and some non-essential (mostly transition) metals influence the translocation of each other [5]. For example, Cd, Ni, Cr, Cu and Al are known to interact with Fe translocation [6, 7, 8]. Several non-essential and trace metals are tought or known to be taken up and translocated by such systems which are involved in essential macro- or micronutrient metal transport. Among others, Sr^{2+} is known to be transported together with Ca^{2+} [9] and Rb^{+} with K^{+} [10]. Ba^{2+} which have similar ionic radius to K^{+}, was shown to intefere with K^{+} metabolism, but competes with Ca^{2+} [11]. Nevertheless, Ba^{2+} moves mostly in the apoplast. Among transition metal ions, Fe^{2+} is known to be taken up and transported by transporters (ZIP and Nramp family transporters) which are also involved in Mn^{2+} and Zn^{2+} transport [12] but even Cr^{3+} is thought to be transported together with Fe. Ni^{2+} and Al^{3+} were

shown to compete for chelators with Fe^{3+} [6, 13] but probably use different transporters. Other transitional metals, like Ti, Sc and Co which are hardly translocated to the shoot were also shown to affect the essential transition metal transport to shoot [14]. Understanding of the ways and control of transition metal uptake and translocation is very important particularly because some of them can be highly toxic when accumulate in the cells [15].

1.2 Impact of cadmium on metal translocation

Cadmium is a non-essential and highly toxic heavy metal for all organisms. When translocated to the shoot, it causes strong oxidative stress and the inhibition of plant metabolism including photosynthesis by direct and indirect mechanisms [16, 17]. The most important indirect effect of Cd is the perturbation of metal uptake and translocation. It is known to interfere, among others, with the Zn, Fe, Mn, Cu and Ca translocation to shoot in plants [18]. Cd itself is often thought to be transported using either Ca [19] or Fe [20] translocation routes. In animal cells as well as plant stomata guard cells, Cd was shown to move through Ca channels/transporters [19, 21]. However, one of the most important causes of Cd toxicity is that it induces strong Fe deficiency in the shoot [22]. The Fe transport system is also known to carry other divalent transition metal ions, but also Cd^{2+} [12]. The translocation of iron from root to shoot through xylem elements needs citrate as chelator. In the presence of Cd, the expression of xylem citrate transporter FRD3 is down-regulated [3]. Though Cd inhibited the Fe reductase in sugar beet [23], Fe accumulation in poplar roots under Cd stress revealed that it is not the Fe acquisition but the translocation that is blocked by Cd [22]. The competition between Fe and Cd neither for the xylem transporters nor the chelators cannot be excluded. Cu translocation is also known to decrease under Cd stress [24]. In the root, there are specific transporters for Cu [25] but there is a competition between Cu and other heavy metals for the chelators as Cu also uses nicotianamin as organic chelator in

the xylem sap [26]. Therefore, there is not agreement whether Cd is transported from the root to the shoot using a translocation system involved in Ca, Fe or other essential metals.

The goal of the present study is to clarify if there is any regularity in the Cd induced perturbation of the accumulation of different metals in the leaves in order to shed more light on Cd translocation pathways.

2. Materials and methods

2.1 Plant material

Experiments were performed on micropropagated poplar (*Populus jacquemontiana var. glauca* (Haines) Kimura, 1982, cv. Kopeczkii) plants, grown in climate chamber [14/10 hours light (120 µE m^{-2} s^{-1})/dark periods, 24/22°C and 70/75% relative humidity] in quarter-strength Hoagland nutrient solution: 1.25 mM $Ca(NO_3)_2$, 1.25 mM KNO_3, 0.5 mM $MgSO_4$, 0.25 mM KH_2PO_4, 0.08 µM $CuSO_4$, 4.6 µM $MnCl_2$, 0.19 µM $ZnSO_4$, 0.12 µM Na_2MoO_4, 11.56 µM H_3BO_3, and 10 µM $Fe^{(III)}$-citrate as Fe source. All chemicals were lab-grade ones (Reanal, Hungary). To prepare nutrient solution, deionized water was used, G<4.0 µS. The nutrient solution was refreshed on every second day to keep the element concentrations at constant level. From their four-leaf stage, plants were further grown under control conditions (Ctrl) or treated by the addition of 10 µM $Cd(NO_3)_2$ for three weeks (Cd-plants). Second leaves, emerged during the treatment were used for element determination.

2.2 Determination of element concentration

Leaves were harvested during the second and third weeks of treatment: three times a week from Cd-plants, and once a week from Ctrl plants. Dried (one week at 60°C) leaves were digested by HNO_3 for 30 min at 60°C and then in H_2O_2 for 90 min at 120°C. After

filtration by MN 640W paper ion contents were measured by ICP-MS (Inductively Connected Plasma Mass Spectrometer, Thermo-Fisher, USA) for microelements and by ICP-OES (Inductively Connected Plasma Optical Emission Spectrometer, Perkin-Elmer, USA) for macroelements. The concentration of aluminium, barium, calcium, cadmium, chromium, copper, iron, potassium, lithium, magnesium, manganese, sodium, nickel, scandium, strontium, titanium and zinc were measured in the samples. The elements not present in the nutrient solution but detectable in samples are contaminants arised from the de-ionized water or laboratory chemicals.

2.3 Statistical analysis

Metal concentrations were measured in two biological repetitions from two leaves on every second day between the 7^{th} and 21^{st} days of treatment. Unpaired t-tests, ANOVAs, and linear regressions were performed by Microsoft Office Excel 2007 and InStat v. 3.00 (GraphPad Software Inc.). The term ‘significantly different’ means that the similarity of samples is less than $P=0.05$.

3. Results

3.1 Leaf development

The dry weight of Ctrl leaves were higher than those of Cd-plants’ leaves, the difference was significant at $P<0.001$ under the experimental period. Leaf element concentration measurements were performed after one week treatment when the surface area growth of second leaves had terminated [18]. During the second and third week of treatment, there was only about 10% increase in the dry weight of both Cd-plants’ and Ctrl second

leaves. The slopes of the increase did not differ significantly in Cd-plants' and Ctrl leaves (not shown). Therefore, we normalized the element contents to the dry weight of the leaves.

3.2 Metal content of the dezionized water and the nutrient solution.

Despite the deionization process, the deionized water (G<4.0 µS) still contained remarkable amount of metals (Table 1A). There was not measurable Cd, Sc and Ti contamination, while the concentrations of the other transition metals were more or less equal in the deionized water. The concentration of some essential metals, like Cu and Mo was close to the theoretical metal concentrations of the nutrient solution, whereas the concentration of Na and Zn exceeded the amount of Na and Zn given in the quarter-strength Hoagland's solution. The amount of non-essential metals, like Al, Ba, Cr, Li, and Sr was comparable to those of essential transition metals (Cu, Mn, Mo).

Lab chemicals were also contained some trace metal contamination as the metal concentrations has increased compared to the theoretical concentrations and has changed compared to dezionized water (Table 1B). However, the concentration of Sc and Ti remained under the detection limit of ICP-MS in the nutrient solution.

Table 1: Metal concentrations (µM) in the dezionized water (A) and in the nutrient solution (B).

	A	B
Al	**3.36**±0.13	**3.65**±1.11
Ba	**0.28**±0.02	**0.29**±0.03
Ca	**17.01**±1.25	**1458.95**±30.07
Cd	nd	**0.19**±0.01
Cr	**0.10**±0.01	**0.13**±0.01
Cu	**0.07**±0.01	**0.13**±0.01
Fe	**0.87**±0.10	**10.07**+0.26
K	**4.75**±1.50	**1686.21**±20.95
Li	**0.22**±0.00	**0.42**±0.01
Mg	**5.55**±0.50	**569.63**±4.86
Mn	**0.04**±0.00	**5.14**±0.04
Na	**27.40**±10.57	**33.62**±0.89
Ni	**0.04**±0.00	**0.05**±0.01
Sc	nd	nd
Sr	**0.04**±0.00	**0.70**±0.01
Ti	nd	nd
Zn	**0.56**±0.02	**0.57**±0.02

3.3 Metal concentrations in second leaves

Despite the largest translocation rates occurred during the leaf developmental phase, both Ctrl and Cd-plants had taken up and translocated measureable amount (>0.1 ppm) of essential and non-essential elements to their leaves during the time of treatment (between the 7^{th} and 21^{st} days). Concerning the macronutrients, the concentration of K and Ca showed tendentious increase in Ctrl leaves (Table 2) during the time of treatment, while Mg concentration did not change significantly. The concentration of most microelements also showed some increase in Ctrl leaves. Contaminating elements behaved similarly to essential metals, the concentration of Al, Ba, Li, Sr showed, however, only tendentious but not significant increase. Ctrl plants contained only a small amount of Cd (1.16±0.2 µg g^{-1} DW) the concentration of which did not change under the time of treatment significantly.

Under Cd treatment, a relatively large amount of Cd was translocated to the Cd-plants' second leaves emerged during the treatment. Seven day old leaves contained considerable amount of Cd (268.0±17.3 μg g^{-1} DW) and there was a continuous, nearly linear increase during the next two weeks of treatment (21 days old leaves contained 373.0±28.2 μg g^{-1} DW Cd). Cd treatment caused significant decrease in Ca and K, but not in Mg concentration, compared to Ctrl. However, both metals showed increase under further treatment (Table 2). Concerning the microelements, the accummulation of Fe was inhibited drastically in the first week as a result of Cd treatment. Further treatment on, there was no significant change in Fe concentration. The concentration of Mn and Cu also decreased in the first period of treatment, but whereas there were not any further tendentious changes in the Cu concentration, the concentration of Mn showed some increase. In contrast to other microelements, the concentration of Zn exceeded that of Ctrl leaves during the treatment. Similarly to K, Na concentration decreased significantly in the first week of Cad treatment compared to the control, but the further treatment did not cause any significant change. Concerning the non-essential elements, Cd treatment decreased continuously the translocation of all measured elements, Al, Ba, Cr, Li, Ni, Sc, Sr and Ti, compared to the Ctrl. However, the level of decrease in translocation was specific for each element. At the end of the Cd treatment, the concentration of Al, Cr, Li and Sc reached the lowest value (<50%) compared to Ctrl leaves, while the concentration of Ba, Ni, Sr and Ti was 60-90% of the Ctrl.

Table 2: Metal concentrations of 7 and 21 days old Cd-treated and 7 days old Ctrl leaves given as the percentage of the concentration of 21 days old control leaves (100%). The data of the 21 days old contol leaves are given in absolute values (µg metal g^{-1} DW).

	7th day		21st day	
	Ctrl (%)	Cd-treated (%)	Ctrl (µg metal g^{-1} DW)	Cd-treated (%)
Al	**83.5**±22.5	**29.1**±3.2	***121.00***±*27.19*	**29.7**±4.7
Ba	**92.7**±11.4	**45.6**±1.5	***316.00***±*35.99*	**61.4**±4.9
Ca	**79.0**±1.6	**70.3**±1.8	***9683.00***±*153.98*	**82.2**±1.5
Cr	**53.8**±3.9	**36.0**±3.7	***2.27***±*0.03*	**49.3**±5.3
Cu	**74.3**±9.4	**52.0**±1.7	***12.70***±*2.49*	**54.5**±4.6
Fe	**65.0**±5.2	**27.8**±1.7	***149.00***±*11.43*	**29.4**±4.1
K	**88.9**±10.8	**77.5**±4.1	***44126.00***±*5947.71*	**83.8**±1.4
Li	**89.6**±7.3	**38.9**±7.2	***0.46***±*0.19*	**40.2**±0.9
Mg	**93.9**±9.1	**114.1**±6.4	***2036.00***±*108.93*	**125.1**±4.0
Mn	**74.4**±9.3	**69.2**±5.4	***95.00***±*8.84*	**87.0**±1.6
Na	**76.0**±8.3	**52.0**±1.3	***903.00***±*21.15*	**57.0**±12.0
Ni	**54.4**±3.1	**60.4**±10.3	***1.04***±*0.10*	**64.2**±41.0
Sc	**77.3**±11.4	**57.5**±0.6	***0.21***±*0.02*	**55.1**±2.0
Sr	**86.0**±12.6	**65.6**±2.7	***27.90***±*3.50*	**76.3**±0.5
Ti	**71.1**±7.5	**64.9**±2.4	***0.37***±*0.04*	**81.2**±25.4
Zn	**63.8**±5.6	**117.9**±7.5	***49.20***±*2.82*	**142.3**±1.9

Therefore, three significantly different groups were found according to the concentration growth (Fig. 1). Alkali and transition metals (except K, Mn and Zn) showed significantly lower increase in concentration (<50% of Ctrl total metal concentration in three-week old leaves) than Sr, Ca, K, Mn and Zn (>50% of total metal concentration). The concentration increase of Ba and Mg even exceeded the level of Ctrl. The similarities within the groups were significantly higher than between groups.

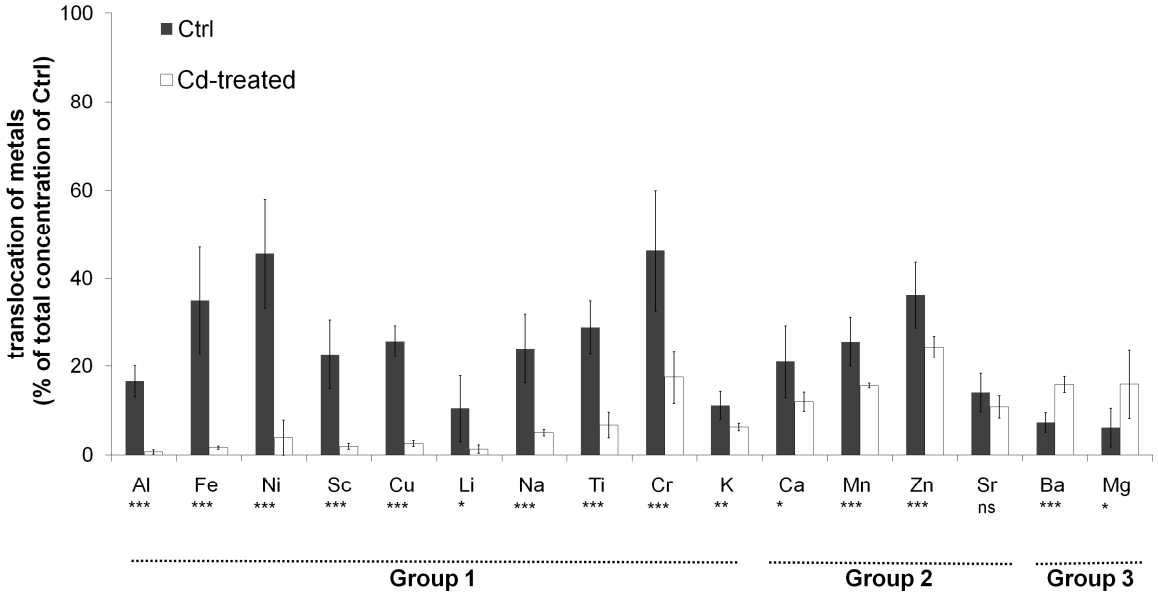

Figure 1: Cd treatment induced changes in the translocation of metals to the leaves between the 7th and 21st day of treatment. Changes were normalized to the maximal metal concentrations in the 21 day old Ctrl leaves (see: Table 2). Differences were analyzed by t-test (ns: non significant, *: P<0.1, **: P<0.05, ***: P<0.01). Three groups of metals were established based upon the Cd treatment to Ctrl rate in translocation percentage. The differences are significant (P<0.05) between the groups but not within the groups (ANOVA with Tukey-Kramer post test).

3.4 The influence of Cd on metal translocation

During two weeks, 28.15±5.69% of the final Cd content translocated to the second leaf, whereas the concentration of Cd in control leaves remained stable. For testing the effect of Cd on the translocation of metals, relative (% of maximal) metal concentrations of leaves were plotted as codomains set against changes in relative Cd concentration as domains ($[Me]_{t,rel} \rightarrow [Cd]_{t,rel}$). Linear regressions were performed on the plots of which slopes were analyzed (Fig. 2). Based on these slopes, three different groups of metals were found. In the

first group, which contained all the studied alkali and transition metals (except of Mn and Zn), the slopes of plots remained below 0.5. However, the concentration of Al, Li and Sc hardly increased (or even decreased). In the second group containing the alkaline earth metals together with Zn and Mn, the slopes of the correlation were over 0.5. In the case of Ba (third group), the slope approached 1.0 The variability of slopes in a group remained much lower than between groups, which means that the groups significantly differed from each other. The variability was higher in the first group compared to the second one.

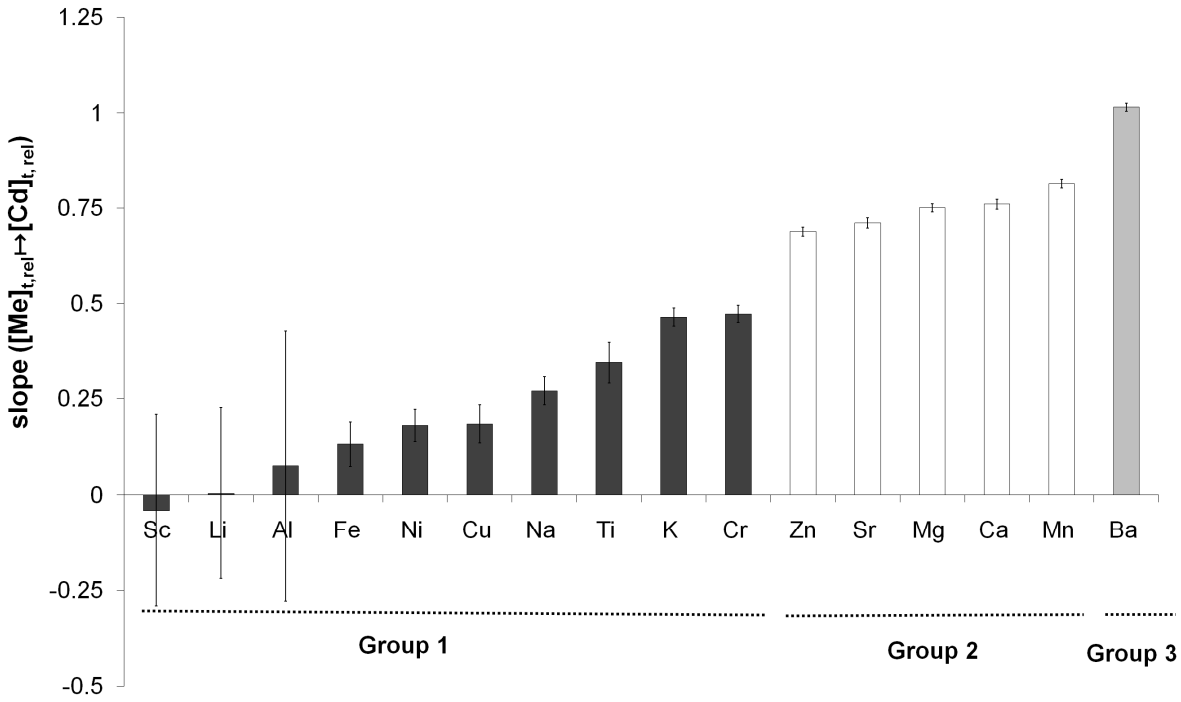

Figure 2: Correlation of metal translocation to Cd accummulation shown by the slopes of the plots of [Me] as the function of [Cd]. Three groups could be established based on the retardation in translocation caused by Cd treatment. The differences are significant ($P<0.05$) between the groups but not within the groups (ANOVA with Tukey-Kramer post test).

4. Discussion

Essential and non-essential elements are known to interact with each other in the root-to-shoot translocation [27, 28]. Cd is known to cause Fe deficiency in the shoot, but it also affects the calcium metabolism [19]. The translocation and metabolism of other macro- and micronutrients, like K, Zn, Mn and Cu were also shown being disturbed [16]. As Cd induced considerable changes in the concentration of nearly all essential and non-essential metals in leaves (Fig. 1), the different changes in translocation of the different groups of metals may also indicate how Cd is translocated in plants. In addition, some pieces of information about the mode of interactions of Cd with metal translocation can be obtained.

4.1 Changes in the metal translocation

In spite of the low concentration of non-essential metals in the nutrient solution originated from both the deionized water and lab chemicals, plants accumulated them effectively in their leaves.

4.1.1 Transition metals

Under Cad treatment, the Fe transport was strongly reduced, there was only little increase in leaf Fe content of Cad plants after the beginning of treatment. Cd is known to interfere with Fe uptake to the root cells, as under Cd treatment the apoplastic Fe concentration is increasing. The expression of IRT1 was shown to be enhanced in the presence of Cd [29]. However, the loading of Fe to the xylem elements and its translocation to shoot tissues is strongly dependent on the presence of citrate in the xylem sap. Under Cd treatment, the citrate transporter FRD3 is known to be down-regulated [3]. The stagnating leaf Fe concentration supports the theory that Fe accumulates in roots and not transported to shoot when the plants is exposed to Cd [22], because Cd acts as regulator on the expression of the

genes involved in root-to-shoot Fe translocation. Cd is reported to interact with Ca-signalization pathway [21] and also to Zn-finger dependent regulation pathways [30]. The interaction of Cd to signalization pathways can be a reason for the phenomena of changed expression of some transporters, but also supports the theory of Ca-like behaviour of Cd. The metals known to be transported more or less the same way as Fe (Cu, Al, Cr, Ni) forming citrate or nicotianamine complexes, show a very similar concentration change profile, indicating that Cd affects their translocation similarly to Fe. However, the translocation profile of Zn and Mn differed from that of other transition metals. Mn is known to form organic or phosphate chelates in the xylem sap [31], while Zn forms Zn-nicotianamine complexes in the symplast but not in the xylem sap [32] suggesting that both Zn and Mn translocation must be less affected by Cd. Cd increases the shoot Zn content but the elevated translocation activity seems to be restricted to the leaf development phase as after that period the translocation of Zn does not differ from other metals. The moderate retardation of leaf Mn accummulation indicates that Mn translocation/Mn complexation are less affected by Cd as the translocation of Mn basically differs from that of Fe and Cu [25, 33]. The translocation profile of Ti and Sc indicates that both of them should be translocated to the shoot using one of the pathways involved in chelator-dependent transition metal translocation [34].

4.1.2 Alkali metals

Changes in the translocation profile of alkali metals under Cad treatment were similar to that of the transition metals. However, alkali metals are transported in plants as free ions. Their transporters are specific, i.e they can discriminate between Na and K. Li may be transported together with Na as it is predicted by the rate of retardation under Cd treatment.

Concerning the effect of Cd on the translocation of alkali metals, some general effect should occur. As a divalent cation, Cd can not use transporters/channels dedicated for monovalent cations, therefore the effect of Cd on alkali metal translocation should also be a

kind of non-competitive inhibition. Ca is also known to interact with K uptake of plants [35, 36]. The similar effect of Cd on potassium uptake and translocation also indicates the analogy between Cd and Ca in plants.

4.1.3 Alkaline earth and earth metals

The Cd treatment also disturbed the translocation of alkaline earth metals - except Mg - to the leaves. The accumulation was, however, significantly less affected than that of most transition metals. Mg is taken up and translocated from the root to shoot separately from other alkaline earth metals. However, Sr and Ba interfere with Ca transport [9]. Among alkaline earth metals, the translocation of Ba was the less affected under Cad treatment. Unlike other alkaline earth metals Ba was shown to move in the apoplast only, not entering in the symplast [37], which could be a reason why Cd affected the Ba tranlocation much less than Ca and Sr. Mg is also not effected by Cd indicating that Mg transporters can discriminate between Mg^{2+} and Cd^{2+} easily. Sr is known to be transported together with Ca in both plants and animals [1]. The middle strength retardation in the translocation of Ca and Sr compared to most transitional and alkali metals indicates that in this case a competitive-like inhibition of translocation is more likely than a non-competitive one. Cd is known to interfere with membrane transport of Ca not only in animal cells [21] but also in stomatal guard cells [19] referring to the disturbance of some Ca-dependent signalling processes. In animal cells, Cd is known to interfere with the activation of some signal transduction pathways based on the Ca-like features of Cd [38] as Cd can bind to some Ca-binding sites. A large amount of Cd in plants does not enter to the symplast but remains in the cell wall. Cd was shown to replace Ca in the cell wall structure on Ca-binding sites [39]. These data seems to support the theory that Cd and Ca share a common translocation system.

The earth metal Al showed strongly decreased translocation under Cd treatment which makes it similar to some of the transition metals. The translocation profile of Al shows close

relationship to those of Fe, Ni and Cu. Al is known to interact with Fe transport by competing for citrate as chelator [6], or even for transporters. Therefore the similarities in Fe and Al translocation also support the theory of indirect effect of Cd on their translocation by inhibiting the accummulation of citrate in the xylem sap.

4.2 Conclusion

Based on the translocation profiles, two groups of metals could be identified in which Cd affects the translocation of metals either Fe-like or Ca-like ways. In the Ca-like group of metals, a competitive type of inhibition of translocation occurs, possibly at the level of the function of transporters, which seems to support the theory that Cd and Ca share a common translocation system. In the second group, which contains most of the alkali and transition (except Zn and Mn) metals together with Al, the inhibition of translocation is non-competitive type. The strongly decreased translocation of chelator-dependent transition metals may indicate Cd related disturbances in signalling pathways and gene expression of xylem transporters or chelators.

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