Short Transcript-derived Fragments from the Metal Hyperaccumulator Model Species *Arabidopsis halleri*

Dörthe B. Dräger, Katrin Voigt, and Ute Krämer*

Max Planck Institute of Molecular Plant Physiology. Am Mühlenberg 1, D-14476 Golm, Germany. Fax: +49(0)331 567 8408. E-mail: kraemer@mpimp-golm.mpg.de

* Author for correspondence and reprint requests

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Phytoremediation of metal contaminated soils requires high-biomass plants exhibiting tolerance to and accumulation of metal contaminants. However, very little is known about the genes controlling these traits. In order to better understand this, *Arabidopsis halleri* ssp. *halleri* (L.) O'Kane and Al-Shehbaz, a naturally selected zinc and cadmium tolerant plant species capable of hyperaccumulating both metals, is a suitable model plant. To date, the scarcity of sequence information from *A. halleri* is still limiting its use as a model organism. Here we report 128 transcript-derived sequence fragments (TDFs) identified in a cDNA-AFLP approach aimed at identifying metal-regulated transcripts in roots. In addition we show that in roots of *A. halleri*, transcript levels of *AhPDR11*, encoding an ATP-binding-cassette (ABC) transport protein, are slightly induced in response to metal exposure.

Key words: cDNA AFLP, Hyperaccumulation, *Arabidopsis halleri*

Introduction

Arabidopsis halleri ssp. *halleri* displays zinc and cadmium tolerance, and in naturally occurring populations plants have been reported to hyperaccumulate zinc to concentrations of up to 2.4%, and Cd to concentrations of up to 0.15%, primarily in above-ground dry biomass (Bert *et al.*, 2000, 2002, 2003). This species has thus naturally evolved traits that are interesting for the development of plant-based phytoremediation technologies, although its biomass production is prohibitively low. However, *A. halleri* is well suited as a model plant to aid the development of a molecular understanding of metal homeostasis in higher plants. *A. halleri* and the closely related non-tolerant and non-accumulating species *Arabidopsis lyrata* are the closest known relatives of the widely established genetic model species *A. thaliana* (Koch *et al.*, 2000). Within coding regions, cDNAs of *A. halleri* and *A. thaliana* have been reported to share approximately 93% sequence identity (Becher *et al.*, 2004; Weber *et al.*, 2004). *A. halleri* is a diploid species with $2n = 16$ chromosomes. It can be crossed with *A. lyrata*, and populations have been obtained that segregate with respect to metal tolerance and accumulation (Macnair *et al.*, 1999; Bert *et al.*, 2003). As a stoloniferous plant, *A. halleri* can be propagated clonally, and genetic transformation is being developed by several research groups. However, the information on gene expression and nucleotide sequence of *A. halleri* is still very limited. For example, the design of primers suitable for expression analysis by quantitative real-time RT-PCR is dependent on precise cDNA sequence information.

The cDNA-AFLP technique is an unbiased approach for the identification of differentially regulated transcripts at the sequence level (Bachem *et al.*, 1996). It requires no prior sequence information and is capable of identifying transcripts that are expressed at very low levels. Here we report the identification and sequence of a number of putatively metal-regulated transcripts from roots of *A. halleri*.

Materials and Methods

Seeds of *Arabidopsis halleri* ssp. *halleri* (L.) O'Kane and Al-Shehbaz (accession Langelsheim), formerly termed *Cardaminopsis halleri* Hayek (L.), were collected at the site (Ernst, 1974). The seeds were placed on a layer of 0.8% (w/v) solidified Noble agar (Bio101, Vista, CA, USA) in clipped black 0.5-ml Eppendorf tubes. These were inserted into holes in floating polystyrene lids. 50 to 60 seedlings were germinated and cultivated in 2-l vessels in a modified 0.25-strength Hoagland solution (Becher *et al.*, 2004) as described in a climate-controlled growth chamber (temperature:

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20 °C day/16 °C night; relative humidity: 60% day/75% night; light: 16 h day/8 h night at 120 μmol m^{-2} s^{-1}). After germination, the solutions were exchanged weekly. Six-week-old plants were exposed to a nutrient solution (controls) or the same solution supplemented with 300 μM $ZnSO_4$ and 30 μM $CdCl_2$ for 6 h. Subsequently, the roots were harvested and immediately frozen in liquid nitrogen. Total RNA was extracted from roots of treated and untreated plants and used for the cDNA-AFLP protocol. RNA extraction, mRNA isolation, synthesis of double stranded cDNA (ds cDNA), restriction of ds cDNA, ligation of adaptors, and cDNA-AFLP analysis were performed according to a previously published protocol (Bachem *et al.*, 1998), which is available at http://www.spg.wau.nl/aflp.htm. Single stranded cDNA synthesis was primed using oligo-dT and the SuperScript™ First Strand Synthesis System (Invitrogen, Carlsbad, CA, USA). The restriction enzymes used were *AseI* and *TaqI* (Roche, Mannheim, Germany). A total of 224 primer combinations with two additional selective nucleotides at the 3′ end of each primer was used for transcript profiling. Each amplification step was performed in two replicates, resulting in a total of four technical replicates per treatment. After separation of PCR products on a 6% (v/v) denaturing polyacrylamide gel, the gel was dried and exposed to an X-ray film for 3 to 5 d. Fragments of interest were excised from the polyacrylamide gel, eluted in 100 μM $T_{0.1}E$ buffer (10 mM Tris/HCl, [tris(hydroxymethyl)aminomethane], 0.1 mM Na-EDTA, pH 7.5) at 4 °C overnight. 1 μl of the eluate was used for reamplification using primers with the respective two-base extensions in a reaction volume of 10 μl. PCR conditions, except the primers, were the same as those of the pre-amplification step in the cDNA-AFLP protocol. PCR products were resolved on a 2% agarose gel to verify the expected size, and subsequently cloned into the TOPO pCR2.1 expression vector (Invitrogen) following the manufacturer's recommendations. Three to eight colonies per isolated fragment were picked and grown in 160 μl of liquid YT medium supplemented with carbenicillin in 96-well plates at 37 °C overnight. The inserts were tested for the correct size performing a colony PCR using the standard reverse primer M13rev and the standard forward primer T7for. The cell suspension culture was diluted 1:3 with double distilled H_2O, 5 μl were used in a total volume of 25 μl in the subsequent amplification reaction. PCR products were resolved on a 2% (w/v) agarose gel for verification of the size of the insert. 3 to 8 clones per isolated fragment were sequenced (AGOWA, Berlin, Germany). The sequences of the fragments were compared with the *A. thaliana* genome and transcriptome in the AGI database using FASTA (Pearson and Lipman, 1988). The cDNA fragments, FASTA search results and accession numbers are listed in Table I. Northern blot (Dräger *et al.*, 2004) and quantitative real-time RT-PCR analysis (Becher *et al.*, 2004), respectively, were performed as described previously.

Results and Discussion

A cDNA-AFLP approach was employed in order to identify genes that were differentially expressed in roots of six-week-old *A. halleri* plants following exposure to a combination of 300 μM $ZnSO_4$ and 30 μM $CdCl_2$ for 6 h. In preliminary experiments, this metal treatment had been determined not to cause any toxicity symptoms or growth impairment in *A. halleri* within 1 week of exposure (data not shown). A cDNA-AFLP analysis was performed using 224 out of 256 possible primer combinations. From a total of approximately 19,000 bands that were visually inspected on polyacrylamide gels, 272 putatively differentially expressed fragments were excised. Of these, 197 eluted fragments could be re-amplified using the same primer pairs as in the second amplification step of the cDNA-AFLP protocol. Following re-amplification, a random selection of 78 fragments were successfully cloned. Initially, three clones per fragment were sequenced. An additional 3 to 5 clones per fragment were sequenced if the first set of 3 clones per fragment yielded two or more different sequences. A total of 329 clones were sent for sequencing. Sequencing of clones obtained from 37 transcript-derived fragments (TDFs) yielded single sequences for each TDF, whereas for 41 cloned TDFs between 2 and 4 different sequences were obtained per TDF. This suggested the presence of several fragments of the same size in about half of the excised bands.

In total, 136 different sequences were obtained. These were used in a homology search against the *Arabidopsis thaliana* and the AGI transcript and genome data bases using FASTA (Pearson and Lipman, 1988). Homologous sequences corresponding to transcripts from *A. thaliana* were ob-

Table I. List of *A. halleri* TDFs. Given are the annotations of the homologous *A. thaliana* sequences identified in a FASTA search and their AIS/MIPS codes, the % sequence identities between the nucleotide sequence of the *A. halleri* TDFs and the respective homologous *A. thaliana* sequences, the length of the *A. halleri* cDNA clones, the putative metal-exposure induced regulation in roots of *A. halleri* as suggested by the cDNA-AFLP experiment, and the EMBL accession numbers of the *A. halleri* sequences.

Annotation	AIS/MIPS	Identity (%)	Length [bp]	Regulation	Accession
Nodulin-26 like protein	At4g19030	89.7	294	up	AJ627047
Putative protein	At4g30010	98	164	up	AJ627048
Ubiquitin-specific protease 24	At4g30890	96.9	229	down	AJ627049
Hypothetical protein	At2g17930	98.7	162	down	AJ627050
F-box protein family	At3g22870	70	91	down	AJ627051
60S ribosomal protein L27A	At1g70600	87.4	200	down	AJ627052
Unknown protein	At5g45490	92.5	259	up	AJ627053
Expressed protein	At2g27230	95.2	105	down	AJ627054
NAM protein – related	At4g01540	96	88	down	AJ627055
Dehydrin protein family	At1g54410	90	110	up	AJ627056
Pectinesterase – related	At4g12390	92.6	393	down	AJ627057
ABC transporter-like protein	At3g53480	95	341	up	AJ627058
Glycine-rich protein	At2g05530	93	155	up	AJ627059
Peroxisomal membrane protein (PMP36)	At2g39970	91	228	up	AJ627060
Acyl CoA binding protein (ACBP) family	At3g05420	95.4	411	up	AJ627061
Ath-B, cellulose synthase catalytic subunit	At5g16910	97	334	down	AJ627062
Putative pectinesterase	At1g76160	93.4	332	up	AJ627063
Glycosyl transferase, putative	At3g25140	95.5	380	down	AJ627064
Serine/threonine-protein kinase-like protein	At5g60550	94	80	up	AJ627065
Hypothetical protein	At3g14070	94.5	454	down	AJ627066
Unknown protein	At1g25550	93.6	110	down	AJ627067
Cystatin	At5g12140	93.5	351	up	AJ627068
Putative serine carboxypeptidase II	At3g02110	97.4	341	down	AJ627069
Hypothetical protein	At4g21250	93.5	109	up	AJ627070
60S ribosomal protein L41 (RPL41G)	At3g56020	95.1	201	down	AJ627071
AtMRP8, a full size ABC transporter	At3g13090	97	525	up	AJ627072
60S ribosomal protein L10	At1g26910	97.5	281	up	AJ627073
Expressed protein	At4g33666	65.8	84	up	AJ627074
Cytosolic triosephosphateisomerase	At3g55440	97.8	93	down	AJ627075
Putative ribosomal protein	At1g74270	95	103	up	AJ627076
Putative protein	At4g33550	95.2	189	up	AJ627077
Serine/threonine protein kinase-like protein	At5g59260	72	180	up	AJ627078
Strong similarity to unknown protein	At5g65560	93	87	up	AJ627079
Unknown protein	At3g01670	95.11	144	down	AJ627080
60S ribosomal protein L7A	At2g47610	91.21	89	down	AJ627081
Unknown protein	At2g38310	93.83	94	down	AJ627082
Cytochrome P450, putative[a]	At3g14610	78.8	152	down	AJ627083
FAD-linked oxidoreductase family	At1g30720	92.6	147	down	AJ627084
Unknown protein	At3g13200	96.7	458	up	AJ627085
Hypothetical protein	At3g08020	97.2	464	up	AJ627086
Mitochondrial carrier protein family	At1g79900	96	456	up	AJ627087
Translationally controlled tumor protein-related	At3g16640	90	450	up	AJ627088
Cytochrome P450 family	At2g27000	86.2	254	down	AJ627089
Bet v I allergen family	At1g70880	66.7	259	down	AJ627090
Putative translationally controlled tumor protein	At3g16640	94.9	215	down	AJ627091
Unknown protein	At1g78150	90.3	219	down	AJ627092
Glutathione transferase, putative	At1g78320	94.9	216	down	AJ627093
Unknown protein	At1g13930	93.8	214	down	AJ627094
Hevein-related protein precursor (PR-4)	At3g04720	93	202	down	AJ627095
Putative protein	At3g61130	95.5	204	down	AJ627096
Expressed protein	At5g65180	94	101	down	AJ627097
Expressed protein	At5g23570	98	100	down	AJ627098
Transcription factor NF-Y, CCAAT-binding-protein	At3g53340	91.2	247	down	AJ627099
Expressed protein	At1g43790	94.6	245	down	AJ627100
Expressed protein	At3g60680	90.1	210	up	AJ627101

Table I. (cont.)

Annotation	AIS/MIPS	Identity (%)	Length [bp]	Regulation	Accession
Protease inhibitor/seed storage/lipid transfer protein (LTP) family	At3g53980	87.7	212	up	AJ627102
Putative pectinesterase	At1g76160	94.7	194	down	AJ627103
Putative laccase	At3g09220	95.9	194	down	AJ627104
Unknown protein	At1g03080	93	172	down	AJ627105
Phytochelatin synthase	At5g60920	94	169	down	AJ627106
Hypothetical protein	At5g52790	93.1	146	up	AJ627107
Unknown protein	At5g15550	75.6	239	down	AJ627108
EREBP-4 like protein	At4g17486	96.2	469	up	AJ627109
Putative cytosolic malate dehydrogenase	At5g43330	96.8	466	up	AJ627110
Expressed protein	At3g15630	93.3	118	down	AJ627111
Auxin-induced protein family	At3g25290	95.6	114	down	AJ627112
Curculin-like (mannose-binding) lectin family	At1g78850	88.4	116	down	AJ627113
Unknown protein	At1g77710	77.7	146	up	AJ627114
Unknown protein	At5g11760	89.3	146	up	AJ627115
Protease inhibitor/seed storage/lipid transfer protein (LTP) family	At3g22600	91.8	232	up	AJ627116
ZAT	At2g46800	97.1	232	up	AJ627117
Putative cytochrome P450	At2g26710	95.4	211	up	AJ627118
Ubiquitin-conjugating enzyme family	At2g36060	96.4	112	up	AJ627119
Luminal binding protein 2 precursor (AtBP2)	At5g42020	62	211	up	AJ627120
Ribosomal protein S18, putative	At4g09800	96.6	209	up	AJ627121
Proline-rich protein family, contains proline-rich extensin domains	At3g54580	78.4	289	down	AJ627122
PSI type III chlorophyll a/b-binding protein (Lhca3*1)	At1g61520	94.3	287	down	AJ627123
Putative Rab geranylgeranyl transferase	At5g41820	75.5	160	down	AJ627124
Glyoxalase family protein (lactoylglutathione lyase family protein)	At1g15380	98	164	down	AJ627125
Putative aquaporin	At2g39010	98.3	116	up	AJ627126
Protease inhibitor/seed storage/lipid transfer protein (LTP) family	At3g22600	94	117	up	AJ627127
mRNA for small GTP-binding protein (ara-3)	At3g46060	89	141	down	AJ627128
60S acidic ribosomal protein P2 (RPP2A)	At2g27721	94.1	238	down	AJ627129
Expressed protein	At4g21540	96.7	382	down	AJ627130
RAN1 small Ras-like GTP-binding nuclear protein	At5g20010	97.3	147	down	AJ627131
Peptide transporter-like protein	At5g13400	94.5	146	down	AJ627132
Dehydration-induced protein-related	At1g19430	87.9	148	down	AJ627133
Expressed protein	At1g31170	71.6	147	down	AJ627134
Putative mitochondrial carrier protein	At4g39460	98.6	142	down	AJ627135
Transcriptional regulator, putative	At1g80790	95.3	151	down	AJ627136
Copine-like protein	At5g07300	95.7	139	up	AJ627137
Unknown protein	At1g72390	97.6	141	up	AJ627138
Long-chain-fatty-acid–CoA ligase (acyl-CoA synthetase)	At4g23850	96.4	139	up	AJ627139
Unknown protein	At1g19960	92.86	182	up	AJ627140
Putative protein	At3g44430	86.93	179	up	AJ627141
phyC Photoreceptor	At5g35840	94.41	305	down	AJ627142
40S ribosomal protein S30	At2g19750	88.27	308	down	AJ627143
AHK2, histidine kinase	At5g35750	98.13	269	down	AJ627144
Unknown protein	At5g49830	96.3	268	down	AJ627145
Putative transcription factor (MYB88)	At2g02820	96.01	280	up	AJ627146
Ribosomal protein L14-like protein	At4g27090	94.6	277	up	AJ627147
Contains similarity to unknown protein	At5g65685	89.9	71	up	AJ627148
40S ribosomal protein S25 (RPS25E)	At4g39200	98.49	69	up	AJ627149
Hypothetical protein	At4g20480	84.26	163	up	AJ627150
Hypothetical protein	At4g14390	90.54	160	up	AJ627151
Unknown protein	At5g43150	87.16	160	up	AJ627152
Actin depolymerizing factor 4, ADF4	At5g59890	61.4	153	up	AJ627153

Table I. (cont.)

Annotation	AIS/MIPS	Identity (%)	Length [bp]	Regulation	Accession
SAR DNA binding protein, putative	At1g56110	94.3	156	up	AJ627154
Glycine-rich RNA binding protein	At2g21660	93.4	153	up	AJ627155
Integral membrane protein, putative	At1g06470	96.69	153	down	AJ627156
60S ribosomal protein L38-like protein	At2g43460	99.1	134	down	AJ627157
Expressed protein	At4g24370	85.8	133	down	AJ627158
Brassinosteroid receptor kinase, putative	At3g13380	84.62	238	down	AJ627159
Glyoxalase II isozyme, putative	At1g06130	94.37	233	down	AJ627160
Unknown protein	At4g01040	92.89	239	down	AJ627161
Putative aconitase	At4g26970	98.1	158	up	AJ627162
Cell division cycle (CDC) protein-related	At2g20000	88.12	158	up	AJ627163
Similar to pectinesterase	At1g11580	88.97	236	down	AJ627164
Glucuronosyl transferase-like protein	At5g05890	82.83	234	down	AJ627165
Hypothetical protein	At4g11670	93.55	253	down	AJ627166
Glycine-rich protein	At4g30450	72.4	254	down	AJ627167
Ribosomal protein S28-like protein	At5g03850	83.61	209	down	AJ627168
Expressed protein	At1g54095	84.9	204	down	AJ627169
AP2 domain transcription factor, putative	At1g22190	89.74	191	up	AJ627170
H^+-pumping ATPase 16 kDa proteolipid, vacuolar (ava-p1)	At2g 16510	85.7	187	up	AJ627171
Translation initiation factor eIF-4A1	At3g13920	91.03	187	up	AJ627172
Glutaredoxin protein family	At1g03020	91.86	172	down	AJ627173
Putative 60S ribosomal protein L35	At2g39390	86.13	174	down	AJ627174

[a] This and below: sequences derived from bands incorporating more than one sequence.

tained for 128 sequences, whereas FASTA searches identified no homologous *A. thaliana* sequences for five sequences, and indicated that three sequences were likely to correspond to genomic DNA. These eight sequences were excluded from further processing. The 128 short cDNA fragments of transcripts that are potentially metal-regulated in roots of *A. halleri* are listed in Table I. Overall, the identity of cDNA fragments with homologous *A. thaliana* sequences was 91.08 ± 0.97%. This is slightly lower than our earlier observations, which is probably explained by the tendency of the used cDNA-AFLP method to yield fragments derived from the 3′ untranslated regions of transcripts (Samuelian *et al.*, 2004).

Initially, confirmation of the differential expression of two candidate genes was attempted by Northern blot analysis. For the generation of the probes two primers each were designed, one of which annealed to the cDNA sequence of the isolated fragments (AJ627047 and AJ627058, respectively), whereas the other was designed on the basis of the cDNA of the respective *A. thaliana* sequence. Amplification of *A. halleri* cDNA using the described primer pairs yielded the expected products of 740 bp for the probe corresponding to At4g19030 (AJ627047), and 390 bp for the probe corresponding to At3g53480 (AJ627058). Total RNA was isolated from root material used also for cDNA-AFLP analysis. Quantification of signal intensities revealed a 2.1-fold up-regulation of the gene corresponding to At3g53480 (Fig. 1). This gene is highly homologous to the *A. thaliana AtPDR11* (Martinoia *et al.*, 2002) encoding an ATP-binding-cassette (ABC) transporter-like protein

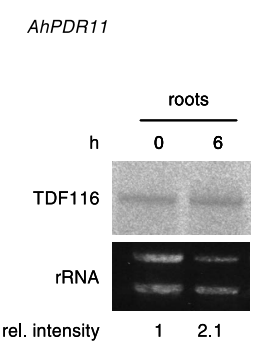

Fig. 1. Northern blot showing expression of *AhPDR11* in roots of *A. halleri* after 0 and 6 h of metal treatment with 300 μM $ZnSO_4$ and 30 μM $CdCl_2$ (top). 5 μg of total RNA were separated by agarose gel electrophoresis and blotted. Ethidium-bromide stained ribosomal RNA served as loading/blotting control (bottom). Relative signal intensities, calculated by normalising the magnitude of the hybridisation signal to the magnitude of the rRNA signal, are shown below the panel.

(At3g53480; AL132966), which is addressed as *AtPDR9* in some studies (Sanchez-Fernandez *et al.*, 2001; van den Brule and Smart, 2002). Transcripts of the homologous gene in *A. thaliana*, *AtPDR11* (or *AtPDR9*) have been reported to be most abundant in roots and to be induced by a toxic concentration of 100 μM $CdCl_2$ (van den Brule and Smart, 2002). For the other gene tested, hybridisation of the Northern blot did not confirm metal regulation (data not shown).

Quantitative real-time RT-PCR experiments performed on plant material grown precisely as for the cDNA-AFLP indicated a 1.54-fold upregulation of transcript levels of *AhPDR11* in response to metal exposure, as well as a 1.54-fold upregulation of the cDNA corresponding to fragment AJ627047 (data not shown). However, real-time RT-PCR did not indicate metal regulation of expression of genes corresponding to cDNA fragments AJ627047, 056, 060, 063, 064, 066, 069, 071, 078, 104, 109, 117, and 144, after 6 h of exposure (data not shown, see Table I).

In summary, metal-dependent regulation of transcript abundance was only confirmed for a small fraction of between one and two out of 14 genes that were arbitrarily selected from a total of 128 putatively metal-regulated genes identified by cDNA-AFLP. Moreover, for the genes confirmed to be metal-regulated the change in transcript abundance was small. This suggests that cDNA-AFLP is a highly sensitive technique, which can, however, result in the identification of false positives. Using microarray chips that cover approximately 80% of transcribed genes of *A. thaliana*, transcript levels of only 4 genes were found to be upregulated at least 3.5-fold (maximum 4.7-fold) in roots of *A. halleri* in response to exposure to 300 μM $ZnSO_4$ for 8 h (Ina Talke and Ute Krämer, unpublished data). Interestingly, according to the microarray data *AhPDR11* was upregulated 1.8-fold in these experiments (data not shown). ABC transporter proteins have previously been implicated in cellular metal detoxification by mediating the sequestration of Cd-glutathione (Song *et al.*, 2003) or Cd-phytochelatin (Ortiz *et al.*, 1995) complexes. Further experiments are required to investigate the biological role of *AhPDR11*. The cDNA-AFLP and the microarray chip data both suggest that there are only minor transcriptional changes in response to metal exposure in *A. halleri* (Becher *et al.*, 2004; Weber *et al.*, 2004). Instead, a number of genes are constitutively highly overexpressed in *A. halleri* at the transcript level. Consistent with this, both metal tolerance and accumulation have been described as "constitutive" in *A. halleri* ssp. *halleri*, implying that these traits are common to individuals from populations growing on metalliferous and on normal soils. More advanced cDNA-AFLP techniques have recently been developed that reduce the complexity of the primary template, and may reduce the occurrence of false positives in cDNA-AFLPs (van der Biezen *et al.*, 2000; Breyne and Zabeau, 2001).

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Bachem C. W., van der Hoeven R. S., de Bruijn S. M., Vreugdenhil D., Zabeau M., and Visser R. G. (1996), Visualization of differential gene expression using a novel method of RNA fingerprinting based on AFLP: analysis of gene expression during potato tuber development. Plant J. **9**, 745–753.

Bachem C. W., Oomen R. J., and Visser R. G. (1998), Transcript imaging with cDNA-AFLP: A step-by-step protocol. Plant Mol. Biol. Rep. **16**, 157–173.

Becher M., Talke I. N., Krall L., and Krämer U. (2004), Cross-species microarray transcript profiling reveals high constitutive expression of metal homeostasis genes in shoots of the zinc hyperaccumulator *Arabidopsis halleri*. Plant J. **37**, 251–268.

Bert V., Macnair M. R., de Laguerie P., Saumitou-Laprade P., and Petit D. (2000), Zinc tolerance and accumulation in metallicolous and nonmetallicolous populations of *Arabidopsis halleri* (Brassicaceae). New Phytol. **146**, 225–233.

Bert V., Bonnin I., Saumitou-Laprade P., de Laguerie P., and Petit D. (2002), Do *Arabidopsis halleri* from nonmetallicolous populations accumulate zinc and cadmium more effectively than those from metallicolous populations? New Phytol. **155**, 47–57.

Bert V., Meerts P., Saumitou-Laprade P., Salis P., Gruber W., and Verbruggen N. (2003), Genetic basis of Cd tolerance and hyperaccumulation in *Arabidopsis halleri*. Plant Soil **249**, 9–18.

Breyne P. and Zabeau M. (2001), Genome-wide expression analysis of plant cell cycle modulated genes. Curr. Opin. Plant Biol. **4**, 136–142.

Dräger D. B., Desbrosses-Fonrouge A. G., Krach C., Chardonnens A. N., Meyer R. C., Saumitou-Laprade P., and Krämer U. (2004), Two genes encoding *Arabidopsis halleri* MTP1 metal transport proteins co-segregate with zinc tolerance and account for high *MTP1* transcript levels. Plant J. **39**, 425–439.

Ernst W. H. O. (1974), Schwermetallvegetationen der Erde. Gustav Fischer Publ., Stuttgart, Germany.

Koch M. A., Haubold B., and Mitchell-Olds T. (2000), Comparative evolutionary analysis of chalcone synthase and alcohol dehydrogenase loci in *Arabidopsis*, *Arabis*, and related genera (Brassicaceae). Mol. Biol. Evol. **17**, 1483–1498.

Macnair M. R., Bert V., Huitson S. B., Saumitou-Laprade P., and Petit D. (1999), Zinc tolerance and hyperaccumulation are genetically independent characters. Proc. R. Soc. London B **266**, 2175–2179.

Martinoia E., Klein M., Geisler M., Bovet L., Forestier C., Kolukisaoglu U., Müller-Röber B., and Schulz B. (2002), Multifunctionality of plant ABC transporters – more than just detoxifiers. Planta **214**, 345–355.

Ortiz D. F., Ruscitti T., McCue K. F., and Ow D. W. (1995), Transport of metal-binding peptides by HMT1, a fission yeast ABC-type vacuolar membrane protein. J. Biol. Chem. **270**, 4721–4728.

Pearson W. R. and Lipman D. J. (1988), Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. USA **85**, 2444–2448.

Samuelian S., Kleine M., Ruyter-Spira C. P., Klein-Lankhorst R. M., and Jung C. (2004), Cloning and functional analyses of a gene from sugar beet up-regulated upon cyst nematode infection. Plant Mol. Biol. **54**, 147–156.

Sanchez-Fernandez R., Davies T. G., Coleman J. O., and Rea P. A. (2001), The *Arabidopsis thaliana* ABC protein superfamily, a complete inventory. J. Biol. Chem. **276**, 30231–30244.

Song W. Y., Sohn E. J., Martinoia E., Lee Y. J., Yang Y. Y., Jasinski M., Forestier C., Hwang I., and Lee Y. (2003), Engineering tolerance and accumulation of lead and cadmium in transgenic plants. Nat. Biotechnol. **21**, 914–919.

van den Brule S. and Smart C. C. (2002), The plant PDR family of ABC transporters. Planta **216**, 95–106.

van der Biezen E. A., Juwana H., Parker J. E., and Jones J. D. (2000), cDNA-AFLP display for the isolation of *Peronospora parasitica* genes expressed during infection in *Arabidopsis thaliana.* Mol. Plant. Microbe Interact. **13**, 895–898.

Weber M., Harada E., Vess C., von Roepenack-Lahaye E., and Clemens S. (2004), Comparative microarray analysis of *Arabidopsis thaliana* and *Arabidopsis halleri* roots identifies nicotianamine synthase, a ZIP transporter and other genes as potential metal hyperaccumulation factors. Plant J. **37**, 269–281.