Journal of Hazardous Materials 199–200 (2012) 309–313

Contents lists available at SciVerse ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

The assembly of metals chelation by thiols and vacuolar compartmentalization conferred increased tolerance to and accumulation of cadmium and arsenic in transgenic *Arabidopsis thaliana*

Jiangbo Guo[a,b], Wenzhong Xu[a], Mi Ma[a,*]

[a] Key Laboratory of Plant Resources, Institute of Botany, The Chinese Academy of Sciences, Beijing 100093, PR China
[b] Inner Mongolia Key Laboratory of Biomass-Energy Conversion, The Institute of Bioengineering and Technology, Inner Mongolia University of Science and Technology, Baotou 040100, PR China

ARTICLE INFO

Article history:
Received 1 September 2011
Received in revised form 2 November 2011
Accepted 3 November 2011
Available online 9 November 2011

Keywords:
Chelation
Phytochelatin synthase
Phytoremediation
Transgenic
Vacuolar compartmentalization

ABSTRACT

Transgenic *Arabidopsis thaliana* were developed to increase tolerance for and accumulation of heavy metals and metalloids by simultaneous overexpression of *AsPCS1* and *YCF1* (derived from garlic and baker's yeast) based on the fact that chelation of metals and vacuolar compartmentalization are the main strategies for heavy metals/metalloids detoxification and tolerance in plants. Dual-gene transgenic lines had the longest roots and the highest accumulation of Cd and As than single-gene transgenic lines and wildtype. When grown on cadmium or arsenic (arsenite/arsenate), Dual-gene transgenic lines accumulated over 2–10 folds cadmium/arsenite and 2–3 folds arsenate than wild type or plants expressing *AsPCS1* or *YCF1* alone. Such stacking modified genes involved in chelation of toxic metals and vacuolar compartmentalization represents a highly promising new tool for use in phytoremediation efforts.

1. Introduction

Phytoremediation, as an emerging, cost-effective, and noninvasive alternative or complementary technology based on the cellular mechanisms for heavy metal/metalloid detoxification and tolerance in plants [1], was used to clean up heavy metals/metalloids from the environment or to render them harmless recently [2]. The cellular mechanisms of plants potentially involved in detoxification, and thus tolerance of heavy metals and metalloids stress, are chelation of metals by thiols [3,4] and vacuolar compartmentalization by transporter families [5–7]. Phytochelatins (PCs) are the main thiol peptides to chelate metals in plant cytoplasm [8,9] and then the complex of these thiols with metals/metalloids are sequestered into vacuole via ATP binding cassette transporters (ABC transporters) such as YCF1 located in *Saccharomyces cerevisiae* tonoplast [10,11].

Many highly effective attempts were performed to develop transgenic plant for phytoremediation by respective overexpression of genes involved in PCs synthesis [3,12–14] and heavy metal vacuolar compartmentalization [6,7], but some reports have found the opposite results [15,16]. Cadmium (Cd) hypersensitivity in transgenic *PCS* lines may be due to the toxicity of PCs at supraoptimal levels when compared to glutathione (GSH) levels, and the supply of GSH in cells limits the synthesis of PCs in *AtPCS*-transgenics under Cd stress [17]. The vacuole is generally considered to be the main storage site for metals in yeast and plant cells and vacuolar compartmentalization of metals is also a part of the tolerance mechanism [5,7]. Hence we hypothesized that perhaps there was another possibility that heavy metals and its complex with thiols were not sequestered into vacuole efficiently and resulted in toxicity to plant cells and hypersensitivity to these metals.

Dhankher et al. [12], Wawrzynski et al. [18] and Guo et al. [14] proved that stacking genes that encode different components of the metal hyperaccumulation system in transgenic plants may produce plants ideally suited for phytoremediation of pollutant metals, such as Cd and arsenic (As). Dhankher and his colleagues showed that plants expressing *SRS1p*/*ArsC* and *ACT2p*/*γ-ECS* together accumulated 2–3 folds more arsenic per gram of tissue than wild type or plants expressing *γ-ECS* or *ArsC* alone. Wawrzynski et al. proved that simultaneous expression of heterologous genes involved in phytochelatin biosynthesis led to about 8-fold elevation of thiols in triple-gene transgenic lines than in wildtype plants. Chelation of metals by thiols and vacuolar compartmentalization are the main strategies of heavy metals/metalloids detoxification and tolerance in plants, and these two strategies are not independent during the detoxification of heavy metals/metalloids and vacuole

* Corresponding author.
E-mail address: mami@ibcas.ac.cn (M. Ma).

0304-3894/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2011.11.008

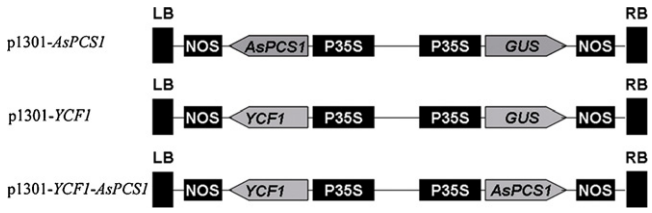

Fig. 1. Schematics of three binary vectors. *YCF1*: sequence encoding *Saccharomyces cerevisiae* YCF1, a transporter belongs to ABC family; *AsPCS1*: sequence encoding *A. sativum* phytochelatin synthase (EC: 2.3.2.15).

is the main storage site for these metals and their complex [1,7]. Therefore there must be the possibility that the amounts of heavy metal ions in transgenic plant cells are too large to be efficiently sequestered into the vacuole when overexpressed genes involved in PCs synthesis, consequently the jam of vacuolar compartmentalization leads to the hypersensitivity to Cd. To test this hypothesis, we constructed a binary vector carrying two genes involved in chelation and vacuolar compartmentalization and analyzed the effects of simultaneous overexpression of the garlic *AsPCS1* gene and the *S. cerevisiae YCF1* gene in *Arabidopsis thaliana* in the present study.

2. Materials and methods

2.1. Genes cloning, binary vectors construct and transgenic plants identification

AsPCS1 was cloned by RACE from garlic (*Allium Sativum* L.) [19,20] and harbored in the plasmids p35S-*AsPCS1-MT*. *YCF1* was amplified from *S. cerevisiae* strain S288C genomic DNA by the following oligonucleotides (YB: 5′GTG GAT CCA TGG CTG GTA ATC TTG TTT CAT GGG CCT3′ and YS: 5′ACG AGC TCT TAA TTT TCA TTG ACC AAA CCA GCC TCC ATG3′). The PCR products (4.6 kb) containing *YCF1* were inserted into *Bam*HI/*Sac*Isites of p35S-*AsPCS1-MT* by displacing the gene *MT* between the 35S promoter and terminator of cauliflower mosaic virus (CaMV), and the constructed binary vector was named p1301-*YCF1-AsPCS1*. Then the above 4.6 kb fragment was introduced into *Bam*HI/*Sac*Isites of pSN1301 (derived from pCAMBIA1301, CAMBIA) between the CaMV35S promoter and terminator of cauliflower mosaic virus (CaMV), namely binary vector p1301-*YCF1*. The schematic maps of three binary vectors were shown in Fig. 1.

Transgenic lines of *Arabidopsis thaliana* (ecotype Columbia) were obtained by Agrobacterium-mediated dip flora transformation as described by Clough and Bent [21]. The transgenic Arabidopsis seeds of T_1 generation were screened through hygromycin (Roche chemicals #843555) selection and then by genomic DNA PCR identification. Furthermore the transformants of *AsPCS1* or *YCF1* were confirmed by GUS assay. The expression of *YCF1* and *AsPCS1* on transcript levels in T_2, T_3 and T_4 transgenic plants were detected by RT-PCR (25–27 cycles). Total RNA isolated from 14-day-old transgenic and wildtype Arabidopsis seedlings using Trizol reagent (Invitrogen, #15596-026), were converted to cDNA using ReverTra Ace (TOYOBO, #TRT-101). *AsPCS1* and *YCF1* cDNA were amplified using the following specific primers, *AsPCS1*: 5′AGT CTT GAG GAT TTC CGC CAG CAT A3′ and 5′TGT GAT TTT GAT GGC GAT GTG GGT AC3′; *YCF1*: 5′ACA TCA AGG GAG TTG CGT CGT T3′ and 5′TCA TTG ACC AAA CCA GCC TCC ATG CAC3′. The *actin* primers were 5′TGT GTC TCA CAC TGT GCC AAT CTA CG3′ and 5′TTC CTG GAC CTG CCT CAT CAT ACT C 3′.

Plants were maintained in culture room at 23 °C under a 12-h photoperiod provided by cool-white fluorescent tubes at a photon flux density of approximately 80 $\mu E\,m^{-2}\,s^{-1}$.

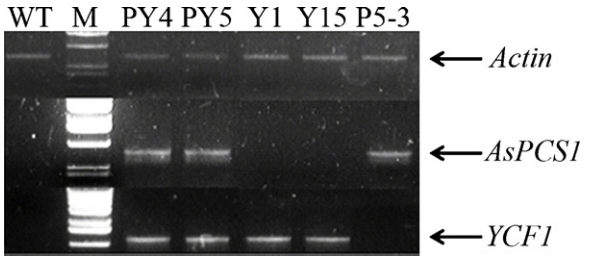

Fig. 2. Transcript expression of *YCF1* and *AsPCS1* in the T_4 generation of transgenic lines. WT: wildtype; M: 1-kb DNA ladders; PY4, PY5: *YCF1* and *AsPCS1* dual-gene transgenic lines; Y1, Y15: *YCF1* single-gene transgenic lines; P5-3: *AsPCS1* single-gene transgenic line.

2.2. Analysis of tolerance to and accumulation of Cd or As

Two-day-old seedlings germinated and grown on half-strength germination medium (GM, 1/2MS + 1% sucrose + 0.05% MES) were transfer to half-strength GM containing different concentration of Cd (supplied with $CdCl_2$) or As (supplied with $NaAsO_2$ and Na_3AsO_4, respectively). The above plates were vertically maintained in the culture room for 14 d to compare the root length of wildtype and transgenics. Every line was chosen at least 10 seedlings. The experiments were repeated three times.

Being kept treated by Cd or As for 7 d in plastic pots, aerial parts of plants grown in anthesis were harvested and washed thoroughly with distilled water three to four times and dried at 80 °C for 48 h. After weighing, the dried tissues were ground and then digested with a mixture of HNO_3/$HClO_4$ (85/15, v/v). The determination of Cd content was performed using an atomic absorption spectrophotometer (model 3300; PerkinElmer, Wellesley, MA, USA). As contents were determined using an Atomic Fluorescence Spectroscopy (model AFS-930; Jitian, CITY, China). The experiments were replicated two times independently. At least 10 seedlings were selected per line in the experiment.

2.3. Statistical analysis

Statistical analyses were accomplished with SPSS 13.0 (SPSS Inc., Chicago, IL, USA) and graphs were produced by SigmaPlot 10.0 (SYSTAT Software Inc.,). One-way ANOVA were performed, and means of data were compared using Duncan test at the 0.05 probability levels. In the figures, letters were used to indicate the levels of significance in the differences between non-modified and transgenic plants for heavy metal treatments. The results were based on at least three replicates from two independent experiments.

3. Results

3.1. Transformation of AsPCS1 and YCF1 into Arabidopsis thaliana

Experienced genomic DNA PCR identification and GUS assay (data not shown), two dual-gene transformed lines (PY4 and PY5, transformed by p1301-*YCF1-AsPCS1*) of T_4 generation and three single gene transformed lines (P5-3 transformed by p1301-*AsPCS1*, Y1 and Y15 transformed by p1301-*YCF1*) of T_4 generation were arbitrarily chosen for the future analysis. As shown in Fig. 2, an electrophoretic band appeared in the lines of PY4, PY5, Y1 and Y15 corresponding to *YCF1* fragment while P5-3 and wildtype were blank. And the same situation happened to *AsPCS1* in two dual-gene transformants while no apparent bands were detected in Y1, Y15 and wildtype. These results revealed that *AsPCS1* and *YCF1* were successfully transformed into *Arabidopsis thaliana* and expressed stably by CaMV 35S promoters.

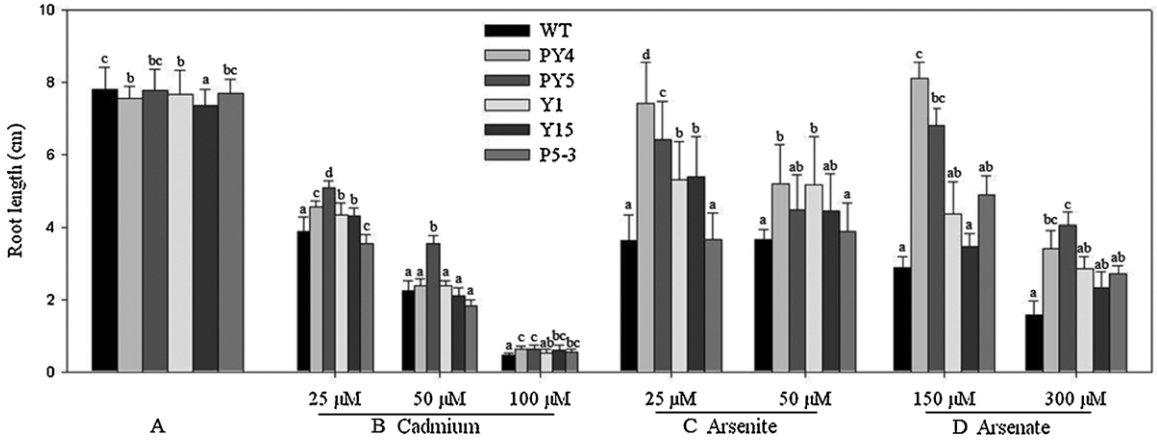

Fig. 3. Root elongation of transgenic lines subjected to cadmium or arsenic. WT: wildtype *Arabidopsis*; PY4, PY5: *YCF1* and *AsPCS1* dual-gene transgenic lines; Y1, Y15: *YCF1* single-gene transgenic lines; P5-3: *AsPCS1* single-gene transgenic line; A: control, 1/2 GM media; B: cadmium, 1/2 GM media containing different concentrations of $CdCl_2$; C: arsenite, 1/2 GM media containing different concentrations of $NaAsO_2$; D: arsenate, 1/2 GM media containing different concentrations of Na_3AsO_4. Values shown are means ± standard error. Bars with different letters are significantly different at $P \leq 0.05$ by Duncan test.

3.2. Transgenic plants exhibited different tolerance to Cd or As stresses

To evaluate the tolerance of transgenic *Arabidopsis*, seedlings were transferred to half-strength GM medium containing different concentrations of Cd or As to compare root elongation. After 14 d of growth on the half-strength GM medium, no visible differences in root length were found between transgenic lines and wildtype *Arabidopsis* (Fig. 3A). Significant differences in root length between transgenic lines and wildtype were detected when plants were subjected to 25 or 50 μM $CdCl_2$ (Fig. 3B). The dual-gene transgenic lines, PY4 and PY5, had longer roots length than single-gene transformed lines and wildtype ($P \leq 0.05$), although all of single-gene transgenic lines except P5-3 were more tolerant of Cd stress than wildtype plants ($P \leq 0.05$). PY5 had the longest root length of 7.7 and 3.6 cm exposed to 25 and 50 μM $CdCl_2$, respectively. When the $CdCl_2$ concentration reached 100 μM, root elongation was severely retarded in all plants.

Transgenic and wildtype *Arabidopsis* were exposed to different As species to evaluate their tolerance of As stress. Though the root growth of dual-gene transgenic lines were severely retarded when exposed to 25 μM arsenite, PY4 and PY5 showed the longest root length of 2.58 and 2.24 cm than other lines (Fig. 3C). There were no obvious differences in root elongation amongst all of transgenic Arabidopsis except P5-3 which still had longer root than wildtype under 50 μM arsenite stress. The root growth of dual-gene transgenic lines were not severely retarded when exposed to 150 μM arsenate while plants of the same lines suffered from severe retardation of root elongation under 300 μM arsenate treatment (Fig. 3D). PY4 and PY5 showed the longest root length of 4.9 and 4.1 cm in exposure to 150 μM arsenate.

3.3. Transgenic plants had increased production of PCs and accumulated higher amounts of Cd or As

PCs contents in leaves collected from transgenic and wild-type plants were determined before and after exposure to Cd for 3 d. Compared to the wildtype plants, PCs production in transgenic lines overexpressed *AsPCS1* increased over 2.5 times and about 2 times in *YCF1* lines under control conditions. Plants subjected to Cd stress had 2.7–3 times more total PCs than plants of the same lines grown without Cd (Fig. 4).

All of transgenic lines had higher accumulation of Cd than wildtype, thereinto three single-gene transgenic lines, P5-3, Y1 and Y15, had 4 to 5 times higher Cd contents than wildtype ($P \leq 0.05$) (Fig. 5A). Dual-gene transgenic lines simultaneously overexpressed *AsPCS1* and *YCF1* had higher amounts of Cd than other transgenic lines and wildtype ($P \leq 0.01$), for example they accumulated more than 10 mg kg^{-1} Cd, which was 8–10 times higher than in wildtype and nearly twice over in three single-gene transgenic lines in average.

Transgenic lines accumulated higher amounts of As than the wildtype when exposed to arsenite or arsenate (Fig. 5B and C). P5-3, Y1 and Y15 had over 3.4 times amounts of arsenic than wildtype under arsenite stress while PY lines accumulated at least 1.7 times than these single-gene transgenic lines. As contents in PY lines were at least 2.6 times higher than in wildtype and were over 1.6 times higher than in Y1 and P5-3 when plants were subjected to arsenate.

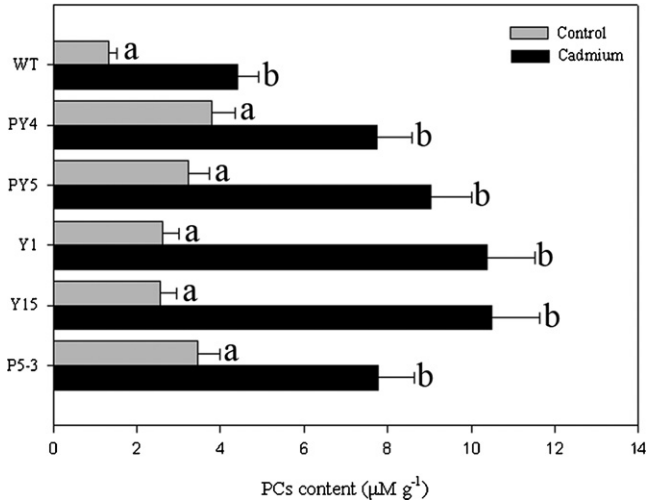

Fig. 4. PCs content in different transgenic lines before and after Cd exposure. WT: wildtype *Arabidopsis*; PY4, PY5: *YCF1* and *AsPCS1* dual-gene transgenic lines; Y1, Y15: *YCF1* single-gene transgenic lines; P5-3: *AsPCS1* single-gene transgenic line; control, 1/4 Hoagland culture solution; cadmium, supplied as $CdCl_2$; values shown are means ± standard error; LSD ≤ 0.05. Plants in anthesis were treated with 30 mg kg^{-1} Cd (supplied with $CdCl_2$) or 28 mg kg^{-1} As (supplied with arsenite and arsenate) for 3 d, respectively. PCs content in aerial parts of plants were determined as the method described in De Vos et al. [26] and He et al. [27]. The experiments were repeated three times. Values shown are means ± standard error. Bars with different letters are significantly different at $P \leq 0.05$ by Duncan test.

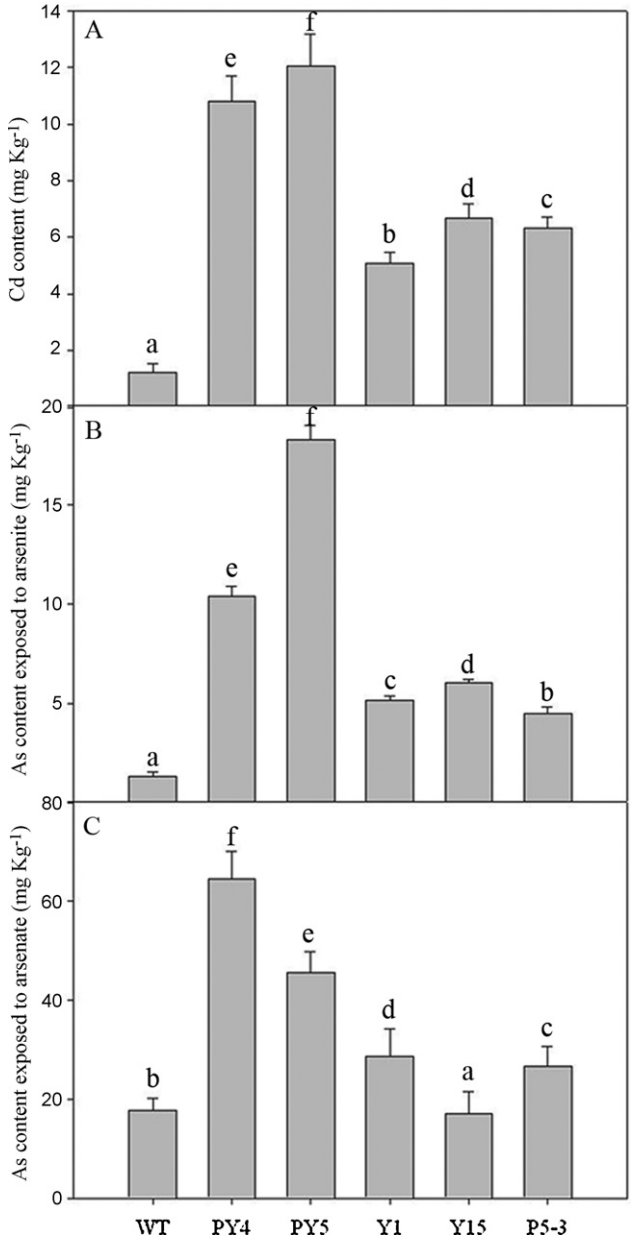

Fig. 5. Comparison of Cd or As contents in different transgenic lines in exposure to different heavy metals. (A) Cd contents under $CdCl_2$ exposure; (B) As contents under arsenite stress; (C) As contents under arsenate stress. WT: wildtype *Arabidopsis*; PY4, PY5: *YCF1* and *AsPCS1* dual-gene transgenic lines; Y1, Y15: *YCF1* single-gene transgenic lines; P5-3: *AsPCS1* single-gene transgenic line. Values shown are means ± standard error. Bars with different letters are significantly different at $P \leq 0.05$ by Duncan test.

4. Discussion

Since this century, many researchers tried to develop engineering plants for phytoremediation. Bang et al. [22] reported that expression of the thiosulfate reductase gene from *Salmonella typhimurium* in *Escherichia coli* led to increase the efficiency of removal heavy metals from solution and accumulation of cadmium up to 150 mM in 98% cells. Heterogeneous overexpression of gene *PCS* in *Nicotiana glauca* R. Graham (shrub tobacco) elevated the production of PCs and higher accumulation of double of lead (Pb) than in wildtype [23]. Song et al. [6] reported that the utility of the yeast protein YCF1 in *Arabidopsis thaliana* led for 1.2–2 folds increase of Pb and Cd contents in transgenic plants than in control lines, which provided a second-generation approach to engineering plants for phytoremediation [5,7]. The results of Cd accumulation in lines overexpressed *YCF1* in our study were in agreement with Song et al. [6] (Figs. 3 and 5). These lines overexpressed *YCF1* had higher contents of PCs (Fig. 4) and GSH (data not shown) than wildtype, maybe due to YCF1 is responsible for vacuolar sequestration of thiols and their complexes with metals [10]. This vacuolar sequestration of thiols perhaps put the balance move to synthesis of PCs and led to produce more amounts of PCs.

However, the reported studies paid more attention on single overexpression of genes involved in chelation or ions transportation, and some publications indicated that overexpression of genes involved in PCs synthesis in plants results in hypersensitivity to Cd [15,16]. In our study, P5-3, the line overexpressed *AsPCS1*, was not tolerant to Cd stress (Fig. 3B) though it had higher content of PCs (Fig. 4) and accumulated higher amounts of this metal (Fig. 5A) than wildtype. This result supported the above reports. Interestingly, the transgenic lines simultaneous overexpressed *AsPCS1* and *YCF1* gene had higher contents of PCs than wildtype before and after Cd exposure due to overexpression of foreign genes (Fig. 4). Dual-gene transgenic lines had the longest root elongation (Fig. 3B–D) and accumulated higher amounts of Cd or As (Fig. 5) than single-gene transformants, which showed that overexpression of genes involved in chelation and vacuolar compartmentalization led to increase the tolerance and accumulation to Cd and As, as well as supported our hypothesis.

Engineering plants for phytoremediation must have higher tolerant to heavy metals and be able to accumulate more amounts of these metals [1,3,4,7,8]. PCs chelate heavy metals to inactivate them while YCF1 pumps toxic metals into vacuole to store [5,11]. Overexpression of genes involved in PCs synthesis leads to increase the amounts of toxic metals in transgenic plants as previous reports [8,14,23–25], but not absolutely increase the tolerance to these metals [15] as the obtained results of P5-3 in our study (Fig. 3B). The substrates that YCF1 pumps into vacuole are organic compounds after their S-conjugation with glutathione and GSH–metal complexes [10]. If there are not enough substrates offered, transgenic plants overexpressed *YCF1* would not accumulate more amounts of toxic metals in their cells, which explained why Y1 and Y15 had lower amounts of Cd and As compared to PY4 and PY5 (Fig. 5) although Y1 and Y15 had higher level of these metals than wildtype as reported in Song et al. [6]. In our study, we put these two processes of chelation and vacuolar compartmentalization into an assembly simultaneously. Thus chelation of toxic metals by thiols will not limit the sequestration of these metals into vacuole because of overexpression of *AsPCS1*, at the same time the jam of vacuolar compartmentalization will not happen due to overexpression of *YCF1*. Tong et al. [5] proposed that vacuolar compartmentalization was a second-generation approach to engineering plants for phytoremediation compared to overexpression of genes involved to chelating peptides. Here our results indicated that simultaneous overexpression of genes involved to these two strategies of heavy metals/metalloids detoxification and tolerance was promising for phytoremediation and nowadays biotechnology for engineering plants was a vital guarantee for this stacking genes transformation.

5. Conclusion

In conclusion, we have demonstrated that simultaneous expression of *AsPCS1* and *YCF1* in Arabidopsis led to elevate the tolerance to Cd and As and have higher amounts accumulation of these metals than corresponding single-gene transgenic lines and wildtype. Such a stacking of modified genes involved in chelation of toxic metals by thiols and vacuolar compartmentalization represents a

highly promising new tool for use in phytoremediation for multiple heavy metals co-contamination.

Acknowledgements

We thank Prof. Yoshiharu Inoue, the Research Institute for Food Science, Kyoto University, for providing *Saccharomyces cerevisiae* strain S288C. This work was supported by the Knowledge Innovation Programs of CAS (KSCX2-YW-G053-4 and KSCX2-EW-J-1) and the National Natural Science Foundation of China (90713030).

References

[1] M. Mohanty, N.K. Dhal, P. Patra, B. Das, P.S. Reddy, Phytoremediation: a novel approach for utilization of iron-ore wastes, Rev. Environ. Contam. Toxicol. 206 (2010) 29–47.
[2] E. Pilon-Smits, Phytoremediation, Annu. Rev. Plant Biol. 56 (2005) 15–39.
[3] R. Blum, K.C. Meyer, J. Wunschmann, K.J. Lendzian, E. Grill, Cytosolic action of phytochelatin synthase, Plant Physiol. 153 (2010) 159–169.
[4] S. Hong-Bo, C. Li-Ye, R. Cheng-Jiang, L. Hua, G. Dong-Gang, L. Wei-Xiang, Understanding molecular mechanisms for improving phytoremediation of heavy metal-contaminated soils, Crit. Rev. Biotechnol. 30 (2010) 23–30.
[5] Y.P. Tong, R. Kneer, Y.G. Zhu, Vacuolar compartmentalization: a second-generation approach to engineering plants for phytoremediation, Trends Plant Sci. 9 (2004) 7–9.
[6] W.Y. Song, E.J. Sohn, E. Martinoia, Y.J. Lee, Y.Y. Yang, M. Jasinski, C. Forestier, I. Hwang, Y. Lee, Engineering tolerance and accumulation of lead and cadmium in transgenic plants, Nat. Biotechnol. 21 (2003) 914–919.
[7] W.Y. Song, J. Park, D.G. Mendoza-Cozatl, M. Suter-Grotemeyer, D. Shim, S. Hortensteiner, M. Geisler, B. Weder, P.A. Rea, D. Rentsch, J.I. Schroeder, Y. Lee, E. Martinoia, Arsenic tolerance in Arabidopsis is mediated by two ABCC-type phytochelatin transporters, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 21187–21192.
[8] P. Brunetti, L. Zanella, A. Proia, A. De Paolis, G. Falasca, M.M. Altamura, L. Sanita di Toppi, P. Costantino, M. Cardarelli, Cadmium tolerance and phytochelatin content of Arabidopsis seedlings over-expressing the phytochelatin synthase gene AtPCS1, J. Exp. Bot. (2011), doi:10.1093/jxb/err228.
[9] O. Zitka, O. Krystofova, P. Sobrova, V. Adam, J. Zehnalek, M. Beklova, R. Kizek, Phytochelatin synthase activity as a marker of metal pollution, J. Hazard. Mater. 192 (2011) 794–800.
[10] Z.S. Li, Y.P. Lu, R.G. Zhen, M. Szczypka, D.J. Thiele, P.A. Rea, A new pathway for vacuolar cadmium sequestration in *Saccharomyces cerevisiae*: YCF1-catalyzed transport of bis(glutathionato)cadmium, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 42–47.
[11] P.D. Adamis, A.D. Panek, E.C. Eleutherio, Vacuolar compartmentation of the cadmium–glutathione complex protects *Saccharomyces cerevisiae* from mutagenesis, Toxicol. Lett. 173 (2007) 1–7.
[12] O.P. Dhankher, Y. Li, B.P. Rosen, J. Shi, D. Salt, J.F. Senecoff, N.A. Sashti, R.B. Meagher, Engineering tolerance and hyperaccumulation of arsenic in plants by combining arsenate reductase and gamma-glutamylcysteine synthetase expression, Nat. Biotechnol. 20 (2002) 1140–1145.
[13] K. Gasic, S.S. Korban, Expression of *Arabidopsis phytochelatin* synthase in Indian mustard (*Brassica juncea*) plants enhances tolerance for Cd and Zn, Planta 225 (2007) 1277–1285.
[14] J. Guo, X. Dai, W. Xu, M. Ma, Overexpressing GSH1 AsPCS1 simultaneously increases the tolerance and accumulation of cadmium and arsenic in *Arabidopsisthaliana*, Chemosphere 72 (2008) 1020–1026.
[15] S. Lee, J.S. Moon, T.S. Ko, D. Petros, P.B. Goldsbrough, S.S. Korban, Overexpression of *Arabidopsis phytochelatin* synthase paradoxically leads to hypersensitivity to cadmium stress, Plant Physiol. 131 (2003) 656–663.
[16] Y. Li, O.P. Dhankher, L. Carreira, R.S. Balish, R.B. Meagher, Arsenic and mercury tolerance and cadmium sensitivity in Arabidopsis plants expressing bacterial gamma-glutamylcysteine synthetase, Environ. Toxicol. Chem. 24 (2005) 1376–1386.
[17] M. Pomponi, V. Censi, V. Di Girolamo, A. De Paolis, L.S. di Toppi, R. Aromolo, P. Costantino, M. Cardarelli, Overexpression of *Arabidopsis phytochelatin* synthase in tobacco plants enhances Cd(2+) tolerance and accumulation but not translocation to the shoot, Planta 223 (2006) 180–190.
[18] A. Wawrzynski, E. Kopera, A. Wawrzynska, J. Kaminska, W. Bal, A. Sirko, Effects of simultaneous expression of heterologous genes involved in phytochelatin biosynthesis on thiol content and cadmium accumulation in tobacco plants, J. Exp. Bot. 57 (2006) 2173–2182.
[19] Y.N. Jiang, B.M. Feng, H.Y. Zhang, M. Ma, Improving the heavy metal tolerance of yeast by transferring a phytochelatin synthase gene from garlic, Acta Phytoecol. Sin. 29 (2005) 57–63 (in Chinese, with English abstract).
[20] H. Zhang, W. Xu, J. Guo, Z. He, M. Ma, Coordinated responses of phytochelatins and metallothioneins to heavy metals in garlic seedlings, Plant Sci. 169 (2005) 1059–1065.
[21] S.J. Clough, A.F. Bent, Floral dip: a simplified method for Agrobacterium-mediated transformation of *Arabidopsis thaliana*, Plant J. 16 (1998) 735–743.
[22] S.W. Bang, D.S. Clark, J.D. Keasling, Engineering hydrogen sulfide production and cadmium removal by expression of the thiosulfate reductase gene (phsABC) from *Salmonella enterica serovar typhimurium* in *Escherichia coli*, Appl. Environ. Microbiol. 66 (2000) 3939–3944.
[23] C. Gisbert, R. Ros, A. De Haro, D.J. Walker, M. Pilar Bernal, R. Serrano, J. Navarro-Avino, A plant genetically modified that accumulates Pb is especially promising for phytoremediation, Biochem. Biophys. Res. Commun. 303 (2003) 440–445.
[24] C.G. Kawashima, M. Noji, M. Nakamura, Y. Ogra, K.T. Suzuki, K. Saito, Heavy metal tolerance of transgenic tobacco plants over-expressing cysteine synthase, Biotechnol. Lett. 26 (2004) 153–157.
[25] Y.L. Zhu, E.A. Pilon-Smits, A.S. Tarun, S.U. Weber, L. Jouanin, N. Terry, Cadmium tolerance and accumulation in Indian mustard is enhanced by overexpressing gamma-glutamylcysteine synthetase, Plant Physiol. 121 (1999) 1169–1178.
[26] C.H. De Vos, M.J. Vonk, R. Vooijs, H. Schat, Glutathione depletion due to copper-induced phytochelatin synthesis causes oxidative stress in *Silene cucubalus*, Plant Physiol. 98 (1992) 853–858.
[27] Z. He, J. Li, H. Zhang, M. Ma, Different effects of calcium and lanthanum on the expression of phytochelatin synthase gene and cadmium absorption in *Lactuca sativa*, Plant Sci. 168 (2005) 309–318.