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Research Article

Arsenate reductase gene from *Pityrogramma calomelanos* L. enhances tolerance to arsenic in tobacco

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Abstract

Arsenic (As) contamination in soil, water and air is an alarming issue worldwide and has serious effects on human health and environment. Arsenic is a naturally occurring element found in rocks, soil, and water, and exposure to high levels of arsenic can lead to a range of health problems. The effects of arsenic contamination can also be felt in the environment, as it can harm plants and animals and disrupt ecological systems. The major purpose of this study was to produce transgenic plants with improved tolerance to and accumulation of arsenic via transformation of arsenate reductase gene (*ArsC*) into tobacco genome. Transgenic plants were screen by PCR and southern blot. Further, their tolerance and accumulation to arsenic were evaluated. In the result, we have cloned, characterized, and transformed the *ArsC* gene from *Pityrogramma calomelanos* L. (*PcArsC*). Its phylogenetic analysis revealed 99% homology to ArsC gene in GenBank (accession number X80057.1). Moreover, Southern blot analysis showed that *ArsC* gene was integrated into the tobacco genome as a single-copy. These single-copy transgenic lines showed much higher tolerance to and accumulation of As than wild type, with no other phenotypes observed. These results demonstrated that *Pityrogramma calomelanos ArsC* gene can improve arsenic tolerance and accumulation in transgenic tobacco lines. Thus, using *Pityrogramma calomelanos* L. *ArsC* gene for genetic engineering has potential implications in the decontamination of arsenic-containing soil.

Keywords: *ArsC*; arsenic tolerance; phytoremediation; *Pityrogramma calomelanos* L.

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Introduction

Contamination of toxic metals in soil, waste areas and groundwater have harmful effects on human health. It is necessary to develop effective technologies to solve this problem. Phytoremediation is a bioremediation process that utilizes plants to remove contaminants without the need to collect and discard them elsewhere (Ashraf *et al*., 2015). This method has gained popularity due to its numerous advantages, including large scale application, low cost, and environmental friendliness. After being proved successful at Chernobyl in 1986, this technology began to be applied more widely (Lyubenova *et al*., 2009). The past decade has witnessed encouraging results that opened up opportunities for developing phytoremediation technologies and for applying genetic engineering to generating heavy metal-hyperaccumulating plants (Dhankher *et al.*, 2002; Yang *et al.*, 2005; Li *et al.*, 2006; Sarma, 2011).

Studying genes involved in the uptake, accumulation and transformation of heavy metals contributes to the understanding of molecular mechanisms underlying these biological processes as well as the development of genetically-modified plants that can be used as phytoremediators. Much progress has been made in the study of heavy metal tolerance in plants. Genetic engineers have already begun to successfully enhance the capacity to tolerate and accumulate heavy metals in plants. For the uptake and transport of heavy metals, critical gene families have been identified, including ion- regulated transporter (IRT) (Milner *et al*., 2013), ZRT, IRT-like Protein and metal transporter gene family members as natural resistance-associated macrophage protein (Ullah *et al*., 2018). Genes reported to play a role in the accumulation and chelation of heavy metals are *phytochelatin* (PC) – *CAD1* (cadinene synthase gene), *GmPCS1* (phytochelatin synthase) and *metallothionein* (MT) – *MT1*, *MT2* genes (Liu *et al.*, 2021). These genes are mostly universal in plants. In Arabidopsis plants, the *CAX1* (Cation exchanger1), *CAX2* were reported to encode the metal transporter (Hirschi *et al*., 1996). *AtACR2* gene of *Arabidopsis thaliana* was transferred into the genome of tobacco (*Nicotiana tabacum* var. 'Sumsun'). These transgenic tobacco plants were significantly more tolerant of arsenic compared to wild type (Nahar *et al.*, 2017). The transgenic *Arabidopsis thaliana* has also been used to test the function of new genes involved in arsenic tolerance. New glutaredoxin gene - *PvGRX5* transferred *Arabidopsis thaliana* increased significantly arsenic tolerant to compared with control lines when arsenic treatment. These results suggested that *PvGRX5* from As hyperaccumulating ferns can be employed as a novel biotechnological solution for decreasing As pollution in crops (Sundaram *et al*., 2009).

Both arsenic (As) and cadmium (Cd) are common, harmful pollutants in Vietnam and worldwide. Previous research has discovered that some plants have the capacity to accumulate As, such as rice, *Holcus lanatus,* watercress, ferns, duckweed, Indian mustard and *Eleusine indica* (Nwaichi and Dhankher, 2016). Transgenic approaches have been developed for As phytoremediation technologies. Characterization of the Ars genotype in 17 bacterial isolates (from Mandovi and Zuariestuarine water systems) revealed ArsA (ATPase), ArsB (arsenite permease) and ArsC (arsenate reductase) genes on their plasmid DNA. Phylogenetic analysis of *ArsB* and *ArsC* genes indicated their close genetic relationship with plasmid-born *Ars* genes of *E. coli* and arsenate reductase of plant origin (Sri Lakshmi Sunita *et al*., 2012). The arsenate reductase gene *PvACR2* was reported to be isolated from *Pteris vittata*, an As hyper-accummulating fern (Ellis *et al.*, 2006). Transgenic seeds could germinate in the presence of 80 µM As (III) or 1200 µM arsenate [As(V)] treatment (Chen *et al*., 2013). Overexpression of garlic *AsPCS1* and yeast *GSH1* in *Arabidopsis thaliana* resulted in transgenic plants with high accumulating capacity of cadmium (Cd) and As. Both single-gene transgenic lines and double transformants exhibited significantly higher tolerance to and accumulated more Cd and As than the wild-type (Guo *et al.*,, 2008). Transfer of *Escherichia coli arsC gene* encoding arsenase reductase with soybean rubisco promoter (SRS1p) into *Arabidopsis* plants produced transgenic plants with higher As tolerance (Dhankher *et al*., 2002).

As the discovery of *P. vittata, P. calomelanos* and other hyperaccumulators is likely to benefit arsenic removal, scientists are focusing on optimizing the ability to accumulate this toxin and detoxify contaminated soil (Chen *et al.*, 2002). In a recent study evaluating heavy metal accumulation in three species *Pityrogramma calomelanos*, *Cynodon dactylon* and *Nephrolepis biserrata,* it was shown that these three species were able to take up As in the shoot and Cu in the root. Aresenate uptake in *Nephrolepis biserrata* was the highest, followed by *Cynodon dactylon* and *Pityrogramma calomelanos* (Hidalgo *et al.*, 2020). In Vietnam, the first detailed investigation of soil contamination at various mine sites was published in 2018. A total of 33 different plant species samples were collected. *P. vittata.* and *P. calomelanos.* were the only two ferns identified as As hyper-accumulators, containing more than 0.1% heavy metals in their shoots. This is a potential genetic resource that could produce plants with large biomass and strong suitability for As-contaminated areas (Anh *et al.*, 2018).

In this study, we isolated a gene belonging to the ArsC family from *P. calomelanos*, a local fern that can strongly accumulate As. The function of ArsC gene in tolerance and accumulation of As in transgenic tobacco lines was evaluated. We also investigated whether ArsC gene integration disturbed the overall growth of transgenic lines.

Materials and Methods

Plant materials and growth conditions

P. calomelanos plants were collected at Ha Thuong mine, Thai Nguyen (Vietnam), whose As level is 202-3690 ppm, 300 times higher than the national safety standard. These plants were used to isolate ArsC gene (Figure 1).

Figure 1. *Pityrogramma calomelanos* L.

Tobacco *Nicotiana tabacum* K326 plants were used for the ectopic overexpression experiments and grown on a modified Murashige and Skoog (MS) medium containing 4.3 g/l MS salt mixture, supplemented with 0.2 mg/l of 2,4-dichlorophenoxyacetic acid (2,4-D), 0.18 g/l KH_2PO_4, 0.1 g/l myo-inositol, 1 mg/l thiamine HCl, and 30 g/l sucrose, which was designated as MST. All tobacco plants, including germinating seeds and seedlings, were grown in a controlled environment greenhouse at 26 °C, with a 16-h light/8-h darkness photoperiod, and a light intensity of 120 mmol m^{-2} s^{-1}.

Cloning of ArsC

Total RNA was isolated using TRI Reagent TM Solution (Ambion, USA). 1 μg of total RNA and an oligo dT18 primer were used for reverse transcription (Thermo Scientific, USA). ArsC full length sequence was amplified from the first strand cDNA obtained from *P. calomelanos*. by polymerase chain reaction (PCR) using ArsC specific primers. The primers used for the amplified genes were *ArsC* (forward) 5′-

TACCATGGACTGATATGAGCAACATTACC-3′ (reverse) 5′-TACACGTGTTATTTCAGGCGCTTA-CC-3′; The amplified product was purified and cloned into the pJET1.2/blunt cloning vector (Thermo Scientific, USA).

Construction of pCambia 1301- ArsC vector

Isolation of the ArsC gene and construction of a binary vector for plant transformation were performed as described by Luong (2016). Briefly, the *ArsC* containing fragment was cut out from the pJET1.2/blunt cloning vector by restriction enzymes *Nco*I and *Eco*721. The pCambia1301 expression vector was digested with *Nco*I and *Eco*721 and ligated to the *ArsC* containing fragment with the T4 DNA ligase (Thermo Scientific, USA). The new plant expression vector was designated pCambia1301- *ArsC*. The pCambia1301 expression vector (Figure 4) contains the ArsC gene driven by the 35S promoter and terminated by nopaline synthase terminator (nos). This vector was introduced into electrocompetent *Agrobacterium tumefaciens* strain C58 (BTX® ECM® 600 electroporator). Transformed cells were selected using medium containing hygromycin B (50 µg/ml) and cefotaxime (500 µg/ml). Successful transformants were confirmed by PCR amplification with ArsC specific primers (Table 1) and stored at –70 °C.

Arsenic tolerance assay in E. coli

Tolerance assay in *E. coli* was evaluated via colony formation assay. *E. coli* containing pCambi1301 or *pCambia1301-ArsC* were grown in LB liquid medium to OD 0.8 – 1, followed by a dilution to OD 0.1 and 0.01. Five microliters of each dilution were placed on the LB plates containing different concentrations of arsenic (0, 25 and 50 µM). The plates were incubated overnight. The surface areas of colonies were measured using a Nikon microscope (Nikon Instech, Co., Ltd.) and iSolution FL Autosoftware (IMT i-solution Inc.). Three replicates were performed.

Transformation and screening

Transformation was performed using *Agrobacterium tumefaciens*-mediated method (Krugel, 2002). Briefly, 2 mm in diameter of K326 leaves were mixed with 100 µl of a fresh overnight culture of *A. tumefaciens* C58. After 48-hour cocultivation at 27 °C in the dark, bacterial cells were washed in sterile liquid MST, and pieces of leaves were plated on MST medium (0.8% agar) containing hygromycin B (50 µg/ml) and cefotaxime (500 µg/ml). Hygromycin B-resistant transformants were visible within 3 weeks and transferred to fresh MST agar containing selective antibiotics. Viable putative transformants were transferred to soil in the greenhouse.

Molecular analysis of transgenic plants

To select tobacco lines transformed with ArsC, putative transgenic plants were primarily screened by PCR. PCR-positive transgenic tobacco lines were further analyzed by southern blot hybridization. Southern blotting was performed using Biotin DecaLabel DNA Labeling kit and Biotin Chromogenic Detection kit (Thermo Scientific, USA). Briefly, genomic DNA of transgenic lines and WT were digested into small fragments using *NcoI*. Digested genomic DNA was separated by gel electrophoresis, and then transferred to nitrocellulose membranes. DNA fragments were fixed onto nitrocellulose via UV light. Target DNA was detected by a labeled probe complementary to the target DNA.

Estimation of arsenic content in transgenic plants

To evaluate the tolerance and accumulation of As in tobacco, T2 seeds from transgenic T1 lines showing strong expression of *ArsC* gene and containing only one copy of the integrated T-DNA, were germinated on MS medium supplemented with hygromycin B (50 µg/ml). Seeds of wild type plants were germinated on hygromycin B - free MS medium. Seedlings expressing *ArsC* gene and harboring a single insertion of integrated transgene as well as wild type seedlings of the same size and age (21 days old) were transferred to pots containing

agar-solidified MS medium or soil in the greenhouse supplemented with different concentrations of AsV (0, 50, 100 and 200 μM) (Nahar et al., 2012; 2017). Growth conditions were the same as described above. Each treatment was done in triplicate. Plants were collected at day 40 and washed thoroughly with distilled water to remove any residual As adhering to the root surface. All ground materials were stored at 4 °C until further use. To quantify As content, dried samples were mixed with 2 mL of HNO_3 (65%, Merck, Darmstadt, Germany) and 6 mL of HCl (37%, Merck). Mixture was heated to 70 °C for 1 h, and then diluted with 10 mL of deionized water. The acid digestion was filtered to remove residual particulates. Arsenic concentration was determined by inductively coupled plasma mass spectroscopy (ICP-MS) method.

Statistical analysis

All experiments were performed in triplicate. Data were expressed as means ± standard error of results. Significance between two groups determined in this study was test by the Student's t-test, and analysis of variance was utilized between three or more groups. P-values less than 0.001 are considered statistically significant. All statistical analyses were performed by Sigma Plot software.

Results

Isolation of ArsC gene from Pityrogramma calomelanos

Total RNA isolation from selected plants was performed according to the manufacturer's protocol. Total RNA with high quality was used as template RNA for the cDNA synthesis reaction (Figure. 2A). PCR results showed that DNA fragment with the expected size (426 bp) was amplified from the first strand cDNA of *P. calomelanos* (Figure 2B). These PCR products were purified and cloned into the pBT cloning vector. The ligation mixture was used directly to transform E. coli DH5α. Colonies containing the expected size (426 bp) was confirmed by PCR amplification with *ArsC* specific primers (Figure 2 C). These results suggested that *ArsC* gene from *P. calomelanos* was successfully cloned into pBT cloning vector.

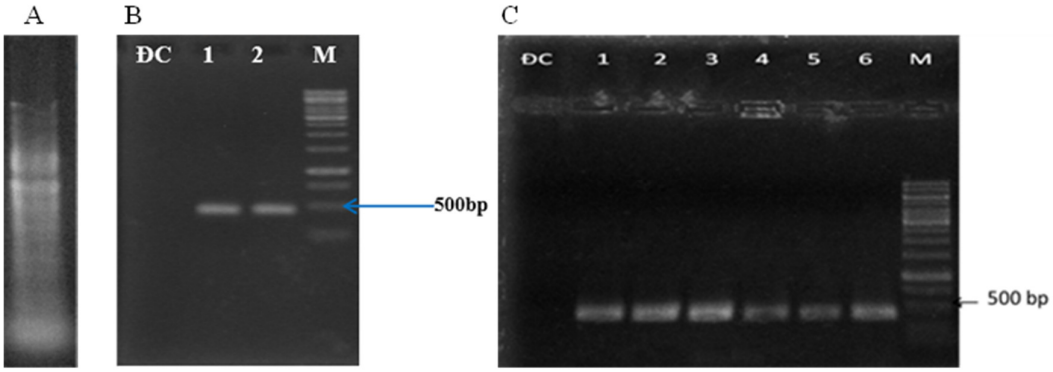

Figure 2. Isolation of ArsC gene from *Pityrogramma calomelanos* (A) Analysis and detection of total RNA by 1% agarose gel electrophoresis; (B) PCR amplification of cDNA with ArsC gene specific primers, ĐC negative control, 1-2 sample, (C) PCR product from colonies containing gene ArsC, ĐC negative control, 1-6 colonies.

Protein and nucleotide sequence of the ArsC genes

The nucleotide sequence of the *ArsC* gene was shown in Figure. 3 A. This sequence comprises 426 nucleotides and contains one open reading frame. The open reading frame encodes a protein of 141 amino acids (Figure 3B). Sequencing analysis revealed that the *ArsC* clone from *P. calomelanos* and *ArsC* gene in GenBank with accession number X80057.1 shared high similarity (99%) with each other. Using BioEdit

software, we compared nucleotide sequences and translated into protein sequence. Compared to the *ArsC* gene in GenBank (accession number X80057.1), *ArsC* gene sequence from *P. calomelanos* differed by one position (32), Thymine instead of Cytosine (Figure 3A). The deduced amino acid at position 11 was Alanine instead of Valine (Figure 3B).

Figure 3. Sequence alignment of ArsC with its homologous sequences, ArsC gen ArsC (X-A) Gene Bank number (X80057.1). (A) nucleotide sequence, (B) amino acid sequence of gene ArsC

Construction of pCambia1301-ArsC

To express target gene in transgenic tobacco lines, we used *pCambia1301* vector that contains a 35S promoter. The 35S promoter is a strong constitutive promoter and has been reported to upregulate gene expression in dicots. To design *pCambia1301-ArsC* vector, *pCambia1301* vector and pBT vector containing *Ars*C gene were both digested by *Nco*I and *Eco72*I. *pCambia1301* vector without *GUS* gene (9823 bp in length) and *ArsC* gene were purified, ligated by T4 ligase and transformed into competent *E. coli* DH5α cells. *pCambia1301* vector containing *Ars*C gene was confirmed by PCR amplification with ArsC-specific primers and restriction enzymes (Figure 4).

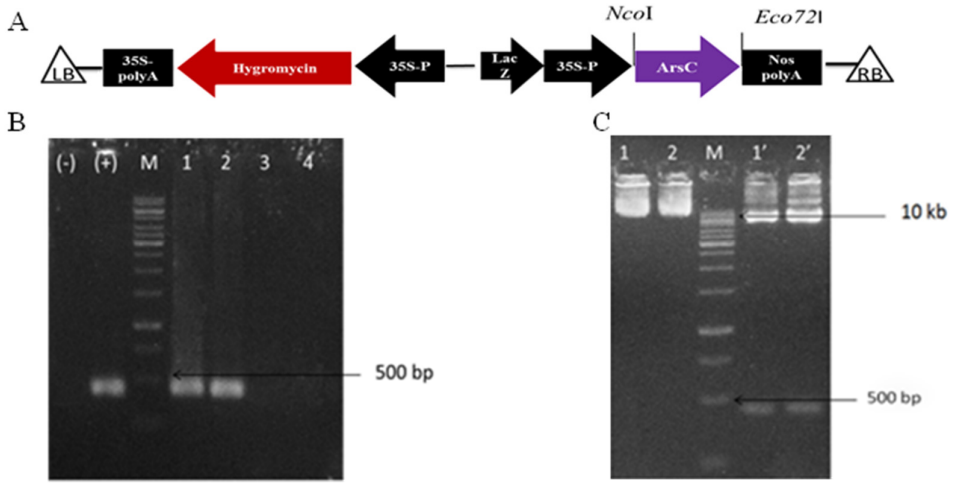

Figure 4. Analysis and detection of pCambia1301-ArsC plasmid structure for plant transformation. (A) schematic diagram of the binary vector pCambia1301 with 35S promoter, hygromycin gene conferring kanamycin resistance. (B) PCR product from coloníes containing ArsC gene (-) negative control, (+) positive control, 1-4 colonies, (C) enzyme digested product of pCambia1301-ArsC vector, 1-2 plasmid without restriction enzyme treatment, 1'-2' plasmid treated with restriction enzyme.

Molecular analysis of transgenic tobacco lines

Tobacco cultivar 'K326' (Wilt type –WT) was selected as the starting material due to its excellent regeneration ability. Transgenic tobacco lines were screened by PCR amplification. PCR results showed that 6 independent transgenic lines with the expected size of DNA fragment were observed. There was no DNA amplicon of interest in the non-transgenic lines or the blank control (Figure 5 A). Sequencing analysis confirmed that this amplified band was precisely the 426 bp partial sequence of *ArsC*. These results suggested that the *ArsC* gene was successfully inserted into tobacco genome.

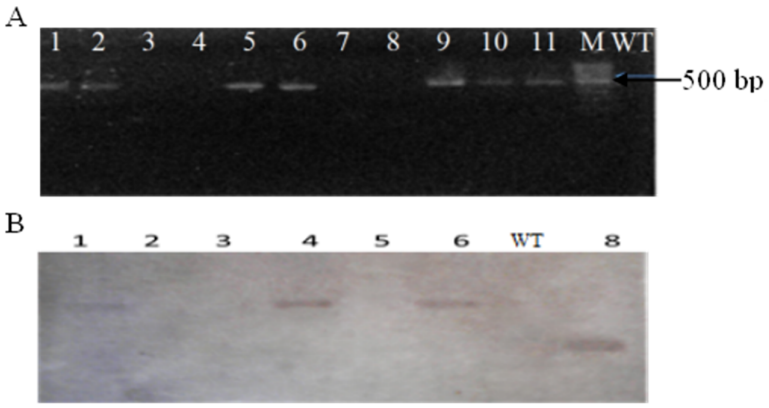

Figure 5. Molecular analysis and detection of ArsC transgenic tobacco plants. (A) PCR amplification of genomic DNA of wild type and transgenic tobacco plants. Lane 1, positive control pBT-ArsC plasmid; lanes 2-11, amplification from individual transgenic tobacco plants; M marker, WT wild type plant DNA; (B) Southern blot results from transgenic and wild type plant, 1-6 transgenic lines, WT wild type plant, 8 lane positive control- ArsC gene PCR product.

To verify the number of gene copies in transgenic lines, we performed southern blot analysis with a specific probe designed to complement target DNA. All 6 transgenic lines (T1) were selected by hygromycin resistance and confirmed by PCR (Figure 5B). There were 3 transgenic lines (K1, K9 and K18) containing a single-copy of integrated T-DNA. No hybridization signal was observed in the three remain lines or the non-transgenic control. In addition, plants in the T2 generation containing a single copy of the transgene showed similar results. These data demonstrated that three transgenic lines were stably inherited. Three transgenic lines with a single copy of integrated transgene were selected for further investigation

Arsenic inhibited plant growth

To evaluate the effect of As exposure on plant growth, plant height, leaf area, and fresh weight in transgenic lines and wild type plants were measured. There was no significant difference in plant height, leaf area, and fresh weight between transgenic lines and wild type plants in the absence of As (control treatment) and at a low concentration (50 µg/ml). However, plant growth was significantly restricted at high As concentrations (100-200 µg/ml) both *in vitro* and in greenhouse experiment; no wild type plants survived (Figure 6 A-B). In particular, As inhibited plant growth in a dose dependent manner. Interestingly, all transgenic lines showed significantly less stunted growth than wild type when plants were treated with 100-200 µg/ml of As (Figure 6C to 6E) and data not shown for *in vitro* experiment). Taken together, these results suggested that As had inhibitory activity against plant growth. However, ArsC gene in transgenic lines conferred higher resistance to As.

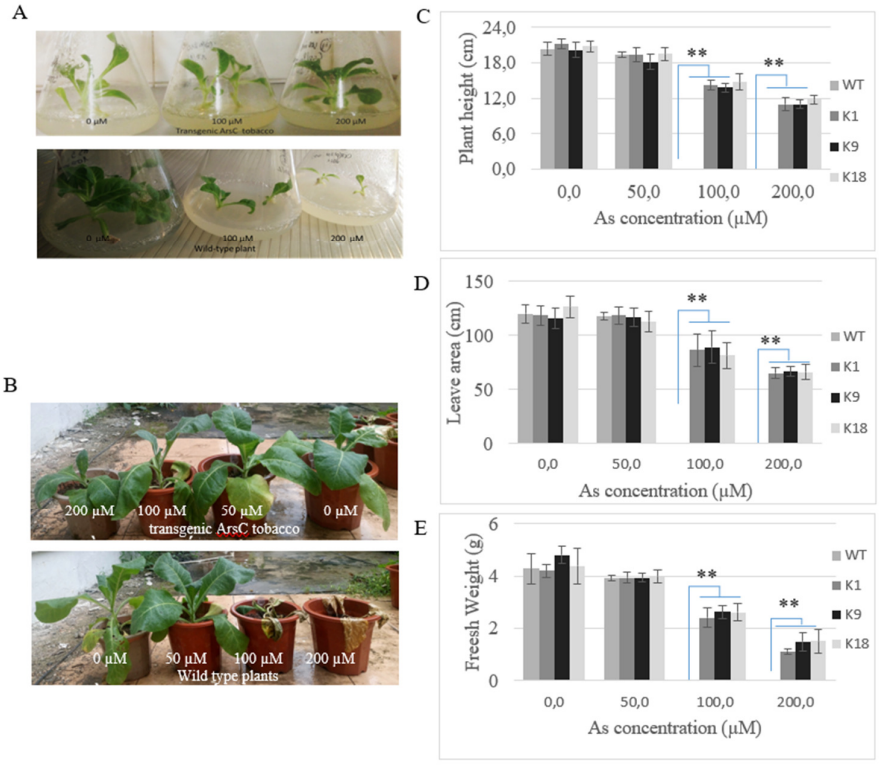

Figure 6. Comparisons of growth parameters between transgenic lines and WT under the different As concentration. (A) transgenic lines and WT in vitro, (B) transgenic lines and WT in greenhouse, (C) Comparisons of plant height, (D) leaf area, (E) fresh weight.

Quantitative data were obtained from three independent experiments, n = 3. Data represent the mean±S.E.M. (standard error of the mean) **p<0.001 compared to treated wild type plants.

Assessment of As accumulation in transgenic tobacco lines

To investigate the role of *Asr*C gene in As accumulation capacity in tobacco, an ICP-MS analysis was conducted on roots of plants grown in the presence or absence of 100, 200 µg/ml As for 3 weeks (Figure 7). When plants were treated with 50 µM arsenate, the amount of arsenate accumulated in the roots of the transgenic lines were approximately three-fold higher (7.133 µg/g dry wt) than that found in the wild type (2.276 µg/g dry wt). In addition, in exposure to 100 or 200 µM arsenate, transgenic lines were able to accumulate up to 17.30 µg/g dry wt of arsenate in their roots, whereas the wild type could not survive. It is important to note that no significant difference in the ability to take up arsenite was observed among three transgenic lines. These results indicated that AsrC gene improved As accumulation capacity in tobacco.

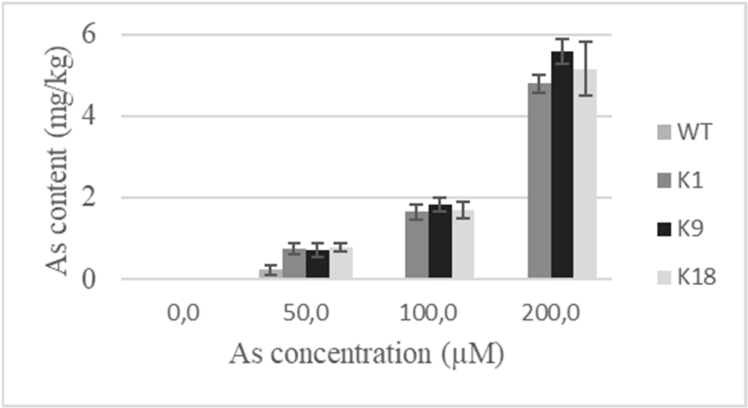

Figure 7. Accumulation of As in root of WT and transgenic lines. Data were taken on the 40th day after starting the experiment

Quantitative data were obtained from three independent experiments, n = 3. Data represent the mean ±S.E.M. (standard error of the mean). **p<0.001 compared to treated wild type plants.

Discussion

In the present study, we cloned and characterized ArsC gene from *P. calomelanos*. Moreover, we have generated transgenic tobacco lines expressing *P. calomelanos* ArsC gene by using Agrobacterium tumefaciens-mediated method. Transgenic tobacco lines significantly enhanced As accumulation capacity in roots. The effect of gene integration into tobacco genome on plant growth was evaluated.

Many efforts have been made to improve the phytoremediation efficiency of plants. Transgenic plants contain potential candidate genes involved in arsenic accumulation, such as PCS1, ACR, MRP1 and MRP2. Of all *ACR* is one of the key genes. It was first isolated from bacteria and yeast (Mukhopadhyay *et al*., 2000). ACR gene encodes arsenate reductase, an enzyme converting arsenate (AsV) to arsenite (As III) and the arsenite form can be sequestered in the vacuoles of aplant cell (Xu et al., 2007; Zhao *et al.*, 2012). The reduction of AsV to AsIII also occurs nonenzymatically via glutathione (Siddiqui *et al*., 2015). In the presence of glutathione, AsV can be hydrolyzed to AsIII and this process depends on intracellular availability of substrates and effectors (Németi *et al*., 2011). Reduction of AsV to AsIII has been known as the major mechanism of As resistance in plants that are able to hyperaccumulate As (Pickering *et al.,* 2006; Schmöger *et al.*, 2000). In addition, hyperaccumulators tend to transfer As immediately to other organs like leaves or shoots instead of retaining As in their roots. This translocation, as seen in *P.vittata*, further enhances As uptake (Singh and Ma 2006). In this study, we isolated *ArsC* gene from *P. calomelanos*. Sequencing results revealed that our ArsC gene sequence was highly similar to previously reported ACR genes. Several homologous proteins from Arabidopsis

(AtAsr/AtACR2), *P. vittata* (PvACR2), and rice (OsACR2.1 and OsACR2.2) have been known to possess ACR activity (Dhankher *et al.*, 2002; Ellis *et al.* 2006).

Southern blotting is a powerful tool to analyze copy number and locus complexity in transgenic plants. In our study, we used southern blot to determine the number of integrated T-DNA copies in transgenic lines. The number of integrated T-DNA copies also affects to the growth of transgenic lines. In this study, the transgenic lines harboring single copy of the integrated transgene were selected.

In this study, transgenic lines and wild-type plants grew well under normal growth condition and at low As concentrations. No significant difference in growth parameters, including plant height, leaf area, and fresh weight, was observed between the transgenic lines and wild-type plants. However, these growth readouts for both plant types, especially wild-type plants, were significantly lower in the presence of high As. Interestingly, transgenic lines containing a single copy gene showed strong resistance to As and higher As accumulation capacity compared to wild type. Molecular mechanisms underlying As resistance in these transgenic lines remain to be investigated.

A better understanding of As tolerance is conducive to generating As-resistant plants and vital to phytoremediation and safe cropping. For soil remediation and contaminated site rehabilitation, a diverse group of resistant plants suitable for growth in a wide range of environments and able to (hyper) accumulate As in harvestable biomass are needed. In contrast, for safe cropping, As-resistant plants that can detoxify the surrounding groundwater and land and prevent As accumulation in plant products of interest are required.

Conclusions

In this study, we successfully generated three transgenic tobacco lines containing *ArsC* gene from *P. calomelanos*. These transgenic accumulated a much higher amount of arsenic in the roots compared to wild-type plants. These results suggested that *P. calomelanos*. ArsC gene has a significant potential in genetic engineering of plants to produce transgenic plants that reduce arsenic content of soil.

Authors' Contributions

TVN and TTBL conceived and designed the experiments; HTH, TTD, NTN, LTTN and LTT performed the experiments; TVN TTBL analyzed the data, TVN, TLTB wrote the paper. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Anh BTK, Minh NN, Ha NTH, Kim DD, Kien N, Trung NQ, Cuong TT, Danh LT (2018). Field survey and comparative study of *Pteris vittata* and *Pityrogramma calomelanos* grown on arsenic contaminated lands with different soil pH. Bulletin of Environmental Contamination and Toxicology 100:720-726. *https://doi.org/10.1007/s00128-018-2325-5*

Ashraf U, Kanu AS, Mo Z, Hussain S, Anjum SA, Khan I, Abbas RN, Tang X (2015). Lead toxicity in rice: effects, mechanisms, and mitigation strategies–a mini review. Environmental Science and Pollution Research 22:18318-18332. *https://doi.org/10.1007/s11356-015-5463-x*

Chen T (2002). Arsenic hyperaccumulator *Pteris vittata* L. and its arsenic ac-cumulation. Chinese Science Bulletin 47:902. *https://doi.org/10.1360/02tb9202*

Chen Y, Xu W, Shen H, Yan H, Xu W, He Z, Ma M (2013). Engineering arsenic tolerance and hyperaccumulation in plants for phytoremediation by a PvACR3 transgenic approach. Environmental Science & Technology 47:9355–9362. *https://doi.org/10.1021/es4012096*

Dhankher OP, Li Y, Rosen BP, Shi J, Salt D, Senecoff JF, Sashti NA, Meagher RB (2002). Engineering tolerance and hyperaccumulation of arsenic in plants by combining arsenate reductase and γ-glutamylcysteine synthetase expression. Nature Biotechnology 20:1140-1145. *https://doi.org/10.1038/nbt747*

Ellis DR, Gumaelius L, Indriolo E, Pickering IJ, Banks JA, Salt DE (2006). A novel arsenate reductase from the arsenic hyperaccumulating fern *Pteris vittata*. Plant Physiology 141:1544-1554. *https://doi.org/10.1104/pp.106.084079*

Guo J, Dai X, Xu W, Ma M (2008). Overexpressing GSH1 and AsPCS1 simultaneously increases the tolerance and accumulation of cadmium and arsenic in *Arabidopsis thaliana*. Chemosphere 72:1020-1026. *https://doi.org/10.1016/j.chemosphere.2008.04.018*

Hidalgo Ancheta M, O Quimado M, L Tiburan Jr C, Doronila AS, Fernando E (2020). Copper and arsenic accumulation of *Pityrogramma calomelanos*, *Nephrolepis biserrata*, and *Cynodon dactylon* in Cu- and Au- mine tailings. Journal of Degraded and Mining Lands Management 7:2201-2208. *https://doi.org/10.15243/jdmlm.2020.073.2201*

Hirschi KD, Zhen RG, Cunningham KW, Rea PA, Fink GR (1996). CAX1, an H+/Ca2+ antiporter from Arabidopsis. PNAS 93:8782-8786. *https://doi.org/10.1073/pnas.93.16.8782*

Li J, Guo J, Xu W, Ma M (2006). Enhanced cadmium accumulation in transgenic tobacco expressing the phytochelatin synthase gene of *Cynodon dactylon* L. Journal of Integrative Plant Biology 48:928-937. *https://doi.org/10.1111/j.1744-7909.2006.00314.x*

Liu J, Zhang J, Kim SH, Lee HS, Marinoia E, Song WY (2021). Characterization of Brassica rapa metallothionein and phytochelatin synthase genes potentially involved in heavy metal detoxification. PLoS One 16:e0252899. https://doi.org/10.1371/journal.pone.0252899

Lyubenova L, Nehnevajova E, Herzig R, Schröder P (2009). Response of antioxidant enzymes in *Nicotiana tabacum* clones during phytoextraction of heavy metals. Environmental Science and Pollution Research 16:573–581. *https://doi.org/10.1007/s11356-009-0175-8*

Milner MJ, Seamon J, Craft E, Kochian L V (2013). Transport properties of members of the ZIP family in plants and their role in Zn and Mn homeostasis. Journal of Experimental Botany 64. *https://doi.org/10.1093/jxb/ers315*

Mukhopadhyay R, Shi J, Rosen BP (2000). Purification and characterization of Acr2p, the *Saccharomyces cerevisiae* arsenate reductase. Journal of Biological Chemistry 275:21149–21157. *https://doi.org/10.1074/jbc.M910401199*

Nahar N, Rahman A, Moś M, Warzecha T, Algerin M, Ghosh S, Johnson-Brousseau S, Mandal A (2012). *In silico* and *in vivo* studies of an *Arabidopsis thaliana* gene, ACR2, putatively involved in arsenic accumulation in plants. Journal of Molecular Modeling 18:4249-4262. *https://doi.org/10.1007/s00894-012-1419-y*

Nahar N, Rahman A, Nawani NN, Ghosh S, Mandal A (2017). Phytoremediation of arsenic from the contaminated soil using transgenic tobacco plants expressing ACR2 gene of *Arabidopsis thaliana*. Journal of Plant Physiology 218:121-126. *https://doi.org/10.1016/j.jplph.2017.08.001*

Németi B, Regonesi ME, Tortora P, Gregus Z (2011). The mechanism of the polynucleotide phosphorylase-catalyzed arsenolysis of ADP. Biochimie 93:624-627. *https://doi.org/10.1016/j.biochi.2010.11.013*

Nwaichi EO, Dhankher OP (2016). Heavy metals contaminated environments and the road Map with phytoremediation. Journal of Environmental Protection (Irvine, Calif) 07:41-51. *https://doi.org/10.4236/jep.2016.71004*

Pickering IJ, Gumaelius L, Harris HH, Prince RC, Hirsch G, Banks JA, Salt DE, George GN (2006). Localizing the biochemical transformations of arsenate in a hyperaccumulating fern. Environmental Science & Technology 40:5010-5014. *https://doi.org/10.1021/es052559a*

Sarma H (2011). Metal hyperaccumulation in plants: A review focusing on phytoremediation technology. Journal of Environmental Science and Technology 4:118-138. *https://doi.org/10.3923/jest.2011.118.138*

Schmöger MEV, Oven M, Grill E (2000). Detoxification of arsenic by phytochelatins in plants. Plant Physiology 122:793-802. *https://doi.org/10.1104/pp.122.3.793*

Siddiqui F, Tandon PK, Srivastava S (2015). Arsenite and arsenate impact the oxidative status and antioxidant responses in *Ocimum tenuiflorum* L. Physiology and Molecular Biology of Plants 21:453-458. *https://doi.org/10.1007/s12298-015-0299-x*

Singh N, Ma LQ (2006). Arsenic speciation, and arsenic and phosphate distribution in arsenic hyperaccumulator *Pteris vittata* L. and non-hyperaccumulator *Pteris ensiformis* L. Environmental Pollution 141:238-246. *https://doi.org/10.1016/j.envpol.2005.08.050*

Sri Lakshmi Sunita M, Prashant S, Bramha Chari P V, Nageswara Rao S, Balaravi P, Kavi Kishor PB (2012) Molecular identification of arsenic-resistant estuarine bacteria and characterization of their ars genotype. Ecotoxicology 21:202-212. *https://doi.org/10.1007/s10646-011-0779-x*

Sundaram S, Wu S, Ma LQ, Rathinasabapathi B (2009). Expression of a *Pteris vittata* glutaredoxin PvGRX5 in transgenic *Arabidopsis thaliana* increases plant arsenic tolerance and decreases arsenic accumulation in the leaves. Plant, Cell & Environment 32:851-858. *https://doi.org/10.1111/j.1365-3040.2009.01963.x*

Ullah I, Wang Y, Eide DJ, Dunwell JM (2018). Evolution, and functional analysis of Natural Resistance-Associated Macrophage Proteins (NRAMPs) from T*heobroma cacao* and their role in cadmium accumulation. Scientific Reports 8:14412. *https://doi.org/10.1038/s41598-018-32819-y*

Xu XY, McGrath SP, Zhao FJ (2007). Rapid reduction of arsenate in the medium mediated by plant roots. New Phytologist 176:590-599. *https://doi.org/10.1111/j.1469-8137.2007.02195.x*

Yang X-E, Jin X-F, Feng Y, Islam E (2005). Molecular mechanisms and genetic basis of heavy metal tolerance/hyperaccumulation in plants. Journal of Integrative Plant Biology 47:1025-1035. *https://doi.org/10.1111/j.1744-7909.2005.00144.x*

Zhao F-J, Stroud JL, Khan MA, McGrath SP (2012). Arsenic translocation in rice investigated using radioactive 73As tracer. Plant and Soil 350:413-420. *https://doi.org/10.1007/s11104-011-0926-4*