




# Root transcripts associated with arsenic accumulation in hyperaccumulator *Pteris vittata*

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Hyperaccumulation of arsenic (As) by brake fern *Pteris vittata* has been described as an important genetic trait that provides an option for development of a sustainable phytoremediation process for As mitigation. Accumulation of very high concentration of arsenic in above-ground tissues may be the result of arsenic vacuole compartmentalization, but the mechanism(s) of arsenic uptake and transport by underground tissues are largely unknown. In this study, we made an attempt towards understanding the molecular mechanism of As hyperaccumulation in this plant. A time-dependent As accumulation study indicates an exponential accumulation of As from 7 to 30 days of arsenic exposure in fronds, and day 3–7 in roots. Root transcriptome analysis identified 554,973 transcripts. Further, subsets of 824 transcripts were differentially expressed between treated and control samples. Many of the genes of critical As-stress response, transcription factors and metal transporters, biosynthesis of chelating compounds involved in uptake and accumulation mechanisms were identified. The genes that were highly expressed such as cysteine-rich RLK, and ABC transporter G family member 26 needs further studies along with arsenite transmembrane transporter. The analysis of generated transcriptome dataset has provided valuable information and platform for further functional studies.

**Keywords.** ABC transporter; differential expression analysis; heavy metal accumulation; transcriptome

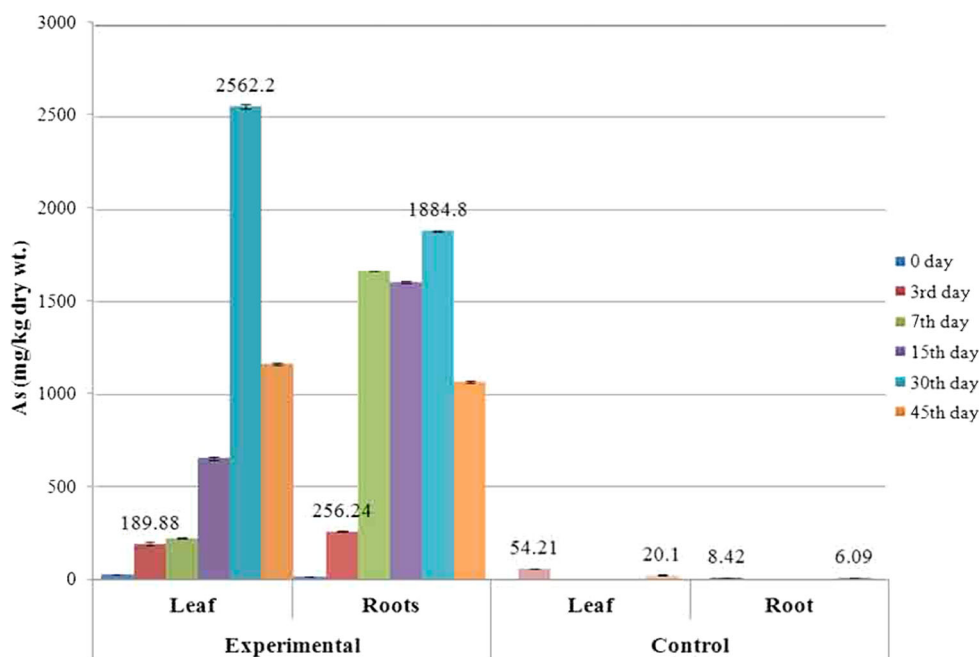
## 1. Introduction

Arsenic (As) has geogenic and anthropogenic origin, widely present in the environment and poses health risk to all forms of life. People living in areas contaminated with As suffer from deformities and diseases occurring due to As toxicity (McCarty *et al.* 2011 and the references therein). Existing technologies for As removal relies on excavation of soil and treating with chemical, which makes it a costly method of remediating contaminated site (Wuana and Okieimen 2011). Phytoremediation is known to be an environment-friendly alternative clean-up method (Rungwa *et al.* 2013). However, it requires hyperaccumulator plants such as *Brassica* (Irtelli and Navari-Izzo 2008; Srivastava *et al.* 2009; Freitas-Silva *et al.* 2016; Rahman *et al.* 2016) and brake fern *Pteris vittata* (Ma *et al.* 2001; Wang *et al.* 2007; Sarangi and Chakrabarti 2008). The hyperaccumulator *P. vittata* can accumulate As to such a high level where other plants die (Gumaelius *et al.* 2004), or *Pteris*

*vittata* has developed such mechanisms that it avoids or excludes the metal (Zhu *et al.* 2011). Whether the plant is resistant or accumulator depends on what happens at the root tissue (Meharg and Hartley-Whitaker 2002). Resistant plants reduce uptake of arsenate through suppression of high-affinity phosphate transporter (Meharg and MacNair 1992). So, *Pteris* must be having a mechanism that the other plants do not possess (Xie *et al.* 2009). Further, *P. vittata* root zone is found to be housed by specific endophytes with ability for As resistance and transformation. These endophytes are reported to play a significant role in promotion of plant growth, ameliorating soil conditions for bio-availability of metal to plants (Tiwari *et al.* 2016). Therefore, symbiotic interaction between plants and microbes for better improvement of metal uptake is receiving attention.

Arsenic gets into the plant root through the inorganic phosphate transport proteins in the form of arsenate (Meharg and Hartley-Whitaker 2002), whereas entry of arsenite

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**Figure 1.** Arsenic accumulation in foliar (above-ground) and root (below-ground) samples.

in rice plant was reported through some root aquaporins such as nodulin 26-like intrinsic membrane proteins (NIPs) (Ma *et al.* 2008) and plasma membrane intrinsic proteins (PIPs) (Mosa *et al.* 2012). Once in the root cell, arsenate is converted into the arsenite in the root through arsenate reductase (Xu *et al.* 2007). This arsenite then gets complexed with glutathione and some phytochelators (Raab *et al.* 2004). These complexes stored in vacuole (Lombi *et al.* 2002). Thus As is sequestered in the vacuole, and its toxic effects are minimized. Target gene identification and expression studies related to As sequestration and detoxification was carried out in plants such as *O. sativa*, *P. vittata* and *Holcus lanatus* (as cited above). Here, the aim of the present study was to look at the molecular level in terms of global transcriptome and proteins that are involved in As hyperaccumulation in Indian ecotype of *P. vittata*. However, the molecular mechanism underlying As hyperaccumulation have not yet been completely understood and requires further elucidation at the gene and protein level, especially the full set of genes that are up-regulated or down-regulated in order to deal with the As stress and accumulation and the transcription factors regulating these genes.

The present study specifically aimed to (1) develop reference transcriptomes of an Indian eco-type of *P. vittata*, and (2) identify root-specific differentially expressed As-stress-related genes (including transcription factors and metal transporters) that are inherent to hyperaccumulator plants.

**Table 1.** Statistics of RNA-Seq reads and assembly from *P. vittata* below-ground tissue

| Attributes                                       | Value      |            |
|--|------------|------------|
|  | Treated    | Control    |
| Total raw reads                                  | 32,562,726 | 46,666,008 |
| Total paired-end clean reads                     | 27,589,850 | 39,312,332 |
| Q30 percentage                                   | 91.87      | 92.24      |
| GC percentage                                    | 56.93      | 54.72      |
| Number of transcripts with FPKM $\geq$ 1.0       | 152,573    | 140,480    |
| All assembled transcripts                        | 561,740    |            |
| Number of transcripts after cd-hit clustering    | 554,973    |            |
| Mean GC % of transcripts after cd-hit clustering | 56.4       |            |

## 2. Materials and methods

### 2.1 Plant materials and arsenic accumulation analysis

Spores of *P. vittata* were germinated in plastic pots with sand and soil at a ratio of 1:1 and were maintained in greenhouse condition. Sporophytes of 6-month-olds were transplanted in As-contaminated (10 mg/kg) soil (further indicated as treated plant sample) and without As soil (control). The plants were allowed to grow for 3, 7, 15, 30 and 45 days under greenhouse condition. The plants were harvested at each time interval and washed thoroughly with Milli-Q water. The plants were separated into two

parts, i.e. above-ground (fronds) and below-ground (roots including rhizomes); these were separately oven-dried at 60°C for 48 h. Dried samples (0.1 g) were acid-digested with concentrated nitric acid (Cai *et al.* 2000) and samples were prepared for total As analysis using ICP-OES iCAP 6300 DUO (Thermo Scientific, USA). Elemental As analyses of the samples were performed under optimized conditions according to U.S. Environmental Protection Agency (US EPA) method (US EPA 1991).

## 2.2 RNA isolation and Illumina sequencing

The below-ground tissues (roots including rhizome) were sampled separately from plants grown in As-treated and control soil. Tissues were thoroughly rinsed in chilled Milli-Q water, excess water wiped with tissue paper, tissues were weighed to 1 gm and immediately ground to fine powder in presence of 50 mg of polyvinylpyrrolidone (PVPP) with liquid nitrogen. Further, total RNA was extracted using extraction buffer (100mM Tris-Cl (pH-8.0), 10 mM EDTA (pH-8.0), 100 mM LiCl, 2 % SDS (v/v), 5 %  $\beta$ -mercaptoethanol) and treated with water saturated Phenol:Chloroform (1:1). RNA was precipitated with isopropanol and 3 M sodium acetate. Extracted total RNA preparation was purified by silica based on column DNA digestion using DNase I and removal of colouring compounds associated with below-ground tissues (humic acid, phenolic compounds) using Zymo-Spin<sup>TM</sup> IV-HRC spin filters (ZR plant RNA MiniPrep, Zymo Research, USA). Quantity and purity of extracted total RNA were determined using NanoDrop (Thermo Fisher Scientific Inc, USA), Qubit 2.0 Fluorometer with Qubit RNA BR Assay kit (Life Technologies, USA) and Agilent 2100 bioanalyzer (Agilent Technologies, USA), respectively. The isolated total RNA was used to enrich mRNA and cDNA library construction. Paired-end sequencing of cDNA libraries was constructed using TruSeq RNA Library Preparation Kit as per the manufacturer's instructions (Illumina Inc., CA) and sequencing with an Illumina HiSeq 2500 sequencing platform (Kukurba and Montgomery 2015).

## 2.3 Sequence analysis, annotation and differential gene expression

RNA-sequencing and *de novo* assembly was accomplished with short reads assembling program – Trinity (version- trinityrnaseq\_r20140717) (Grabherr *et al.* 2011). The transcripts of length  $\geq 200$  bp were only used to determine the transcript expression using Bowtie2 program (ver. 2.2.2.6). Transcripts with  $\geq 1$  FPKM (fragments per kilobase of exon model per million fragments mapped) were used for further downstream annotations. BLASTX search was performed with obtained transcript sequences against several databases, including NCBI Nr, UniProt, Gene Ontology, using a cut-off E-value of

$10^{-5}$  and % identity cut-off of 40%. Further, these transcripts were classified based on GO annotation for molecular function, biological process and cellular component (Conesa *et al.* 2005). KEGG (Kyoto encyclopaedia of genes and genomes) pathway analysis was based on the comparative results between annotated transcripts and the current KEGG database (Kanehisa *et al.* 2016). Differential gene expression analysis between treated and control libraries were performed using DEseq program (ver. 1.16.0) (Anders and Huber 2010).

## 2.4 Reverse transcriptase-PCR (RT-PCR) validation of RNA-sequencing analysis

To validate the mRNA abundance of arsenic related genes, we randomly selected 6 genes identified in RNA-seq analysis to perform semi-quantitative RT-PCR analysis. Total RNA (1  $\mu$ g) from control and treated samples were used to synthesize cDNAs using SuperScript IV first-strand synthesis kit (Thermo Inc., USA) according to manufacturers guidelines. The specifics of genes and their respective primers for RT-PCR are listed in (supplementary table 4). We used elongation factor (EF-1 $\alpha$ ) as an internal control for normalization.

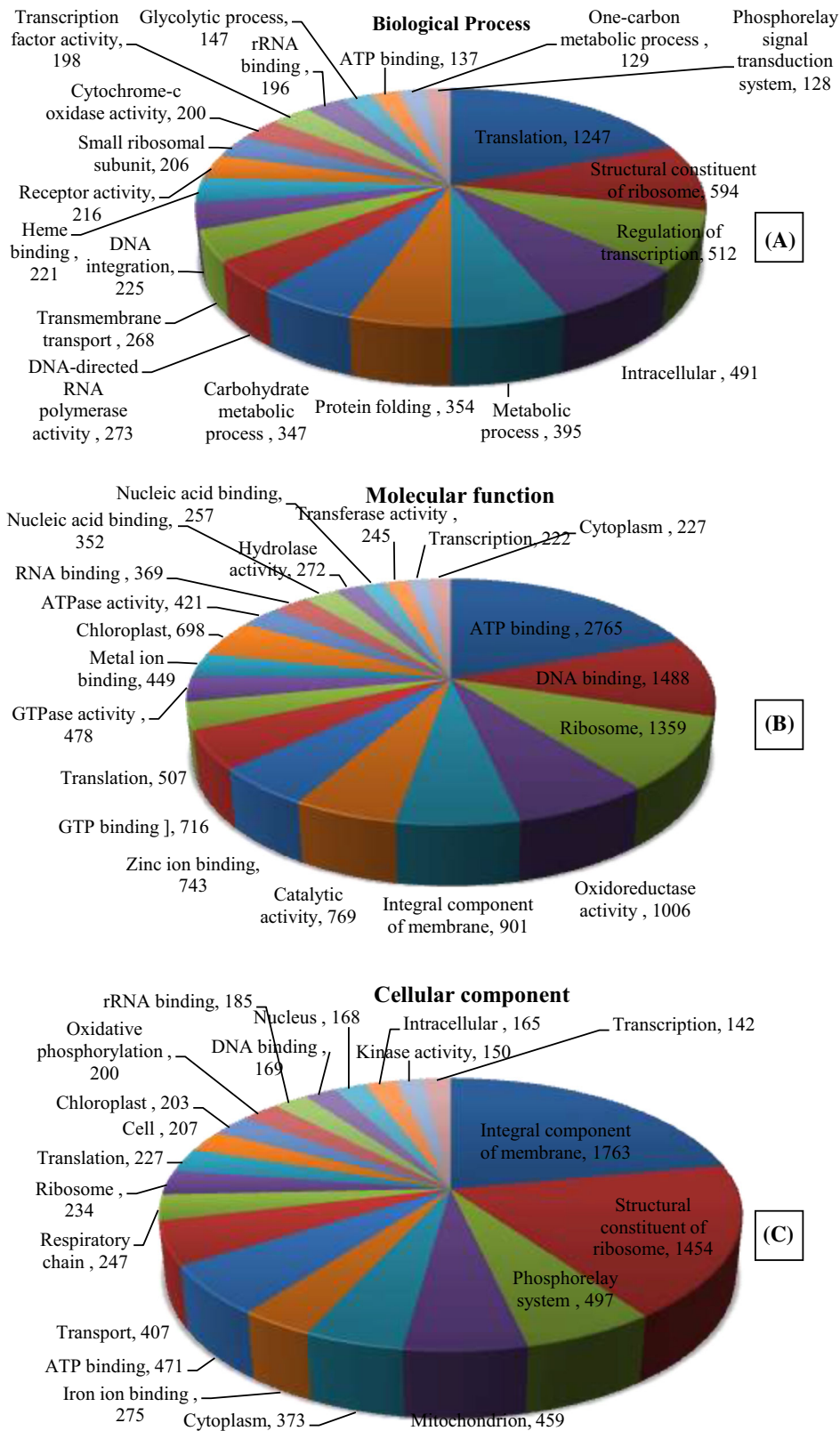
## 3. Results

### 3.1 Time-dependent As accumulation

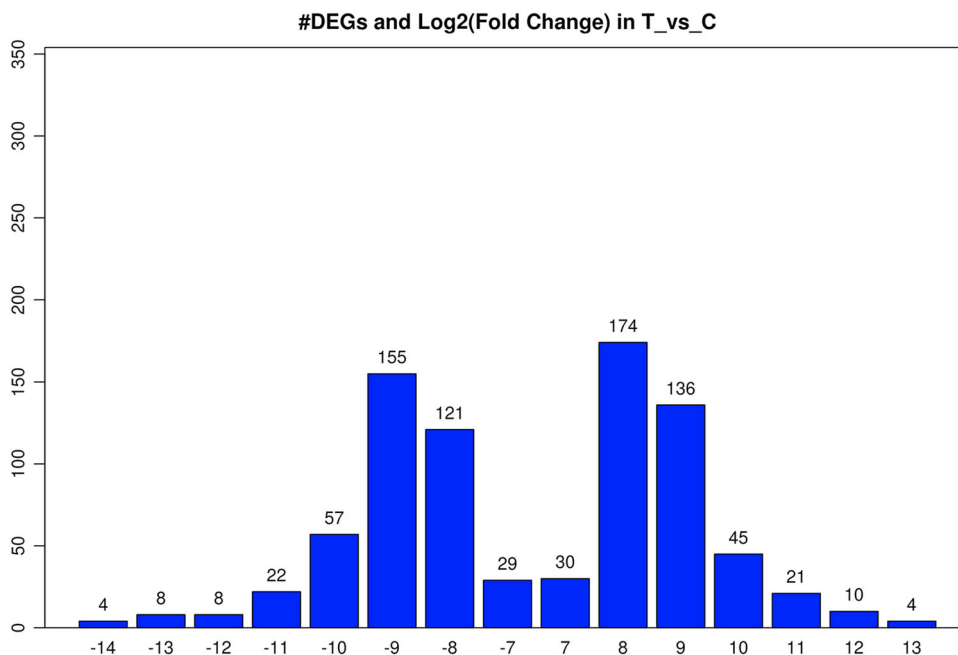
The below-ground tissue samples (roots including rhizome) were analysed to study the time of maximum influx of As into *P. vittata* upon As exposure vis-à-vis induction of expression of As-related genes. Total As concentration in both above-ground (fronds) and below-ground tissues were increased with increasing exposure time from 0 to 30 day. Time-dependent As accumulation indicated that the exponential accumulation of As starts from day 7 to day 30 in fronds, while in roots, sudden increase of As from day 3 to 7 was observed. However, there were no much drastic change in the As uptake rate from day 7 onwards was detected in roots. After 30 days, decrease in As content was detected both in leaf and root tissues as measured on 45 day, while 50 days onwards plants did not survived. Highest As uptake in fronds (2571.07  $\mu$ g gm<sup>-1</sup> dry wt.) and in roots (1885.93  $\mu$ g gm<sup>-1</sup> dry wt.) was detected on 30 day of As exposure (figure 1). Based on time-dependent As accumulation study, below-ground tissues were harvested and pooled from 3 and 7 day for isolation of total RNA.

### 3.2 RNA sequencing and de novo assembly

In the present study, tissue-specific libraries from below-ground tissues of *P. vittata* were constructed for understanding



**Figure 2.** Gene Ontology annotation of transcriptome. Top 20 GO terms with number of transcripts are summarized for each category as follows: (A) Biological process, (B) Molecular function and (C) Cellular components.



**Figure 3.** Bar diagram depicting differential gene expression between As-treated and control libraries.

the molecular mechanism underlying As hyperaccumulation. To capture the maximum transcript diversity expressed in below-ground tissues upon As exposure, pooled tissues from roots and rhizome were used to isolate total RNA. This total RNA from control and treated samples were used to construct cDNA libraries for sequencing. These libraries were subjected to sequencing on Illumina HiSeq 2500 platform that generated a total of 4666.6 Mb and 3256.28 Mb raw reads from the control and treated library, respectively. The obtained fastq files comprising raw reads were trimmed for adapters and low-quality reads prior to performing *de novo* assembly to avoid sequence-specific biasness. The clean reads so obtained had average GC content for control and treated library sequences of 54.72% and 56.93%, respectively, indicating marginal GC richness of the transcripts. The trimmed reads were aligned to the assembled transcriptome (length  $\geq 200$ bp) that resulted in 140,480 unique transcripts for control and 1,52,573 for treated library with expression  $\geq 1.0$  FPKM. Overall, a total of 5,61,740 transcripts were assembled using Trinity with default options, whereas 5,54,973 transcripts obtained after cd-hit clustering with 56.4 mean GC % (table 1). The raw reads and the assembly data for treated library were deposited at the SRA section of NCBI under the accession SRX2354811.

### 3.3 Transcripts annotation and pathway analysis

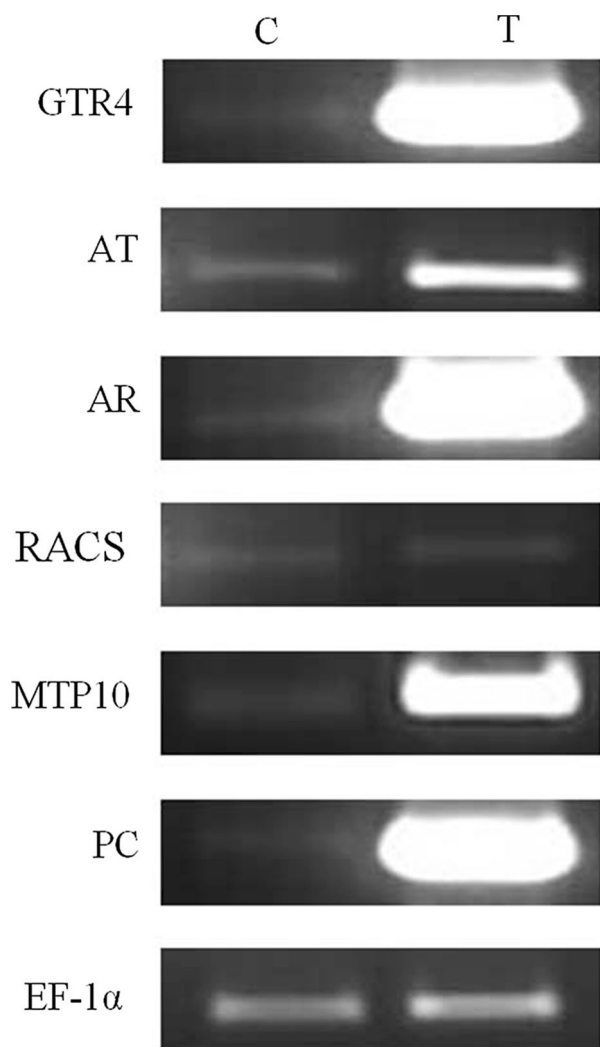
The filtered, assembled transcripts were primarily compared with NCBI non-redundant (Nr) protein database using BLASTX program. Matches with E-value cut-off of  $10^{-5}$  and

% identity cut-off of 40% were retained for further annotation using UniProt, Gene Ontology, KEGG databases. The BLASTX search result indicated that 217,344 transcripts have similarity of  $\geq 60\%$  at protein level. Out of these, a total of 67,596 (31.1 %) were annotated against Nr, 46,438 (21.36 %) against UniProt, 10,324 (4.75 %) against GO, and 5,665 (2.6 %) against KEGG database. Based on NR database annotation, the top BLASTX hit of each transcript return with most frequent organisms were *Ricinus communis* (7,020), *Dorco-ceras hygrometricum* (5,847), *Physcomitrella patens* subsp. *patens* (3,716), *Marchantia polymorpha* subsp. *polymorpha* (2,870), and *Selaginella moellendorffii* (2,313).

The Gene Ontology (GO) terms were assigned to 10,324 transcripts and the terms were summarized into the following GO categories; biological processes, molecular functions and cellular components. The top 20 terms of each category are shown in figure 2A, B and C. Among the biological processes category, highest transcripts belong to translation (1,247); in molecular function category, maximum transcripts belong to ATP binding (2,480), whereas in cellular component category, maximum number of transcripts belong to integral component of membrane and structural constituent of ribosome (1,541 and 1,205, respectively).

To better understand the function of sequenced transcripts, these were searched against the KEGG database. KEGG pathway analysis showed that 5,665 transcripts were assigned to 96 pathways, including metabolic pathways with 611 (10.78%) transcripts, biosynthesis of secondary metabolites 295 (5.2%), microbial metabolism in diverse environments 135 (2.3%), and oxidative phosphorylation 60





**Figure 4.** Semi-quantitative RT-PCR assays of arsenic-responsive genes in arsenic treated (T) and control (C) tissues. Glutaredoxin like protein 4 (GTR4), arsenite transporter like protein (AT), arsenate reductase (AR), response to arsenic containing substance (RACS), Metal tolerance protein 10 (MTP10), phytochelatin (PC) and elongation factor 1 alpha (EF1 $\alpha$ ).

(1%), (supplementary table 1). Further exploration of stress-related pathway, such as oxidative phosphorylation (KEGG pathway ID: ko00190), revealed that most genes including proton channel and cytochrome redox complexes were prominently identified.

### 3.4 Identification of abiotic stress-related transcription factors

Regulation of gene expression upon As exposure was of interest in the present study. Alignment and analysis of differential gene expression of annotated transcripts of

treated and control libraries resulted in the identification of transcripts belonging to 21 transcription factor families. The members of MYB, Orphan, AP2, Dof, WRKY, bHLH, HOMEBOX, NAC, Trihelix, MADS, C3HDZ and Tubby-like F-box protein were dominant (supplementary table 2).

### 3.5 Transporter proteins

Large number of metal transporter families including transmembrane transporter, ABC-type, aquaporins, NRAMP and ZIP family proteins were reported to be involved in heavy metal (HM) uptake, transport and distribution. Significant number of transporters including ABC transporter family proteins, sulphate transporters, inorganic phosphate transmembrane transporter, efflux family proteins, HM-associated domain containing proteins, and aquaporins were identified as differentially expressed genes (DEGs) upon As-stress. Among the differentially expressed metal transporters, the major portion belongs to inorganic phosphate transmembrane transporter; ABC-type, aquaporins, NRAMPs, ZIPs, and arsenite transporters were identified in the present study (supplementary table 3). Further, the genes encoding MT-like proteins 2 and phytochelatins (PCs)-related genes were also identified.

### 3.6 Differential gene expression analysis

By comparing the transcripts from As-treated and control libraries, 5,54,973 transcripts were subjected to DESeq program for identification of DEGs. Relatively large numbers of differentially expressed transcripts were identified. Among all the identified DEGs, 420 were identified to be up-regulated and 404 were down-regulated (figure 3). Two of the unique transcript c110929\_g1\_i1 and c215021\_g1\_i1 were highly up-regulated ( $\log_2FC$ : 13.2995 and 13.0142, respectively), whereas the unique transcript c113888\_g1\_i1 was found to be highly down-regulated ( $\log_2FC$ : -14.2293).

Further, semi-quantitative RT-PCR analysis of six arsenic/metal-related genes showed significant level of differential expression. Among these six genes, only the gene RACS did not show any difference in expression, whereas the other genes were significantly up-regulated (figure 4).

## 4. Discussion

Arsenic is ubiquitous in the earth's crust in the form of arsenopyrite (Zhao *et al.* 2010). Atmospheric flux of As is due to volcanic action, erosion of rocks and forest fires, or through anthropogenic activities (Duker *et al.* 2005; Neumann *et al.* 2010). Compared to physico-chemical and mechanical As remediation method, phytoremediation is

**Table 2.** Significantly up-regulated top 10 annotated DEGs in As-treated samples compared to control

| Transcript ID  | Description   | Log2FC  | P-value | Kegg ID |
|----------------|---|---------|---------|---------|
| c211017_g10_i1 | Interferon regulatory factor 8 (IRF8)   | 9.6015  | 0.0171  | K10155  |
| c213821_g1_i3  | U3 small nucleolar RNA-associated protein 6 (UTP6)                            | 10.2989 | 0.0096  | K14557  |
| c213661_g1_i1  | Hypothetical protein BVRB_025990 [Beta vulgaris subsp. vulgaris]              | 12.3954 | 0.0049  |         |
| c215357_g1_i2  | Cysteine-rich RLK (receptor-like protein kinase) 8 [Dorcoceras hygrometricum] | 10.0617 | 0.0129  |         |
| c209426_g2_i2  | Ubiquinol-cytochrome c reductase cytochrome b subunit                         | 9.5934  | 0.0172  | K00412  |
| c212679_g3_i2  | Hypothetical protein  | 9.8803  | 0.0144  | K07006  |
| c213724_g8_i1  | Hypothetical protein  | 11.5176 | 0.0063  | K04165  |
| c208712_g28_i2 | PREDICTED: ABC transporter G family member 26 [Phoenix dactylifera]           | 9.8208  | 0.0149  |         |
| c211017_g3_i2  | Putative site-specific DNA endonuclease [Monomastix sp. OKE-1]                | 9.9041  | 0.0141  | K00412  |
| c213138_g1_i3  | ATPeF0A; F-type H <sup>+</sup> -transporting ATPase subunit a                 | 9.4460  | 0.0189  | K02126  |

**Table 3.** Down-regulated top 10 annotated DEGs in As-treated compared to control samples

| Transcript ID | Description  | Log2FC   | P-value | Kegg ID |
|---------------|--|----------|---------|---------|
| c283834_g1_i1 | gatB; aspartyl-tRNA(Asn)/glutamyl-tRNA(Gln) amidotransferase subunit B | -9.5515  | 0.0197  | K02434  |
| c194625_g1_i1 | RNA-dependent RNA polymerase   | -12.655  | 0.0038  | K00052  |
| c204507_g2_i2 | iron complex outer membrane receptor protein                           | -9.6352  | 0.0186  | K02014  |
| c281977_g1_i1 | Mechanosensitive ion channel protein 1, mitochondrial                  | -9.8304  | 0.0162  |         |
| c203616_g1_i1 | orf189; hypothetical protein   | -12.915  | 0.0036  | K03500  |
| c213747_g3_i4 | tolB; TolB protein   | -9.503   | 0.0143  | K03641  |
| c198470_g1_i2 | methyl-accepting chemotaxis protein                                    | -11.3399 | 0.0064  | K03406  |
| c211836_g1_i1 | HSP20; HSP20 family protein  | -9.8555  | 0.0159  | K13993  |
| c184173_g2_i1 | tartrate-resistant acid phosphatase type 5                             | -11.6653 | 0.0055  | K14379  |
| c213798_g2_i3 | ibpA; molecular chaperone IbpA   | -11.2983 | 0.0065  | K04080  |

being evaluated as an eco-friendly, cost-effective, sustainable method. The findings of As-hyperaccumulating fern species (Ma *et al.* 2001) and investigation of the molecular mechanisms essential to effectively control HM uptake, translocation and accumulation by plant genetic manipulation and genomic research (Wang *et al.* 2013) has received considerable attention.

#### 4.1 Arsenic accumulation in below-ground tissue

In the tissue-specific As accumulation study, majority of As accumulated in the above-ground biomass (Potdukhe *et al.* 2015), which supports the findings of Ma *et al.* (2001) that 93% of the total As accumulated in the above-ground biomass was concentrated in the fronds. Low As was recorded in below-ground tissue, but rapid uptake of As was higher in below-ground tissue within short time (3 to 7 day) of As exposure compared to above-ground tissue (figure 1). It was observed that As concentrations increased with time up to 30 days in fronds, while there was rapid uptake up to day 7 and there was not much change observed from day 7 onwards in below-ground tissues. The decrease in As concentration in both above- and below-ground tissues after 30 days of As exposure could be attributed to the dilution factor resulting

from an increase in the plant biomass, which was also noted by Cai *et al.* (2004).

#### 4.2 Root transcripts associated with As stress and regulatory network

Based on time-dependent As accumulation study and pooling the below-ground tissues (roots and rhizome) from two different time frames (day 3 and 7) resulted in 1,40,480 unique transcripts for control and 1,52,573 for treated library with expression  $\geq 1.0$  FPKM. Further, overall 5,54,973 transcripts were obtained after cd-hit clustering and about 2,17,344 transcripts were shown similarity of more than 60% at protein level in BLASTX search, these results indicating the representation of high genes diversity in constructed libraries. In this study, we found that a broad set of unique and novel genes was significantly up- or down-regulated in response to As exposure. A global analysis of transcriptome was reported to assists in identification of critical genes, expression and understanding the regulatory mechanisms in response to abiotic stresses in plant such as high-salinity, drought and cold stresses (Shan *et al.* 2013) and HM stress (Li *et al.* 2014). Arsenate and phosphate are reported to have striking similarities and share the same

**Table 4.** Some crucial arsenic stress responsive DEGs in As-treated samples compared to control below-ground tissue

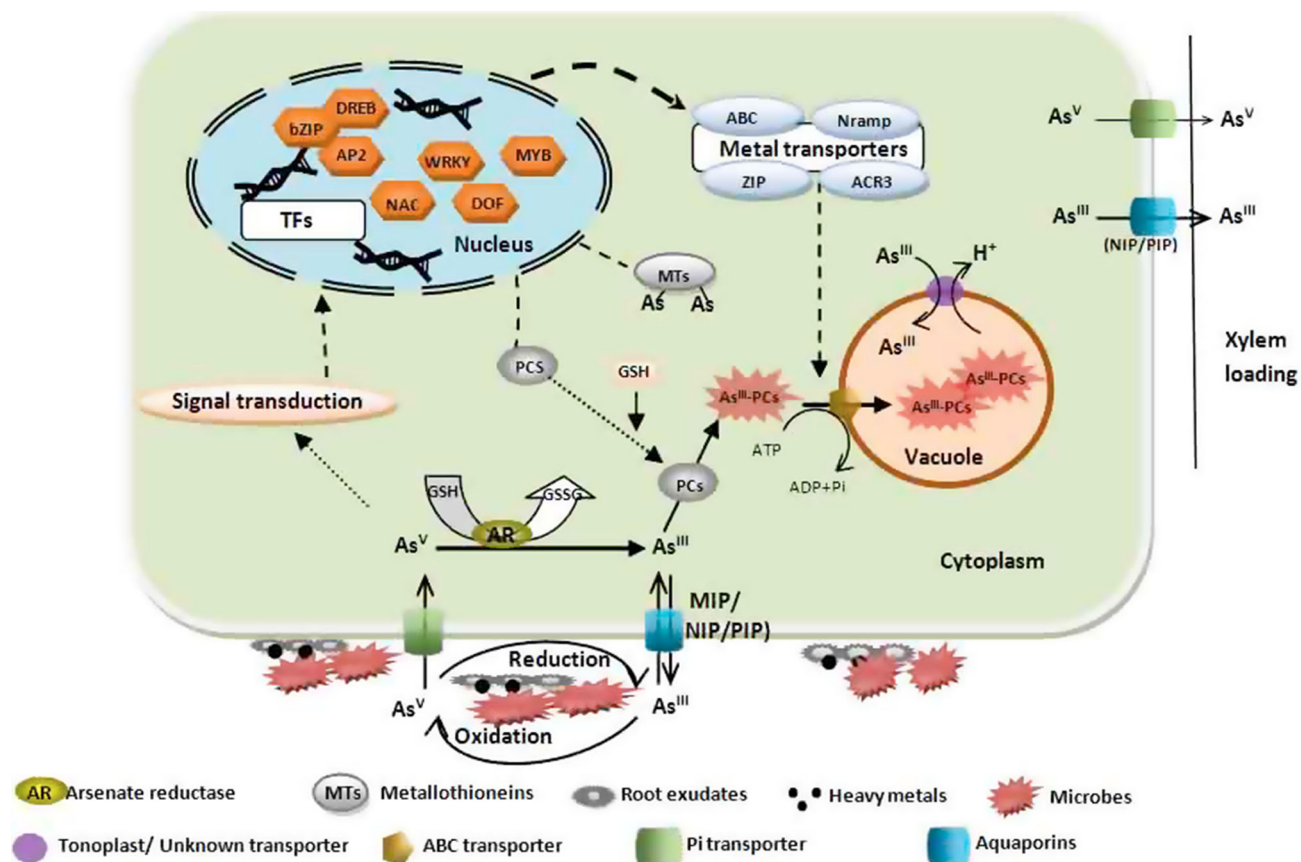
| Genes                              | Transcript abundance | Representative transcript | Description   | Log2FC | P-value |
|------------------------------------|----------------------|---------------------------|---|--------|---------|
| Arsenate reductase                 | 15                   | c99039_g1_i1              | arsenate reductase, putative                                | 3.4918 | 0.437   |
| Arsenical-resistance protein       | 2                    | c94028_g1_i1              | arsenical-resistance protein, putative                      | 3.1293 | 0.443   |
| Arsenite transporter               | 3                    | c201177_g1_i1             | arsenite transporter-like protein                           | 0.4024 | 0.926   |
| Arsenite transmembrane transporter | 2                    | c189323_g4_i1             | arsenite transmembrane transporter activity                 | 6.4199 | 0.147   |
| Metal tolerance protein            | 2                    | c73091_g1_i1              | cation transmembrane transporter activity                   | 1.4436 | 0.725   |
| Phytochelatin synthase             | 1                    | c206906_g1_i1             | phytochelatin synthase [ <i>Pteris vittata</i> ]            | 1.3319 | 0.675   |
| Gamma-glutamylcysteine synthetase  | 5                    | c210624_g1_i2             | gamma-glutamylcysteine synthetase [ <i>Pteris vittata</i> ] | 1.0406 | 0.772   |

transport pathway in higher plants (Ullrich-Eberius *et al.* 1989). The uptake of As in the form of arsenate involves inorganic phosphate transport system as shown in *P. vittata* (Wang *et al.* 2002) and *Arabidopsis thaliana* (Shin *et al.* 2004; Gonzalez *et al.* 2005), whereas arsenite can be actively taken up by plant roots through the subfamilies of aquaporins (Meharg and Jardine 2003; Bienert *et al.* 2008a). In the present study, the transcripts of inorganic phosphate transporter (PiT) and a great number of transcripts for different subfamilies of aquaporins, such as plasma membrane intrinsic proteins (PIPs), nodulin 26-like intrinsic proteins (NIPs), tonoplast intrinsic proteins (TIPs) and major intrinsic proteins (MIPs), were identified (supplementary table 3). Along with Pi transporters, higher transcripts of aquaporins as identified in the present study might be responsible for direct influx of arsenite (Abedin *et al.* 2002; Meharg and Jardine 2003) and higher accumulation of As in the form of arsenite. Entry of As in root cells activates stress including oxidative stress responsive signalling molecules via protein kinase cascades (Rao *et al.* 2011), and oxidative phosphorylation system.

In the present investigation, cysteine-rich RLK (receptor-like protein kinase) 8 was found to be highly up-regulated. Interestingly, the up-regulation of CRK (cysteine-rich RLK) were also reported for Cr stress as was elucidated from microarray data of expression of signalling genes in rice roots (Trinh *et al.* 2014). This indicates that changes in cysteine-rich RLK expression might be related to the response of metals/metalloids stress. High up-regulation of cysteine-rich RLK 8, which is reported to be induced by reactive oxygen species (ROS) (Wrzaczek *et al.* 2010; Burdiak *et al.* 2015) and ubiquinol-cytochrome c reductase cytochrome b subunit (table 2), might be induced by As-stress. Further, these cascades induce different transcription factors (TFs) such as WRKY, bZIP, MYB families, in the nucleus to regulate the expression of some functional genes (Huang *et al.* 2012; Thapa *et al.* 2012). These genes might encode transporters (arsenite transporters, ABC-type, aquaporins, ZIP and Nramps) and metal chelators such as MTs and PCs, which

would detoxify and accumulate As in plant cells. In the present study, about 67 transcripts belonging to different TFs families and 54 transcripts encoding HM transporter proteins including arsenite transporter were identified (supplementary tables 2 and 3). Among the major transporters, ABCs transporter are full size and contain forward-oriented nucleotide binding domain (NBD) and a trans-membrane domain (TMD). Members of this family generally play some role in detoxification (Verrier *et al.* 2008). Proteomic data obtained for *Arabidopsis* vacuoles suggest that most ABCs of *Arabidopsis* reside in the tonoplast, and in addition to AtABCC1/2, other ABCs, may contribute to the overall glutathione conjugate transport activity (Jaquinod *et al.* 2007). Expression of ABC-type multidrug resistance-associated proteins (MRPs) in the present study indicates high activity localization near the tonoplast of the vacuole. Song *et al.* (2010) have shown extreme sensitive of *A. thaliana* to arsenic and arsenic-based herbicides in the absence of two ABC-type transporters, AtABCC1 and AtABCC2. In differential gene expression analysis, some critical genes were prominently up-regulated, which includes cysteine-rich receptor-like protein kinase 8 (CRK8), ubiquinol-cytochrome c reductase cytochrome b subunit, ABC transporter G family member 26 and F-type H<sup>+</sup>-transporting ATPase subunit (table 2). The down-regulated genes were identified as iron complex outer-membrane receptor protein, mechanosensitive ion channel protein 1, and tartrate-resistant acid phosphatase type 5 (table 3). The genes that are directly related to As uptake, transport and accumulation were moderately up-regulated (table 4). Moreover, up-regulation of GTR4, AT, AR, MTP10 and PC genes as observed in RT-PCR assay corroborate the reported literature. Based on the DEGs enrichments in the present study and reported literature (Shin *et al.* 2004; Raab *et al.* 2004; Ma *et al.* 2008; Ellis *et al.* 2006; Bienert *et al.* 2008b; Thapa *et al.* 2012; Cesaro *et al.* 2015; Tiwari *et al.* 2016), a possible regulatory network of As uptake, transport and accumulation was put forward in the below-ground tissue of *P. vittata* (figure 5). The present investigation reports root transcriptome data and provides useful information about the root-specific As-stress-





**Figure 5.** Schematic diagram of root uptake, accumulation and vascular transport of arsenic.

related genes that may pave the way for future functional studies.

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