[Journal of Bioresource Management](https://corescholar.libraries.wright.edu/jbm)

Volume 3 | Issue 4 Article 4

2016

Characterization and Analysis of *PCS1* Gene from Wheat

Haris Ahmed Khan
University of Agriculture, Faisalabad, Pakistan, harisniazi005@gmail.com

Ayaz Ahmad
University of Agriculture, Faisalabad, Pakistan

Sehar Nawaz
University of Agriculture, Faisalabad, Pakistan

J. Bioresource Manage. (2016) 3(4): 24- 36.

CHARACTERIZATION AND ANALYSIS OF *PCS1* GENE FROM WHEAT

Haris Ahmed Khan*, Ayaz Ahmad, Sehar Nawaz

Department of Biotechnology, University of Agriculture Faisalabad, Pakistan

*Email: harisniazi005@gmail.com

ABSTRACT

Wheat (*Triticum aestivum*) is an important cereal crop grown worldwide on large areas. Yields of wheat are decreasing worldwide due to heavy metal contamination. A number of genes have been characterized as those that confer tolerance to cadmium stress. *PCS1* is one of them. Phytochelatins have a key role in the detoxification of heavy metals in plants. However, the mechanism behind the detoxification remains unknown and wheat genes related to detoxification have not yet been identified. Here we report the isolation of cDNA encoding of phytochelatin synthase (PCS) from wheat (var. Galaxy 2013). The *AtPCS1* cDNA sequence contained 833 bp and encodes 277 amino acid proteins having a molecular weight of 55kDa. Multiple alignment and phylogenetic analyses were conducted by using bioinformatic tools. *AtPCS1* was most related to the *Hordeum vulgare PCS1 gene* (AK372435.1) as they were clustered in the same clade. This gene could be helpful in making transgenic crops which will help in phytoremediation.

Keywords: Cadmium, glutathione, heavy metal, phytochelatin synthase (PCS)

INTRODUCTION

Wheat (*Triticum aestivum*) is of immense importance as a cereal crop and has been used as a staple food for many countries on the globe for the last eight thousand years. There is a range of factors which limit its maximum productivity, including heavy metal toxicity. Increasing soil pollution is also contributing significantly to low yields in peri-urban areas (Munzuroglu and Geckil 2002, Kahl, et al. 2015). The final yield of wheat decreases when grown in cadmium stress (Savaghebi, et al. 2002). The accumulation of heavy metals in the food chain can cause the death of an organism. Cadmium is highly toxic due to its highly soluble nature in water (Lockwood 1976, Ming et al. 2016).

The occurrence of phytochelatins is ubiquitous in plants (Gekeler, et al. 1989, Ariani, et al. 2015). They have the ability to link with metal ions, specifically with cadmium. Pytochelatins are also produced in higher plants in response to cadmium exposure. Phytochelatins, which are peptides which bind with heavy metals produced enzymatically from glutathione, have an important part in providing protection from heavy metal stresses (Chaurasia, et al. 2008). The basic structure of PCs is (γ-Glu-Cys)n-Gly, here n = 2 to 11 (Ramos, et al. 2008). They are cysteine rich peptides (Grill, et al. 1985, Rauser 1990). PCs also have a role in protecting the plant from the dangerous effects of other heavy metals, i.e. cadmium (Cd), arsenic (As) and lead (Pb). The phytochelatin biosynthesis pathway is shown in Fig.1 (Inouhe 2005). Phytochelatins have also been identified in *Arabidopsis thaliana*, *S. pombe*, *Triticum aestivum*, *C. elegans*, (Clemens, et al. 1999, Ha, et al. 1999, Vatamiunik, et al. 1999, Cobbett 2000a, b; Clemens 2001) algae, fungi (including *Schizosaccharomyces pombe*), in worms (*Caenorhabditis elegans*) (Ha, et al. 1999), *Brassica juncea* (Heiss, et al. 2003, Jung, et al. 2014), and in other species, such as the slime mould, *Dictyostelium. discoideum* (Cobbett 1998), *Lactuca sativa* (He, et al. 2005), and *Lotus japonicus* (Ramos et al. 2008). The synthesis of phytochelatins is accomplished by the help of the phytochelatin synthase enzyme (PCS) from substrate

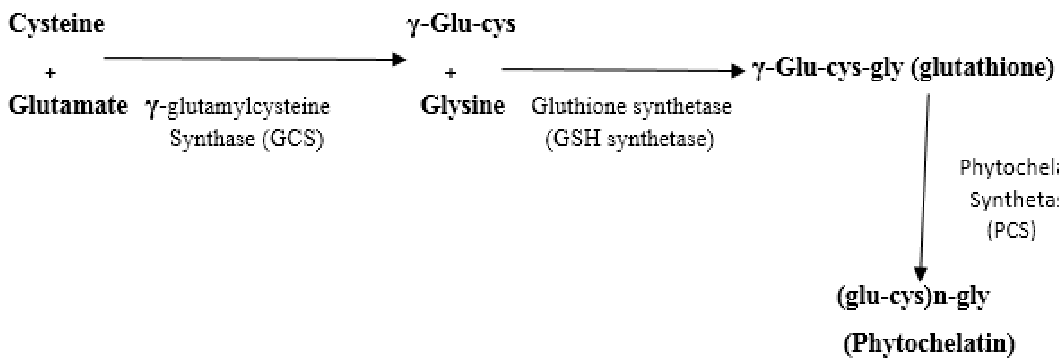


Figure 1: Simplified schematic representation of the Phytocheatin biosynthesis pathway (Inouhe 2005).
GCS:Gama-Glutamylcysteine Synthase; GSH:Glutahthione Synthetase; PCS:Phytochelatin Synthase.

glutathione (GSH) (Thangavel et al. 2007). After synthesis of phytochelatins, they bind with heavy metal ions and help in the facilitation and transportation in the form of complexes into the vacuoles of the cell (Clemens 2006, Ming, et al. 2016). In the vacuoles, they form high molecular weight complexes with phytochelatins, which is the mechanism of homeostasis in response to metal stress (DalCorso et al. 2008).

With a goal of identifying the genes involved in the detoxification of heavy metals, we isolated and cloned the cDNA of the PCS1 gene from *T. aestivum*. The main objective of this research was to find out the sequence of the required fragment of the PCS1 gene for multiple alignment and phylogenetic analysis. Sequence analysis showed close relationships with *Triticum aestivum*, *Hordeum vulgare*, and *Oryza sativa Japonica*. This information will be useful for the development of transgenic plants with improved tolerance to cadmium stress by over expressing this gene. Such transgenic plants will be helpful in the phytoremediation of Cd polluted soil.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Seeds of Wheat (*Triticum aestivum*) Galaxy 2013 were collected from the Ayub Agricultural Research Institute, Faisalabad, Pakistan, and were rinsed thoroughly with distilled water and covered by sand under 4°C for 60 days. After sterilizing with NaClO at 50% (v/v) (5% active Cl2) for ten min, seeds were planted in pots with quartz sand as the matrix. When the fourth true leaf fully expanded, uniform plantlets were transferred to deionized water containing 20 µM cadmium sulfate ($CdSO_4$, Sigma-Aldrich, St. Louis, MO), 20µM zinc sulfate ($ZnSO_4$, Sigma-Aldrich), 20µM copper sulfate ($CuSO_4$, Sigma-Aldrich), 200µM BSO (SigmaAldrich), 200µM GSH (Sigma-Aldrich), and 20µM $CdSO_4$ for 24 hrs. (25°C). Plantlets grown in deionized water for 24 hrs. were used as a control. The roots, stems and leaves were then collected for gene isolation (Chang, et al. 2012).

RNA Extraction

Total RNA was extracted from leaf, stem and root samples using RNA-Sepasol (NACALAI TESQUE, INC.) following the manufacturer's instructions and was followed by chloroform extraction and isopropanol precipitation.

cDNA Synthesis and Amplification:

RT-PCR was manipulated using mRNA Selective PCR Kit (Thermo Scientific kit). PCR reactions using equal amounts of RNA samples were performed. First strand cDNA was synthesized using Oligonucleotide primers; PCS1LP (5'CAGACCACCATCCACGACTT3'), PCS1-RP (5' AAAGGGAATGAACAGGCTGT 3') of the PCS1 gene was designed from a previously reported sequence (accession no. AF093752.1) for its amplification from wheat. The cDNA synthesized from isolated RNA was used as a template. PCR was performed with denaturing for 1 min at 94°C, annealing for 1 at 55°C and extending for 1 min at 72°C. The final extension was at 72°C for 3 min. The cycle was repeated 30 times using cDNA.

The PCR product was electrophoresed in TAE buffer (0.5X) (0.45 mM Tris-acetate, 1 mM EDTA) and 1% agarose gel and the amplified fragments were visualized under the Gel Documentation apparatus. The desired fragment was excised from the gel with a sterilized surgical knife (Feather Safety razor, Japan) under a UV Transilluminator.

Cloning

The desired DNA fragment was eluted from the gel by using a Thermo Scientific Gel elution kit (Kit # ICO691) according to the manufacturer's instructions. The top 10 strains of *E. coli* were used to prepare competent *E. coli* cells. Amplified and purified fragments were cloned into a TA- vector (TA- Cloning kit Fermentas). Transformation of competent cells of E. coli was done by using the heat shock method of transformation. For this purpose, 3 µl of the ligation mixture was added in 50 µl of competent cells and placed on ice for 30 minutes. Then the mixture was placed on a heat block adjusted to 42°C for 60-90 seconds. Again it was placed on ice for 10 minutes and 200 µl of liquid LB was added to the mixture. This mixture was incubated at 37°C for 45 minutes to let the cells grow. These cells were spread over the surface of the LB agar plates containing antibiotic ampicillin, X-gal and IPTG using a sterile bent glass rod. The plates were left at room temperature until the liquid had been absorbed. The plates were sealed, inverted and incubated at 37°C overnight. Blue white colonies appeared in 12 hours. Pure white colonies were picked and streaked to get fresh colonies. These transformed colony samples were used to perform colony PCR. Denaturation proceeded at 94ºC for 1 minute, annealing 55ºC for 1 minute, extension 72ºC for 1 minute, and final Extension 72ºC for 3 minutes. PCR positive colonies were selected and used for plasmid DNA isolation by using a plasmid isolation kit (QiaGen Science, USA). Plasmid DNA was purified by using the polyethylene glycol precipitation method with some modifications (Sambrook et al. 1989).

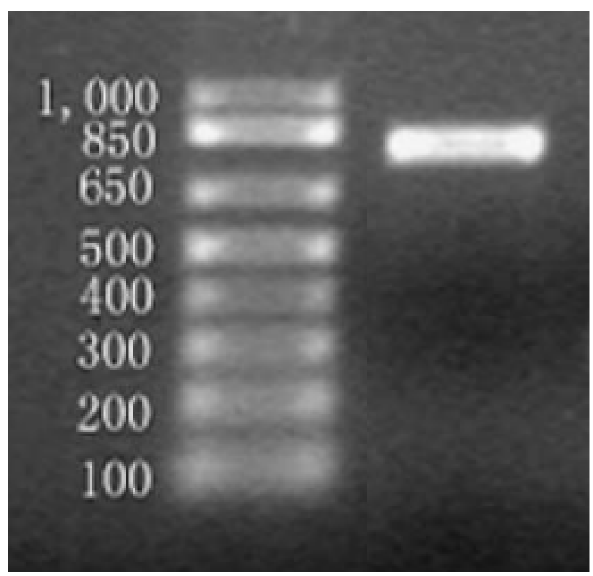
Computer analyses

Sequencing was performed at the Molecular Biology Product Incorporation, Pakistan. Plasmid DNA samples were sequenced by different primers. The sequence of these standard primers is given as follows: VectorF (TATAAAACGACGGCCAGT) and VectorR (CAGGAAACAGCTATGACC). Comparisons were conducted by using bioinformatics tools such as: BLAST (nucleotide and amino acid) at the National Centre for Biological Information (http://www.ncbi.nlm.nih.gov), ClustalW, JustBio and Expasy softwares. A phylogenetic tree was constructed by using phylogeney.fr software.

RESULTS AND DISCUSSION

The toxic effects of Cd on plant morphological and physiological processes have been extensively studied using different species or different varieties (cultivars) of the same species (Sanita di Toppi and Gabbrielli, 1999, Wu, et al. 2007, Ekmekci, et al. 2008). Exposure of plants to toxic metals can lead to numerous physiological and biochemical disorders. The inhibition of plant seedling growth can be regarded as general responses associated with heavy metal toxicity (Kopyra et al. 2003). To overcome such toxicity, plants use phytochelatin biosynthetic pathways to adapt to Cd stress. Numerous studies have investigated the mechanisms of the Cd tolerance of plants (Clemens, 2001, Hall 2002, Li et al. 2016).

For the amplification of the PCS1 gene, RNA was isolated from wheat (*Triticum astivum*). Quantitative and qualitative analysis of extracted RNA were performed by using a spectrophotometer and gel electrophoresis, respectively. From this RNA, cDNA was produced, which was then used as a template for PCR amplification using gene specific primers. The result showed an amplification of PCS1 gene fragments of 833bp (compared with 1kb gene ruler) as shown in figure 2. Amplified DNA fragments were eluted from gel cloned into T.A cloning vector. After that, the vector was inserted into *E. coli* competent cells. For the blue white selection, colonies were grown and incubated over night at 37°C as shown in figure 3. Blue and white colonies appeared within twelve hours on L.B agar plates. Colony PCR was performed to analyze and confirm white colonies carrying recombinant plasmid. All of the white colonies were marked with consecutive numbers and positive clones were confirmed by colony PCR using PCS1 specific primers. The result confirmed PCS1 clones (Fig. 3). PCS1 recombinant colonies were cultured in the LB medium containing



ampicillin for eight hours. Plasmid DNA was isolated from these E. coli cells and used for the sequencing purpose.

Figure 2: Gel electrophoresis of PCR amplified PCS1 fragment. Gene specific

J. Bioresource Manage. (2016) 3(4): 24- 36.

primers were used for the amplification. PCR product was electrophoresed in 1.5% agarose gel in TBE buffer. Lane 1: 1000 bp ladder marker, Lane 2: PCR product.

The amplified nucleotide and deduced amino acid sequence of wheat PCS1 gene is given in Fig 4. Protein and Nucleotide Blast (NCBI) and Justbio translator were used for this purpose. For this fragment, similarity to the corresponding genes from other species was detected. In comparison with some related, previously reported sequences, each fragment exhibited high homology to the corresponding gene from other species in nucleotide sequences. The alignment of the deduced nucleotide and amino acid sequence of the PCS1 clone showed homology to the wheat PCS1 gene, *Hordeum vulgare* PCS1 gene (AK372435.1), and *Oryza sativa* PCS1 gene (AK071958.1). Multiple alignment is also shown in figure 5. There was no intron in the deduced amino acid sequence.

Figure 3: Blue white screening of *PCS1* clone fragment after cloning.

```
CAG ACC ACC ATC CAC GAC TTC CGC GCC CAC CTC ACG CGC TGC GCC TCC TCC CAG GAC TGC
 Q   T   T   I   H   D   F   R   A   H   L   T   R   C   A   S   S   Q   D   C

CAT CTC ATC TCC TCC TAC CAC AGG AGC CCC TTC AAG CAG ACT GGG ACT GGC CAT TTC TCA
 H   L   I   S   S   Y   H   R   S   P   F   K   Q   T   G   T   G   H   F   S

CCG ATC GGC GGG TAT CAT GCC GAG AAA GAC ATG GCG CTC ATC TTG GAT GTT GCG CGC TTC
 P   I   G   G   Y   H   A   E   K   D   M   A   L   I   L   D   V    A   R   F

AAA TAC CCT CCT CAT TGG GTT CCA TTG ACG CTT CTC TGG GAT GCC ATG AAC ACG ACT GAT
 K   Y   P   P   H   W   V   P   L   T   L   L   W   D   A   M   N   T   T   D

GAA GCA ACT GGG CTT CTC AGG GGG TTC ATG CTT GTA TCA AGG CGC AGT TCA GCT CCT TCA
 E   A   T   G   L   L   R   G   F   M   L   V   S   R   R   S   S   A   P   S

TTG CTC TAC ACA GTG AGT TGC GGC CAT GGA AGT TGG AAA AGC ATG GCA AAG TAT TGT GTG
 L   L   Y   T   V   S   C   G   H   G   S   W   K   S   M   A   K   Y   C   V

GAA GAT GTG CCC AAT CTA CTG AAG GAT GAG AGT CTA GAC AAT GTT ACA ACA CTT CTG TCC
 E   D   V   P   N   L   L   K   D   E   S   L   D   N   V   T   T   L   L   S

CGC CTA GTG GAA TCT CTC CCA GCA ATG CTG GAG ATT TGA TCA AAT GTG TCA TTG AAG TTA
 R   L   V   E   S   L   P   A   N   A   G   D   L   I   K   C   V   I   E   V

GGA GAA AAG AGG AAG GTG AAT CAA GCT TGA GTA AAG AGG AGA AAG AAA GGC TTT TTT TGA
 R   R   K    E   E   G   E   S   S   L   S   K   E   E   K   E   R   L   F   L

AGG AAA AAG TAT TAC AGC AAA TCC GTG ATA CTG ATC TTT TCA GAG TAG TCC ACG AAC TGC
 K   E   K   V   L   Q   Q   I   R   D   T   D   L   F   R   V   V   H   E   L

AAT ATC CCA AGG GGC TAT GTG GTA GTT GCT CGT CTT CAA GTG ATG AAG ATT CGC TTG CCG
 Q   Y   P   K   G   L   C   G   S   C   S   S   S   S   D   E   D   S   L   A
```

```
AGA TTG CAG CCA CTG TGT GCT GTC AAG GAG CTG CAT TCC TAT CTG GTA ACC TTG TAT CTA
 E   I    A   A   T   V C   C   Q   G   A   A   F   L   S   G   N   L   V   S

GAG ATG GGT TCT GCT GCC GAG AAA CAT GTA TCA AAT GTA TAG AAG CAA ATG GTG ATG           R
D   G    F   C   C   R    E   T   C   I   K   C    I   E   A   N   G   D    N

GAC TAA AGA CTG TTA TCT CAG GAA CCG TGG TAT CTA AAG GGA ATG AAC AGG CTG T
G    L   K   T   V   I   S   G   T   V   V   S   K   G   N   E   Q   A
```

Figure 4: Nucleotide and amino acid sequence of *PCS1* clone. The amino acid sequence is shown in the single letter code below the nucleotide sequence. Justbio software was used for this analysis. Gene specific primers are shown with an arrow.

```
Oryza          ----------------------------------------GATTTCAAGGTCTTAATAAC
Triticum       ATCCACGACTTCCGCGCCCACCTCACGCGCTGCGCCTCCTCCCAGGACTGCCATCTCATC
Hordeum        ATCCACGACTTCCGCGCCCACCTCGCGCGCTGCGCCTCCTCCCAGGACTGCCATCTCATC
                                                             *  * * *   * *

Oryza          CAACCAATTTATCATTGCATTGCATTTCAGACTGGAACCGGCCATTTCTCTCCAATCGGC
Triticum       TCCTCCTACCACAGGAGCCCCTTCAAGCAGACTGGGACTGGCCATTTCTCACCGATCGGC
Hordeum        TCATCCTACCACAGGAGCCCCTTCAAGCAGACTGGGACTGGCCATTTCTCGCCCATCGGC
                   *     *     **         ******** ** *********** ** ******

Oryza          GGCTACCATGCCGGCCAAGACATGGCGCTTATCCTGGATGTCGCCCGCTTCAAATACCCT
Triticum       GGGTATCATGCCGAGAAAGACATGGCGCTCATCTTGGATGTTGCGCGCTTCAAATACCCT
Hordeum        GGCTACCACGCCGAGAAAGACATGGCGCTCATCTTGGATGTGGCGCGCTTCAAATACCCT
               ** ** ** ****   ************* *** ******* ** ***************

Oryza          CCTCACTGGGTTCCACTCCCACTGCTTTGGGAAGCCATGAATACAACTGATGACGCAACT
Triticum       CCTCATTGGGTTCCATTGACGCTTCTCTGGGATGCCATGAACACGACTGATGAAGCAACT
Hordeum        CCTCATTGGGTTCCATTGACGCTTCTCTGGGATGCCATGAACACGACCGATGAAGCAACT
               ***** ********* *  * ** ** ***** ******** ** ** ***** ******

Oryza          GGTCTACTCAGGGGGTTCATGCTTATCTCAAGGCACACTGCAGCTCCTTCATTGCTCTAC
Triticum       GGGCTTCTCAGGGGGTTCATGCTTGTATCAAGGCGCAGTTCAGCTCCTTCATTGCTCTAC
Hordeum        GGGCTTCTCAGGGGGTTCATGCTTGTATCAAGACGCAGTTCAGCTCCTTCATTGCTCTAC
               ** ** ****************** * ***** * ** * ********************

Oryza          ACAGTGAGTTGCAGAGATGAAAGCTGGAAAAGCATGGCGAAGTATTGCATGGAAGATGTA
Triticum       ACAGTGAGTTGCGGCCATGGAAGTTGGAAAAGCATGGCAAAGTATTGTGTGGAAGATGTG
Hordeum        ACAGTGAGTTGCGGCGATGGAAGTTGGAAAAGCATGGCAAAGTATTGTGTGGAAGATGTA
               ************ *  *** *** ************** ********  **********

Oryza          CCCGATCTTCTTAAGGATGAGAGTGTAGACAATGTTCCAGCACTTCTGTCCCGCTTAGTG
Triticum       CCCAATCTACTGAAGGATGAGAGTCTAGACAATGTTACAACACTTCTGTCCCGCCTAGTG
Hordeum        CCCAATCTACTGAAGGATGAGGGTCTAGACAATGTTACAACACTTCTGTCCCGCCTAGTG
               *** **** ** ********* ** *********** ** ************** *****

Oryza          AAATCCCTTCCTGCCAATGCTGGAAATTTGATCAAATGGGTTATTGAAGTTAGGAGACAA
Triticum       GAATCTCTCCCAGCCAATGCTGGAGATTTGATCAAATGTGTCATTGAAGTTAGGAGAAAA
Hordeum        GAATCTCTCCCAGCCAATGCTGGAGATTTGATCAAATGTGTCATTGAAGTTAGGAGAAAA
                **** ** ** ************ ************* ** *************** **

Oryza          GAGGAAGGAGGATCAGGATTAAGCAAAGAGGAGGAAGAAAGGCTTATTTTGAAGGAAATG
Triticum       GAGGAAGGTGAATCAAGCTTGAGTAAAGAGGAGAAAGAAAGGCTTTTTTTGAAGGAAAAA
```

```
Hordeum         GAGGAAGGTGGATCAAGCTTGAGCAACGAGGAGAAAGAAAGGCTTGCTTTGAAGGAAAAA
                ******** * **** * ** ** ** ****** ***********  ***********

Oryza           ATACTACAGCAAGTCCGTGATACTGAGCTTTTTAGATTAGTCCGTGAACTGCAATTCACT
Triticum        GTATTACAGCAAATCCGTGATACTGATCTTTTCAGAGTAGTCCACGAACTGCAATATCCC
Hordeum         GTATTACAGCAAATCCGTGATACTGATCTTTTCAGAATAGTTCACGAACTGCAATATCCC
                 ** ******** ************* ***** *** **** *  **********   *

Oryza           AAGCAGCCATGTTGTAGTTGCTCATATTCAAGTGATGATGATTCCTTTACCCGGATTGCA
Triticum        AAGGGGCTATGTGGTAGTTGCTCGTCTTCAAGTGATGAAGATTCGCTTGCCGAGATTGCA
Hordeum         AAGGGGCTATGTGATAGTTGCTCGTTTTCAAGAGATGAAGACTCGCTTGCCCAGATTGCA
                ***  ** ****  ********* * ****** ***** ** **  ** **  *******

Oryza           GCCTCTGTGTGCTGTCAAGGGGCCGCATTGCTAACAGGGAATCTTTCATCAAAAGATGGG
Triticum        GCCACTGTGTGCTGTCAAGGAGCTGCATTCCTATCTGGTAACCTTGTATCTAGAGATGGG
Hordeum         GCCACTGTATGCTGTCAAGGAGCTGCATTCCTATCTGGCAACCTTGTATCTAGAGATGGG
                *** **** *********** ** ***** *** * ** ** ***  *** * *******


Oryza           TTCTGCTGCAGAGAAACTTGCTTCAAATGTGTACAAGTGGATGGTGATGGGCCTAAGACT
Triticum        TTCTGCTGCCGAGAAACATGTATCAAATGTATAGAAGCAAATGGTGATGGACTAAAGACT
Hordeum         TTCTGCTGCCGAGAAACATGTATCAAATGTATACAAGCAAATGGTGATGGACTAAAGACT
                ********* ******* **  ******** ** ***   ********** *  ******

Oryza           GTCGTTACAGGCACAGCGGTTTCAGGAGTCAATGAACAAAGTGTTGATATGCTTCTACCG
Triticum        GTTATCTCAGGAACCGTGGTATCTAAAGGGAATGAACAGGCTGTTGATTTGCTTTTACCA
Hordeum         GTTATCTCAGGCACCGTGGTATCTAAAGGGAATGAACAGGGTGTTGATTTGCTATTACCA
                **  *  **** ** * *** **   **  ********   ******* ****  ****

Oryza           ATATCCACATTGGAAACAAGCGTGTGCAATTCAAATTCAAGCAACGAGGTTGTCAAATAT
Triticum        ACATCCTCGTCAAAAACAAGCTTATGCAATTCAAACTTGAAGAGCAAGATTGTCAAGTAT
Hordeum         ACTTCTTCATCGGAAACAAGCTTATGCAATTCAAACTTGAGGAGCAAGATTGTCAAGTAT
                *  **  * *   ******** * *********** *  *  * * ** ******* ***

Oryza           CCATCTAGAACAGATATTTTAACTGTTCTATTGCTGGCTTTACATCCTAGCACATGGGTG
Triticum        CCATCAAGCACAGATGTTCTAACTGTCCTACTGCTGGTTTTACAGCCTAACACATGGCTT
Hordeum         CCATCAAGCACAGATGTTCTAACTGTCCTATTGCTGGTTTTACAGCCTAACACATGGCTT
                ***** ** ****** ** ******* *** ****** ****** **** ******* *

Oryza           GGCATTAAAGACGAGAGGCTGAAAGCTGAATTCCAGAGTCTTATTTCAACAGACATTCTT
Triticum        GGCATAAAAGACGAGAACGTGAAAGCTGAATTTCAGAGTCTTGTTTCAACAGACAATCTT
Hordeum         GGCATAAAAGACGAGAAAGTGAAAGCTGAATTCCAGAGTCTTGTTTCAACAGACAACCTT
                ***** **********   ************* ********* ************  ***

Oryza           CATGATGATCTTAAACGAGAGATATTGCATCTAAGACGGCAACTCCATTATGTGAGGTCC
Triticum        CCTGATCTTCTTAAACAGGAGATACTGCATCTAAGGCGGCAGCTCCATTATTTGGCTGGT
Hordeum         CCTGATCTTCTTAAACAGGAGATACTGCATCTGAGGCGGCAGCTCCATTATTTGGCGGGC
                * ****  ********  ****** ******* ** ***** ********* **

Oryza           TGTAAAGAGGAGGAATATGGAGATCCTGTGCCACAATCCCATTAACAATGATGCAA---A
Triticum        TGTAAAGGACAGGAGGCATGTCAAGAGCCTCCATCCCCTTAGGGACGCTGCTGCGACAAT
Hordeum         TGTAAAGGACAGGAGTCATGTCAAGAGCCTCCATCTCCTTAGTGATGGTGCCGCGACAAT
                *******   ****     *  *       ***    *  *   *   **  ** *

Oryza           TCGCGCAGTTGGTTA-----------CCCTGGAGATGCAAAAAAAAGGGGTTAGAGGAGG
Triticum        CTGCTCACTTGGTTAGGAGATAAGGCCCTTGGAGATCCCACGAGCATACTATCGAGGCAA
Hordeum         CTGCTGACATGATTAGGAGAGGAGGCCCTTGGAGATCCCGCGAGCATACTATTGAGGCAA
                  **  *  ** ***           ** ******* *    *  *     * ****

Oryza           AACTACATACTCCGTA-------TTACCTTTGTTTCGAGTGAGGACTTCTCATTTTTGAG
Triticum        AAATATATGATTCAATAAACAGACTTACTTCGTGAGGTAGGAGGACATACTAAGGATCAA
Hordeum         AATTATATGATTCAATAAACAGACTTACTTCGTGAGGTAGCAGGACATGCTAAGGATCAA
```

```
                ** ** **  * *           *  *** **   *    ***** *   *    * *

Oryza           -ACACCTGACCTGAGACGGATCCGTGTAGACATG-TTCATGTTCATCACCTGTGGTCGTT
Triticum        AGTAATAGGATTTTGAGAGAGTATTGGAGCCAAGATAGCTGGTAACATCCTGGTTACCGG
Hordeum         -GTAATATGATTTTGAGAGAGTATTGGAGCCAAGATAGCTGGTAACATCCTGGTTTTGGT
                   *       *  **  **    ** ** ** * *   ** * *   ****

Oryza           TCTCTTGTTAGTGACAAC---TGACAACTAGCGGGGAGGCACACGCTAATTGTGCGG---
Triticum        TCTCTTTTGGTTCACTATATATGTAAATTCTTTTGCGTTTATATTATTTTCTTCGACTAT
Hordeum         -----------GCACTATATATGTAAATTCTTTTGTGTTTATATAATTTTCTTGGACTGT
                             ** *    **  ** *     *     * *   *  *  *
```

Figure 5: Multiple alignment of the nucleotide sequence of *PCS1* clone with *Hordeum vulgare PCS1* and *Oryza sativa Japonica PCS1* genes.

To investigate the evolutionary relationships among PCS1 and other related genes in different crop species in plants (as revealed by Shen, et al. 2010 and Li, et al. 2009), the phylogenetic tree was constructed on the basis of a nucleotide similarity using phylogeny fr. software (Fig.6). The nucleotide sequence homology is shown in table 1. The result showed that the plant PCS1 genes are clearly divided into three distinct clusters. For PCS1, monocots: *Zea mays*, *Brassica rapa* and *Pteris vittata*. For Dicots: *Nicotiana tabacum*, *Populus trichocarpa*, *Allium sativum*, *Typha latifolia*, *Oryza sativa Japonica*, *Cynodon dactylon*, *Paspalum vaginatum*, *Setaria italica*, *Phragmites australis*, *Triticum aestivum*, and *Hordeum vulgare*. AtPCS1 was closely related to the Hordeum vulgare PCS1 gene (GenBank accession no AK372435.1) and was clustered in same clade. Citrus, clementine (XM_006443729.1) and Glycine max (NM_001248647.1) were at a distance from these groups. It is concluded that genes which have been cloned from wheat share a common evolutionary ancestor with the other related PCS1 gene in plants (*Hordeum vulgare*, *Oryza sativa*, *Pteris vittata*).

Table 1. Nucleotide Sequence homology of wheat *PCS1* gene with genes of other crops.

Crop Name	Gene	Accession No.	Nucleotide Identity
Triticum aestivum	PCS1	AF093752.1	100%
Hordeum vulgare	PCS1	AK3735.1	96%
Oryza sativa Japonica	PCS1	AK071958.1	88%
Pteris vittata	PCS1	HM559480.1	88%
Phragmites australis	PCS1	JX826285.1	84%
Zea mays	PCS1	Eu975366.1	82%

J. Bioresource Manage. (2016) 3(4): 24- 36.

Brassica rapa	PCS1	GU971084.1	82%
Cynodon dactylon	PCS1	AF384111.2	79%
Paspalum vaginatum	SPCT2	KT203454.1	79%
Setaria italica	PCSI	KM_004968528.3	79%
Nicotiana tabacum	CAD1	AY235426.1	72%
Typha latifolia	PCS1	AF308658.3	70%
Populus trichocarpa	PCS1	XM_002320590.2	69%
Allium sativum	PCS1	AF384110.1	69%
Citrus clementine	PCS1	XM_006443729.1	69%
Glycine max	PCS1	NM_001248647.1	69%

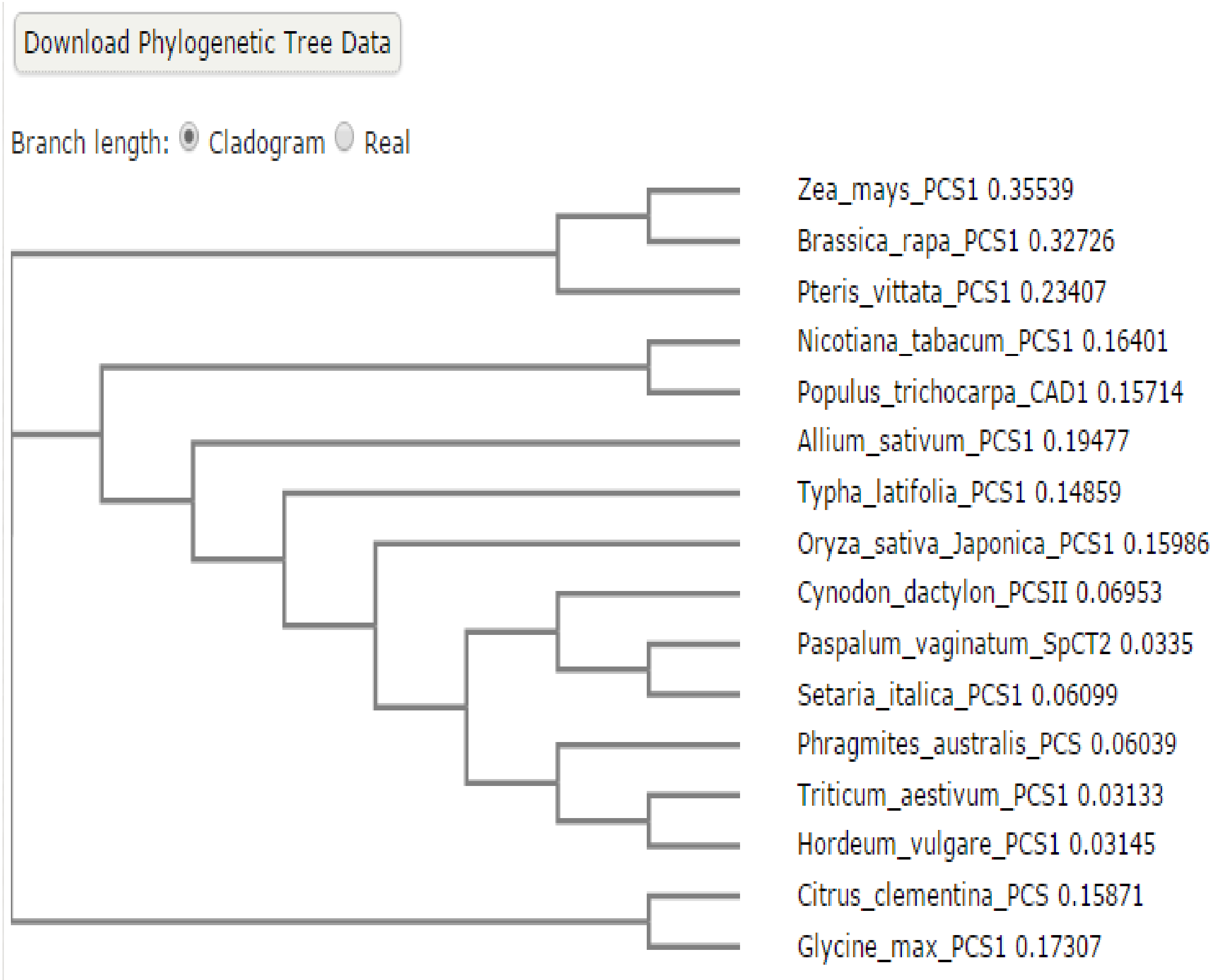


Figure 6: Phylogenetic tree of nucleotide sequences among the genes from other crops similar to Wheat *PCS1* gene. The tree was constructed using phylogeny.fr.software

It will be helpful to understand the role of this gene in the metabolic pathway of phytochelatin biosynthesis in wheat. It will also provide an opportunity to keenly study the phytochelatin biosynthesis pathway and its metabolic engineering for the beneficial purposes of human and animal health care (Zhu, et al. 1999, Kramer and Chardonnens, 2001, Sadi, et al. 2008, Song, et al. 2010, Pal and Rai 2010, Cozatl, et al. 2011, Liu, et al. 2012, Shukla, et al. 2013).

Despite the major role of PCs in Cd detoxification, the overexpression of the phytochelatin synthase gene (PCS) to engineer enhanced Cd tolerance and accumulation led to contrasting plant phenotypes. Numerous studies have demonstrated that it can have either no effect on Cd tolerance (Wawrzynski, et al. 2006), increased Cd tolerance (Gisbert, et al. 2003, Martínez, et al. 2006, Pomponi, et al. 2006, Gasic and Korban, 2007, Greger, et al. 2016) or, surprisingly, result in Cd hypersensitivity (Lee, et al. 2003, Li, et al. 2004, Chen, et al. 2016). Over expression of PCS1 is helpful in phytoremediation and will be helpful in improving yields of crops under heavy metal stress.

Conclusion

This analysis clearly demonstrated that a cloned and sequenced fragment is the wheat PCS1 gene which has a close relationship with Hordeum vulgare, *Oryza sativa Japonica*, *Pteris vittata*, *Zea mays*, *Brassica rapa*, and *Phragmites australis*. *Glycine max* and *Citrus clementina* showed less homology with cloned wheat PCS1. The cloned wheat PCS1 gene will provide opportunities to understand its role wheat phytochelatin biosynthesis pathway, and open new ways to improve wheat tolerance to heavy metal stresses. By manipulating this gene and genetically engineering the crop for the benefits of human and animals, PCS1 may prove helpful in phytoremediation purposes.

Acknowledgements

This study was funded by University of Agriculture Faisalabad, Center of Agricultural Biochemistry and Biotechnology (CABB) Pakistan.

REFERENCES

Ariani A, Alessandra F, Andrea A, Luca S (2015). Over-expression of AQUA1 in Populus alba Villafranca clone increases relative growth rate and water use efficiency, under Zn excess condition. Plant Cell Rep. DOI 10.1007/s00299-015-1883-9. 22:299-313.

Chang YH, Hui L,Yu C, Jing L, Bao LS (2012). Characterization and exression of a phytochelatin synthase gene in Brich leaf pear. Plant. Mol. Biol. Rep. 30: 1329–1337.

Chaurasia N, Mishra Y, Rai LC (2008). Cloning expression and analysis of phytochelatin synthase (PCS) gene from Anabaena sp. PCC 7120 offering multiple stress tolerance in Escherichia coli. Biochem. Biophys. Res. Commun. 376: 225-230.

Chen Y, Chuanming C, Zhiqun T, Jun L, Lili Z, Zhimin Y, Bingru H (2016). Functional Identification and Characterization of Genes Cloned from Halophyte Seashore Paspalum Conferring Salinity and Cadmium

Tolerance. ORIGINAL RESEARCH. doi:10.3389/fpls.2016.00102. 7: 102-114.

Clemens S, Kim EJ, Neumann D, Schroeder JL (1999). Tolerance to toxic metals by a gene family of phytochelatin synthases from plants and yeast. EMBO. J. 18: 3325-3333.

Clemens S (2001). Molecular mechanisms of plant metal tolerance and homeostasis. Planta 212: 475–486.

Clemens S (2006). Evolution and function of phytochelatin synthases. J. Plant Physiol. 163: 319-332.

Cobbett CS, May MJ, Howden R, Rolls B (1998). The glutathione deficient, Cd-sensitive mutant, cad2–1, of Arabidopsis thaliana is deficient in c-glutamylcysteine synthetase. Plant J. 16: 73-78.

Cobbett CS (2000a). Phytochelatins and their roles in heavy metal detoxification. Plant Physiol. 123: 825–832.

Cobbett CS (2000b). Phytochelatins and heavy metal tolerance in plants. Curr. Opin. Plant Biol. 3: 211-216.

Cozatl DGM, Jobe TO, Hauser F, Schroede JI (2011). Long-distance transport, vacuolar sequestration, tolerance, and transcriptional responses induced by cadmium and arsenic. Curr. Opin. Plant. Biol. 14: 554-562.

DalCorso G, Farinati S, Maistri S, Furini A (2008). How plants cope with cadmium: staking all on metabolism and gene expression. J. Integr. Plant Biol. 50: 1268-1280.

Ekmekci Y, Tanyolac D, Ayhan, B (2008). Effects of cadmium on antioxidant enzyme and photosynthetic activities in leaves of two maize cultivars. J. Plant Physiol. 165: 600–611.

Gasic K, Korban SS (2007). Expression of Arabidopsis phytochelatin synthase in Indian mustard (Brassica juncea) plants enhances tolerance for Cd and Zn. Planta. 225: 1277–85.

Gekeler W, Grill E, Winnacker EL, Zenk MH (1989). Survey of the plant kingdom for the ability to bind heavy metals through PCs. Z. Natur. Forsch. 44: 361-369.

Gisbert C, Ros R, De Haro A, Walker D, Bernal M, Serrano R et al. (2003). A plant genetically modified that accumulates Pb is especially promising for phytoremediation. Biochem. Biophys. Res. Commun. 303: 440–5.

Greger M, Ahmad HK, Tommy L, Pooja JM, Sylvia L (2016). Silicate reduces cadmium uptake into cells of wheat. Environmental Pol. 211:90-97.

Grill E, Winnacker EL, Zenk MH (1985). Phytochelatins: the principal heavy-metal complexing peptides of higher plants. Sci. 230: 674-676.

Ha SB, Smith AP, Howden R, Dietrich WM, Bugg S, Connell MJO, Goldsbrough PB, Cobbetta CS (1999). Phytochelatin Synthase Genes from Arabidopsis and the Yeast Schizosaccharomyces pombe. The Plant Cell. 11: 1153-1163.

Hall JL (2002). Cellular mechanisms for heavy metal detoxification and tolerance. J. Exp. Bot. 53: 1–11.

He Z, Li J, Zhang H, Ma M (2005). Different effects of calcium and lanthanum on the expression of Phytochelatin synthase gene and cadmium absorption in Lactuca sativa. Plant Sci. 168: 309-318.

Heiss S, Wachter A, Bogs J, Cobbett C, Rausch T (2003). Phytochelatin synthase (PCS) protein is induced in Brassica juncea leaves after prolonged Cd exposure. J. Exp. Bot. 54: 1833-1839.

Inouhe M (2005). Phytocheltins.Braz. J. Plant Physiol. 17: 65-78.

Jung B, Jungwook P, Hokyoung S, Yin-Won L, Young-Su S, Jungkwan L (2014). A Putative Transcription Factor pcs1 Positively Regulates Both Conidiation and Sexual Reproduction in the Cereal Pathogen Fusarium graminearum. Plant Pathol. J. 30: 236-244.

Kahl J, Nicolaas B, Gaby M, Jens-Otto A, Paul D, Aumaporn A, Angelika P (2015). Standardization and Performance Test of Crystallization with Additives Applied to Wheat Samples. Food Anal. Methods. DOI 10.1007/s12161-015-0142-6. 10:142-150.

Kopyra M, Gwozdz EA (2003). Nitric oxide stimulates seeds germination and counteracts the inhibitory effect of heavy metals and salinity on root growth of Lupinus luteus. Plant Physiol. Biochem. 41: 1011–1017.

Kramer U, Chardonnens AN (2001). The use of transgenic plants in the bioremediation of soils contaminated with trace elements. Appl. Microbiol. Biotechnol. 55: 661-672.

Lee S, Petros D, Moon J, Ko T, Goldsbrough P, Korban S (2003). Higher levels of ectopic expression of Arabidopsis phytochelatin synthase do not lead to increased cadmium tolerance and accumulation. Plant Physiol. Biochem. 41: 903–10.

Li AM, Yu BY, Chen FH, Gan HY, Yuan JG, Qiu R, Huang JC, Yang ZY, Xu ZF (2009). Characterization of the Sesbania rostrata Phytochelatin Synthase Gene: Alternative Splicing and Function of Four Isoforms. Int. J. Mol. Sci. 10: 3269-3282.

Li LZ, Chen T, Willie JGMP, Yong-Ming L (2016). Characteristics of cadmium uptake and membrane transport in roots of intact wheat (Triticum aestivum L.) seedlings. Environmental Pol. 155: 1-8.

Li Y, Dhankher OP, Carreira L, Lee D, Chen A, Schroeder JI et al. (2004). Overexpression of phytochelatin synthase in Arabidopsis leads to enhanced arsenic tolerance and cadmium hypersensitivity. Plant Cell Physiol. 45: 1787–97.

Liu Z, Gu C, Chen F, Yang D, Wu K, Chen S, Jiang J, Zhang Z (2012). Heterologous Expression of a Nelumbo nucifera Phytochelatin Synthase Gene Enhances Cadmium Tolerance in Arabidopsis thaliana. Appl. Biochem. Biotechnol. 166: 722–734.

Lockwood (1976). Longevity and reproduction of Daphnia pulex (de Geer) exposed to cadmium-contaminated food or water. Environmental Pollution. 19: 295-305.

Martínez M, Bernal P, Almela C, Vélez D, García-Augustín P, Serrano R et al. (2006). An engineered plant that accumulates higher levels of heavy metals than Thlaspi caerulescens, with yields of 100 times more biomass in mine soils. Chemosphere. 64: 478–85.

Ming C, ZHAO J, LÜ J, REN Z, WU H (2016). Homologous cloning, characterization and expression of a new halophyte phytochelatin synthase gene in Suaeda salsa. Chinese J. Oceanol. Limnol. http://dx.doi.org/10.1007/s00343-016-4382-0.

Munzuroglu O, Geckil H (2002). Effects of metals on seed germination, root elongation, and coleoptile and hypocotyls growth in Triticum aestivum and Cucumis sativus. Arch. Environ. Cont. Tox. 43: 203-213.

Pal R, Rai JPN (2010). Phytochelatins: Peptides Involved in Heavy Metal Detoxification. Appl. Biochem. Biotechnol. 160: 945-963.

Pomponi M, Censi V, Di Girolamo V, De Paolis A, di Toppi LS, Aromolo R et al. (2006). Overexpression of Arabidopsis phytochelatin synthase in tobacco plants enhances Cd2+ tolerance and accumulation but not translocation to the shoot. Planta. 223: 180–90.

Ramos J, Naya L, Gay M, Abian J, Becana M (2008). Functional characterization of an unusual Phytochelatin synthase, LjPCS3, of Lotus japonicus. Plant Physiol. 148: 536-545.

Rauser WE. 1990. Phytochelatins. Annual Review of Biochemistry. 59: 61-86.

Sadi BBM, Vonderheide AP, Gong JM, Schroeder JI, Shann JR, Caruso JA (2008). An HPLC-ICP-MS technique for determination of cadmium Phytochelatins in genetically modified Arabidopsis thaliana. J. Chromatogr. 861: 123-129.

Sanita di Toppi L, Gabbrielli R (1999). Response to cadmium in higher plants. Environ. Exp. Bot. 41: 105–130.

Savaghebi GH, Ardalan MM, Malekouti MJ (2002). Effects of combined application of cadmium and Zinc in Calcareous soil on response of wheat plant, Irainan. J. Agric. Sci. 33: 2-9.

Shen GM, Zhu C, Du QZ (2010). Genome-wide identification of Phytochelatin and Phytochelatin synthases domain-containing Phytochelatin family from rice. Electronic J. Biol. 6: 73-79.

Shukla D, Tiwari M, Tripathi RD, Nath P, Trivedi PK (2013). Synthetic Phytochelatins complement a Phytochelatin-deficient Arabidopsis mutant and enhance the accumulation of heavy metal(loid)s. Biochem. Bioph. Res. Co. 434: 664-669.

Song WY, Parka J, Cozatlc DGM, Grotemeyerd MS, Shima D et al. (2010). Arsenic tolerance in Arabidopsis is mediated by two ABCC-type phytochelatin transporters. PNAS. 107: 21187-21192.

Thangavel P, Long S, Minocha R (2007). Changes in phytochelatins and their biosynthetic intermediates in redspruce (Picea rubens Sarg) cell suspension cultures under cadmium and zinc stress. Plant Cell Tiss. Org. 88: 201-216.

Wawrzynski A, Kopera E, Wawrzynska A, Kaminska J, Bal W, Sirko A (2006). Effects of simultaneous expression of heterologous genes involved in phytochelatin biosynthesis on thiol content and cadmium accumulation in tobacco plants. J. Exp. Bot. 57: 2173–82.

Wu F, Zhang G, Dominy P, Wu H, Bachir DML (2007). Differences in yield components and kernel Cd accumulation in response to Cd toxicity in four barley genotypes. Chemosphere 70: 83–92.