African Journal of Biotechnology Vol. 10(69), pp. 15470-15476, 7 November, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.422
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Molecular cloning and phylogenetic analysis of a novel BURP domain-containing gene from *Camellia sinensis*

Peng Wang, Rui-Rui Su, Ji-Yang Zheng, Shuang-Huai Cheng and Guo-Ping Zhu*

The Key Laboratory of Molecular Evolution and Biodiversity, Institute of Molecular Biology and Biotechnology, College of Life Sciences, Anhui Normal University, No.1 Beijing East Road Wuhu 241000, Anhui, China.

Accepted 29 July, 2011

BURP domain-containing proteins are a new class of plant-specific proteins that play important roles in the growth, development and stress response of plants. In this paper, a novel BURP domain-containing gene from *Camellia sinensis* (*CsBDP*, GenBank accession No. EU715397) was isolated and cloned using reverse transcription-polymerase chain reaction (RT-PCR) followed by 5′ and 3′ rapid amplification of cDNA ends (RACE) reactions. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot were then applied to detect the expression of *CsBDP* gene in *Escherichia coli*. Sequence comparison showed that the deduced protein sequence of *CsBDP* gene shared high sequence similarity to other BURP domain-containing proteins and contained four typical modules: an N-terminal hydrophobic region, a short conserved segment, a variable region containing two repeats of about 19 amino acids, which is unique for the RD22-like proteins of BURP family and a C-terminal BURP domain. Furthermore, phylogenetic tree revealed that the BURP protein family can be classified into eight subfamilies, and CsBDP was distinctly clustered into subfamily III with all RD22-like proteins. Therefore, *CsBDP* gene might be a stress-inducible gene and important for the stress tolerance of tea plant.

Key words: *Camellia sinensis*, BURP domain-containing protein, cloning, sequence comparison, phylogenetic analysis.

INTRODUCTION

The BURP domain-containing proteins are ubiquitous in the plants. This family is termed as such based on the conservation of the deduced amino acid sequences of four typical members (Hattori et al., 1998): BNM2, a microspore protein of rapeseed/canola (*Brassica napus*) (Treacy et al., 1997); USP, an unknown seed protein of bean (Vicia faba) (Bassüner et al., 1988); RD22, a dehydration-responsive protein of *Arabidopsis thaliana* (Yamaguchi-Shinozaki and Shinozaki 1993); and PG1β, a β-subunit of polygalacturonase isozyme 1 of tomato (*Lycopersicon esculentum*) (Zheng et al., 1992). BURP domain-containing proteins consist of three or four conserved modules: (i) an N-terminal hydrophobic domain with a presumptive transit peptide; (ii) a short conserved segment or other short segment; (iii) an optional segment consisting of repeated units, which is unique to each member; and (iv) the C-terminal BURP domain (Hattori et al., 1998). The BURP domain is a motif of about 230 amino acids and usually contains several highly conserved sequences, including two phenylalanine residues (FF), two cysteine (C) residues and four repeated cysteine-histidine (CH) motifs. The sequence between CH motifs is always $CHX_{10}CHX_{25\text{-}27}CHX_{25\text{-}26}CH$, where X represents any amino acid (Hattori et al., 1998).

Although evidence suggests that the BURP proteins have diverse functions in plants (Hattori et al., 1998), only a few BURP genes have been isolated and the functions of most members are still obscure. It is reported that PG1β forms a PG1 complex with catalytic PG2 polypeptide. The PG1 complex accumulates during fruit ripening and plays a role in pectin solubilization and depolymerization (Pogson et al., 1991). BNM2 is originally expressed in microspore-derived embryos of *B. napus*, but accumulates in the protein storage vacuoles (PSV) (Treacy et al., 1997). The overexpression of BNM2

*Corresponding author. E-mail: gpz1996@yahoo.com Tel/Fax: + 86 553 3883592.

Table 1. Primers used in this study.

Primer name	Sequence (5' to 3')
C1	GGAGAGGARAARTATTGTGC
C2	TGTGGCANACNGCANCTGCT
5′-GSP	GCATCGCTTGCCATCTTCTTCACTCCTT
3′-GSP	AGCCGTAGTGTGCCATAAGCAAAAC
E1	AATATCGCGGATCCATGGAGTTCCACTTCCTACCCATCC
E2	ATAAGAATGCGGCCGCGTTATGAACCCAAACTATATGATCC

causes the distortion of PSV (Teerawanichpan et al., 2009). USP plays similar roles in the growth and development of plants. It is found that the ectopic expression of AtUSPL1 from A. *thaliana* causes the shrunken of seeds (Van Son et al., 2009). RAFTIN, an anther-specific BURP protein from rice (*Oryza sativa*) and wheat (*Triticum aestivum*), is essential for the late phase of pollen development. Suppression of *RAFTIN1* in rice causes mature anthers nondehiscent and the collapse of pollen grains (Wang et al., 2003).

In addition, BURP domain-containing proteins have been found to be responsive to stress treatments, such as *BnBDC1* from *B. napus* upregulated by mannitol, and NaCl and ABA (Yu et al., 2004). Recent genome-wide studies systematically characterize 17 BURP genes in rice, 23 BURP genes in soybean, 15 BURP genes in maize and 11 BURP genes in sorghum (Ding et al., 2009; Xu et al., 2010; Gan et al., 2011). These genes have diverse structures and expression patterns under different stress conditions. Stress treatments show that 15 of 17 BURP genes in rice, 17 of 23 BURP genes in soybean and 8 of 15 BURP genes in maize respond to the stress treatments, including drought, salt, cold and abscisic acid (ABA) treatment (Ding et al., 2009; Xu et al., 2010; Gan et al., 2011). These results suggest that BURP genes are crucial for plant development and stress adaptation. In this study, a novel BURP domain-containing gene from *Camellia sinensis* (*CsBDP*) was isolated, cloned and expressed in *Escherichia coli* as a fused protein. Furthermore, the relationships of CsBDP protein to other BURP domain-containing proteins were analyzed by the phylogenetic tree.

MATERIALS AND METHODS

Plant materials

The young and most recently emerged developing leaves from flush shoots of tea plants (*C. sinensis* cv Longjing43) growing at the tea plantation of Anhui Agriculture University were collected and frozen in liquid N_2, and then stored at -70°C until RNA extraction.

Amplification of the conserved fragment of *CsBDP*

Total RNA was extracted using SV total RNA isolation system (Promega, Madison, WI) and used as templates to synthesize first strand cDNA by reverse transcription PCR (RT-PCR) with PrimeScript™ 1st Strand cDNA synthesis kit (TaKaRa, Japan). The degenerated primers C1 and C2 (Table 1) were used to amplify the conserved region of *CsBDP*, which were designed based on the highly conserved motifs of four plant RD22-like proteins. PCR was carried out in a thermal cycler (Bio-Rad iCycler) by initial denaturation at 94°C for 3 min followed by 35 cycles of 30 s at 95°C, 45 s at 56°C, 1 min at 72 °C with a final extension at 72°C for 10 min. The resulting PCR product was cloned into pMD19-T (TaKaRa, Japan) vector by TA cloning method and then sequenced.

Isolation of the full-length cDNA of *CsBDP*

Rapid amplification of cDNA ends (RACE) was carried out using the Invitrogen GeneRacer kit (Invitrogen, USA). Briefly, RNA was treated with calf intestinal phosphatase to remove the 5′-phosphates of the truncated mRNA and non-mRNA forms of total RNA. Dephosphorylated RNA was then treated with tobacco acid phosphatase to remove the 5′ cap structure from intact full-length mRNA. A RNA oligonucleotide was ligated onto the 5′ end of the intact mRNA using T4 RNA ligase to provide a priming site for subsequent 5′ RACE. SuperScript III RT and GeneRacer Oligo (dT) were used for reverse-transcribing the RNA oligonucleotide-ligated mRNA to single strand cDNAs. Gene specific primers 5′-GSP and 3′-GSP (Table 1) for RACE PCR were designed from the conserved fragment of *CsBDP*. PCR parameters for 5′- and 3′-RACE was 2 min at 94°C followed by 35 cycles of 30 s at 95°C, 45 s at 64°C, 1 min at 72°C with a final extension at 72°C for 10 min. PCR product was TA-cloned into pMD19-T and sequenced. Then, 5′- and 3′-ends and the conserved fragment of *CsBDP* were assembled with Sequencher version 4.0.5 (Gene Codes Corporation).

Cloning and expression of *CsBDP* gene

The GB1-fusion expression vector, pGBTNH, was used in this study, which was constructed based on the pET-22b vector and GB1 as a partner protein at N-terminus and 6His tag at C-terminus (Bao et al., 2006). The coding sequence of *CsBDP* gene was amplified with primers E1 and E2 and then inserted into pGBTNH at the cloning sites of *Bam*HI and *Not*I, generating pGBTNH-*CsBDP*. The successful cloning was confirmed by DNA sequencing. Then, *E. coli* Rosetta (DE3) harboring pGBTNH-*CsBDP* was cultured overnight at 37°C and then inoculated into the fresh LB medium containing 100 μg/ml ampicillin and 20 μg/ml chloramphenicol until the density reached an OD_{600} of 0.4 to 0.6. Isopropyl β-D-1-thiogalactopyranoside (IPTG) was then added at a final concentration of 0.5 mM with subsequent cultivation for 5 h. Cells were harvested and sonicated, and the debris was removed by centrifugation at 15,000 *g* for 15 min at 4°C. The supernatant was

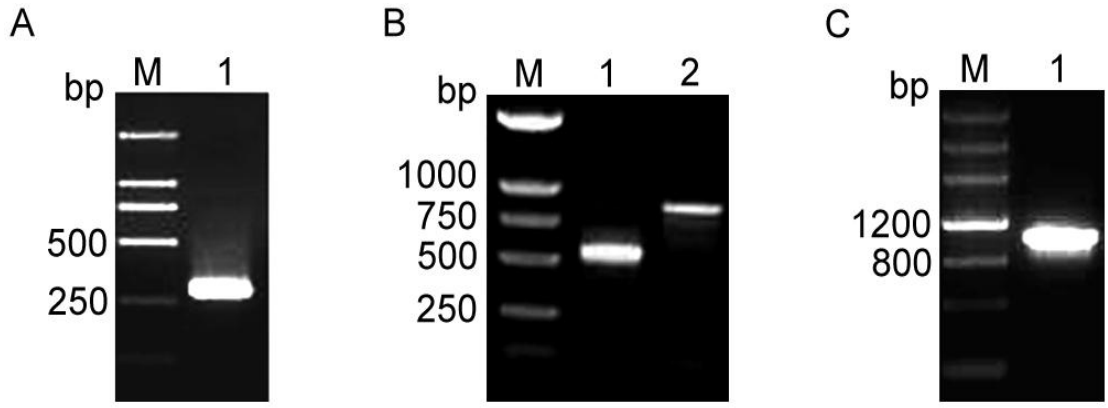

Figure 1. PCR product of amplified *CsBDP* gene. (A) Amplification of the conserved fragment of *CsBDP*; M, DNA marker; lane 1, amplified conserved fragment (291 bp); (B) amplification of 5′- and 3′- fragments of *CsBDP* by RACE PCR. M, DNA marker; lane 1, amplified 3′- fragment (659 bp); lane 2, amplified 5′- fragment (918 bp); (C) amplification of the ORF of *CsBDP*: M, DNA marker; lane 1, amplified ORF of *CsBDP* (1011 bp).

detected by SDS-PAGE.

Western immunoblotting

Protein samples were separated by 12% SDS-PAGE and transferred to nitrocellulose membranes (Amersham Biosciences. Germany) by electroblotting and blocked for 1 h at room temperature in TBS-T (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.2% Tween-20) containing 5% nonfat milk. Then, His-tag polyclonal antibody (Cell Signaling Technology Inc., Beverly, MA, USA) and alkaline phosphatase conjugated anti-rabbit IgG (Promega, Madison, WI, USA) were applied to the blocked membrane at room temperature. The blots were then incubated with the Lumi-Phos^{TM} WB Chemiluminescent Substrate (Pierce Biotechnology, U.S.A.). The blots were exposed to X-ray film for the appropriate time period to visualize the chemiluminescence signal corresponding to the specific antibody-antigen reaction in the dark room.

Phylogenetic analysis

The deduced amino acid sequence of *CsBDP* was used as a protein query for homology comparison with BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/). Other BURP protein sequences were downloaded from GenBank. Structure-based amino acid sequence alignment was conducted with ClustalX program (ftp://ftp.ebi.ac.uk/pub/software/clustalw2) (Larkin et al., 2007) and ESPript 2.2 web tool (http://espript.ibcp.fr/ESPript/ESPript/) (Gouet et al., 1999). Bootstrapped neighbor joining tree was constructed with MEGA 4 software (Tamura et al., 2007).

RESULTS

Cloning and expression of *CsBDP* gene

A 291-bp conserved DNA fragment was obtained by amplification with the degenerate primers C1 and C2 (Figure 1A). Then, 5′- and 3′-ends (918 and 659-bp) were obtained using 5′- and 3′-RACE PCR, respectively (Figure 1B). After joining three sequences, the full-length cDNA of this novel BURP-containing gene was acquired. The full-length cDNA of *CsBDP* (GenBank accession No. EU715397) was 1333-bp long. The 5′- and 3′-untranslated regions were 71 and 248-bp, respectively. The open reading frame (ORF) of *CsBDP* was 1011 bp from the start codon ATG at 72 base position to the stop codon TGA at 1083 base position (Figure 1C).

The *CsBDP* ORF encoded a 337-amino acid polypeptide (GenBank accession No. ACH97124). SDS-PAGE suggested a molecular mass of approximately 46 kDa (Figure 2A), which compared well with the molecular mass for the fusion protein as predicated (~ 9 kDa for tags and 36.9 kDa for CsBDP). Western blot analysis revealed one specific protein band using the anti-6×His antibody as probe (Figure 2B).

Sequence comparison

A BLAST search suggested that the deduced amino acid sequence of *CsBDP* shared high sequence identity with other BURP proteins, especially RD22-like proteins from diverse sources, such as *Gossypium hirsutum* (65%; GenBank accession No. AAL67991) (Li et al., 2002), *Vitis vinifera* (64%; AAV36561), and *Prunus persica* (60%; AF319165) (Callahan et al., 1993). Protein multiple sequence alignment revealed that CsBDP protein contained four conserved modules as other BURP proteins (Figure 3): (i) an N-terminal hydrophobic region (1 to 21 amino acids), which might be a signal peptide and cleaved off latterly by specific signal peptidases predicted using the SignalP 3.0 server (Bendtsen et al., 2004); (ii) a conserved segment (22 to 46 amino acids); (iii) a variable region containing two repeats of about 19 amino acids (TXVXVGKGGVXVX$_{4\sim5}$GKP, X may be any amino acid), which is the unique consensus sequence pecific for

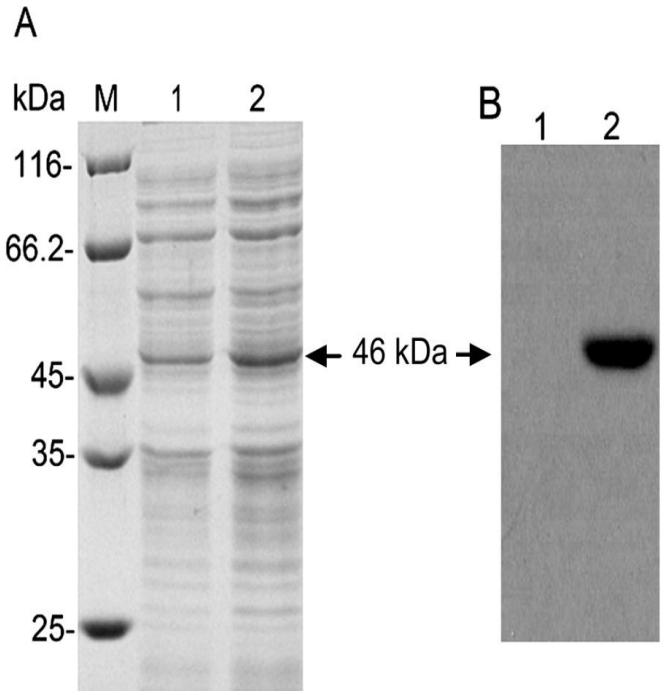

Figure 2. Expression of *CsBDP* gene and Western blot analysis. (A) SDS-PAGE analysis of *CsBDP* expression in *E. coli*; M, protein molecular weight marker; lane 1, soluble fractions of cells harboring pGBTNH-*CsBDP* without IPTG induction; lane 2, soluble fractions of cells harboring pGBTNH-*CsBDP* with 0.5 mM IPTG induction; (B) Western blot analysis using his-tag polyclonal antibody as probe: lane 1, negative control; lane 2, one specific protein band from the supernatant of *CsBDP* expression.

RD22-like proteins; (iv) a C-terminal BURP domain (128 to 337 amino acids).

Phylogenetic tree

To investigate the evolutionary relationships of CsBDP, a phylogenetic tree was generated from the deduced amino acid sequences of 69 BURP domain-containing proteins from various plant species. The phylogenetic tree was analyzed by MEGA 4 using the Neighbor-Joining method with 1000 bootstrap replicates. The evolutionary distances were calculated by Poisson correction method. The phylogenetic tree clearly showed that the BURP domain-containing protein family can be classified into eight subfamilies (Figure 4). Subfamilies I-IV corresponded to BNM2-like, USP-like, RD22-like and PG1β-like-like subfamilies (Granger et al., 2002), respectively. Subfamilies V-VII corresponded to subfamilies BURP V-VII as previously reported (Ding et al., 2009). Subfamily VIII was firstly identified in this study and all of the members were from *Physcomitrella patens* subsp. *patens*. The phylogenetic analysis demonstrated that CsBDP protein was clustered into the subfamily III with all RD22-like proteins (Figure 4).

DISCUSSION

BURP domain-containing proteins comprise a novel and broadly distributed protein family. The members of this strictly plant specific BURP domain protein family are growing rapidly and over 270 deduced proteins from about 40 kinds of the plants were deposited in GenBank database before now. The function of all BURP domain proteins are poorly understood, but thought to be involved in cellular secretion pathway during embryogenesis, seed, fruit and root development.

In this study, the *CsBDP* from *C. sinensis*, a novel gene encoding a BURP domain-containing protein was isolated using a RT-PCR and RACE based cloning strategy. A BLAST search shows that the deduced protein sequence of *CsBDP* shares high amino acid sequence identity to other RD22-like proteins in BURP family. The coding sequence of *CsBDP* encodes a 337 amino acid polypeptide that preserves the general modular construction of BURP family. The BURP domain of CsBDP protein consisted of 209 amino acids and has typical characteristics, such as two phenylalanines (FF) and four cysteine-histidine (CH) motifs (Figure 3).

The signal peptide was present in almost all the putative BURP proteins, and the sequences of the signal peptide and BURP domain were very similar to each other. However, the variable internal regions following the signal peptide distinctly vary by the family (Hattori et al., 1998). It suggests that the gene duplication and the amplification of the repeated sequence in the variable region may play an important role in the evolution of BURP genes (Ding et al., 2009). The variable region of RD22-like proteins contains several repeats of ~19 amino acids that are unique for them. The repeat sequences from different plants are diverse. For example, RD22-like protein from *A. thaliana* has four repeats of TXVXVGXGGVX$_6$KGK, RD22-like protein from *G. arboretum* has four repeats of THVNVGGKGVGV-NTGKX$_2$ and RD22-like protein from *O. sativa* has six repeats of TTVGVGKGGVGVNVKPGYG. The sequence comparison shows that the variable region of CsBDP protein from *C. sinensis* contains two repeats of TXVXVGKGGVXVX$_{4\sim5}$GKP (Figure 3), which indicates that CsBDP is a member of RD22-like proteins. Furthermore, the difference in the copy number of the repeated unit between CsBDP and the homologues may be attributed to their functional differences.

In addition, more and more putative genes of RD22-like proteins from all kinds of plants have been submitted to the GenBank database. Some of them have been isolated and are stress-responsive. *RD22* from *A. thaliana* was induced by water deficit, abscisic acid (ABA) and salt stress (Yamaguchi-Shinozaki and Shinozaki, 1993). Four genes from *Bruguiera gymnorrhiza* (*BgBDC*1, 2, 3, and 4) were responsive to salt, ABA, and drought stresses (Banzai et al., 2002). *PpRD22* from *Prunus persica* and *GhRDL* from *Gossypium hirsutum*

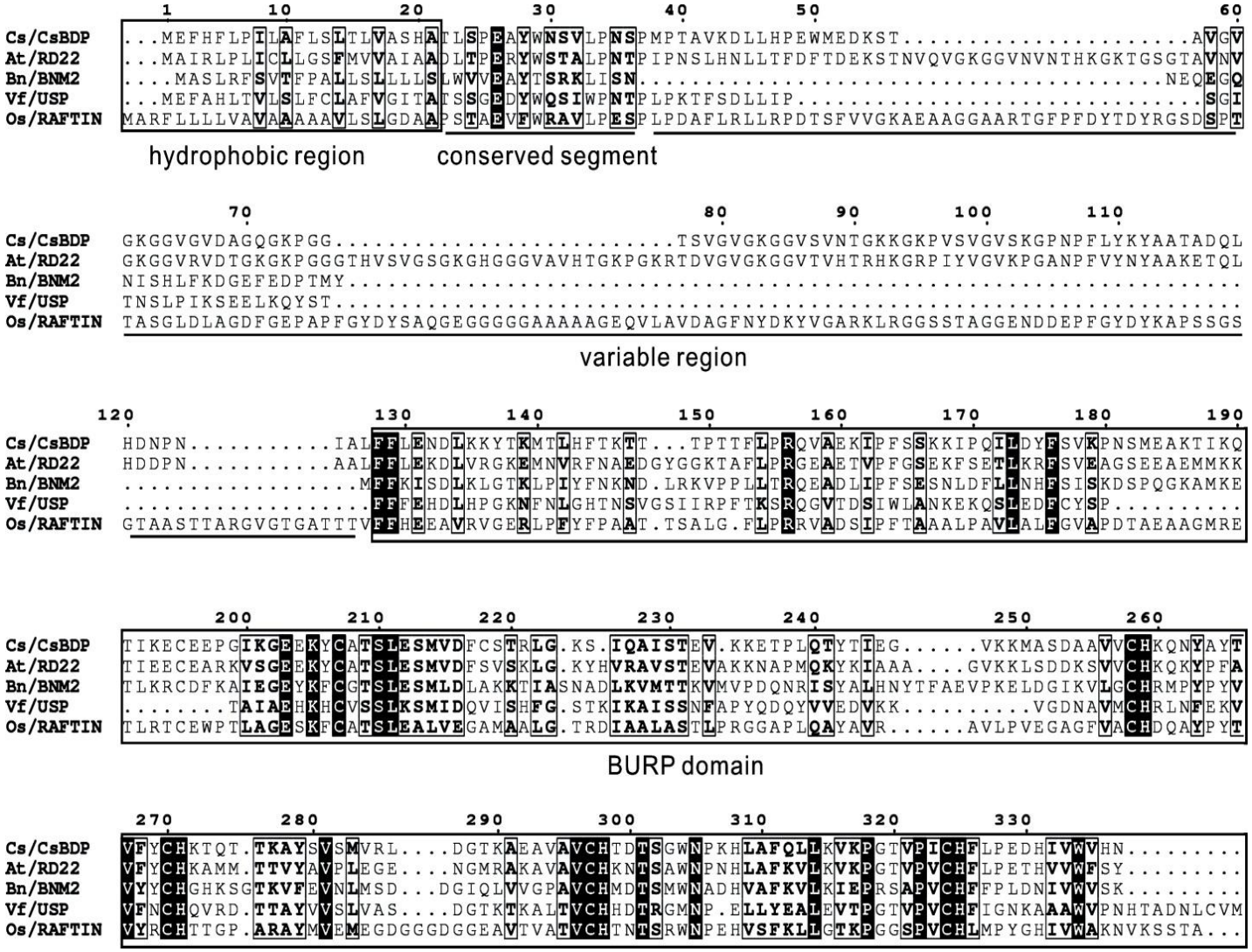

Figure 3. Multiple sequence alignment of CsBDP protein with other four typical members of BURP domain-containing protein family. The completely conserved amino acid residues are highlighted as shaded black boxes; two phenylalanine residues (FF), one cysteine (C) residues and four repeated cysteine-histidine (CH) motifs. Four structure modules of CsBDP protein are shown; Cs/CsBDP, CsBDP from *C. sinensis* (ACH97124); At/RD22, RD22 from *A. thaliana* (BAA01546); Bn/BNM2, BNM2 from *B. napus* (AAC15700); Vf/USP, USP from *Vicia faba* (CAA39696); Os/RAFTIN, RAFTIN from *O. sativa* (CAE02618).

were also upregulated by stress (Callahan et al., 1993; Li et al., 2002). The functions of CsBDP protein are yet to be elucidated. However, phylogenetic analysis reveals that the protein clustered into the clade of subfamily III with all RD22-like proteins (Figure 4). Therefore, the *CsBDP* gene might be a stress-inducible gene and encode a dehydration-responsive protein, which is important for the adaptation of tea plant.

Since plant growth and yield are greatly affected by environmental conditions, numerous stress-responsive genes from various protein families have been studied and used in genetic engineering to improve the stress tolerances of economic plants. BURP domain-containing proteins have shown great potential in the utilizations for stress-resistant transgenic crops. Therefore, the isolation and phylogenetic analysis of *CsBDP* gene would provide a fundament for further investigation of its functions and utilizations in the plants.

ACKNOWLEDGEMENTS

We thank Prof. Hongyu Hu for the numerous contributions towards the expression vector pGBTNH. This work was supported by grants from the National Natural Science Foundation of China (30870062; 31040003), Scientific Research Foundation for the Returned Overseas Chinese Scholars from State Education Ministry and Program for Innovative Research Team in Anhui Normal University.

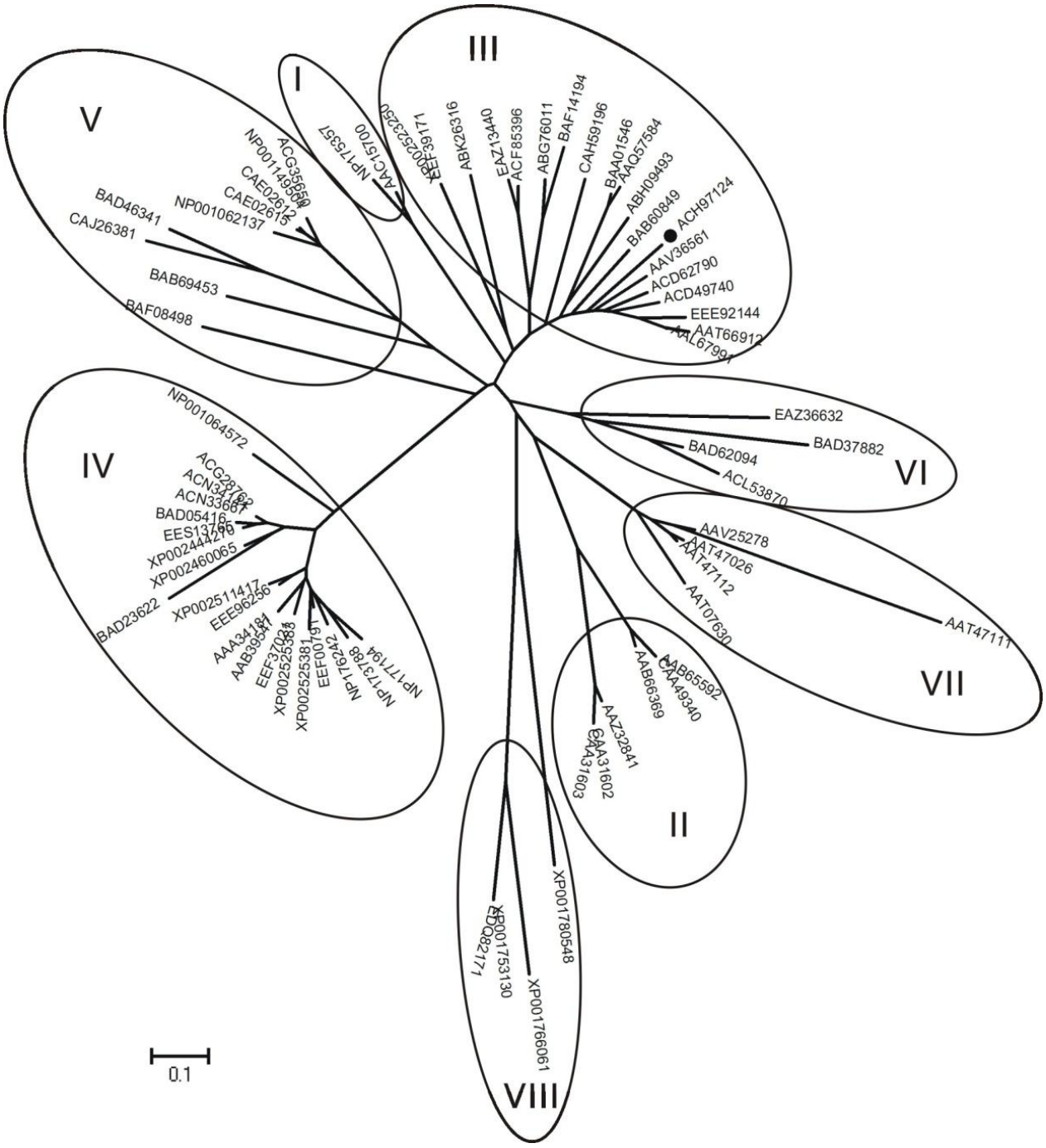

Figure 4. Unrooted phylogenetic tree of BURP domain-containing protein family. The CsBDP protein from *C. sinensis* was marked by a blackspot "•". The phylogenetic tree was generated using MEGA 4 program. Protein sequences are annotated with GenBank accession number. The scale represents the frequency of amino acid substitution between sequences as determined by Poisson evolutionary distance method.

Abbreviations

***CsBDP*,** BURP domain-containing gene from *Camellia sinensis*; **BNM2,** *Brassica napus* microspore protein; **USP,** unknown seed protein; **RD22,** *Arabidopsis thaliana* dehydration-responsive protein; **PG1β,** β-subunit of polygalacturonase isozyme 1 from *Lycopersicon esculentum*; **RT-PCR,** reverse transcription polymerase chain reaction; **RACE,** rapid amplification of cDNA ends; **ORF,** open reading frame; **cDNA,** complementary DNA; **IPTG,** isopropyl β-D-1-thiogalactopyranoside; **BLAST,** The Basic Local Alignment Search Tool.

REFERENCE

Banzai T, Sumiya K, Hanagata N, Dubinsky Z, Karube I (2002). Molecular cloning and characterization of genes encoding BURP domain-containing protein in the mangrove, *Brugniera gymnorrhiza*. Trees, 16: 87-93.

Bao WJ, Gao YG, Chang YG, Zhang TY, Lin XJ, Yan XZ, Hu HY (2006). Highly efficient expression and purification system of small size protein domains in *Escherichia coli* for biochemical characterization. Protein Exp. Purif. 47: 599-606.

Bassüner R, Bäumlein H, Huth A, Jung R, Wobus U, Rapoport TA, Saalbach G, Müntz K (1988). Abundant embryonic mRNA in field bean (*Vicia faba L.*) codes for a new class of seed proteins: cDNA cloning and characterization of the primary translation product. Plant Mol. Biol. 11: 321-334.

Bendtsen JD, Nielsen H, von Heijne G, Brunak S (2004). Improved prediction of signal peptides: SignalP 3.0. J. Mol. Biol. 340: 783-795.

Callahan AM, Morgens PH, Cohen RA (1993). Isolation and initial characterization of cDNAs for mRNAs regulated during peach fruit development. J. Am. Soc. Hort. Sci. 118: 531-537.

Ding X, Hou X, Xie K, Xiong L (2009). Genome-wide identification of BURP domain-containing genes in rice reveals a gene family with diverse structures and responses to abiotic stresses. Planta, 230: 149-163.

Gan D, Jiang H, Zhang J, Zhao Y, Zhu S, Cheng B (2011). Genome-wide analysis of BURP domain-containing genes in maize and sorghum. Mol. Biol. Rep., 38: 4553-4563.

Gouet P, Courcelle E, Stuart DI, Metoz F (1999). ESPript: analysis of multiple sequence alignments in PostScript. Bioinformatics, 15: 305-308.

Granger C, Coryell V, Khanna A, Keim P, Vodkin L, Shoemaker RC (2002). Identification, structure, and differential expression of members of a BURP domain containing protein family in soybean. Genome, 45: 693-701.

Hattori J, Boutilier KA, van Lookeren Campagne MM, Miki BL (1998). A conserved BURP domain defines a novel group of plant proteins with unusual primary structures. Mol. Gen. Genet. 259: 424-428.

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007) Clustal W and Clustal X version 2.0. Bioinformatics, 23(21): 2947-2948.

Li CH, Zhu YQ, Meng YL, Wang JW, Xu KX, Zhang TZ, Chen XY (2002). Isolation of genes preferentially expressed in cotton fibers by cDNA filter arrays and RT-PCR. Plant Sci. 163: 1113-1120.

Pogson BJ, Brady CJ, Orr GR (1991). On the occurrence and structure of subunits of *endopolygalacturonase* isoforms in mature-green and ripening tomato fruits. Aust. J. Plant Physiol. 18: 65-79.

van Son L, Tiedemann J, Rutten T, Hillmer S, Hinz G, Zank T, Manteuffel R, Bäumlein H (2009) The BURP domain protein AtUSPL1 of *Arabidopsis thaliana* is destined to the protein storage vacuoles and overexpression of the cognate gene distorts seed development. Plant Mol. Biol. 71: 319-329.

Tamura K, Dudley J, Nei M, Kumar S (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24: 1596-1599.

Teerawanichpan P, Xia Q, Caldwell S J, Datla R, Selvaraj G (2009). Protein storage vacuoles of *Brassica napus* zygotic embryos accumulate a BURP domain protein and perturbation of its production distorts the PSV. Plant Mol. Biol. 71: 331-343.

Treacy BK, Hattori J, Prud'homme I, Barbour E, Boutilier K, Baszczynski CL, Huang B, Johnson DA, Miki BL (1997). *BNM1*. A *Brassica* pollen-specific gene. Plant Mol. Biol. 34: 603-611.

Wang A, Xia Q, Xie W, Datla R, Selvaraj G (2003). The classical Ubisch bodies carry a sporophytically produced structural protein (RAFTIN) that is essential for pollen development. Proc. Natl. Acad. Sci. USA. 100: 14487-14492.

Xu H, Li Y, Yan Y, Wang K, Gao Y, Hu Y (2010). Genome-scale identification of Soybean BURP domain-containing genes and their expression under stress treatments. BMC Plant Biol. 10: p. 197.

Yamaguchi-Shinozaki K, Shinozaki K (1993). The plant hormone abscisic acid mediates the drought-induced expression but not the seed-specific expression of *rd22*, a gene responsive to dehydration stress in *Arabidopsis thaliana*. Mol. Gen. Genet. 238: 17-25.

Yu S, Zhang L, Zuo K, Li Z, Tang K (2004). Isolation and characterization of a BURP domain-containing gene *BnBDC1* from *Brassica napus* involved in abiotic and biotic stress. Physiol. Plantarum. 122: 210-218.

Zheng L, Heupel RC, DellaPenna D (1992). The beta subunit of tomato fruit polygalacturonase isoenzyme 1: isolation, characterization, and identification of unique structural features. Plant Cell. 4: 1147-1156.