Analysis of common bean (*Phaseolus vulgaris* L., genotype BAT93) calmodulin cDNA using computational tools

Kassim Amelia¹², Jasvin Singh², Farida Habib Shah¹³, Subhash J. Bhore¹²

¹Department of Molecular Biology, Melaka Institute of Biotechnology, Lot 7, Melaka International Trade Centre City, 75450 Ayer Keroh, Melaka, ²Department of Biotechnology, Faculty of Applied Sciences, AIMST University, Semeling 08100, Kedah, ³Department of Research and Development, Novel Plants Sdn. Bhd., 27C Jln Petaling Utama 12, 7.5 Miles Old Klang Road, 46000 Petaling Jaya, Malaysia

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**INTRODUCTION**

The animal products such as eggs, meat and milk are sources of dietary protein. But, legumes are a well-known and widely used as a source of dietary proteins, particularly by poor people.[¹] By understanding the importance of common beans (*Phaseolus vulgaris* L.) in food supply chain, the Phaseomics international consortium was developed to establish the necessary framework of knowledge and materials for the advancement of bean genomics, transcriptomics, and proteomics. The major goal of this Phaseomics international consortium is to help in generating new common bean varieties suitable and desired by farmers and consumers.[²] As a part of this consortium, research work was undertaken for the generation of *P. vulgaris* expressed sequence tags (ESTs).[³]

So far, 5972 ESTs has been generated, and while processing and analyzing generated ESTs, calmodulin (CaM) EST was identified. CaM is known to play an important regulatory role in a bimodular mechanism of calcium...
control in eukaryotes. Therefore, to elucidate the \textit{P. vulgaris} CaM (\textit{PvCaM}) cDNA clone sequence, it was fully sequenced, and cDNA and deduced amino acid (protein) sequences were analyzed and annotated in this study using computational tools. The \textit{PvCaM} gene cDNA sequence, deduced protein sequence, predicted secondary structures and three-dimensional (3D) structure is reported in this paper.

**MATERIALS AND METHODS**

\textit{Phaseolus vulgaris} L. (genotype BAT93) seeds were provided by Patricia Lariguet, Laboratoire de Biologie Moléculaire des Plantes Supérieures, Department of Plant Biology, University of Geneva, Geneva, Switzerland. Seed germination and seedlings maintenance was done as stated by Bhore \textit{et al.}\[3\]

The \textit{PvCaM} cDNA clone was identified from the ESTs generated from 5-day-old (days after anthesis) bean-pod-tissue cDNA library. The cDNA library was constructed (unpublished data) using “CloneMiner cDNA library construction kit” obtained from Invitrogen Corporation.

Escherichia coli cells harboring recombinant plasmid with \textit{PvCaM} cDNA were cultivated in 10 ml Luria-Bertani (LB) medium supplemented with 40 µg/ml Kanamycin. Plasmid DNA was isolated and purified using Wizard® Plus SV Minipreps DNA purification system (Promega). Plus and minus strand of \textit{PvCaM} cDNA clone was sequenced using M13 (forward) and M13 (reverse) primer.\[3\]

The basic alignment analysis of cDNA sequence was carried out using blast (bl2seq) program available online at National Center for Biotechnology Information and the finalized cDNA sequence was analyzed using online bioinformatics tools. The similarity search was performed using blast programs (blastN and blastP). Bioinformatics tools available at JustBio (http://www.justbio.com/) were used to deduce the protein sequence, and to find out the general features of \textit{PvCaM} cDNA and deduced protein sequence.

The deduced \textit{PvCaM} protein sequence was used as a blast (blastP) input to find the most analogous protein sequence and or structure in protein data bank.\[3\] However, for the prediction of secondary structures and the 3D structure of \textit{PvCaM}, Phyre2, a free web-based service for protein structure prediction was used.\[8\]

**RESULTS**

Full-length \textit{PvCaM} cDNA clone was isolated from 5-day old bean-pod-tissue cDNA library. Plus (+) and minus (−) strands sequence was aligned and after elimination of the adaptor sequence, cDNA sequence was finalized. Analysis of the results showed that \textit{PvCaM} cDNA is 818 bp in length. Identity of cDNA was confirmed by analyzing nucleotide (cDNA) and deduced amino acid sequences. Annotated nucleotide and deduced protein sequence of \textit{PvCaM} is deposited in GenBank/DDBJ/EMBL under the accession number JX869966. The basic annotated features of cDNA nucleotide and deduced protein sequence are summarized in Table 1, and cDNA nucleotide sequence along with its deduced amino acid sequence is shown in Figure 1.

The amino acid sequence analysis results showed that \textit{PvCaM} protein is rich in glutamic acid (13.4%) and aspartic acid (12.1%). Results also showed that \textit{PvCaM}

| Table 1: The basic features of \textit{PvCaM} cDNA and its deduced protein sequence |
|---------------------------------------------------------------|
| **General features**                                           | \textit{PvCaM} |
| Size, bp                                                      | 818           |
| Molecular weight (daltons)                                    | 253,227       |
| 5’UTR, bp                                                    | 66            |
| CDS                                                          | 450           |
| 3’UTR, bp                                                    | 302           |
| Stop codon                                                   | TGA (UGA)     |
| G+C content %                                                | 43            |
| Protein sequence                                             |               |
| Length, amino acids                                          | 149           |
| Molecular weight (dalton)                                    | 16845.73      |
| Isoelectric point (theoretical)                              | 4.12          |

\textit{PvCaM}=\textit{Phaseolus vulgaris} L. calmodulin; CDS=Coding sequence; cDNA= complementary DNA; TGA/UGA= codon; UTR= Untranslated Region

\textbf{Figure 1:} Nucleotide and deduced amino acid sequence of \textit{Phaseolus vulgaris} L. calmodulin cDNA (GenBank Accession No: JX869966). An open reading frame (ORF) and noncoding regions are shown in capital and small letters, respectively. The deduced amino acid sequence is given below the nucleotide sequence, which is numbered at both ends of each sequence line. The ORF encodes for a protein containing 149 amino acid residues. Initiation and termination codons are shown in green and red colour, respectively; *represents stop codon.
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does contain less (<1%) cysteine, histidine and tyrosine. Interestingly, there was not a single residue of the tryptophan in \textit{PvCaM} protein. BlastP (domain enhanced lookup time accelerated basic local alignment search tool) results showed the presence of putative conserved domains in \textit{PvCaM} protein.

The topology of \textit{PvCaM} protein to show secondary structures is depicted in Figure 2. The predicted 3D structure produced for \textit{PvCaM} protein by homology modeling using Phyre2 is shown in Figure 3.

**DISCUSSION**

The full-length gene or its cDNA is required for the over-expression of gene in order to increase either the production of a desired important protein or natural products.\cite{9} To understand the secondary and tertiary structural features of the proteins, molecular modeling is commonly used.\cite{10-12} The main goal of this brief-study was to annotate \textit{PvCaM} gene cDNA and its deduced protein (amino acid) sequence. The \textit{PvCaM} cDNA clone was isolated from 5-day-old-pod tissue cDNA library; hence, it reflects that it is expressed in bean’s 5-day-old developing-pod-tissue. However, its level of expression and its pattern of expression are not clear at this moment as we have not characterised its expression.

The guanine-cytosine (GC) content in \textit{PvCaM} cDNA is 43%. It is close to, but significantly higher than the GC content (39.4%) reported in nuclear DNA of broad bean.\cite{13} BlastP results showed the presence of EFh (EF-hand) domains as specific hits. This EFh, calcium binding motif is a diverse superfamily of calcium sensors and calcium signal modulators. Ca2+ binding induces a conformational change in the EF-hand motif, leading to the activation or inactivation of target proteins.\cite{14-16} Results suggest that the secondary structures of \textit{PvCaM} protein are mainly alpha helices (60%) [Figure 2]. The predicted 3D structure of the \textit{PvCaM} protein is based on the best template, 1RFJ. This template is of potato (\textit{Solanum tuberosum}) CaM protein which shows the highest (97%) similarity (figure not shown) with \textit{PvCaM} protein when compared with other templates available in a database of protein.\cite{17} The reported, potato CaM structure was determined using X-ray diffraction data in the resolution range 8.0–2.0 Å.\cite{18} Therefore, we strongly believe that the 3D structure predicted for \textit{PvCaM} protein in this study should be closer to its real structure. However, we suggest the further wet lab experimental work to validate the predicted structure.
Calmodulin is an important protein; because it decodes Ca\(^{2+}\)–dependent and-independent signals and could be helpful in in-depth understanding of the biological molecular mechanisms and signals.\(^{19-22}\)

However, very little is known about \(P_CaM\). Therefore, further research is required to understand more about \(P_CaM\) protein.

**CONCLUSION**

This brief-study has annotated the basic features of \(P_CaM\) gene cDNA and deduced protein. Comparative molecular modelling suggests that the deduced \(P_CaM\) protein is analogous to Potato CaM protein. However, in order to have a comprehensive understanding of \(P_CaM\) protein further studies are required to validate the predicted 3D structure, and to understand its expression and regulation in beans.

**REFERENCES**

1. Massey LK. Dietary animal and plant protein and human bone health: A whole foods approach. J Nutr 2003;133:862S-5.
2. Broughton WJ, Hernandez G, Blair M, Beebe S, Gepts P, Vanderleyden J. Beans (\textit{Phaseolus} spp.) – Model food legumes. Plant Soil 2003;252:55-128.
3. Bhore SJ, Amelia K, Wang E, Priyadharsini S, Shah FH. Computational analysis of common bean (\textit{Phaseolus vulgaris} L. genotype BAT93) lycopene \(\beta\)-cyclase and \(\beta\)-carotene hydroxylase gene's cDNA. Bioinformation 2013;9:197-206.
4. Tidow H, Poulsen LR, Andreeva A, Knudsen M, Hein KL, Wiuf C, et al. A bimodular mechanism of calcium control in eukaryotes. Nature 2012;491:468-72.
5. Strehler EE, Filoteo AG, Penniston JT, Caride AJ. Plasma-membrane Ca(2+) pumps: Structural diversity as the basis for functional versatility. Biochem Soc Trans 2007;35:919-22.
6. Ishida H, Vogel HJ. The solution structure of a plant calmodulin and the CaM-binding domain of the vacuolar calcium-ATPase BCA1 reveals a new binding and activation mechanism. J Biol Chem 2010;285:38502-10.
7. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, et al. The Protein Data Bank. Nucleic Acids Res 2000;28:235-42.
8. Kelley LA, Sternberg MJ. Protein structure prediction on the Web: A case study using the Phyre server. Nat Protoc 2009;4:363-71.
9. Kudo T, Makita N, Kojima M, Tokunaga H, Sakakibara H. Cytokinin activity of cis-zeatin and phenotypic alterations induced by overexpression of putative cis-Zeatin-O-glucosyltransferase in rice. Plant Physiol 2012;160:319-31.
10. Kountouris P, Agathocleous M, Pomononas VJ, Christodoulou G, Hadjicostas S, Vassiliades V, et al. A comparative study on filtering protein secondary structure prediction. IEEE/ACM Trans Comput Biol Bioinform 2012;9:731-9.
11. Wang E, Chinni S, Bhore SJ. Three-dimensional (3D) structure prediction of the American and African oil-palms \(\beta\)-ketoacyl-[ACP] synthase-II protein by comparative modelling. Bioinformation 2014;10:130-7.
12. Sehar U, Mehmood MA, Nawaz S, Nadeem S, Hussain K, Sohail I, et al. Three dimensional (3D) structure prediction and substrate-protein interaction study of the chitin binding protein CBP24 from B. thuringiensis. Bioinformation 2013;9:725-9.
13. Baxter R, Kirk JT. Base composition of DNA from chloroplasts and nuclei of \textit{Phaseolus vulgaris}. Nature 1969;222:272-3.
14. Kretsinger RH. EF-hands embrace. Nat Struct Biol 1997;4:514-6.
15. Ikura M. Calcium binding and conformational response in EF-hand proteins. Trends Biochem Sci 1996;21:14-7.
16. Akke M, Forsén S, Chazin WJ. Solution structure of (Cd2+) 1-calbindin D9k reveals details of the stepwise structural changes along the Apo- and g(Ca2+) II1- and g(Ca2+) II2 binding pathway. J Mol Biol 1995;252:102-21.
17. Fox NK, Brenner SE, Chandonia JM. SCOPe: Structural Classification of Proteins – Extended, integrating SCOP and ASTRAL data and classification of new structures. Nucleic Acids Res 2014;42:D304-9.
18. Yun CH, Bai J, Sun DY, Cui DF, Chang WR, Liang DC. Structure of potato calmodulin PCM6: The first report of the three-dimensional structure of a plant calmodulin. Acta Crystallogr D Biol Crystallogr 2004;60:1214-9.
19. Villarroel A, Taglialetela M, Bernardo-Seisdedos G, Alaimo A, Agirre J, Alberdi A, et al. The ever changing moods of calmodulin: How structural plasticity entails transductional adaptability. J Mol Biol 2014;426:2717-35.
20. Jia L, Chu H, Wu D, Feng M, Zhao L. Role of calmodulin in thermotolerance. Plant Signal Behav 2014;9:pii: e28887. [Epub ahead of print]
21. Tang XH, Chen J, Yang XL, Yan LF, Gao J. Preservation on thermotolerance. Plant Physiol 2012;160:319-31.
22. Saini AS, Taliyar R, Sharma PL. Protective effect and mechanism of Ginkgo biloba extract-EGb 761 on STZ-induced diabetic cardiomyopathy in rats. Pharmacogn Mag 2014;10:172-8.

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