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Computational analysis of common bean (*Phaseolus vulgaris* L., genotype BAT93) lycopene β-cyclase and β-carotene hydroxylase gene's cDNA

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Abstract:
The identification of genes and understanding of genes' expression and regulation in common bean (*Phaseolus vulgaris* L.) is necessary in order to strategize its improvement using genetic engineering techniques. Generation of expressed sequence tags (ESTs) is useful in rapid isolation, identification and characterization of the genes. To study the gene expression in *P. vulgaris* pods tissue, ESTs generation work was initiated. Early stage and late stage bean-pod-tissues cDNA libraries were constructed using CloneMiner cDNA library construction kit. In total, 5972 EST clones were isolated using random method of gene isolation. While processing ESTs, we found lycopene β-cyclase (*PvLCY-β*) and β-carotene hydroxylase (*PvCHY-β*) gene's cDNA. In carotenoid biosynthesis pathway, *PvLCY-β* catalyzes the production of carotene; and *PvCHY-β* is known to function as a catalyst in the production of lutein and zeaxanthin. To understand more about *PvLCY-β* and *PvCHY-β*, both strands of both cDNA clones were sequenced using M13 forward and reverse primers. Nucleotide and deduced protein sequences were analyzed and annotated using online bioinformatics tools. Results showed that *PvLCY-β* and *PvCHY-β* cDNAs are 1639 and 1107 bp in length, respectively. Analysis results showed that *PvLCY-β* and *PvCHY-β* gene's cDNA contains an open reading frame (ORF) that encodes for 502 and 305 amino acid residues, respectively. The deduced protein sequence analysis results also showed the presence of conserved domains needed for *PvLCY-β* and *PvCHY-β* functions. The phylogenetic analysis of both *PvLCY-β* and *PvCHY-β* proteins showed it's closeness with the *LCY-β* and *CHY-β* proteins from *Glycine max*, respectively. The nucleotide sequence of *PvLCY-β* and *PvCHY-β* gene's cDNA and it's annotation is reported in this paper.

Keywords: Expressed sequence tags, Genetic engineering, Health, Human population, Malaysia, Natural products, Nutrition, *Phaseomics*, Proteins, Vegetables.

Background:
We certainly do not know how many people are malnourished; but, FAO report indicates that there are about 925 million undernourished people in the world **[1]**. The animal products (eggs, meat, milk, etc.) are a source of dietary proteins; but, proteins are usually derived from legumes (plants from the bean and pea family) especially by poor people **[2]**. There are thousands of legume species, but common beans (*Phaseolus vulgaris* L.) are cultivated on the large scale. By understanding the importance of *P. vulgaris*, the *Phaseomics* international

consortium was developed to establish the necessary framework of knowledge and materials for the advancement of bean genomics, transcriptomics, and proteomics; and the main goal of it is to help in generating new common bean varieties suitable and desired by farmers and consumers **[3]**. As a part of the international consortium for *Phaseolus* genomics **[3]**, research work on generation of *P. vulgaris* expressed sequence tags (ESTs) was initiated at Melaka Institute of Biotechnology, Malaysia.

The randomly isolated anonymous cDNA clones (on a large scale) are treated as ESTs and used extensively in the gene's expression and regulation studies **[4]**. The generated ESTs data is also used in the evaluation of the genomes for genes content and its structure, in comparative gene expression analysis between different plant tissues using computational tools **[5]**, and in discovery of new and novel genes **[6].** In monocot and dicot plants, various new and novel genes have been identified by using random method of cDNA clones isolation and their nucleotide sequencing **[7-11]**. Hence, ESTs were generated to study the gene's expression and regulations in bean– pod-tissue in-line-with the agenda of the international consortium for *Phaseolus* genomics **[3].**

To this point, we have generated 5972 ESTs; and annotated ESTs were deposited into ESTs database hosted by National Center for Biotechnology Information (NCBI) GenBank / DDBJ / EMBL (our unpublished work). While processing and analysing generated ESTs, we found lycopene β-cyclase and β-carotene hydroxylase gene's cDNA [**12, 13**]. The source of lycopene β-cyclase and β-carotene hydroxylase cDNA is *P. vulgaris;* hence, lycopene β-cyclase and β-carotene hydroxylase cDNAs were designated as *PvLCY-β* and *PvCHY-β*, respectively. In carotenoids biosynthesis pathway, *PvLCY-β* catalyzes the production of carotene (α-carotene and β-carotene) **[12, 14];** and *PvCHY-β* is known to function as a catalyst in the production of lutein and zeaxanthin **[13].**

Due to antioxidant properties of carotenes (β-carotene), several health benefits associated with its consumption are reported elsewhere **[15].** Similarly, the benefits of lutein and zeaxanthin consumption are reported by many researchers; and their reports are reflecting the importance of these (carotenes, lutein and zeaxanthin) natural products in human health **[16-22].**

Both, *PvLCY-β* and *PvCHY-β* cDNA clones do have potential applications in genetic engineering of *P. vulgaris* and other plants. That is why, both clones were fully sequenced. These two cDNA clones could be used in manipulating *P. vulgaris* and level of carotene, lutein and zeaxanthin could be elevated. Hence, in order to understand more about *PvLCY-β* and *PvCHY-β*, their cDNA clones were analysed and annotated. The nucleotide and deduced protein sequence of *PvLCY-β* and *PvCHY-β* gene's cDNA are analyzed and annotated in this study using computational tools. The nucleotide sequence of *PvLCY-β* and *PvCHY-β* gene's cDNA and its annotation is reported in this paper.

Methodology:

Plant Materials

The seeds of *P. vulgaris* genotype BAT93 were kindly provided by Patricia Lariguet, Laboratoire de Biologie Moléculaire des Plantes Supérieures, Department of Plant Biology, University of Geneva, Geneva, Switzerland. Seeds were germinated in soil obtained from a nursery (Melaka, Malaysia), and seedlings were maintained to grow in the open area at Melaka Institute of Biotechnology, Malaysia.

PvLCY-β and *PvCHY-β* cDNA clones isolation

The *PvLCY-β* and *PvCHY-β* cDNA clones were identified from the ESTs generated using random method of gene isolation [7, 8, 23]. The cDNA clone encoding *PvLCY-β* was isolated from 20-day-old [days after anthesis (DAA)] bean-pod-tissue cDNA Entry Library; and the cDNA clone encoding *PvCHY-β* was isolated from 5-day-old bean-pod-tissue cDNA Entry Library. The cDNA libraries were constructed (our unpublished data) using 'CloneMiner cDNA library construction kit' procured from Invitrogen Corporation.

Plasmid DNA isolation

The individual cultures of *Escherichia coli* strain DH5α cells harbouring recombinant plasmids with *PvLCY-β* and *PvCHY-β* cDNA clones were cultivated in 10 ml LB medium supplemented with 40μg/ml Kanamycin. Cultures were incubated in dark at 37ºC, 160 rpm for 18 h. From harvested *E. coli* cells, plasmid DNA was isolated using Wizard® *Plus* SV Minipreps DNA purification system, a commercial kit (Promega).

Nucleotide sequencing

Purified plasmid DNA was used in sequencing reactions. Both strands of both *PvLCY-β* and *PvCHY-β* cDNA clones were sequenced using M13 (Forward) [5'-GTAAAACGACGGCCAG-3'] and M13 (Reverse) [5'-GGATAACAATTTCACACAGG-3'] primers.

cDNA and deduced protein sequence analysis

For both *PvLCY-β* and *PvCHY-β* cDNA clones, the nucleotide sequence of plus (+) and minus (-) strands were aligned using Blast (bl2seq) program available at NCBI [http://blast.ncbi.nlm.nih.gov/]. The 5' and 3' ends of the cDNA sequences were edited to eliminate adaptor and vector sequences. The finalized cDNA sequences were analyzed using online bioinformatics tools.

The similarity searches were performed using blast programs (BlastN and BlastP) available at NCBI. Online bioinformatics tools available at JustBio [http://www.justbio.com/] were used to deduce the protein sequence, to find out the general features of *PvLCY-β* and *PvCHY-β* cDNA and deduced protein sequences. The EMBOSS Water - Pairwise Sequence Alignment [http://www.ebi.ac.uk/Tools/emboss/align/] was used to compare cDNA and deduced protein sequences to find out similarity% with their counterparts from other species. Guanine and cytosine (GC %) content calculation was carried out by using 'DNA/RNA base composition calculator'. Alignment of multiple protein (amino acids) sequences was carried out using multiple sequence alignment by ClustalW program, and the phylograms were constructed using BioEdit and TreeView programs **[24, 25].** Proteins sequences were aligned by using CLUSTAL 2.1 multiple sequence alignment program to find out conserved residues in both *PvLCY-β* and *PvCHY-β* deduced proteins.

Discussion:

PvLCY-β and PvCHY-β cDNA clones isolation

The full-length *PvLCY-β* and *PvCHY-β* cDNA clones were isolated from 20-day-old and 5-day-old bean-pod-tissues cDNA libraries, respectively. The isolated *PvLCY-β* and *PvCHY-β* cDNA clones were designated as *PvLCY-β* and *PvCHY-β* to indicate their precise identity and the source of the plant to which they belong.

Nucleotide sequencing

Both, sense (+) and antisense (-) strands of both cDNA clones were sequenced where M13 forward and M13 reverse primers were used. After elimination of the vector and adaptor sequence, the sequence of sense and antisense strand of individual cDNA was compared using blast (bl2seq) program. Analysis of the results showed that *PvLCY-β* and *PvCHY-β* cDNAs are 1639 and 1107 bp in length, respectively.

```
   1 - ATGTTGCTCAAAACACCTAACAAGCTCGAGTTTTTGCACCCACTTCACGGTTATTCAGAGAAATTAAGCAGTTCAGGCTCCTCTAGGGTG - 90
   1 - M  L  L  K  T  P  N  K  L  E  F  L  H  P  L  H  G  Y  S  E  K  L  S  S  S  G  S  S  R  V  - 30

  91 - CAAAATCAGGATTTTAGATTTGGTTCTAAGAAATCGCTTGTGAGATGGGGTAAATGTGGTGGTTTGCGGGCTAGTAGTAGTGCCCTTTTG - 180
  31 - Q  N  Q  D  F  R  F  G  S  K  K  S  L  V  R  W  G  K  C  G  G  L  R  A  S  S  S  A  L  L  - 60

 181 - GAGCTTGTTCCTGAATTCAAGAAGGAGAATCTGGATTTTGAACTTCCTCTGTATGATTCGTCAAAGGGGGCTGTGGTGGACCTTGCTGTT - 270
  61 - E  L  V  P  E  F  K  K  E  N  L  D  F  E  L  P  L  Y  D  S  S  K  G  A  V  V  D  L  A  V  - 90

 271 - GTGGGAGGGGGGCCTGCGGGGCTTGCAGTTGCACAGCAGGTTTCTGAGGCAGGGCTTTCAGTTTGTGCTATTGATCCAAACCCTAGGTTG - 360
  91 - V  G  G  G  P  A  G  L  A  V  A  Q  Q  V  S  E  A  G  L  S  V  C  A  I  D  P  N  P  R  L  - 120

 361 - ATTTGGCCCAATAATTATGGGGTTTGGGTGGATGAGTTTGAGGCCATGGATTTGCTGGACTGCCTTGACACCACCTGGTCTGGCGCAGTT - 450
 121 - I  W  P  N  N  Y  G  V  W  V  D  E  F  E  A  M  D  L  L  D  C  L  D  T  T  W  S  G  A  V  - 150

 451 - GTCTACATCGACGATAAAACAAAGAAGGATCTTGATAGGCCTTATGGTAGAGTGAATAGGAAGCTGCTGAAGTCGAAGATGATGCAGAAA - 540
 151 - V  Y  I  D  D  K  T  K  K  D  L  D  R  P  Y  G  R  V  N  R  K  L  L  K  S  K  M  M  Q  K  - 180

 541 - TGTATCTCAAATGGTGTCAAGTTTCACCAGGCCAAAGTTATCAAGATTATTCACGAGGATACCAAATCTTTGCTGATTTGTAATGATGGT - 630
 181 - C  I  S  N  G  V  K  F  H  Q  A  K  V  I  K  I  I  H  E  D  T  K  S  L  L  I  C  N  D  G  - 210

 631 - GTCACTGTTCAGGCCACAGTTGTTCTTGATGCCACTGGCTTTTCAGGATGCTTGGTTCAGTATGATAAACCGTACAACCCGGGTTACCAA - 720
 211 - V  T  V  Q  A  T  V  V  L  D  A  T  G  F  S  G  C  L  V  Q  Y  D  K  P  Y  N  P  G  Y  Q  - 240

 721 - GTTGCTTATGGGAATCTGGCTGAAGTTGATGAGCACCCGTATGATGTAGATAAAAGGGCTTTTCTGGACTGGAGAGATTTTCATCTGGAC - 810
 241 - V  A  Y  G  N  L  A  E  V  D  E  H  P  Y  D  V  D  K  R  A  F  L  D  W  R  D  F  H  L  D  - 270

 811 - AATGACAAGGAGCTGAAGCAGAGAAATAGTAGGATACCCACCTTTCTGTATGCAATGCCCTTTTCATCCACTAAGATATTTCTTGAAGAA - 900
 271 - N  D  K  E  L  K  Q  R  N  S  R  I  P  T  F  L  Y  A  M  P  F  S  S  T  K  I  F  L  E  E  - 300

 901 - ACATCCCTTGTAGCACGGCCTGGATTACGAATGGAAGATATACAGGAAAGAATGGTTGCCAGGTTGAAACATTTGGGTATTAGAGTGAAA - 990
 301 - T  S  L  V  A  R  P  G  L  R  M  E  D  I  Q  E  R  M  V  A  R  L  K  H  L  G  I  R  V  K  - 330

 991 - AGTATAGAAGAAGATGAGCACTGTGTGATTCCCATGGGTGGCCCCCTCCCAGTTTTTCCCCAAAGAGTTGTTGGCATTGGCGGTACCGCT - 1080
 331 - S  I  E  E  D  E  H  C  V  I  P  M  G  G  P  L  P  V  F  P  Q  R  V  V  G  I  G  G  T  A  - 360

1081 - GGGATGGTGCATCCTTCAACTGGGTATATGGTTGCAAGGACCCTGGCTGCAGCTCCTATTGTTGCTAATGCTATTGTTCAGAGCCTGGGC - 1170
 361 - G  M  V  H  P  S  T  G  Y  M  V  A  R  T  L  A  A  A  P  I  V  A  N  A  I  V  Q  S  L  G  - 390

1171 - TTTGATAGAGGACTTTCCGGAGGAGATATATTTGCACAAGTGTGGAAAGATTTATGGCCCATCCAAAGGCGGCGACAAAGGGAGTTTTTT - 1260
 391 - F  D  R  G  L  S  G  G  D  I  F  A  Q  V  W  K  D  L  W  P  I  Q  R  R  R  Q  R  E  F  F  - 420

1261 - TGTTTTGGTATGGACATTTTACTCAAGCTTGATTTACCTGGCACAAGGAGATTTTTTGATGCGTTTTTCGATCTGGAACCGCATTACTGG - 1350
 421 - C  F  G  M  D  I  L  L  K  L  D  L  P  G  T  R  R  F  F  D  A  F  F  D  L  E  P  H  Y  W  - 450

1351 - CATGGATTTTTATCATCAAGACTGTTTTTTCCTGAGCTTTTATTTTTTGGACTGTCTTTATTTTTTTATGCTTTTAATACATTTAGGATA - 1440
 451 - H  G  F  L  S  S  R  L  F  F  P  E  L  L  F  F  G  L  S  L  F  F  Y  A  F  N  T  F  R  I  - 480

1441 - GAGATTATGGCGAAGGGAACACTTCCCTTGGCAAACATGATCAGCAACTTGGTAAAAGATACAGAATAAgattacttctttttaatctgt - 1530
 481 - E  I  M  A  K  G  T  L  P  L  A  N  M  I  S  N  L  V  K  D  T  E  *                         - 502

1531 - tgtatgctatggtttgtgcttccccatgtagtttcctactcacattatagatttccaataatgtataatggtgtatatttatttaaaaaa - 1620
1621 - aaaaaaaaaaaaaaaaaaa                                                                         - 1639
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Figure 1: Nucleotide and deduced amino acid sequences of *Phaseolus vulgaris* lycopene β-cyclase (*PvLCY-β*) cDNA clone. Open reading frame (ORF) and 3' non-coding region of cDNA are shown in capital and small letters, respectively. The deduced amino-acid sequence is given below the nucleotide sequence, and numbered at both ends of each sequence line. The ORF encodes for a protein of 502 amino acid residues (blue). Amino acid residues are numbered beginning with the initial Methionine (M) till last Glutamic acid (E) residue. Initiation and termination codons are shown in green and red colour, respectively. *represent the termination codon. This cDNA clone was isolated from *P. vulgaris* 20-day-old-pods tissue cDNA library.

cDNA and Deduced Protein Sequence Analysis

The identity of both cDNA clones was confirmed by analyzing finalized respective cDNA sequence and its deduced amino acid sequence. Annotated nucleotide sequences of both *PvLCY-β* and *PvCHY-β* cDNA were deposited in GenBank/DDBJ/EMBL under the accession numbers

HQ199604 and JN255133, respectively. Annotated general features of cDNA nucleotide and protein sequences are summarized in **Table 1 (see supplementary material)**; and nucleotide sequence of *PvLCY-β* and *PvCHY-β* cDNA along with its deduced amino acid sequence is shown in **Figure 1 & Figure 2**, respectively.

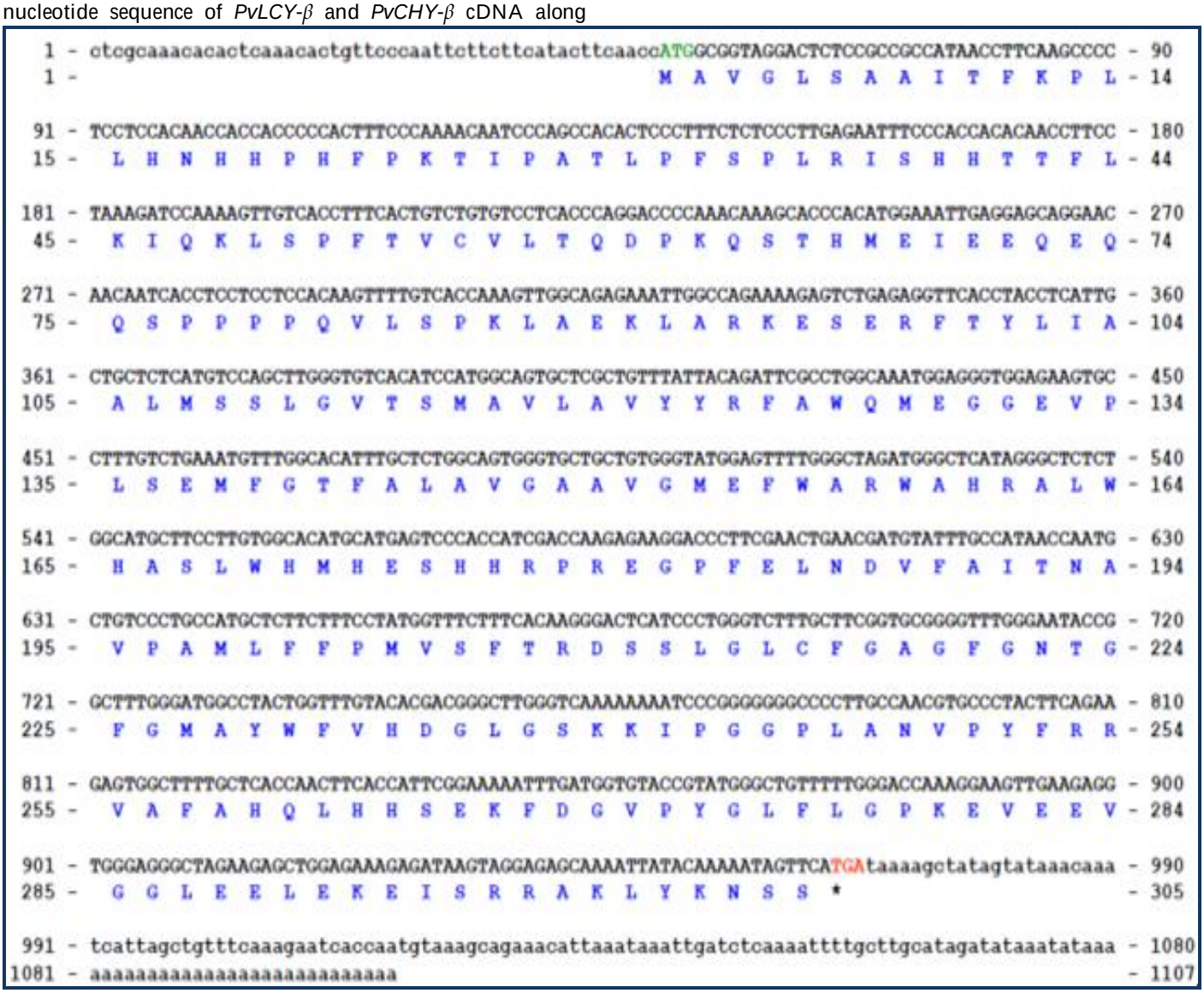

Figure 2: Nucleotide and deduced amino acid sequences of *Phaseolus vulgaris* beta-carotene hydroxylase (*PvCHY-β*) cDNA clone. Open reading frame (ORF) and non-coding regions of cDNA are shown in capital and small letters, respectively. The deduced amino-acid sequence is given below the nucleotide sequence, and numbered at both ends of each sequence line. The ORF encodes for a protein of 305 amino acid residues (blue). Amino acid residues are numbered beginning with the initial Methionine (M) till the last Serine (S) residue. Initiation and termination codons are shown in green and red colour, respectively. *represent the termination codon. This cDNA clone was isolated from *P. vulgaris* 5-day-old-pods tissue cDNA library.

The similarity% of both *PvLCY-β* and *PvCHY-β* cDNA nucleotide and deduced protein sequence with their counterparts from other species are shown in **Table 2** & **Table 3 (see supplementary material)**, respectively. The amino acid Sequence analysis results showed that both *PvLCY-β* and *PvCHY-β* proteins are Leucine (L) rich (**Supplementary Figure 1 & 2)**. The comparison of the *PvLCY-β* protein with its counterparts from other species showed that 217 (out of 502) residues (43.23%) are fully conserved**.** But, in case of the *PvCHY-β* protein, results showed that only 67 (out of 305) residues (21.97%) are fully conserved**.** The consecutive search for conserved domains in *PvLCY-β* and *PvCHY-β* protein sequences resulted in the detection of their conserved domains, and the results are summarised in **Table 4 (see supplementary material)**. The phylograms were constructed in order to understand phylogenetic relationship of *PvLCY-β* and *PvCHY-β* proteins with their counterparts from other species. The phylograms for *PvLCY-β* and *PvCHY-β* proteins are shown in **Figure 3 & Figure 4,** respectively.

The understanding of the identified genes, their expression patterns and regulation is crucial in order to strategize the approach to manipulate any biosynthesis pathway of interest in the plants. For the suppression of a gene expression, partial sequence of that gene can be utilized to induce post-transcriptional gene silencing (PTGS) **(26-28]**. However, the full

length gene or its cDNA is required for its over-expression in order to increase either the production of desired vital proteins or natural products **[29]**. Therefore, understanding of gene of interest and it's cDNA is prerequisite before it can be used in recombinant DNA (rDNA) technology to manipulate genetically, any plant of interest or organism **[30].**

The main goal of this study was to annotate *PvLCY-β* and *PvCHY-β* gene's cDNA and deduced respective protein (amino acid sequence). The *PvLCY-β* cDNA clone was identified in 20-day-old-pod tissue cDNA library, and it indicates that *PvLCY-β* is expressed in bean's 20-day-old developing pod tissue. However, the *PvCHY-β* cDNA clone was identified in 5-day-old-pod tissue cDNA library; and it reflects that *PvCHY-β* is expressed in bean's 5-day-old developing pod tissue. However, the level of both gene's expression, pattern of expression, and tissue-specificity is not clear at this moment as we have not characterised these two gene's expression. It can be done by using either Northern hybridization technique or microarray technique **[31]**.

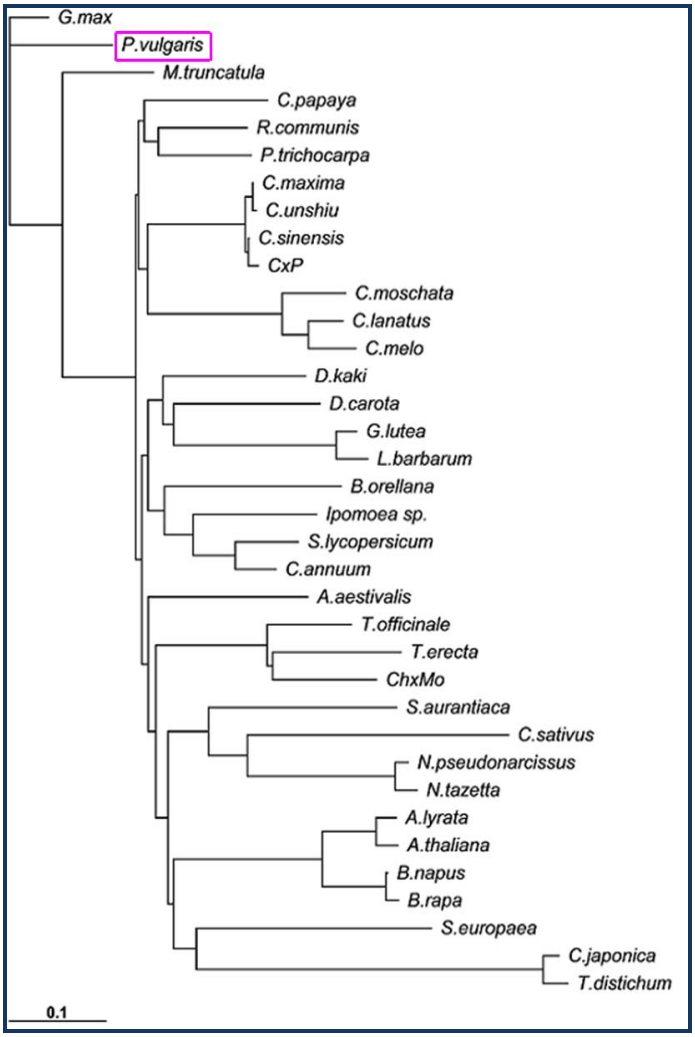

Figure 3: The phylogram showing phylogenetic relationship of common bean (*Phaseolus vulgaris* L.) lycopene β-cyclase (*PvLCY-β*) protein with its counterparts from other species. Available 35 full-length *LCY-β* protein sequences were retrieved from NCBI database (see supplementry Table 2). The location of *PvLCY-β* protein in phylogram is shown in a pink box.

The GC content in *PvLCY-β* and *PvCHY-β* cDNAs is 42% and 46%, respectively. The GC% in both *PvLCY-β* and *PvCHY-β* cDNAs is significantly higher than that of the GC% (39.4 %) reported in nuclear DNA of the broad bean **[32]**.

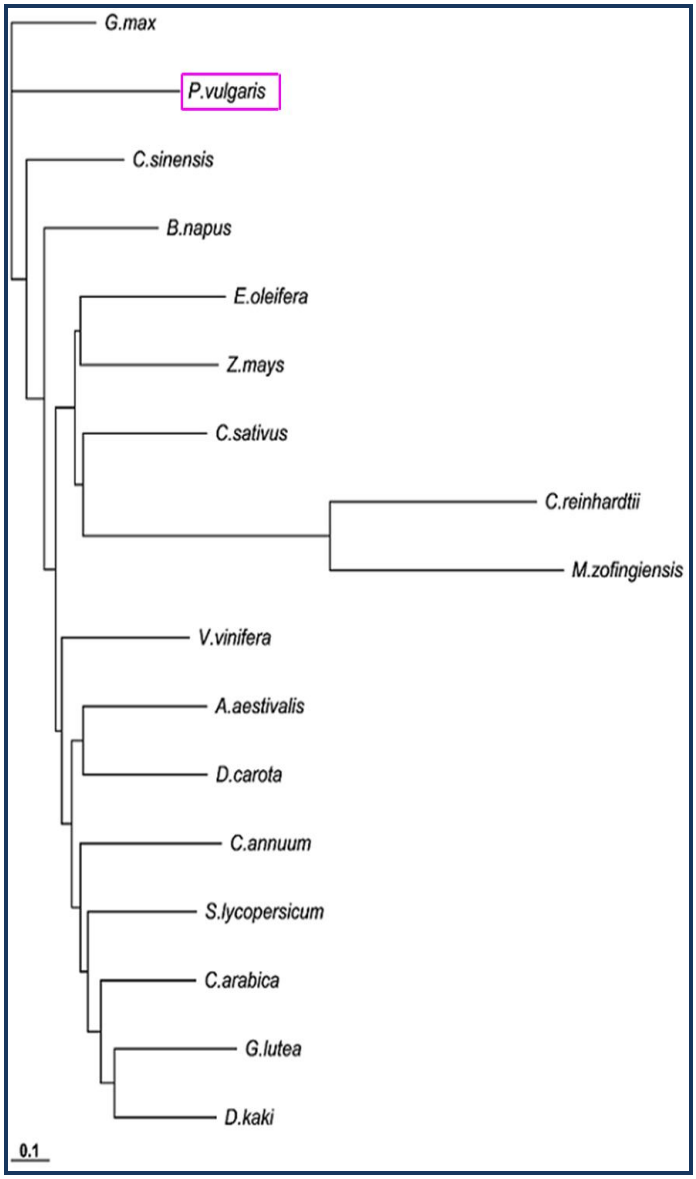

Figure 4: The phylogram showing phylogenetic relationship of common bean (*Phaseolus vulgaris* L.) β-carotene hydroxylase (*PvCHY-β*) protein with its counterparts from other species. Available 16 full-length *PvCHY-β* protein sequences were retrieved from NCBI database (see supplementary Table 3**).** Location of *PvCHY-β* protein in phylogram is shown in a pink box.

The *LCY-β* protein of *G. max* showed the maximum similarity (95.2%) with inferred *PvLCY-β* protein. Whereas, *LCY-β* protein from *Cryptomeria japonica* and *Taxodium distichum* showed less similarity (83.8%) with inferred *PvLCY-β* protein. Both, *Cryptomeria japonica* and *Taxodium distichum* are species that belong to gymnosperms; the relatively low level of *PvLCY-β* similarity with *LCY-β* from Gymnosperm members is in line with evolution in plant species **[33].** Interestingly, *LCY-β* protein of *Salicornia europaea* and *Crocus sativus* showed lowest

similarity with *PvLCY-β*. While analysing *PvCHY-β*, we noticed that *Glycine max CHY-β* protein shows the maximum (78%) similarity with inferred *PvCHY-β* protein. On the contrary, *Muriella zofingiensis CHY-β* protein showed less (58%) similarity with *PvCHY-β* protein. *Muriella zofingiensis* is an algal member, and the relatively low level of similarity between *PvCHY-β* and *CHY-β* from *M. zofingiensis* is along the lines of the evolution in plants. These results are similar to the results reported by Bhore *et al*. **[23]**. Both, *PvLCY-β* and *PvCHY-β* proteins showed highest similarity with *LCY-β* and *CHY-β* of *G. max*, respectively. This makes logical sense because both *P. vulgaris* and *G. max* belongs to the same family, Fabaceae **[34]**.

The *PvCHY-β* protein contains conserved domain for beta-carotene hydroxylase which is a member of the Fatty acid hydroxylase superfamily (Accession No: cl01132) **[35]**. In *PvLCY-β*, two main conserved domains namely, NADB_Rossmann super family and lycopene beta cyclase were detected. The NADB domain does exist in numerous dehydrogenases of metabolic pathways; for example glycolysis. The lycopene cyclase family protein conserved domain was detected in *PvLCY-β*, and lycopene beta and epsilion cyclases are part of this protein family **[36-38]**.

Phaseolus vulgaris is a valuable source of proteins in the human diet; and it is important to increase the yield of this essential crop **[3, 39]**. Several research teams are using GM technology approach to improve yield of the beans **[30, 40]**. For instance developing *P. vulgaris* resistant to the herbicide **[41]** and viral infection **[42]**. In addition to this, there is a vast scope to modify beans genetically for improving the nutritional quality of its pods and seeds. This type of genetic manipulation is possible; because, rice (*Oryza sativa*) has been genetically engineered and β-carotene content in it has been increased for use as a source of vitamin A **[43]**. Similarly, β-carotene content can be increased in beans by over-expression of *PvLCY-β* in its carotenoid biosynthesis pathway **(Supplementary Figure 3).** Furthermore, therapeutically beneficial lutein and zeaxanthin content increment in beans is also possible by over-expression of *PvCHY-β* **[44].**

Genetic modification of agricultural crop plants to improve yield and nutritional quality is a viable option, and it is absolutely important as far as human wellbeing is concerned **[30, 40].** Both, isolated *PvLCY-β* and *PvCHY-β* gene's cDNA are reasonably well annotated in this study, and we believe that the available annotated cDNA sequences could be useful in designing the strategy for the construction of transformation vectors. Further research is needed in this line to achieve the ultimate goal of generating new common bean varieties suitable and desired by farmers and consumers.

Conclusion:

This study has annotated the salient features of *PvLCY-β* and *PvCHY-β* gene's cDNA clones. The computational analysis of deduced *PvLCY-β* and *PvCHY-β* proteins revealed the presence of conserved domains. Furthermore, the comparative analysis of deduced *PvLCY-β* and *PvCHY-β* protein sequences with their counterparts from other species revealed the fully conserved amino acid residues. However, further study is required to understand *PvLCY-β* and *PvCHY-β* gene's expression and its regulation in bean-pods. Both genes' over-expression in bean-pods can be considered for futher research to explore the possibility of nutritional quality improvement of the bean-pods and bean-seeds.

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Conflict of Interests:

Authors attest that there are no conflicts of interest to declare.

References:

[1] http://www.fao.org/
[2] Massey LK, *J Nutr*. 2003 **133** : 862S [PMID: 12612170]
[3] Broughton WJ *et al*. *Plant and Soil*. 2003 **252**: 55
[4] Lange BM *et al*. *Proc Natl Acad Sci U S A*. 2000 **97**: 2934 [PMID: 10717007]
[5] Ronning CM *et al*. *Plant Physiol*. 2003 **131**: 419 [PMID: 12586867]
[6] Ohlrogge J & Benning C, *Curr Opin Plant Biol*. 2000 **3**: 224 [PMID: 10837264]
[7] Liu J *et al*. *Plant Mol Biol*. 1995 **29**: 685 [PMID: 8541495]
[8] Keith CS *et al*. *Plant Physiol*. 1993 **101**: 329 [PMID: 8278499]
[9] Park YS *et al*. *Plant Physiol*. 1993 **103**: 359 [PMID: 8029332]
[10] Höfte H *et al*. *Plant J*. 1993 **4**: 1051 [PMID: 8281187]
[11] Umeda M & Uchimiya H, *Plant Physiol*. 1994 **106**: 1015 [PMID: 12232382]
[12] al-Babili S *et al*. *Novartis Found Symp*. 2001 **236**: 219 [PMID: 11387982]
[13] Ampomah-Dwamena C *et al*. *J Exp Bot*. 2009 **60**: 3765 [PMID: 19574250]
[14] Rodríguez-Sáiz M *et al*. *Appl Environ Microbiol*. 2004 **70**: 5589 [PMID: 15345447]
[15] Jiang YH *et al*. *Poult Sci*. 1994 **73**: 1137 [PMID: 7937475]
[16] Adluri RS *et al*. *Cell Biochem Biophys*. 2011 [Epub ahead of print] [PMID: 21960420]
[17] Holtan SG *et al*. *Int J Cancer*. 2012 **131**: 1158 [PMID: 22038870]
[18] Kabat GC *et al*. *Am J Clin Nutr*. 2009 **90**: 162 [PMID: 19474140]
[19] Gao YY *et al*. *Br J Nutr*. 2012 **108**: 1746 [PMID: 22273061]
[20] Roberts RL *et al*. *Clin Dermatol*. 2009 **27**: 195 [PMID: 19168000]
[21] Ma L & Lin XM, *J Sci Food Agric*. 2010 **90**: 2 [PMID: 20355006]
[22] Polidori MC *et al*. *Dtsch Med Wochenschr*. 2012 **137**: 305 [PMID: 22318842]
[23] Bhore SJ *et al*. *Bioinformation*. 2010 **5**: 104 [PMCID: PMC3040485]
[24] Thompson JD *et al*. *Nucleic Acids Res*. 1994 **22**: 4673 [PMID: 7984417]
[25] Page RD *et al*. *Comput Appl Biosci*. 1996 12: 357 [PMID: 8902363]
[26] Smith NA *et al*. *Nature*. 2000 **407**: 319 [PMID: 11014180]
[27] Liu Q *et al*. *Plant Physiol*. 2002 **129**: 1732 [PMID: 12177486]

[28] Bhore SJ & Shah FH, *Bioinformation*. 2011 **6**: 212 [PMID: 21738318]
[29] Kudo T *et al*. *Plant Physiol*. 2012 **160**: 319 [PMID: 22811434]
[30] Bhore SJ, *Current Science*. 2008 **95**: 1391
[31] Khraiwesh B, *Methods Mol Biol*. 2012 **847**: 25 [PMID: 22350996]
[32] Baxter R & Kirk JT, *Nature*. 1969 **222**: 272 [PMID: 5778394]
[33] Goremykin VV *et al*. *Syst Biol*. 2012 **62**: 50 [PMID: 22851550]
[34] McClean PE *et al*. *BMC Genomics*. 2010 **11**: 184 [PMID: 20298570]
[35] Vences-Guzmán M *et al*. *Mol Microbiol*. 2011 **79**: 1496 [PMID: 21205018]
[36] Marchler-Bauer A & Bryant SH, *Nucleic Acids Res*. 2004 **32**: W327 [PMID: 15215404]
[37] Marchler-Bauer A *et al*. *Nucleic Acids Res*. 2009 **37**: D205 [PMID: 18984618]
[38] Marchler-Bauer A *et al*. *Nucleic Acids Res*. 2011 **39**: D225 [PMID: 21109532]
[39] Peralta H *et al*. *Appl Environ Microbiol*. 2004 **70**: 3272 [PMID: 15184121]
[40] Beyer P, *N Biotechnol*. 2010 **27**: 478 [PMID: 20478420]
[41] Nifantova SN *et al*. *Tsitol Genet*. 2011 **45**: 41 [PMID: 21568221]
[42] Bonfim K *et al*. *Mol Plant Microbe Interact*. 2007 **20**: 717 [PMID: 17555279]
[43] Tang G *et al*. *Am J Clin Nutr*. 2012 **96**: 658 [PMID: 22854406]
[44] Ma L *et al*. *Am J Ophthalmol*. 2012 **154**: 625 [PMID: 22835510]

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Supplementry material:

Table 1: The general features of *Phaseolus vulgaris* lycopene beta-cyclase (*PvLCY-β*) and β-carotene hydroxylase (*PvCHY-β*) cDNAs and their deduced protein sequence.

General features	*PvLCY-β*	*PvCHY-β*
cDNA sequence		
i. Size, bp	1639	1107
ii. Molecular weight (Daltons)	508009	341098
iii. 5' UTR, bp	0	50
iv. Coding sequence	1509	918
v. 3' UTR, bp	130	139
vi. Stop Codon	TAA(UAA)	TGA (UGA)
vii. G+C content %	42 %	46%
Protein sequence		
viii. Length, amino acids	502	305
ix. Molecular weight (Dalton)	56469.44	34127.52
x. Isoelectric point	7.16	7.90

Table 2: Comparison of *Phaseolus vulgaris* lycopene beta-cyclase (*PvLCY-β*) cDNA nucleotide and deduced amino acid sequence with *LCY-β* from monocot, dicot and gymnosperm members.

Species	GenBank Accession No.	Length compared		Similarity (%)	
		nucleotide (bp)	amino acid	nucleotide (bp)	amino acid
Monocots					
Crocus sativus	GQ202143	1503	497	69.9	82.1
Narcissus pseudonarcissus	GQ327929	1515	504	70.9	86.0
Narcissus tazetta	JQ797381	1515	504	71.5	85.2
Sandersonia aurantiaca	AF489520	1696	495	67.0	84.9
Dicots					
Adonis aestivalis	AF321534	2026	502	71.3	88.4
Arabidopsis lyrata	XM_002884738	1953	501	66.2	85.9
Arabidopsis thaliana	NM_111858	1964	501	66.2	85.2
Bixa orellana	AJ549288	1500	499	71.5	86.5
Brassica napus	HM989810	1739	493	67.9	85.9
Brassica rapa	FJ606828	1758	493	67.8	85.3
Capsicum annuum	GU085266	1497	498	75.1	86.6
Carica papaya	DQ415894	3937	503	69.0	90.6
Chrysanthemum x morifolium	AB205041	1705	498	71.3	84.3
Citrullus lanatus	EF183521	2146	504	73.9	86.8
Citrus maxima	AY217103	1678	504	76.5	90.4
Citrus sinensis	DQ496222	1542	504	75.8	90.6
Citrus unshiu	AY166796	1822	504	76.6	90.4
Citrus x paradisi	JF907401	1515	504	78.6	90.4
Cucumis melo	GU457407	1871	504	73.7	86.1
Cucurbita moschata	JN559395	2121	497	70.3	85.9
Daucus carota	DQ192190	2022	508	71.7	88.8
Diospyros kaki	FJ940723	2231	504	73.5	89.2
Gentiana lutea	AB017366	2147	508	70.4	86.5
Glycine max	XM_003554083	1928	507	88.0	95.2
Ipomoea sp.	AB499055	1649	501	74.1	88.5
Lycium barbarum	AY906864	1527	508	71.3	86.3
Medicago truncatula	XM_003624977	1497	498	83.6	91.6
Populus trichocarpa	XM_002308867	1566	504	78.5	90.4
Ricinus communis	XM_002531452	1968	514	74.1	89.4
Salicornia europaea	AY789516	1937	498	71.1	83.8
Solanum lycopersicum	NM_001247297	1503	500	75.0	87.6
Tagetes erecta	AF251017	1906	511	67.8	86.2
Taraxacum officinale	AB247456	1718	504	69.5	87.6
Gymnosperm members					
Cryptomeria japonica	AB161829	1653	525	67.5	83.8
Taxodium distichum	AB096608	1692	525	67.7	83.8

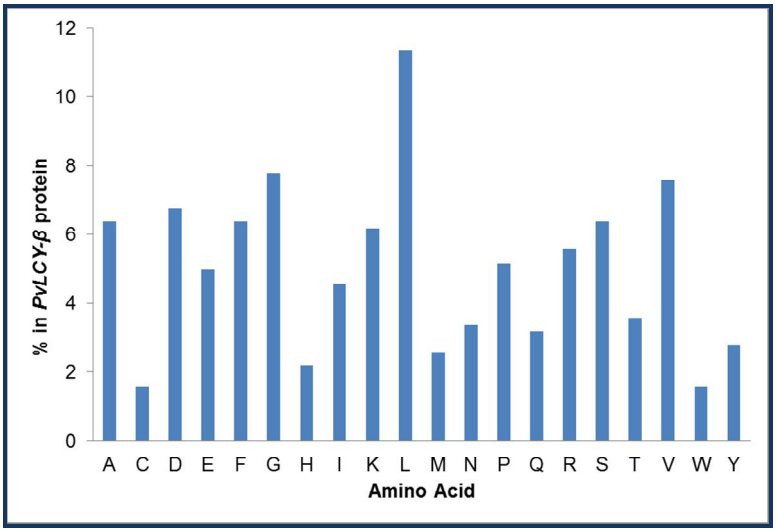
Table 3: Comparison of *Phaseolus vulgaris* beta-carotene hydroxylase (*PvCHY-β*) cDNA nucleotide and deduced amino acid sequence with *CHY-β* from monocot, dicot and algal members.

Species	GenBank Accession No.	Length compared		Similarity (%)	
		nucleotide (bp)	amino acid	nucleotide level	amino acid level
Monocots					
Crocus sativus	AJ416711	918	305	59.8	72.0
Elaeis oleifera	EU057623	1414	325	51.1	68.2
Zea mays	AY844956	1392	308	49.0	69.2
Dicots					
Adonis aestivalis	EF120636	1187	309	58.7	73.5
Brassica napus	EF026098	903	300	64.9	74.3
Capsicuum annuum	Y09225	1112	315	61.8	70.8
Citrus sinensis	DQ228870	936	311	71.1	75.3
Coffea arabica	DQ157169	933	310	62.5	70.4
Daucus carota	DQ192193	1231	309	58.9	68.6
Diospyros kaki	FJ790215	1365	312	61.3	70.1
Gentiana lutea	AB027187	1519	320	50.4	73.2
Glycine max	AY575953	1164	334	70.7	78.0
Solanum lycopersicum	Y14809	1125	309	60.8	73.6
Vitis vinifera	AF499108	900	299	62.1	72.0
Algal members					
Chlamydomonas reinhardtii	XM_001698646	1757	297	48.0	60.3
Muriella zofingiensis	EU016205	1545	299	45.6	58.0

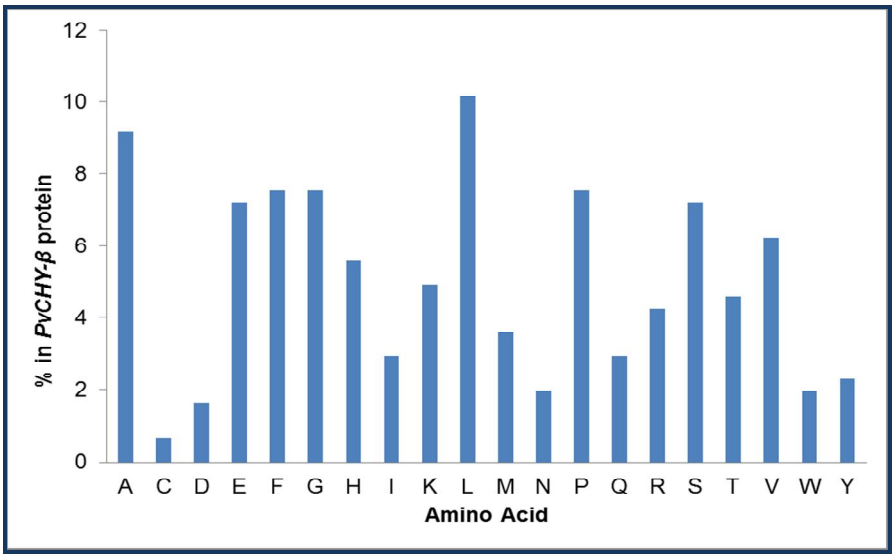
Table 4: The conserved domains detected in *Phaseolus vulgaris* lycopene beta-cyclase (*PvLCY--β*) and beta-carotene hydroxylase (*PvCHY-β*) protein sequences.

No	Conserved Domain [GenBank's code]	Location within protein (amino acids)
	PvLCY-β	
1	Lycopene cyclase protein [pfam05834]	85 - 480
2	Lycopene cyclase [TIGR01789]	87 - 482
3	Lycopene beta cyclase [PLN02463]	58 - 502
4	Lycopene epsilon cyclase [PLN02697]	1 - 502
5	Lycopene cyclase family protein [TIGR01790]	87 - 482
6	Dehydrogenases [COG0644]	83 - 479
7	2P6MH&RFADDO• [COG0654]	84 - 481
8	geranylgeranyl reductase family [TIGR02032]	53 - 380
9	FAD dependent oxidoreductase [pfam01266]	1 - 448
	PvCHY-β	
1	Beta carotene hydroxylase [PLN02601]	1 - 305

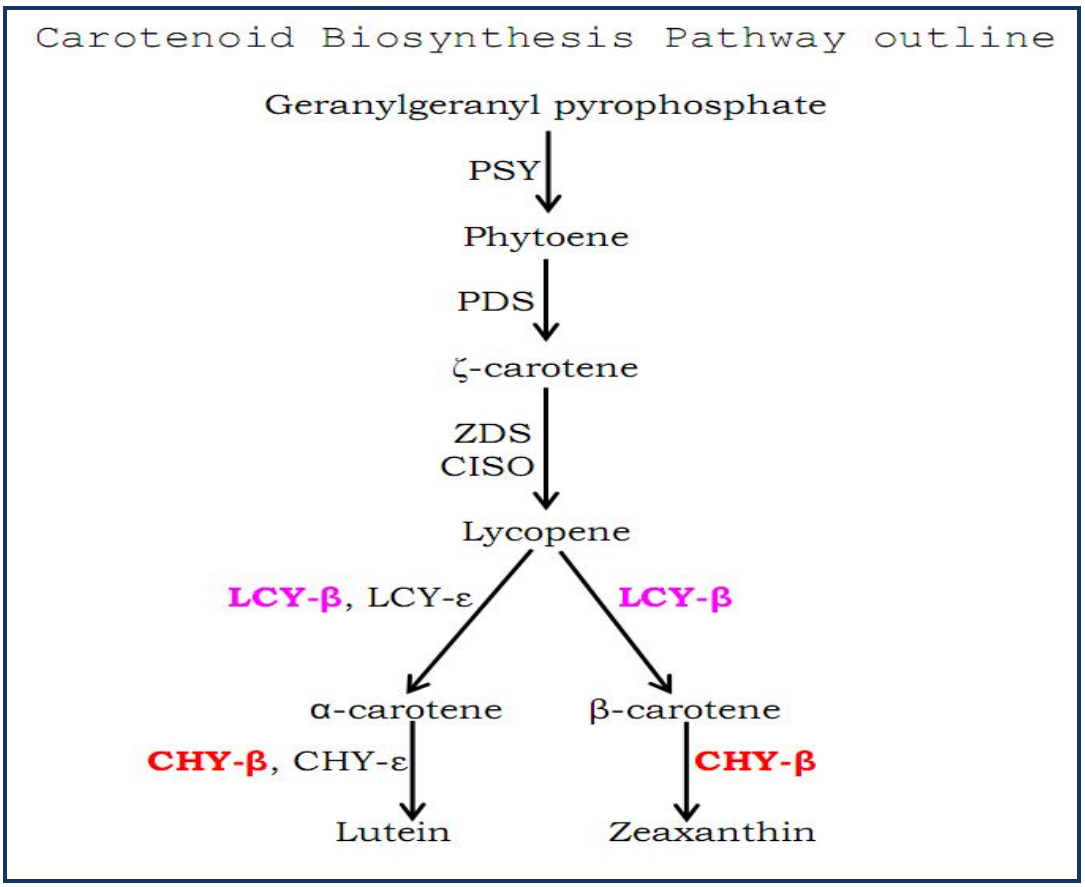
•stands for 2-polyprenyl-6-methoxyphenol hydroxylase and related FAD-dependent oxidoreductases



Supplementary Figure 1: Individual amino acids% in the *P. vulgaris* lycopene β-cyclase (*PvLCY-β*) protein. On X axis, the amino acids are represented by a single letter code. The amino acid composition was calculated online using ProtCalc program of JustBio.



Supplementary Figure 2: Individual amino acids% in the *P. vulgaris* β-carotene hydroxylase (*PvCHY-β*) protein. On X axis, the amino acids are represented by a single letter code. The amino acid composition was calculated online using ProtCalc program of JustBio.



Supplementary Figure 3: Schematic diagram of carotenoid biosynthesis pathway in plants. Enzymatic conversion of the respective substrate is shown by arrows with the enzyme/s responsible for it. PSY, phytoene synthase; PDS, phytoene desaturase; ZDS, zeta-carotene desaturase; CISO, carotene isomerase; LCY-β, lycopene beta-cyclase; LCY-ε, lycopene epsilon-cyclase; CHY-β, beta-carotene hydroxylase; CHY-ε, epsilon-carotene hydroxylase. The LCY-β and CHY-β are shown in pink and red color respectively to highlight their location in the pathway.