

***In-silico* analysis of 7-dehydrocholesterol reductase gene (SL7-DR2) in tomato: Linking cholesterol metabolism to vitamin-D pathway**

ABSTRACT:

Tomatoes are the second most important vegetable crop globally. Most foods contain little vitamin D, and plants are very poor sources. The 7-dehydrocholesterol reductase gene (SL7-DR2) is involved in the synthesis of 7-dehydrocholesterol, which is a precursor for vitamin D₃ synthesis. This gene is present in different plant species and plays a role in cholesterol metabolism. Using MEGA11 software, the phylogenetic analysis of this gene was done and it shows a close resemblance with *Solanum tuberosum* and *Datura stramonium*. The physiochemical characteristics of SL7-DR2 were analyzed by the ExPasy protparam tool. Functional annotation and secondary structure analysis of SL7-DR2 were done using the Pfam, STRING, and SOPMA databases. These tools characterized the protein function of the SL7-DR2 gene and studied the role of 7-dehydrocholesterol reductase in converting 7-dehydrocholesterol to vitamin D₃ and its role in cholesterol metabolism. The pathway analysis using the Reactome database helps in characterizing the network between the cholesterol and vitamin D pathway. The 3D structure of the SL7-DR2 protein was predicted using an *ab initio* approach, Robetta, and the predicted structure was validated using the SAVESv6.0 (PROCHECK) server. The results obtained for the analysis of the SL7-DR2 show that the protein is stable and has a low GRAVY Index indicating its hydrophobicity and the secondary structure analysis indicates the robust nature of this protein. The predicted model has 90.8% of total residues present in the most favoured regions and shows its high readability and quality. These analyses demonstrate the significance of this gene in the vitamin D pathway and its potential for human health in preventing various diseases.

Keywords: Cholesterol; Vitamin D; 7-Dehydrocholesterol reductase gene; Pathway analysis; STRING; SOPMA; Reactome; Modelling; Robetta; PROCHECK

INTRODUCTION:

Tomato (*Solanum Lycopersicum*) is an excellent vegetable that complements any diet because of its health advantages as it contains minerals, phytochemicals, lycopene, potassium, iron, folate, and vitamin C [1,2]. Tomato is suitable for understanding various plant metabolic pathways, including the cholesterol pathway that can be manipulated for accumulating vitamin D₃, a crucial human steroid hormone. The deficiency of vitamin D is widely prevalent among the human population. Vitamin D deficiency in humans causes inflammation, micronutrient deficiencies, cancer, depression, dementia, and rheumatoid arthritis. The major reason for the increase in vitamin D deficiency is largely because of inadequate dietary availability. The best dietary sources of vitamin D are fish and dairy products. Most foods contain little vitamin D, and plants are very poor sources of vitamin D₃.

The Vitamin D content in the blood serum is estimated by knowing the concentration of the 25-hydroxy vitamin D₃. Calcifediol (25(OH)D₃) is taken as a secondary biomarker for analyzing the vitamin D status in humans [3]. A level of 20 ng/ml to 30 ng/mL is considered deficient in people [4,5]. The sufficient amount of 25(OH)D₃ required by humans is 50 nmol/L [6] and the optimal amount of 25(OH)D₃ required is at a concentration of 80 nmol/L [6,7]. The National Institute of Health implemented the vitamin D standardization program (VDSP) [8,9], which establishes deficiency as anything below 50 nmol/L [10]. Moreover, vitamin D deficiency increases the risk of developing COVID-19 [11].

Vitamin D synthesis is linked to the cholesterol pathway with the two metabolisms having a common role in their synthesis [12]. The biosynthesis of the active form of vitamin D [1,25(OH)D₃], involves a series of metabolic processes that occur across various cellular compartments including extracellular regions, cytosol, lysosomes, endoplasmic reticulum, and mitochondria [13]. The metabolic pathways involved in the synthesis of vitamin D comprise the mevalonate pathway and the Kandutsch-Russel branch of the cholesterol pathway and these pathways are finally involved in the transition of 7-dehydrocholesterol to pre-vitamin D₃ [12] by exposure to UV-B light at wavelength range of 290-315 nm by cleaving the bond between C9 and C10 carbons of 7-Dehydrocholesterol. Vitamin D produced in the skin enters the circulation and is transported to the liver by vitamin D binding protein (DPB) and converted to 25-hydroxyvitamin D₃ [25(OH)D₃] by CYP2R1 (cytochrome P450 2R1) [14,15]. Finally, in the

Kidneys, 25(OH)D₃ is transformed to its active form 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃], through CYP27B1 (cytochrome P450 family 27 subfamily B member 1) [16](Fig.1).

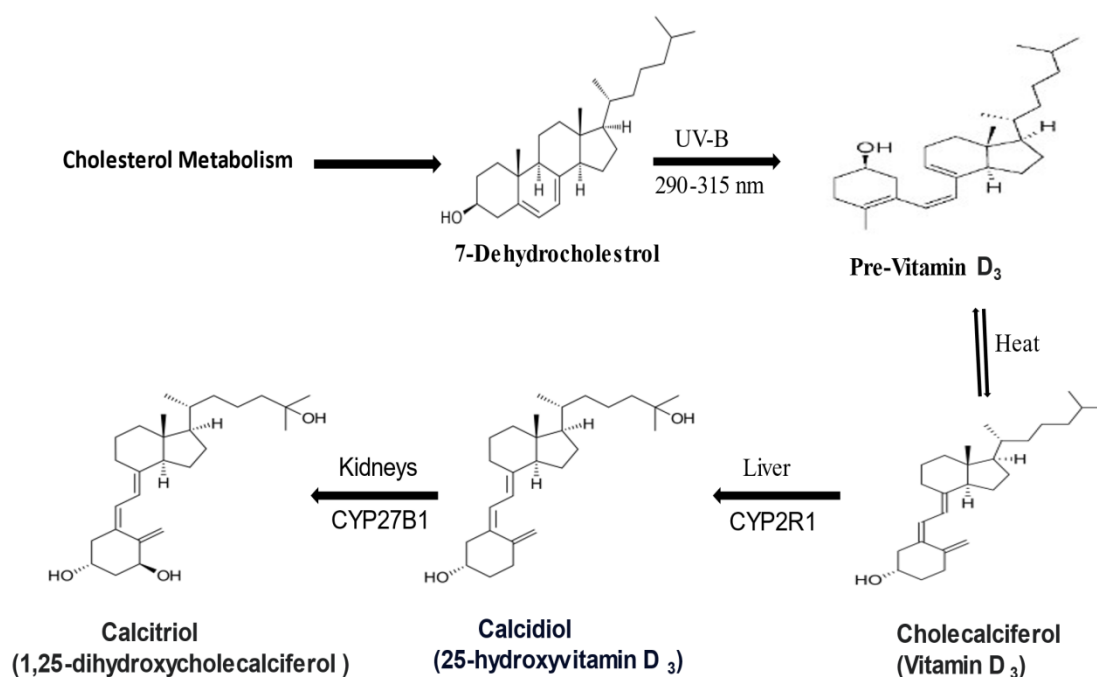


Fig.1. Vitamin D synthesis from 7-dehydrocholesterol in human

Plants producing steroidal glycoalkaloids (SGAs), such as α -solanine in potatoes and α -tomatine in tomatoes, are part of the Solanaceae family and accumulate cholesterol as a major precursor [17]. These plants can serve as unique models for studying plant cholesterol metabolism [18] and manipulating the functional role of this gene through genome editing helps in the synthesis of vitamin D without conversion into cholesterol and this gene is present in different plant species and was studied to know the importance of it in the accumulation of 7-dehydrocholesterol.

In this paper the role of the *7-dehydrocholesterol reductase (Sl7-DR2)* gene which codes dehydrocholesterol reductase protein in tomatoes, employing a series of interconnected analyses was studied and the gene annotation and sequencing analysis between the interspecies helps in characterization and its genetic constitution. The phylogenetic analysis and the functional characters of this gene were studied and helped in finding its genomic position and its nature in involving various biosynthetic pathways. The computational modeling of the pathway involved in vitamin D synthesis and cholesterol synthesis helps in studying the relation between the two pathways. The protein structure prediction and the modeling of the protein helped in studying the gene significance in tomatoes and also helped in finding its enzymatic potential.

2. MATERIALS AND METHODS:

2.1 Gene Annotation and Sequence Analysis:

The *7-Dehydrocholesterol reductase gene* and protein sequence of different plant species *Solanum lycopersicum* (NP_001353043, NP_001353044), *Solanum tuberosum* (NP_001305624), *Arabidopsis thaliana* (NP_001077693), *Capsicum annuum* (XP_016577971), *Withania somnifera* (AOX15301), *Datura stramonium* (MCD7446296), *rosa chinensis* (XP_024181789), *Nicotiana tobaccum* (XP_016480318) and *Homo sapiens* (NP_001157289) were retrieved in FASTA format from NCBI [19] and they are aligned to study the sequence similarity. The conserved protein domains and functional motifs within the protein sequence were studied by using the InterProScan tool [20].

2.1.1 Phylogenetic analysis :

The 7-Dehydrocholesterol reductase homologous gene sequences from different plant species (NP_001353043, NP_001353044, NP_001157289, NP_001077693, NP_001305624, XP_016577971, AOX15301, MCD7446296, XP_024181789, XP_016480318) were chosen and then they were aligned using the MEGA software and the phylogenetic tree was constructed among these species to infer the evolutionary relationship and branching pattern among the species. The bootstrap analysis was done to analyze the robustness of the phylogenetic tree.

2.2 Physio-Chemical Characterization:

The physio-chemical properties of protein sequences and amino acid composition of the gene from different species were determined using the ProtParam server [21] from the ExPASy tool. The isoelectric point (pI), the total number of negative (-R) and positive (+R) residues, extinction coefficients (EC), instability index (II), aliphatic index (AI), and grand average hydropathy were all calculated.

2.3 Functional Annotation and protein interaction analysis of the gene:

The retrieved protein sequence of the gene was used for finding the conserved regions and similarities among sequences, the multiple sequence alignment among different organisms was characterized and the gene potential functions (biological, molecular, and cellular) were predicted by using the UniProt database [22] and STRING database. The protein family, domain, and functional site data were computed using the Prosite server [23] and for characterization of the chosen protein in relation to the particular protein family was done using Pfam [24]. The STRING database was used to find the proteins that are involved in the interaction with 7-dehydrocholesterol reductase and pathways related to the gene.

2.4 Prediction of Secondary Structures:

The secondary structure of the 7-dehydrocholesterol reductase gene was predicted by using the Self-Optimized Prediction Method with Alignment (SOPMA) [25] server. The default parameters of SOPMA, which encompass a similarity threshold of 8 and a window width of 17, were employed. SOPMA algorithmically assesses the content of α -helix, β -sheets, turns, random coils, and extended strands based on the amino acid sequence.

2.5 Computational Modeling of Vitamin D and Cholesterol Pathway:

The computational modeling helps in examining the convergence of cholesterol and vitamin D metabolism [26]. The interplay between these two pathways was studied using the system biology and helped in finding their biochemical interactions. This study helps in finding the role of other metabolisms involved in synthesizing the vitamin D from the cholesterol pathway.

2.6 Molecular modeling of 7-dehydrocholesterol reductase:

The three-dimensional structure of the full-length 7-dehydrocholesterol reductase from *S. Lycopersicum* was not present in the PDB database. In light of this, the tertiary structure of the protein was generated through the utilization of the Robetta web server [27] using the query sequence. The quality of the predicted 3D model was subsequently assessed utilizing the SAVES v6.0 server [28]. This was done to evaluate the overall stereochemical properties of the protein, specifically, energetically allowed regions used for docking studies to know the relation of this gene to Vitamin D₃ conversion.

3. RESULTS AND DISCUSSION

3.1 Sequence and Phylogenetic Analysis:

The gene sequences of different plant species (NP_001353043, NP_001353044, NP_001157289, NP_001077693, NP_001305624, XP_016577971, AOX15301, MCD7446296, XP_024181789, XP_016480318) were retrieved from the NCBI database (Annexure 1). The Phylogenetic tree predicts that *S. Lycopersicum* has a close resemblance with *D. stramonium* and *S. tuberosum* having a bootstrap value of 43 and 40 respectively. This gene present in Homo sapiens and Arabidopsis have 100% resemblance. Fig.(2).

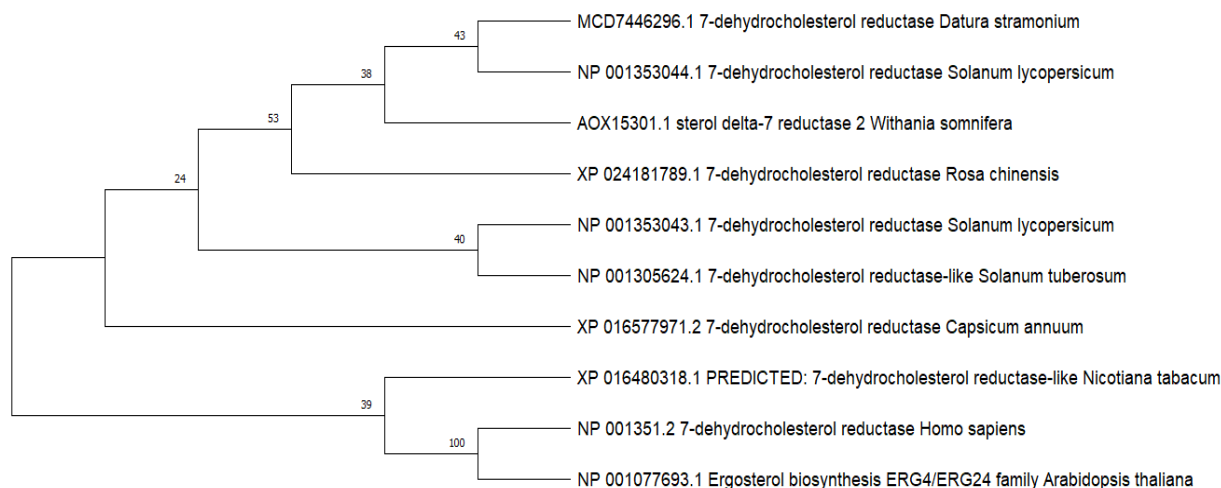


Fig.2. Phylogenetic analysis of 7-Dehydrocholesterol reductase in different species

3.2 Physio-Chemical characterization:

The utilization of ExPASy's ProtParam tool allowed for the computation of the amino acid composition and physio-chemical properties of 7-dehydrocholesterol reductase from various species (Annexure 2.). The protein sequence of the gene exhibits a variance in length, ranging from 431 to 475 amino acids, and displays differing molecular weights (Table 1). The isoelectric point (pI), which measures protein solubility and electrophoretic separation [29], was found to be greater than 7 for all the plant species and human, indicating an elevated level of protein solubility and electrophoretic separation. The aliphatic index, which is representative of a protein's stability across a broad temperature spectrum [30], was observed to be higher in *Capsicum annuum* and then in *Nicotiana tabacum*, *Solanum lycopersicum* (tomato) than that of other species. Lastly, the instability index (II), which is a parameter for measuring protein stability, denotes a stable protein if the value is below 40 [31]. The gene in all these species has a lower instability index and denotes its stability in various species.

The 7-dehydrocholesterol reductase protein found in all four species exhibits a low isoelectric point, suggesting that these proteins are stable. The hydrophobicity of proteins can be measured using the GRAVY index, whereby lower values indicate greater affinity for water molecules and higher values suggest greater hydrophobicity [32]. In comparison to the other species, *S. lycopersicum* displays a lower GRAVY index, indicating a potential for weaker interactions with water molecules. Based on these physio-chemical properties, it is possible that the protein sequence of 7-dehydrocholesterol reductase in tomato has high stability due to its higher aliphatic index and lower GRAVY index denoting its reduced hydrophobicity.

Table 1 : Parameters computed using ExPASy's ProtParam tool

Protein Name	No. of Amino acids	MW	pI	-R	+R	EC	II	AI	GRAVY
<i>S. lycopersicum-C6</i>	434	49786.32	8.77	28	36	132295	31.67	100.16	0.225
<i>S. tuberosum</i>	434	49715.25	8.85	27	36	130805	33.82	99.49	0.239
<i>S. lycopersicum C1</i>	434	49671.37	8.92	28	38	131835	31.84	102.19	0.309
<i>H. sapiens</i>	475	54489.43	8.95	28	39	153960	29.94	96.08	0.232
<i>A. thaliana</i>	431	49404.87	8.65	28	35	137460	31.82	98.84	0.371
<i>C. annuum</i>	434	49859.52	8.75	28	36	129440	28.06	103.73	0.294
<i>W. somniferum</i>	434	49755.39	9.02	28	39	131835	32.78	99.72	0.298
<i>R. chinensis</i>	434	49722.13	8.97	26	36	139855	34.47	97.95	0.285
<i>N. tabacum</i>	434	49768.33	8.66	27	34	136430	32.44	103.09	0.309
<i>D. Somniferum</i>	434	49699.35	8.95	27	38	133450	33.81	99.72	0.286

*MW:Molecular weight,*pI:Isoelectric Point,*-R:Number of negative residues,*+R:Number of positive residues,*EC:Extinction Coefficient at 280nm, *II:Instability Index,*AI:Aliphatic Index,.*GRAVY:Grand Average Hydropathicity

3.3 Functional Annotation and protein interaction analysis of the gene:

The gene's potential functions biological, molecular, and cellular were characterized using UniProt and String database(Table.2).The domain identified by profile for the 7-dehydrocholesterol reductase enzyme was the cytoplasmic domain (position;1-11;89-108;158-228;280-290;401-434residues) and the results of pfam, Interpro is well characterized in table.3.The *S17-DR2*gene is involved in the sterol biosynthetic process(GO:0016126),lipid metabolic process and has oxidoreductase activity,acting on the CH-CH group of donors,NAD or NADP as acceptor and it is a cellular component has seven to eight transmembrane membrane(GO:0016020) regions.The pattern and profile of the protein were assessed using ExPASy'sProsite, which stores annotations for motif descriptors that can be used to identify protein families and domains[33]. The protein was also subjected to Pfam analysis, and the gene that codes this protein belongs to the ergosterol biosynthesis ERG4/ERG24 family.This analysis helps in studying the efficiency of genome annotation and curating information on known protein families[34].The STRING database predicted the interaction between 7-dehydrocholesterol reductase protein(A0A3Q7H0B6) to otherproteins(A0A3Q7IVA9, A0A3Q7HGH7, A0A3Q7F016, A0A3Q7FC39, SSR2, , SISSR1, CYP51, A0A3Q7H150, A0A3Q7F823, A0A3Q7G0R2),with similar domains and families and helps in correlatingthe characteristics of this protein(fig.3). The biological pathway related to the 7-dehydrocholesterol reductase gene was analyzed using the Kyoto Encyclopedia of Genes and Genomes(KEGG) and Reactome database (Table.3).This information can be used to study a wide range of biological phenomena, from the functioning of individual cells to the evolution of entire ecosystems.

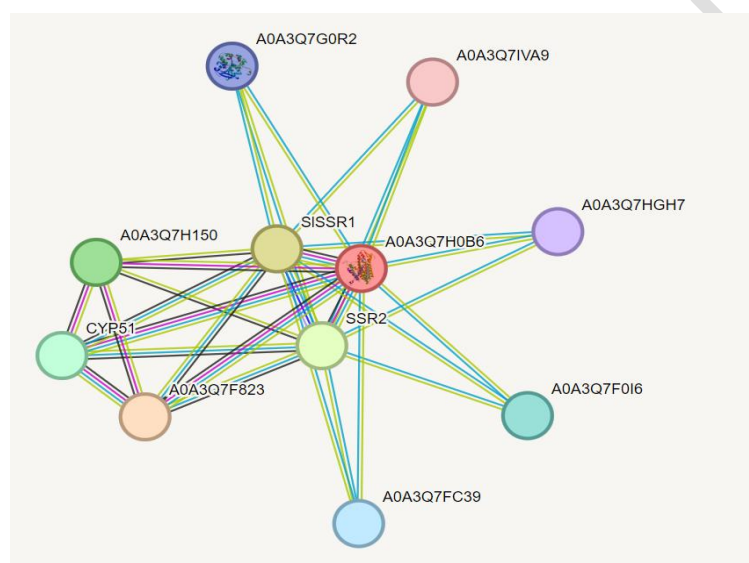


Fig.3 Interaction of 7-dehydrocholesterol reductase with other proteins using STRING database

Table.2 Biological ,Molecular and Cellular function of *S17-DR2* gene characterized through String database

BiologicalFunction	Molecular Function	CellularFunction
Defense response to insect	delta 24-sterol reductase activity	Endoplasmic reticulum
Brassinosteroid biosynthetic process	Triglyceride lipase activity	
Sterol biosynthesis process	Oxidoreducataase activity ,acting on the CH-CH group of donars	
Steroid biosynthesis process	Hydrolase activityon ester bonds	
Plant-type secondary cell wall biogenesis	Catalytic activity	

Organic hydroxy compound biosynthesis process
 Undimensional cell growth
 Lipid catabolic process
 Regulation of **hormone** (hormone) levels
 Lipid metabolic process
 Metabolic process

Table 3 Network analysis,protein domains,pathways of 7-dehydrocholesterol reductase gene

Local network cluster (STRING)	Protein Domains (Pfam)	Protein Domains and Features (Interpro)	KEGG pathways	Reactome Pathways
Delta(24)-sterol reductase, and Sterol delta 7 reductase activity	Putative esterase domain	Lipase, eukaryotic	Steroid biosynthesis	Formation of cornified envelope
Cholesterol biosynthesis via lathosterol and Sterol reductase	FAD binding domain	Delta(24)-sterol reductase	Biosynthesis of secondary metabolites	Cholesterol biosynthesis via lathosterol
Steroid biosynthesis, and Squalene cyclase, N-terminal	Alpha/beta hydrolase fold	Partial AB-hydrolase lipase domain Serine aminopeptidase	Metabolic pathways	Cholesterol biosynthesis via desmosterol
Mixed, Phosphatase complex, and Epoxide hydrolase		Alpha/Beta hydrolase fold		Keratinization Cholesterol biosynthesis

3.4 Prediction of Secondary Structures:

The secondary structure of dehydrocholesterol reductase protein of *S.lycoperscicum* and other plant species and Human were predicted using SOPMA (Table.4). The results show that *S.lycoperscicum* has a higher percentage of alpha helices and it dominates the other species followed by random coils, extended sheets, and beta strands. High alpha helix percentage demonstrating the robust nature of the 7-Dehydrocholesterol reductase protein.

Table4: Predicted secondary structures of different species that codes this gene

Different species (Locus id)	Amino acids	Alpha helix	Extended sheets	Beta turns	Random coils
<i>Solanum lycoperscicum</i> (Chr-6)	434	47.74%	16.36%	5.53%	30.65%
<i>Solanum tuberosum</i>	434	44.24%	18.20%	5.53%	32.03%
<i>Solanum lycoperscicum</i> (Chr-1)	434	44.01%	19.35%	6.91%	29.72%
<i>Homo sapiens</i>	475	43.16%	17.89	5.53%	33.89%
<i>Arabidopsis thaliana</i>	431	42.46%	18.79	5.80%	32.95%
<i>Withiana somnifera</i>	434	40.78%	20.28%	6.68%	32.26%
<i>Datura stramonium</i>	434	46.77%	17.97%	6.22%	29.03%
<i>Capsicum annum</i>	434	39.63%	21.66%	5.99%	32.72%

<i>Rosa chinensis</i>	434	45.39%	18.66%	5.76%	30.18%
<i>Nicotiana tobaccum</i>	434	42.63%	20.28%	6.68%	30.41%

3.5 Molecular pathway analysis of vitamin D and Cholesterol :

Using the Reactome database the cholesterol and vitamin D biosynthesis pathway was analyzed as well as the feedback mechanisms that involved in regulating their synthesis. The system biology graphical notation maps were helpful in studying the entire role of different pathways, enzymes, and proteins that are involved in vitamin D biosynthesis. Systems Biology Graphical Notation (SBGN) data describes the detailed vitamin D and cholesterol pathway[35].

The pathway starts in the cytosol with the conversion of acetyl coenzyme A (AC-CoA) by the enzyme acetyl-coenzyme A acetyltransferase 2 (ACAT2) and then progresses through the endoplasmic reticulum, where key steps involve the conversion of 3-hydroxy-3-methylglutaryl-CoA (bHMG-CoA) to mevalonic acid (MVA) catalyzed by HMGCR[12]. This step is targeted by statin drugs for treating high cholesterol levels. The pathway branches into the Bloch pathway (forming cholesterol) and the Kandutsch–Russell pathway, leading to vitamin D biosynthesis (fig.4). The vitamin D-forming branch starts with the conversion of 7-dehydrocholesterol to cholecalciferol (Vitamin D₃) through UV radiation in the skin layers[36]. The feedback regulation involves (Sterol regulatory element binding protein) SREBP complexes and high cholesterol binds to them and retains them in the endoplasmic reticulum, while low cholesterol levels activate SREBP complexes, upregulating enzymes responsible for early pathway steps subsequently transformed into **calcidiol** (calcidiol) and finally transforming to **calcitriol** (calcitriol)[1,25α(OH)D₃][12]. In this way, these pathways help in regulating the formation of cholesterol to vitamin D.

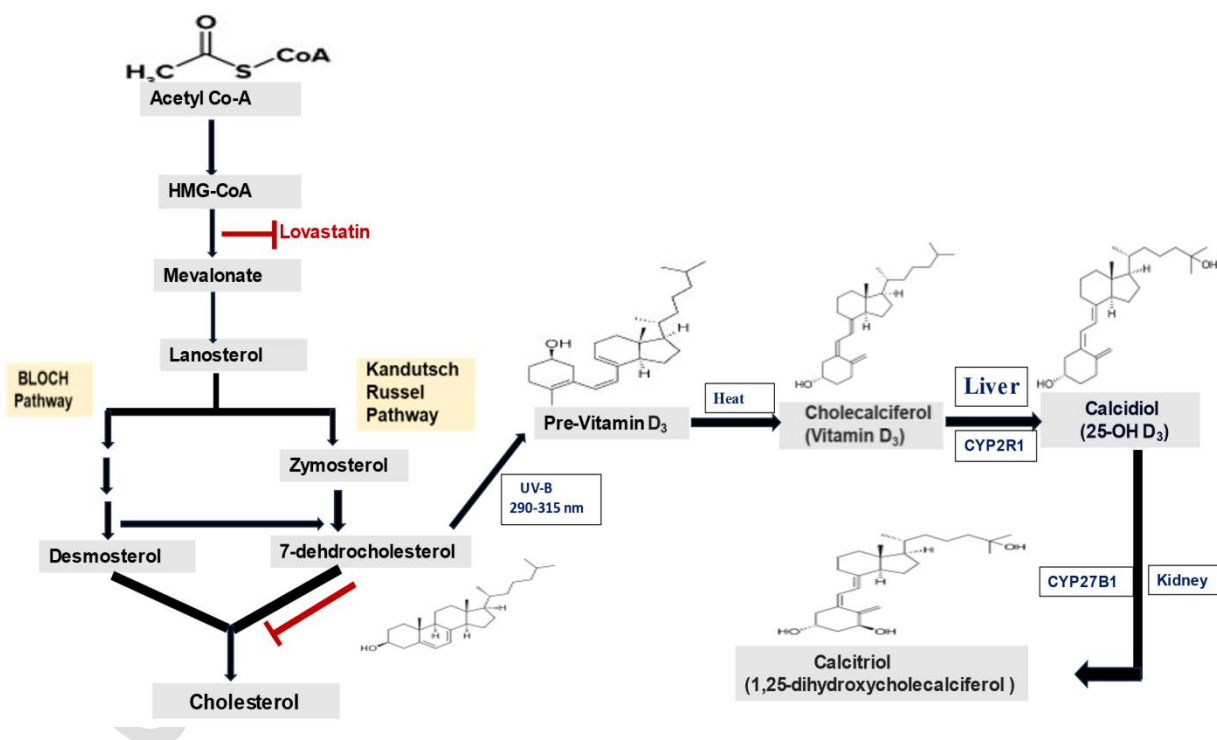


Fig.4 Network connecting the Cholesterol pathway to the Vitamin D biosynthesis pathway

3.6 3D-Modeling and validation of 7-dehydrocholesterol :

The tertiary structure is very important for studying the protein's function and active sites. The Robetta server was used to create the 3D model of the 7-dehydrocholesterol reductase protein by giving the query sequence and a 3D model for this protein was created. The predicted model for this protein was visualized by using the PyMOL molecular visualization tool (Fig.5). For evaluating the predicted model SAVES server 6.0 was used and the model of this protein underwent PROCHECK analysis, which led to the generation of a Ramachandran plot (Fig.6). The Ramachandran plot analysis categorized residues into quadrangle regions. The graph's red sections denote the most permissible areas, while yellow areas indicate permissible areas. The use of Ramachandran map calculations, in conjunction with the PROCHECK tool, facilitated the assessment of the stereochemical quality of predicted models after

refinement. The model structure's reliability and high quality are evidenced by the distribution of approximately 90.8% of the total residues in the most widely distributed region. (Table.5)

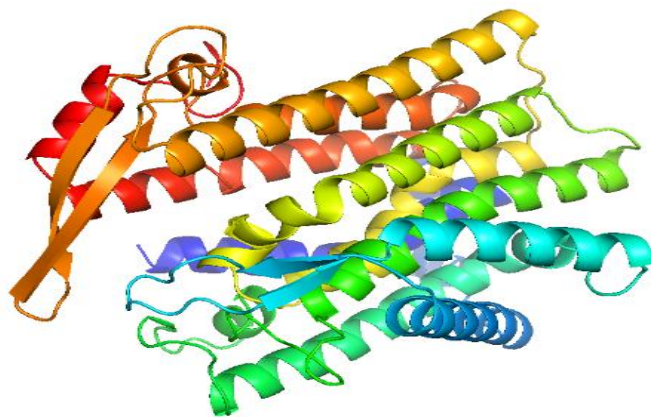


Fig.5. 3D structure of 7 -dehydrocholesterol reductase protein predicted by Robetta server and visualized through PyMOL

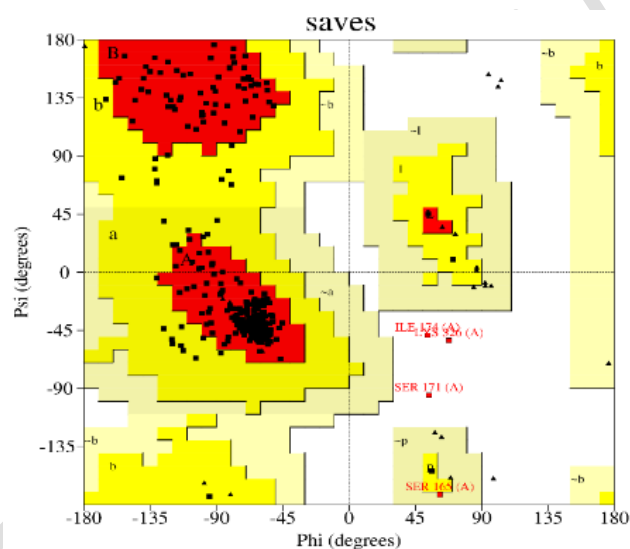


Fig. 6. Ramachandran plot showing the phi -psi torsion angles for all the residues of the 7-Dehydrocholesterol reductase protein: the distribution of amino acid residues to β -sheets (+psi, -phi), right-handed α -helix (-psi, -phi) and left-handed α -helix (+phi, +psi)

Table 5. Ramachandran plot calculation with the PROCHECK program

Ramachandran plot statistics	Distribution
Residues in most favoured regions	90.8%
Residues in additionally allowed regions	8.2%
Residues in generously allowed regions	0.3%
Residues in disallowed regions	0.8%

4.CONCLUSION:

The present study has undertaken an examination of the function of the 7-dehydrocholesterol reductase gene in the process of vitamin D synthesis in the tomato plant. When the gene is upregulated, the 7-Dehydrocholesterol is converted into cholesterol, but due to the downregulation of the gene the 7-DHC content will increase and the pathway is shifted from cholesterol synthesis to vitamin D₃ synthesis and Kandutsch Russel pathway clearly shows the transformation of 7 dehydrocholesterol to previtamin D₃. The gene's conservation across different species, in particular its similarity to the human sequence, serves to underscore its significance in the vitamin D₃ biosynthesis pathway that is observed in various plant species. The close resemblance of the protein sequence with *Solanum tuberosum* and *Datura*

sominifera helps in studying the role of this gene in vitamin D conversion. The gene's extensive tissue expression in the tomato plant, including its presence in leaves, stems, and fruit suggests its involvement in the synthesis of vitamin D₃ throughout the plant. Furthermore, a physio-chemical analysis has brought to light the tomato protein's stability and its potential for the conversion of 7-DHC to vitamin D₃. Pathway analysis shows that the gene has a role in cholesterol metabolism, and also points to its role in the synthesis of other compounds by changing its pathway. The functional analysis of this protein represents its stability, hydrophilic nature, and solubility nature and molecular modeling has revealed a β -barrel fold of the protein that is indicative of enzymatic function in the conversion of 7-DHC to vitamin D₃ and the Ramachandran plot validated this model and represents 90.8 % of amino acid residues in the favoured region. Overall, this study's results have clearly demonstrated this gene's significant role in the synthesis of vitamin D₃, which has important implications for human health, immunity, and crop enhancement. From this study, we can conclude that this gene in other species also has a role in vitamin D conversion and we further study the role of this gene in different plant species in the conversion of dehydrocholesterol to vitamin D₃ by genome editing. Subsequent research could focus on exploring the gene's role in other plant species, delving into the detailed synthesis mechanisms, and devising strategies to enhance the vitamin D content in crops.

REFERENCES

1. Kumar KS, Paswan S, Srivastava S. Tomato-a natural medicine and its health benefits. *Journal of Pharmacognosy and Phytochemistry*. 2012;1(1):33-43.
2. Toor RK, Savage GP. Antioxidant activity in different fractions of tomatoes. *Food research international*. 2005 Jun 1;38(5):487-94.
3. Cashman KD, van den Heuvel EG, Schoemaker RJ, Prévéraud DP, Macdonald HM, Arcot J. 25-Hydroxyvitamin D as a biomarker of vitamin D status and its modeling to inform strategies for prevention of vitamin D deficiency within the population. *Advances in nutrition*. 2017 Nov;8(6):947-57.
4. Cashman KD, Dowling KG, Škrabáková Z, Gonzalez-Gross M, Valtueña J, De Henauw S, Moreno L, Damsgaard CT, Michaelsen KF, Mølgaard C, Jorde R. Vitamin D deficiency in Europe: pandemic?. *The American journal of clinical nutrition*. 2016 Apr 1;103(4):1033-44
5. Bouillon R, Carmeliet G. Vitamin D insufficiency: Definition, diagnosis and management. *Best practice & research Clinical endocrinology & metabolism*. 2018 Oct 1;32(5):669-84
6. Dawson-Hughes B, Heaney RP, Holick MF, Lips P, Meunier PJ, Vieth R. Estimates of optimal vitamin D status. *Osteoporosis international*. 2005 Jul;16:713-6.
7. Henry HL, Bouillon R, Norman AW, Gallagher JC, Lips P, Heaney RP, Vieth R, Pettifor JM, Dawson-Hughes B, Lamberg-Allardt CJ, Ebeling PR. 14th Vitamin D Workshop consensus on vitamin D nutritional guidelines. *The Journal of steroid biochemistry and molecular biology*. 2010 Jul;121(1-2):4-6.
8. Sempos CT, Vesper HW, Phinney KW, Thienpont LM, Coates PM, Vitamin D Standardization Program (VDSP)*. Vitamin D status as an international issue: national surveys and the problem of standardization. *Scandinavian Journal of Clinical and Laboratory Investigation*. 2012 Apr 1;72(sup243):32-40.
9. Binkley N, Sempos CT, Vitamin D Standardization Program (VDSP). Standardizing vitamin D assays: the way forward. *Journal of Bone and Mineral Research*. 2014 Aug;29(8):1709-14.
10. Sempos CT, Heijboer AC, Bikle DD, Bollerslev J, Bouillon R, Brannon PM, DeLuca HF, Jones G, Munns CF, Bilezikian JP, Giustina A. Vitamin D assays and the definition of hypovitaminosis D: results from the First International Conference on Controversies in Vitamin D. *British journal of clinical pharmacology*. 2018 Oct;84(10):2194-207.
11. Lopez L, Hart LH, Katz MH. Racial and ethnic health disparities related to COVID-19. *Jama*. 2021 Feb 23;325(8):719-20.
12. Warren T, McAllister R, Morgan A, Rai TS, McGilligan V, Ennis M, Page C, Kelly C, Peace A, Corfe BM, Mc Auley M. The interdependency and co-regulation of the vitamin D and cholesterol metabolism. *Cells*. 2021 Aug 6;10(8):2007.
13. Christakos S, Ajibade DV, Dhawan P, Fechner AJ, Mady LJ. Vitamin D: metabolism. *Rheumatic Disease Clinics*. 2012 Feb 1;38(1):1-1.
14. Bhattacharyya MH, DeLuca HF. The regulation of rat liver calciferol-25-hydroxylase. *Journal of Biological Chemistry*. 1973 May 1;248(9):2969-73.

15. Christakos S, Ajibade DV, Dhawan P, Fechner AJ, Mady LJ. Vitamin D: metabolism. *Endocrinol Metab Clin North Am*. 2010 Jun;39(2):243-53.
16. Bikle DD, Patzek S, Wang Y. Physiologic and pathophysiologic roles of extra renal CYP27b1: Case report and review. *Bone reports*. 2018 Jun 1;8:255-67.
17. Milner SE, Brunton NP, Jones PW, O'Brien NM, Collins SG, Maguire AR. Bioactivities of glycoalkaloids and their aglycones from *Solanum* species. *Journal of agricultural and food chemistry*. 2011 Apr 27;59(8):3454-84.
18. Choi S, You MK, Jeon YA, Lee J, Kim J, Park YJ, Kim J, Park J, Kim JK, Choe S. Metabolic Engineering to Enhance Provitamin D3 Accumulation in Edible Tomatoes. *GEN Biotechnology*. 2023 Jun 1;2(3):219-27.
19. Available: <https://www.ncbi.nlm.nih.gov/>
20. Available: <https://www.ebi.ac.uk/interpro/search/sequence/>
21. Available: <https://web.expasy.org/protparam/>
22. Available: <https://www.uniprot.org/>
23. Available: <https://prosite.expasy.org/>
24. Available: <http://pfam-legacy.xfam.org/>
25. Available: https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa%20_sopma.html
26. Mc Auley MT, Proctor CJ, Corfe BM, Cuskelly GJ, Mooney KM. Nutrition research and the impact of computational systems biology. *Journal of Computer Science & Systems Biology*. 2013 Oct 7;6(5):271-85.
27. Novère NL, Hucka M, Mi H, Moodie S, Schreiber F, Sorokin A, Demir E, Wegner K, Aladjem MI, Wimalaratne SM, Bergman FT. The systems biology graphical notation. *Nature biotechnology*. 2009 Aug;27(8):735-41.
28. Available: <https://saves.mbi.ucla.edu/>
29. Audain E, Ramos Y, Hermjakob H, Flower DR, Perez-Riverol Y. Accurate estimation of isoelectric point of protein and peptide based on amino acid sequences. *Bioinformatics*. 2016 Mar 15;32(6):821-7.
30. Sivakumar K, Balaji S, Gangaradhakrishnan. In silico characterization of antifreeze proteins using computational tools and servers. *Journal of Chemical Sciences*. 2007 Sep;119:571-9.
31. Gamage DG, Gunaratne A, Periyannan GR, Russell TG. Applicability of instability index for in vitro protein stability prediction. *Protein and peptide letters*. 2019 May 1;26(5):339-47.
32. Magdeldin S, Yoshida Y, Li H, Maeda Y, Yokoyama M, Enany S, Zhang Y, Xu B, Fujinaka H, Yaoita E, Sasaki S. Murine colon proteome and characterization of the protein pathways. *BioData mining*. 2012 Dec;5(1):1-4.
33. Magdeldin S, Yoshida Y, Li H, Maeda Y, Yokoyama M, Enany S, Zhang Y, Xu B, Fujinaka H, Yaoita E, Sasaki S. Murine colon proteome and characterization of the protein pathways. *BioData mining*. 2012 Dec;5(1):1-4.
34. Liu Z, Ma H, Goryanin I. A semi-automated genome annotation comparison and integration scheme. *BMC bioinformatics*. 2013 Dec;14:1-2.
35. Novère NL, Hucka M, Mi H, Moodie S, Schreiber F, Sorokin A, Demir E, Wegner K, Aladjem MI, Wimalaratne SM, Bergman FT. The systems biology graphical notation. *Nature biotechnology*. 2009 Aug;27(8):735-41.
36. MacLaughlin J, Holick MF. Aging decreases the capacity of human skin to produce vitamin D3. *The Journal of clinical investigation*. 1985 Oct 1;76(4):1536-8.

Annexure 1:

Protein Sequence of 7 -dehydrocholesterol reductase gene in different species

>XP_016577971.2 7-dehydrocholesterol reductase [Capsicum annuum]
MGDSQLVHPPLFTYISMLTLLTIVPPFVILMWYTNVHADGSVLQTFNFKENGLQGLIDIWPRPTAVAGK
IIICYALFEAILQLLLPGKRVEGPISPTGHRPVYKANGVAAYIVTLITYLSLWWFGIFNPTIVYDHLGEI
LSTLNFGSLIFCLFLYIKGHVAPSSDTHGSSGNIIDYYWGMELYPRIGKHFDIKVFTNCRFGMISWAVL
PITYCIKQYEEYGNLSDSMLVHTIITLVYVTKFFWWEAGYWNMTMDIAHDRAGFYICWGCLVFLPCIYTSP
GMYLVKHPVNLGPQLAISILVAGILCVYINYDCDRQRQEFRTNGKALVWGRPPSKIVASYTTTTGETKT
SLLLTSGWWGLSRHFHYVPEILASFFWCVPALFNHFIPYFYVIYLVLLLDRAKRDDERCKSKYKGYWKK
YCDKVPYRVIPGIY

>AOX15301.1 sterol delta-7 reductase 2 [Withania somnifera]
MGETKLVHSPLLYASMLTLLSFTPPFVILMWYTNVHADGSVLKTFNHLREHGLQGLIDIWPKPTAIAGK
IIIFYALFEAALQLLLPGKRVEGPISPTGHRPVYKANGMAAYAVTLITYISLWWFGIFTNPAIVYDHLGEI
LSTLVFGSLVFCVFLYIKGHVAPSSDTHGSSGNIIVDFYWGMELYPRIGKHFDIKVFTNCRFGMMSWAVL
AVTYCIKQHEEYGRVSDSMLVNTILMLVYITKFFWWEAGYWNMTMDIAHDRAGFYICWGCLVWVPSIYTSP
GMYLVKQPVNGLGLQSLYILVAGLLCIYINYDCDRQRQEFRTNGKCTVWGKAPSKIVATYTTTTSGEKKT
SLLLTSGWWGLARHFHYVPEILAAFFWSVTALFNHFIPYFYVIFLTILLFDRAKRDDDRCKAKYKGYWKL
YCEKVPYRIIPGVY

>MCD7446296.1 7-dehydrocholesterol reductase [Datura stramonium]
MAETKLVHSPLITYGSMLTLLSFTPPFVILMWYTNVHADGSILKTFNHLRENGLQGLINIWPKPTAIAGK
LIICYALFEAALQLLLPGKRVEGPISPTGHRPVYKANGMAAYAVTLITYISLWWFGIFNPAIVYDHLGEI
FSTLIFGSLVFCVFLYIKGHVAPSSDTHGSSGNIIVDFYWGMELYPRIGKHFDIKVFTNCRFGMMSWAVL

AVTYCIKQHEEYGSVSDSMLVNTILMLVYVTKFFWWEAGYWNTMDIAHDRAGFYICWGCLVWLPCIYTSP
GMYLVKQPVNLGLQLSLYILVAGLLCIYINYDCDRQRQEFRRRTNGKCTVWGKAPSKIVASYTTTSGEKKT
SLLLTSWWGLARHFHYVPEILASFFWTVPALFNHFIPYFYVIYLTILLLDRAKRDDDRCKAKYGKYWKL
YCEKVPYRVVPGIY

>NP_001351.2 7-dehydrocholesterol reductase [Homo sapiens]
MAAKSQPNIPKAKSLDGVTNDRITASQGQWGRAWEVDWFSLASVIFLLLFAPFIVYFIMACDQYSCALTG
PVVDIVTGHARLSDIWAKTPPIRKAALYTLVWTFQVLLYTSPLDFCHKFLPGYVGGIQEGAVTPAGVV
NKYQINGLQAWLLTHLLWFANAHLWSFSPTIIFDNWIPLWCANILGYAVSTFAMVKGYFFPTSARDCK
FTGNFFYNYMMGIEFNPRIGKWFDFKLFNNGRPGIVAWTLINLSFAAQRELHSHVTNAMVLNVLQAIY
VIDFFWNETWYLKTIIDICHDFGWYLGWDCVWLPYLYTLQGLYLVYHPVQLSTPHAVGVLLGLVGYI
FRVANHQKDLFRRTDGRCLIWGRKPKVIECSYTSADQRHHSKLLVSGFWGVARHFNYVGDLMGSLAYCL
ACGGHLLPYFYIYMAILLTHRCLRDEHRCASKYGRDWERIYTAAPYRLLPGIF

>NP_001077693.1 Ergosterol biosynthesis ERG4/ERG24 family [Arabidopsis thaliana]
MAETVHSPIVTYASMLSLLAFCPFFVILLWYTMVHQDGSVTQTFFGFFWENGVOGLINIWRPRTLIWAKII
FCYGAFAEAILQLLLPGKRVEGPISPAGNRPVYKANGLAAYFVTLATYLGWFGIFNPAIVYDHLGEIFSA
LIFGFSFIFCVLLYIKGHVAPSSSDSGSCGNLIIDFYWGMELYPRIGKSFDIKVFNTNCRFGMMSWAVLAVT
YCIKQYEINGKVSMSMLVNTILMLVYVTKFFWWEAGYWNTMDIAHDRAGFYICWGCLVWVPSVYTSPGMY
LVNHPVELGTQLAIYILVAGILCIYINYDCDRQRQEFRRRTNGKCLVWGRAPSKIVASYTTTSGETKTSLL
LTSWWGLARHFHYVPEILSAFFWTVPALFDNFLAYFYVIFLTLFFFDRAKRDDDRCRSKYKGYWKL
KVKYRIIPGIY

>NP_001353043.1 7-dehydrocholesterol reductase [Solanum lycopersicum]
MAESQLVHPPFTYISMITLLTLVPPFVILMWYTNVHADGSVLQTYNYLKENGLQGLIDIWPRPTAIAGK
IICYALFEATLQLLLPGKRVEGPISPAGNRPVYKANGMAAYTVTLITYLSLWVWFGIFNPTIVYDHLGEI
LSTLNFGSLIFCLFLYIKGHVAPSSDHDGSSGNIIVDYWGMELYPRIGKHFDIKVFNTNCRFGMISWGLL
PITYCIKQYEEYGSLSMSMLIHTIITLVYVTKFFWWEAGYWNTMDIAHDRAGFYICWGCLVFLPCMYS
GMYLVKHPVNLGPQLAISILVAGILCVYINYDCDRQRQEFRRRTNGKALVWGKAPSKIVASYTTTGETKT
SILLTSWWGLSRHFHYVPEILASFFWSVPALLNHFMPYIYVIYLTGLLLDRAKRDDDRCKSKYKGYWKK
YCEKVPYRVVPGIY

>NP_001305624.1 7-dehydrocholesterol reductase-like [Solanum tuberosum]
MAESQLVHPPFTYISMLALLTLVPPFVILMWYTNVHADGSVLQTFNYLKENGLQGLIDIWPRPTAIAGK
IICYALFEATLQLLLPGKRVQGPISPAGNRPVYKANGMAAYTVTLITYLSLWVWFGIFNPTIVYDHLGEI
LSTLNFGSLIFCLFLYIKGHVAPSSDHDGSSGNIIVDYWGMELYPRIGKHFDIKVFNTNCRFGMVS
PITYCIKQYEEYGSLSMSMLIHAITLVYVTKFFWWEAGYWNTMDIAHDRAGFYICWGCLVFLPCMYS
GMYLVKHPVNLGPQLAISILVAGILCVYINYDCDRQRQEFRRRTNGKALVWGKAPSKIVASYTTTGETKS
SLLLTSWWGLSRHFHYVPEILASFFWSVPALFNHIMPYFYVIYLTGLLLDRAKRDDDRCKSKYKGYWKK
YCEKVPYRVIPGIY

>NP_001353044.1 7-dehydrocholesterol reductase [Solanum lycopersicum]
MVENKLVHSPLITYGSMLSLLSFTPPFVILMWYTNVHADGSILKTFNHLRENGLQGLIDIWPKPTAIAGK
LIICYALFEAALQLLLPGKTVEGPISPAGNRPVYKANGMAAYAVTLITYLSLWVWFGIFNPAIVYDHLGEI
FSTLIFGSLVFCVLLYIKGHVAPSSDSDGSSGNIIVDFYWGMELYPRIGKHFDIKVFNTNCRFGMMSWAVL
AVTYCIKQHEEYGRVSDSMLVNTILMLVYVTKFFWWEAGYWNTMDIAHDRAGFYICWGCLVWVPSIYTSP
GMYLVKQPVNLGLQLSLYILVAGLLCIYINYDCDRQRQEFRRRTNGKCTVWGKTPSKIVAAYTTTSGEKKT
SLLLTSWWGLARHFHYVPEILAAFFWSVPALFNHFIPYFYVIFLIILLLDRAKRDDDRCKAKYGKYWKL
YCEKVPYRVIPGIY

>XP_024181789.1 7-dehydrocholesterol reductase [Rosa chinensis]
MAESKTVHSPLVTYASMLSLLTLCPPFVILLWYTMVHADGSLFQTWEYLRQNGLQGFIDIWPRPTAIWAK
IIACYAAFEAALQLLLPGRVVEGPISPAGNRPVYKANGMAAYFVTLTYYLGLWVWFGIFNPTIVYDHLGEI
FSALIFGSLVFCVLLHIKGLAPSSDSDGSSGNVIIDFYWGMELYPRIGKNFDIKVFNTNCRFGMMSWAVL
AVTYSIKQYELNVRVADSMVNTILMLVYVTKFFWWEAGYWNTMDIAHDRAGFYICWGCLVWVPSIYTSP
GMYLVNHPINLGTQLALFILVAGILCIYINYDCDRQRQEFRRRTNGKCLVWGRAPSKIVASYTTTSGETKT
SLLLTSWWGLSRHFHYVPEISAAFFWTVPALFNHFIPYFYVFLTILLLDRAKRDDDRCRSKYKGHKWL
YCQKVPYRVIPGIY

>XP_016480318.1 PREDICTED: 7-dehydrocholesterol reductase-like
[Nicotiana tabacum]

MAESQLVHPPLVTYLSMIALLTLPFFVILMWYTNVHADGSVLQTFNYLRENGLQGLIDIWPRPSAVAGK
IIICYALFEAALQLLLPGKTVQGPISPTGHRPVYKANGMAAYIVTLITYLSLWWFGIFNPTIVYDHLGEI
LSTLNFGSLIFCLFLYIKGHVAPSSDHDGSSGNIIDFYWGMELYPRIGKHFDIKVFTNCRFGMISWGL
PITYCIKQYEEYGLSDSMLVNTILTLVYVTKFFWWEAGYWNTMDIAHDRAGFYICWGCLVWLPICIYTSP
GMYLVKQPVNLGLQLAIYILVAGVLCVYINYDCDRQRFRRRTNGKCLVWGKAPSKIVASYTTTTGETKT
SLLLTSGWWGLARHFHYVPEILASFFWSVPALFNHFIPYFYVIYLTILLFDRAKRDDDRCKSKYGKYWKL
YCEKVPYRVIPGIY

UNDER PEER REVIEW

Annexure 2:Amino acid composition of 7-dehydrocholesterol reductase in different plant species

Amino acids	Solanum lycopersicum (C-6)	Solanum tuberosum	Solanum lycopersicum Chr-1	Homo sapiens	Arabidopsis thaliana	Capsicum annum	Withiana somniferum	Datura stromatium	Nicotiana tobaccum	Rosa chinensis
Ala (A)	4.80%	5.30%	6.00%	7.80%	6.50%	4.6%	6.2%	6.0%	5.5	6.9
Arg (R)	3.50%	3.50%	3.50%	4.40%	3.90%	3.5%	3.7%	3.5%	3.5	4.6
Asn (N)	3.00%	3.00%	3.20%	3.60%	3.20%	3.2%	2.5%	3.2%	3.2	3.5
Asp (D)	3.20%	3.20%	3.50%	4.20%	3.50%	3.7%	3.5%	3.2%	3.5	3.5
Cys (C)	2.50%	2.50%	2.50%	2.70%	3.00%	2.8%	2.3%	2.8%	2.8	2.5
Gln (Q)	1.80%	2.10%	1.60%	3.20%	1.90%	1.8%	1.6%	1.6%	2.3	2.1
Glu (E)	3.20%	3.00%	3.00%	1.70%	3.00%	2.8%	3.0%	3.0%	2.8	2.5
Gly (G)	7.60%	7.60%	7.40%	7.80%	7.70%	7.4%	7.4%	7.4%	7.4	7.1
His (H)	3.00%	3.00%	2.80%	3.60%	1.90%	3.0%	3.0%	2.8%	2.5	2.5
Ile (I)	8.80%	8.10%	8.30%	5.90%	8.10%	9.4%	7.8%	7.8%	8.5	7.1
Leu (L)	11.10%	11.30%	11.10%	11.60%	10.20%	10.8%	10.8%	11.1%	11.5	11.1
Lys (K)	4.80%	4.80%	5.30%	3.80%	4.20%	4.8%	5.3%	5.3%	4.4	3.7
Met(M)	2.50%	2.50%	2.50%	1.70%	2.30%	1.8%	2.5%	2.5%	2.1	2.5
Phe (F)	4.60%	5.10%	5.50%	6.70%	7.00%	5.5%	6.0%	5.5%	5.3	6.0
Pro (P)	5.50%	5.50%	4.80%	4.40%	4.40%	5.8%	4.4%	4.8%	5.3	4.8
Ser (S)	5.80%	6.00%	5.80%	4.40%	5.80%	5.3%	5.8%	5.8%	5.5	6.0
Thr (T)	6.90%	6.20%	5.80%	5.30%	5.60%	6.2%	6.7%	6.2%	6.2	6.2
Trp (W)	3.50%	3.50%	3.70%	4.20%	3.90%	3.5%	3.7%	3.7%	3.7	4.1
Tyr (Y)	7.60%	7.40%	6.70%	6.10%	6.70%	7.1%	6.7%	6.9%	7.4	6.2
Val (V)	6.20%	6.50%	7.10%	6.90%	7.20%	6.9%	7.1%	6.9%	6.7	6.9
Pyl (O)	0.00%	0.00%	0.00%	0.00%	0.00%	0.0%	0.0%	0.0%	0.0	0.0
Sec (U)	0.00%	0.00%	0.00%	0.00%	0.00%	0.0%	0.0%	0.0%	0.0	0.0

UNDER PEER REVIEW