International Journal of Science and Research (IJSR)
ISSN: 2319-7064
ResearchGate Impact Factor (2018): 0.28 | SJIF (2019): 7.583

Analysis of HMG-CoA Reductase Protein in *Poaceae* Family and Animals Samples by using Bioinformatics Tools

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Abstract: ***Chemically Terpenoids are made up of a basic five-carbon isoprene unit (2-methyl-1, 3-butadiene) and are among the first known biomolecules, with hopanoids having been recouped from residues as old as 2.5 billion years. The isoprenoids are the biggest category of organic products, enveloping more than 55,000 known compounds till date with various biochemical abilities, of which some are quinones in electron transport chains, as segments of membrane, in cell regulation, as photosynthetic colours, as hormones, and as plant defence compounds. Dimethylallyl diphosphate and isopentenyl diphosphate (IPP) are two fundamental compounds required for production of Terpenoids. Plants utilize two specific process of IPP biosynthesis- the mevalonate pathway and deoxyxylulose 5-phosphate pathway. The developmental history of the compounds associated with the two courses and the phylogenetic relation of their qualities across genomes propose that the mevalonate pathway has its origin accredited to archae bacteria, and the DXP pathway has its roots of origin to eubacteria, and that eukaryotes have acquired their properties for IPP biosynthesis from prokaryotes. The HMG-CoA Reductase enzyme catalyses the transformation of HMG-CoA to mevalonate, which is the initial stage in the mevalonate pathway for biosynthesis of trepenoids in plants. In this examination, multiple sequence alignment of an aggregate of ninety nine HMG-CoA Reductase protein sequences from Poaceae family and three protein sequences from animal samples including Homo sapiens (humans),Mus pahari (mouse) and Drosophila mojavensis (fruit fly)was done and they were studied by free to use bioinformatics softwares available on internet. The protein properties of the sequences was studied (molecularmass, pI, signal peptide, transmembrane helices and conserved domains, secondary and 3D structures). The study of HMG-CoA Reductase protein sequences uncovered that there is high identity between the HMG-CoA Reductase Protein present in plants and animals. PROCHECK tool was used to draw Ramchandran plot of the Triticum aestivum HMG-CoA Reductase protein (Accession Number: AAB29929.1) and the structure of the protein was studied. The study demonstrated that most of the residues of the protein sequence were situated in the most preferred areas in Ramachandran plot, showing that the simulated three-dimensional structure was authentic. Evolutionary investigation demonstrated that there is a relationship betweenthe HMG-CoA Reductase proteins in species of Poaceae family and other animal samples under study. As indicated by theanalysis, HMG-CoA Reductases ought to be derived from a common predecessor.***

Keywords: HMG-CoA, Terpenoids, Bioinformatics

1. Introduction

According to structural and functional characteristics, plants have two important groups of metabolites, which are termed as primary metabolites (DNA, RNA, amino acids, unsaturated fatty acids) and secondary metabolites (not required for the growth or reproduction of an organism but are produced by plants for having a selective advantage in the environment). Under the class of secondary metabolites, terpenoids contribute to being the biggest groups of organic compound which contribute to significant biological functions of plants, for example, terpenoids contribute to the colour, scent and flavour (carotenoid and chlorophyllin) in plant and also play a role in the defence mechanism of plant. Terpenoids are also an important part of traditional herbal medicine and has been used for its digestive, anti-inflammatory, anticancer, antiseptic, antioxidant, astringent, digestive, diuretic properties.

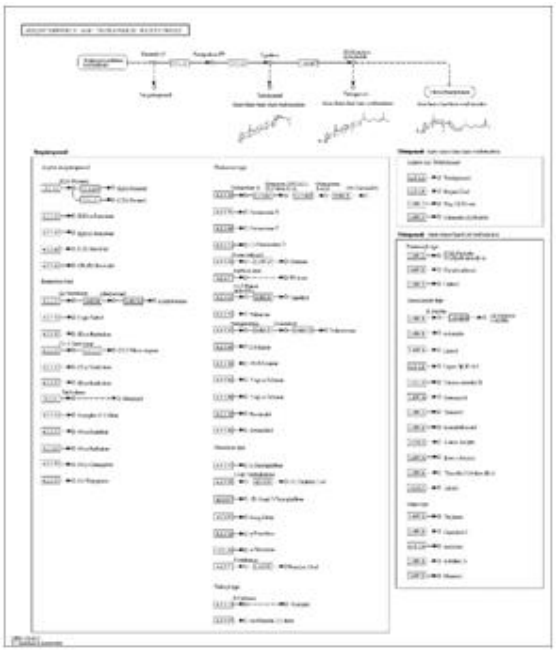

Figure 1: Triterpenoid biosynthetic pathway (Kegg pathway),

Table 1: The seventeen species studied in this bioinformatic analysis

Index	Species	Family
1	*Triticum aestivum*	*Poaceae*
2	*Zea mays*	*Poaceae*
3	*Dichanthelium oligosanthes*	*Poaceae*
4	*Aegilops tauschii*	*Poaceae*
5	*Setaria italica*	*Poaceae*
6	*Oryza brachyantha*	*Poaceae*
7	*Panicum hallii*	*Poaceae*
8	*Panicum miliaceum*	*Poaceae*
9	*Pediococcus acidilactici*	*Poaceae*
10	*Brachypodium distachyon*	*Poaceae*
11	*Triticum urartu*	*Poaceae*
12	*Oryza sativa*	*Poaceae*
13	*Sorghum bicolor*	*Poaceae*
14	*Drosophila mojavensis*	*Drosophilidae*
15	*Homo sapiens*	*Hominidae*
16	*Hordeum vulgare*	*Poaceae*
17	*Mus pahari*	*Muridae*

Trepenoids biosynthesis can occur through two routes in plants: the Methylerythritol Phosphate Pathway, which is non-mevalonate pathway and the second is mevalonate pathway. The Methylerythritol Phosphate Pathway occurs in the cytoplasm which results in synthesis of Triterpenoids and sesquiterpenoids, while mevalonate pathway takes place in the plastid which results in production of monoterpenoids, diterpenoid, and tetraterpenoids. In the synthesis of trepenoid, the 2, 3-oxidosqualene undergoes cyclization reaction in presence of oxidosqualene cyclase, which acts as a catalase. Only one form of oxidosqualene cyclase which islanosterol synthase (sterol biosynthesis) is present in fungi and animals. Higher plants of kingdom plantae have a more than one form of oxidosqualene cyclase. The molecular variety of oxidosqualene cyclases results in around 100 varieties of triterpenoids in plants. Dozens of oxidosqualene cyclise are isolated from model plants as well as harvests.

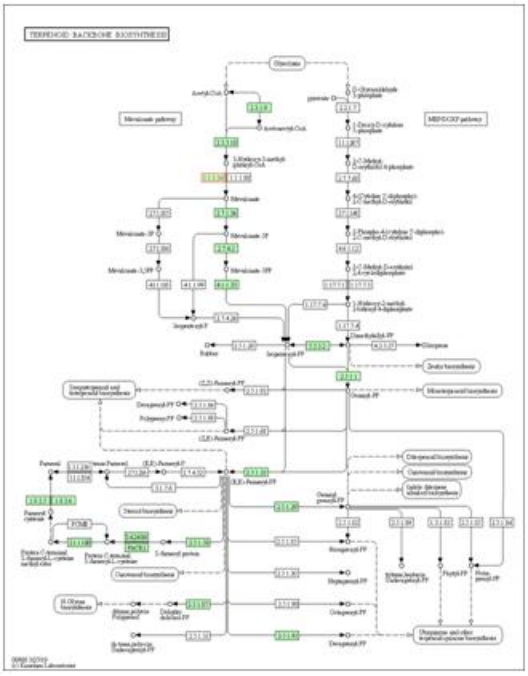

Figure 2: Mevalonate biosynthesis

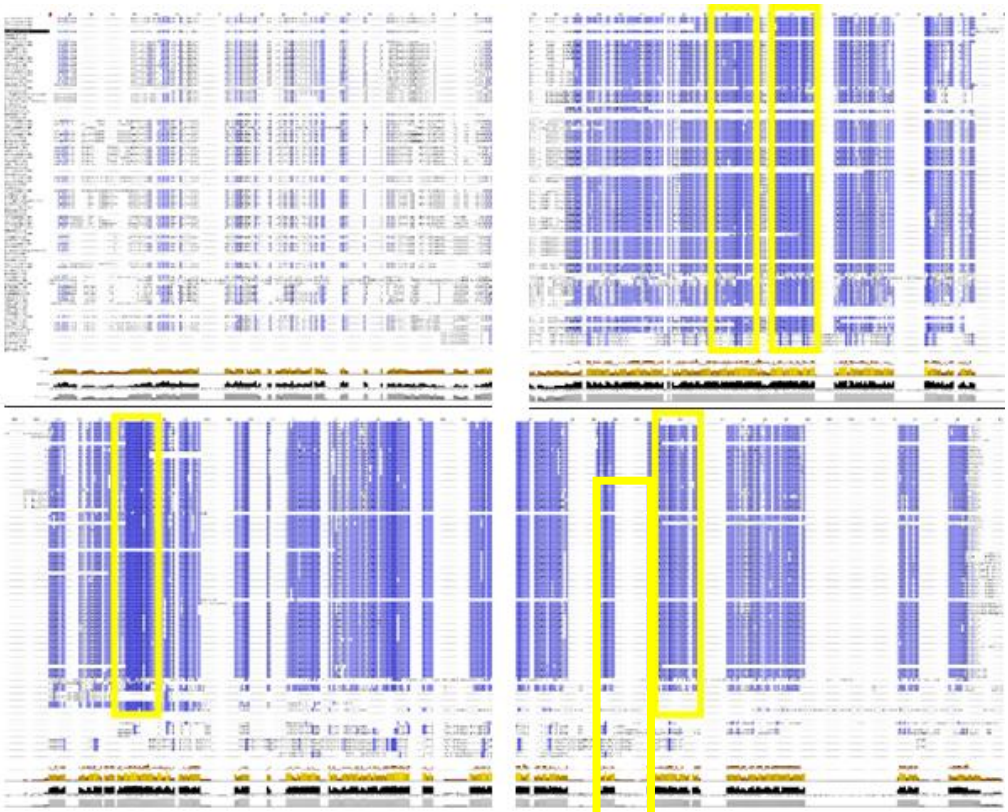

Figure 3: Alignment of multiple sequences of HMG-CoA Reductase protein in *Poaceae* and other animal samplesunder study. Increasing shades of blue residues: match the percentage of identity (conserved domains); multiple sequence alignment was performed with Clustal Omega using CLUSTAL-W method.

As appeared in Fig. 2 (Kegg Pathway Database), the HMG-CoA is a fundamental protein required in production of mevalonate, which is required in the mevalonate pathway for synthesis of terpenoids. HMG-CoA Reductaseis a rate-restricting protein, thus lovastatin is used for its repression. HMG-CoA Reductaseis deeply studied in archaebacterial family, microbial family, kingdom Plantae and Animalia. Upregulation of HMG-CoA reductase in the trepenoid pathway in transgenic plants has resultedin increment in production of triterpenoid. Though it is observed that random metabolic imbalances tend to cause hindrance growth of transgenic plants. Further studies found that catalytic domain of HMG-CoA Reductase can be co-over expressed, to reduce the growth inhibition brought by the individual overexpression of the catalytic domain of HMG-CoA Reductase protein.

HMG-CoA Reductase protein present in plants has 3 motif present in its catalytic domains, HMG Co-A Reductase protein in plants has a prism like structure, and this is because of the presence of 3 domains in the catalytic region of the protein.Studies have suggested that these 3 motifs are highly conserved sequences inHMG-CoA Reductaseprotein family. Analysis of these domains can be helpful in primer designing for amplification and isolation of HMG-CoA Reductase proteins. For this particular research, I used bioinformatics tools for investigation and examination ofstructural, functional and phylogenetic connectionsof the HMG-CoA Reductase protein sequences in Poaceae family and 3 animal samples.

Figure 4: Similarity/identity matrices of proteins sequences in Poaceae family and protein sequences of 3 animal samples (analysed in Fig3.) were analysed using MatGat software

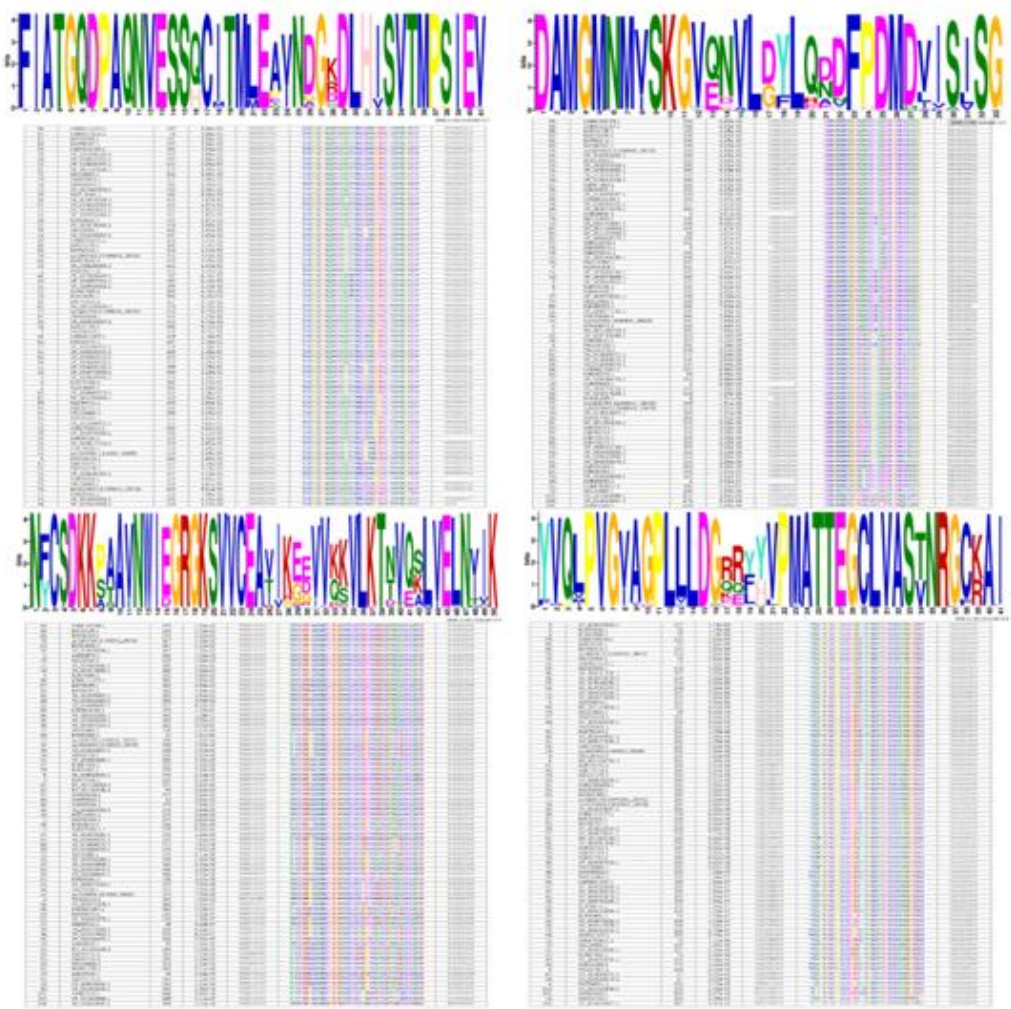

Figure 5: MEME (Multiple EM for Motif Elicitation) for HMG-CoA proteins sequences under study- Motif 1: FIATGQDPAQNVESSQCITMLEAVNDGKDLHISVTMPSIEV; Motif 2: DAMGMNMVSKGVQNVLDYLQDDFPDMDVISISG Motif 3: NFCSDKKPAAVNWIEGRGKSVVCEAVIKEDVVKKVLKTNVQSLVELNVIK Motif 4: YVQLPVGIAGPLLLDGQRYYVPMATTEGCLVASTNRGCKAI

2. Materials and Techniques

Retrieval of HMG-COA Reductase Protein Sequence

Entirety of protein sequences (HMG-CoA Reductase) of Poaceae and 3 samples including *Homo sapiens*, *Mus pahari* and *Drosophila mojavensis* (Total Ninety Nine Sequences) were retrieved from protein database of NCBI (http://www.ncbi.nlm.nih.gov; accessed on September 2020) as recorded in Table 1.

Bioinformatics Examinations

Free to use internet based bioinformatic tools and software's were utilized for examinations of the HMG-CoA reductase in Poaceae family and 3 different animal samples. The homology and analogy of protein arrangement of HMG-CoA reductase protein of Poaceae family with the effectively studied sequences of NCBI database was checked through BLASTp (NCBI), and multiple sequence alignment for related or similar HMG-CoA reductase protein sequences was done byClustal omega utilizing CLUSTAL-W (with character counts)method with default setting. The protein sequences of HMG-CoA Reductases were analysed utilizing Multiple Em for Motif Elicitation tool (MEME; adaptation 5.1.1) for discovering the sequence specific motifs. (http://meme-suite.org/tools/meme). MEME analysis was done using default settings and the motifs to find was increased to 4 per protein sequence.

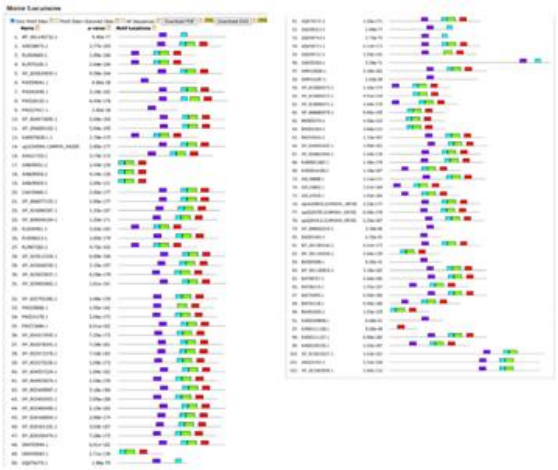

Figure 6: Motifs identified by MEME tool forHMG-CoA reductase proteins. Different-coloured rectangles represent the sequence specific MEME motifs.

Phylogenetic tree was performed by Jalview programming from aligned sequences. The Neighbor Joining (NJ) strategy and blossum62 were used for planning the evolutionary tree. SWISS-MODEL Homology Modelling Report (https://swissmodel.expasy.org/) (SMTL ID 1dq9.1) bioinformatics tool was used for the fully automated protein structure homology-modelling of *Triticum aestivum* HMG-CoA reductase protein (Accession: AAB29929.1). Rasmol software was used for 3D structure perception. Relative Spatial arrangement of atoms (stereochemistry) of 3D model was studied using PROCHECK tool, (http://www.ebi.ac.uk/thornton-srv/informationbases/cgi-canister/pdbsum/GetPage.pl?pdbcode=index.html) which helped in making the Ramachandran plot.

Figure 7: The highest percent similarity between HMG-CoA reductase proteins sequence of *Homo sapiens* and *Triticum aestivum*.

3. Results and Discussion

Study of the multiple sequence alignment indicated that HMG-CoA Reductase protein in Poaceae family and different samples have high rate of homology with one another. Protein sequences of HMG-CoA Reductase in plant have4 conserved motifs (EMPVGYVQLPVGV; TTEGCLVA; DAMGMNM and GTVGGGT).

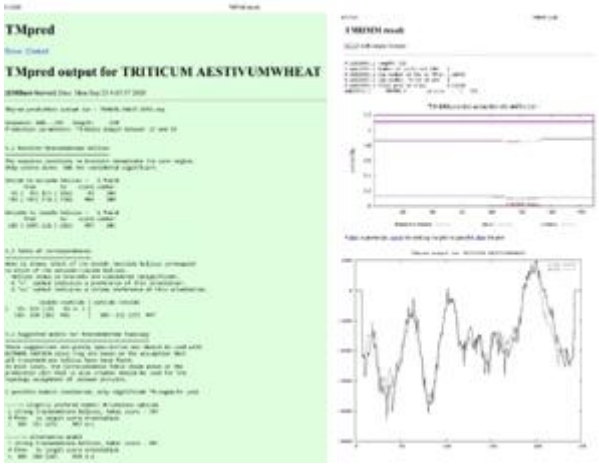

Figure 8: Transmembrane helices in Triticum aestivum HMG-COA REDUCTASE (Accession: AAB29929.1) were predicted using TMpred and TMHMM tools.

Multiple sequence alignment of Ninety Nine HMG-COA Reductase protein sequences belonging to Poaceae family and 3 samples including *Drosophila mojavensis*, *Homo sapiens*, *Mus pahari* (referenced in table 1) was done by Clustal Omega using CLUSTAL-W (with word counts) method. The samples under study demonstrated high homology in the protein sequences aligned by multiple sequence alignment. By study of the results of Fig. 3, 3rd and 4thmotif were found to be conserved more than 1st and 2nd motifs of Poaceae family and 3 animal samples. Triticum aestivum (common wheat) of Poacea family and protein sequence of Homo sapiens showed 61.59 % identity, which was the highest homology in HMG-CoA Reductase protein sequences under study. (Fig 7). I further performed motif examinations of these sequences by

Multiple EM for Motif Elicitation software for identification of motif specific to the protein sequences of Poaceae and 3 animal samples (Figs. 5, 6). Triticum aestivum HMG-CoA Reductase protein (GenBank Accession Number: AAB29929.1) of Poaceae family was chosen to study the protein structure of HMG-CoA Reductase protein and thus was studied usingbioinformatics methods. Triticum aestivum HMG-CoA Reductase (length: 150 aa) has a molecular weight of 15. 76 kilo daltons and the quantity of its isoelectric point (pI) is 4. 90 was found out using https://web.Expasy.Org/cgibin/protparam/protparam. On the amino acid level, Triticum aestivum HMG-CoA Reductase protein issimilar to numerous different HMG-CoA Reductase proteins presentin numerous plants under study.

For prediction of the presence of signal peptides and the location of their cleavage sites in proteins sequence, Triticum aestivum HMG-CoA Reductase protein was further examined by SignalPtool (Fig 11) (http://www.cbs.dtu.dk/administrations/SignalP/) and the results predicted that there is 0.002 probability of presence of Sec signal peptide (Sec/SPI) signal peptide protein sequence.

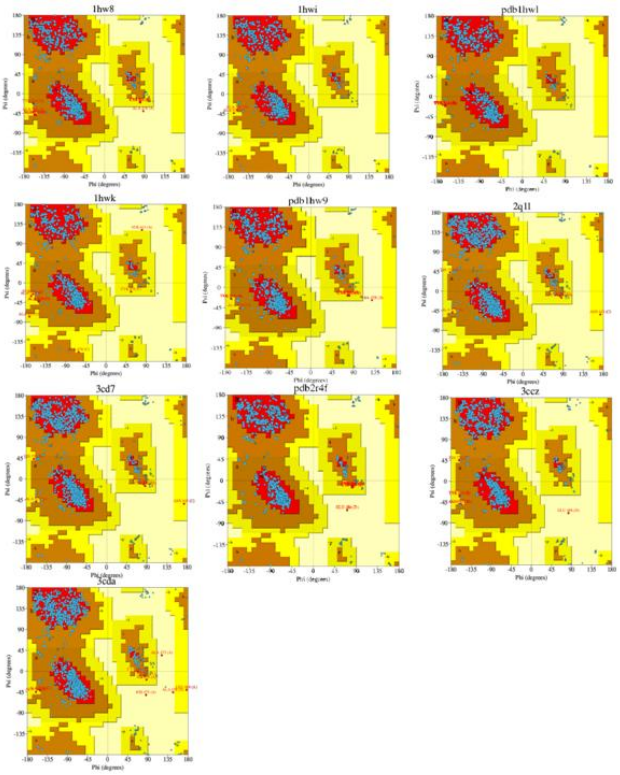

Figure 9: Ramachandran plot of Triticum aestivum HMG-CoA Reductase protein (Triticum aestivum HMG-CoA Reductase; AAB29929.1) (SMTL ID 1dq9.1). 7 different amino acid sequences are seen in the above given Ramchandran plot.

Triticum aestivum HMG-CoA Reductase protein was examined using TMpred and TMHMM tool (Fig 8) (http://www.cbs.dtu.dk/administrations/TMHMM) and the results indicated presence of 3 transmembrane helices, of which 2 are inside to outside helices (one of them is between acid amine 95 to 113 and the second one is between amino acid 185 and amino acid 210), third one is outside to inside helices (between acid amine 185 to 211). SOPMA (Fig 12) examination of Triticum aestivum HMG-CoA Reductase was performed for studying the secondary structure of HMG-CoA Reductase protein sequence and the results stated that the peptide of Triticum aestivum HMG-CoA Reductase had 58 % of alpha helices, 5.33 % of beta turns, 12.67 % of extended strands, and 24 % of random coil.

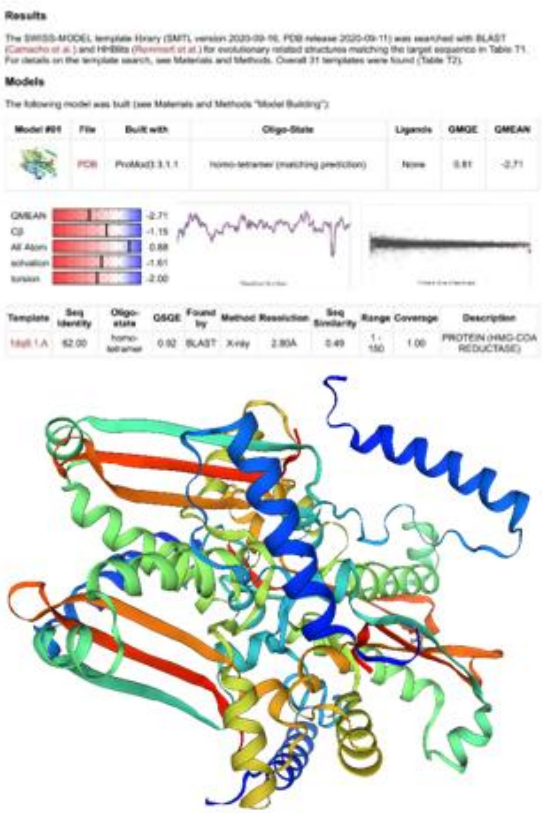

Figure 10: The 3D structure of Triticum aestivum HMG-CoA Reductase (Triticum aestivum HMG-CoA Reductase; AAB29929.1), established by homology-based modelling (SMTL ID 1dq9.1). SWISS-MODEL Homology Modelling Report tool was used for modelling of the 3D structure

SOPMA results indicate that extended strand had values lower than alpha helices and random coil in Triticum aestivum HMG-CoA Reductase protein. PROCHECK tool was used to study the quality of stereochemistry of Triticum aestivum HMG-CoA Reductase protein by generating the Ramachandran plot (Fig. 9), which suggested Triticum aestivum HMG-CoA Reductase protein has good stereochemistry. SWISS-MODEL Homology Modelling Report tool was used (https://swissmodel.expasy.org/). (Fig 10)for prediction of 3D structure of Triticum aestivum HMG-CoA Reductase protein

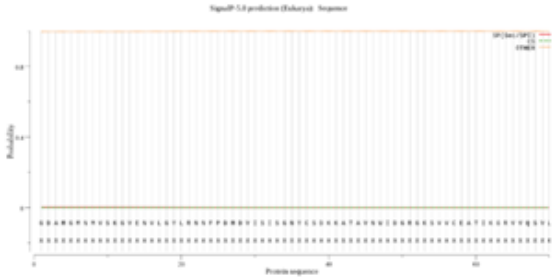

Figure 11: Investigation of Signal PTriticum aestivum HMG-COA REDUCTASE protein

The consequence of atomic demonstrating indicated that Triticum aestivum HMG-CoA Reductase has a spatial engineering that is fundamentally the same as HMG-CoA Reductaseof human. For studying the evolutionary relationship, protein sequences of HMG-CoA Reductases in Poaceae and various species under study were used and a phylogenetic tree was generated. The outcome demonstrated that HMG-CoA Reductases come from one precursor gene and formed into various branches. As indicated by the phylogenetic tree, HMG-CoA Reductases have relationship with one another (Fig. 13).

4. Conclusion

The knowledge of bioinformatics is used to understand the nature of pathways associated with trepenoid synthesis. Methylerythritol Phosphate Pathway and Non-mevalonate pathway have been studied and the proteins associated with it are identified. The characterisation of proteins have helped in biological engineering of trepenoid synthesis pathways.

Figure 12: SOPMA analysis for secondary structure of Triticum aestivum HMG-COA REDUCTASE protein.

Plant metabolism studies have revolutionised due to novel bioinformatics techniques. The new innovations, including designing the terpenoid based defence products, making transgenic plants, modifying a pathway in microorganisms to deliver drugs. The better comprehension of the elements of genes in terpene synthesis could prompt finding novel pathways of terpenoid production or production of new terpenoid compounds, which may lead to new entryway for future remedial products containing terpenoids. Examination demonstrated that HMG-CoA Reductaseprotein family has extremely conserved regions among plants and different organism. Thus this states that this protein sequence has played an important part in evolution. HMG-CoA Reductase protein sequences can be studied and genetic engineering of terpenoid pathways can open a door for industrial intervention employing terpenoids.

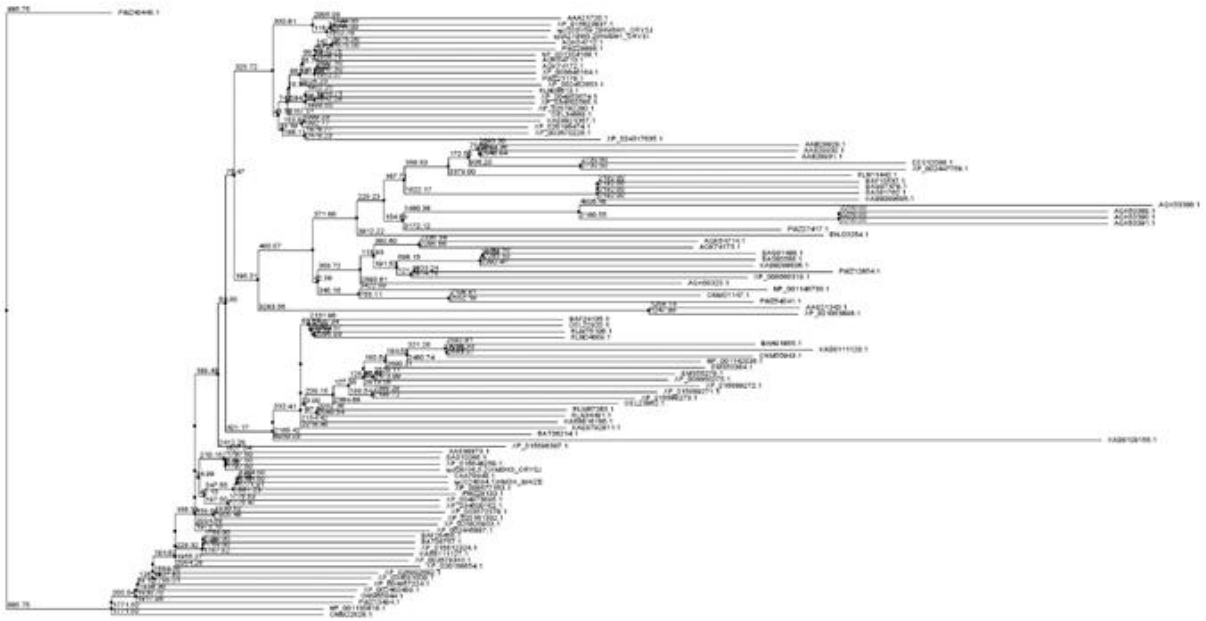

Figure 13: Evolutionary tree of HMG-CoA Reductases from Poaceae family and animals species under study was constructed using neighbour joining method using Jal view software.

5. Competing Interests Disclaimer

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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