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Research Article

COMPUTATIONAL ANALYSIS OF JUVENILE HORMONE EPOXIDE HYDROLASE (JHEH) PROTEIN SEQUENCES AMONG FIVE MAJOR LEPIDOPTERAN PESTS

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ABSTRACT

India is a global agricultural powerhouse. Agriculture, with its allied sectors, is the largest livelihood provider in India and plays a vital role in India's economy. Lepidopteran species are the most threatening pests of major annual and perennial crops, forests, and stored products throughout the world. The larvae of many lepidopteran species like *Manduca sexta, Heliothis virescens, Spodoptera exigua, Helicoverpa armigera and Tricoplusia ni* have become major problem to many food and commercial crops. On this agricultural facade, the main aim should be the control of insect pest population, rather than their eradication, which is neither possible nor ecologically desirable. Juvenile hormone (JH) a key regulator is primarily metabolized by two hydrolytic enzymes namely Juvenile Hormone Esterase (JHE) and Juvenile Hormone Epoxide Hydrolase (JHEH). Hydration of epoxide moiety of JH by JHEH is very crucial in JH metabolism in insects because of its irreversible reaction with JH. Many studies indicated that blocking JH metabolic pathway can result in immediate death or other severe consequences in early instars of insect pest control. Henceforth, the present study is focused on the analysis of putative functions of JHEH across the five serious lepidopteran pests. The functional features, Domains of JHEH protein sequences are identified by HMMR and Batch CDD searcher. The protein family identification and important sites in JHEH protein sequence are interpreted. Gene Ontologies of the sequences are predicted. The pathway in which JHEH is involved has been studied. Protein - protein interactions of the JHEH homologous sequences with reference to *Bombyx mori* are analyzed. The understanding of the functional imminent of JHEHs will divulge more alleyways for the biological lepidopteran pest control in deriving advanced molecular strategies.

INTRODUCTION

Juvenile hormone (JH) regulates various key physiological events in insect's life cycle. It controls a number of physiological activities in different species including behavior, coloration, diapauses and other biological processes[7]. In larval insects, the growth and development as well as reproduction in adult insects are regulated by juvenile hormone. Juvenile Hormone Epoxide Hydrolase (JHEH)[3,5] play a major role in regulating insect JH titer along with Juvenile Hormone Esterase (JHE). The major routes of JH metabolism in Lepidoptera are ester hydrolysis by JH esterase (JHE) and epoxide hydration by JH epoxide hydrolase (JHEH), generating the hormonally inactive JH acid and JH diol, respectively [8]. The JH degradation pathway was shown in Figure:1.

Five major food crop pests were considered in this study targeting JHEH in them. The five serious Indian food crop pests are *Heliothis virescens, Manduca sexta, Spodoptera exigua, Helicoverpa armigera* and *Trichoplusia ni. Manduca sexta* commonly called as Tobacco hornworm can readily defoliate an entire tobacco plant and thus a population of these insects can cause considerable damage to a tobacco crop. This pest mainly attacks the Solanaceae family plants. *Heliothis virescens* is found only in the America, from Canada to Argentina. Due to its high reproductive potential, *H. virescens* may cause considerable losses, especially in cotton, tobacco and soybean, and some ornamentals, but also causing serious damage on Alfalfa, Cabbage, Lettuce, Okra, Pea, Pepper, Squash, Tomato and many others crops. *Helicoverpa armigera* is a very serious pest that causes extensive damage to most of the pulses and vegetable crops including Red gram, Bengal gram, Tomato and other food and cash crops like Mulberry,

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Cotton etc. *Spodoptera exigua* commonly called as Beet army worm occur as a serious pest of vegetable, field and flower crops. Susceptible vegetable crops are Asparagus, Bean, Beet, Broccoli, Cabbage, Cauliflower, Celery, Chickpea, Corn, Cowpea, Eggplant, Lettuce, Onion, Pea, Pepper, Potato, Radish, Spinach, Sweet Potato, Tomato, and Turnip. Field crops damaged include Alfalfa, Corn, Cotton, Peanut, Safflower, Sorghum, Soybean, Sugar beet, and Tobacco. *Trichoplusia ni* also called as Cabbage looper has been reported damaging Broccoli, Cabbage, Cauliflower, Chinese Cabbage, Collards, Kale, Mustard, Radish, Rutabaga, Turnip, and Watercress. Other vegetable crops injured include Beet, Cantaloupe, Celery, Cucumber, Lima Bean, Lettuce, Parsnip, Pea, Pepper, Potato, Snap Bean, Spinach, Squash, Sweet Potato, Tomato, and Watermelon. Additional hosts are flower crops such as Chrysanthemum, Hollyhock, Snapdragon, and Sweet Pea, and field crops such as Cotton and Tobacco

In view of the great importance of the harvest index of food crops and cash crops, it is the hour of need to protect them from these devastating pests. Uses of chemical insecticides are widely adopted control measure against various insect pests. Use of insecticides can effectively control the pests, but it is always associated with negative concerns like toxicity dangers, natural enemy destruction, resistance to pesticides and host plant resistance. Nevertheless, toxicity levels on the environment are of serious concern.

These methods are not potential enough to the threshold limits of pests exceed. Under these circumstances, there is always a need to explore new strategies to combat the pest menace in an eco-friendly way. As juvenile hormone (JH) plays an important role in insect's physiology and reproduction, the systems (metamorphosis, moulting and reproduction) related to JH are potential targets of pest management [9]. Study on JHEH in *Spilosoma obliqua* (*So*JHEH) indicated that 47% homology with *Bombyx mori* followed by *Trichoplusia ni* and *Papilio xanthus*. The important active sites of an epoxide hydrolase that plays an important role namely catalytic triad and oxyanion hole are also highly conserved in *So JHEH* protein sequence indicating the epoxide hydration activity of JHEH on JH metabolism in *Spilarctia obliqua* [10]. On the basis of its high affinity for JH-III, it's structurally conserved domains and trends in enzyme hydrolysis, it is understood that *So*JHEH plays a crucial role in maintaining JH concentrations by converting it into JH-diols in an irreversible manner and is the biologically active JHEH of *Spilarctia obliqua* [11]. The full length sequence in *Heliothis virescens* and its comparative assessments showed that it encodes epoxide hydrolase and suggested it as microsomal EH (Hv-mEH1) whose specific activity was more than 25- and 3900-fold higher than that for the general EH substrates cis-stilbene oxide and trans-stilbene oxide, respectively[12]. Literature suggests that several insilico approaches have been adopted in order to assign functional information for sequences in various organisms[21]. In the current study JHEH gene from theses serious major insect pests subjected to extensive computational study to determine its structural and Gene ontologies along with its pathways

MATERIALS AND METHODS

The JHEH protein sequences belonging to *Heliothis virescens, Manduca sexta, Spodoptera exigua, Helicoverpa armigera* and *Trichoplusia ni* were retrieved from UNIPROT protein database and subjected to HMM Scan and Batch CDD search to identify the PFAM domain information of the sequences. Further PROTPARAM and SOPMA tools were used to find out physico-chemical properties and secondary structural features of the selected protein sequences. Functional gene ontology terms of the protein sequences were predicted by BLAST2GO. Then KEGG Automatic Annotation Server (KAAS) was used to recognize the pathway analysis of the protein sequences. Finally these sequences are submitted to STRING db V10 for identifying the direct and indirect associations with reference to their homologous. The methodology is given below in detail.

Sequences retrieval

The amino acid sequence of Juvenile hormone epoxide hydrolase (JHEH) from *Manduca Sexta, Heliothis virescens* [12], *Spodoptera exigua, Helicoverpa armigera and Trichoplusia ni.* [Accession numbers: Q25489, L7R9X8, Q1W696, C0KH33 & Q94806] were retrieved from the protein database of UNIPROT-KB (Release 2014_02) [22]. These sequences are used for further analysis in the current study.

Physico-Chemical Properties of the preferred JHEH protein sequences

The five JHEH protein sequences were submitted to PROTPARAM tool to identify the physico-chemical properties including molecular weight, theoretical pI, amino acid composition, atomic composition, instability index, grand average of hydropathicity.

Secondary Structural Analysis

The secondary structural analysis for the following selected JHEH sequences of five major lepidopteran are performed by using Self Optimized Prediction Method with Alignment (SOPMA) tool. The protein secondary structural features including α helix, 310 helix, Pi helix, Beta Bridge, Extended strand, Bend region, Beta turns, Random coil, Ambiguous states and other states were predicted. [21]

>*M.sexta*_JHEH_Q25489
MYKILSSFVAGVAIGSGLVITYVLYNVPEPPELDLQRWW
GIGTRPTEEDKSIRPFSIDFNDTVILDLKERLKNRRPFTKP
LEGINSEYGMNTEYLETVLEYWLNEYNFKKRAELLNKF
PHYKTRIQGLDLHFIRVKPEIKEGVQVLPLLMMHGWPSS
SKEFDKVIPILTTPKHEYNIVFEVVAVDLPGYGFSEGTNK
PGLNPVQIGVMMRNLMLRLGFEKFYIQAGDWGSQCAT
HMATLFPDQVLGLHTNMPLSSRPLSTVKLFIGALFPSLIV
DAKYMDRIYPLKNLFSYILRETGYFHIQATKPDTIGVALT
DSPAGLAGYLIEKMAICSNRDQLDTPHGGLENLNLDDV
LDTVTINWINNCIVTSTRLYAEGFSWPEVLIVHRIPSMVP
TAGINFKYEVLYQPDWILRDKFPNLVRSTVLDFGGHFA
ALHTPQALADDIFASAVQFLKFHDRKRNQKSS

>*H.virescens*_JHEH_L7R9X8
MGFLVKAVLVAALGVTAWFVLKCSKPHTIPHFDSEEW
WGPKELKETKQDQSIRPFKIKFDEEMIKDLKYRLKNHRK
FTPPLEGVAFEYGFNTAQLDSWLTYWADKYNFSEREAF
LNKFPHFKTKIQGLDVHFIRVKPQVPKDVEVIPLLMIHG
WPGSVREFYEAIPPLTRQTPGYNFVFELIMPSIPGYGFSD
PAARPGLGLPEVSVIFKNLMNRLGYKKFYVQGGDWGA
AIVSTMSTLFPEDILGSHSNMMVTQNTCAMLRWFLGSFF

PSLVVEDHLADRLYPLSKMFAHFMEEFGYMHIQATKPD
TVGVPLNDSPAGLLAYILEKFSTWTKNEYKHKPDGGLG
SRFTKDQLIDNLMIYWSTSSITTSMRFYAENMGDRVRSL
ALDQITTPVPSWALQAKEELFYQPPSILKTKFVNLLGNT
VLDDGGHFLAFELPEVLSADVFKAIKVFREWHDKNKKT
EL

>*S.exigua*_JHEH_Q1W696
MGFIVKAVLVAALGVAAWYYFIGCCPKTIPKLDNNEW
WGPKELVGKQDNAIRPFKVKFDEAMIKDLKLRLKNHR
AFRPPLEGVGFEYGFNTAQIDSWINYWADKYNFSEREA
FLNKFPHFKTNIQGLDIHFIRVKPEVPKNVEVLPLLMIHG
WPGSVREFYEAIPLLTRQTAGYNFVFELIIPSIPGYGFSDP
AVRPGLGMPQVAVIFRNLMNRLGHKKYYVQGGDWGA
GIVSTMSTLFPEDILGHHSNMLFTQHTCATVRTLVGAFL
PSLIIEEHLASRIYPLSSFFAYVLEEFGYMHQATKPDTVG
VPLSDSPAGLLAYILEKFSTWTKKEYKFKAGGGLSNRFT
KDQLIDNLMIYWSTNSITTSMRFYAENFSHKIMSLNLDQ
IPTDVPTWGLQAKEELFYQPPAVLSAKFKNLIGTTVLDD
GGHFLAFELPQVLSADVFKAVKAFKEWHQANKKTEL

>*H.armigera*_JHEH_C0KH33
MVRLLFIAPILAVILVPIYFVFLQGPPPLPDIDLNEWWGP
ESLKAKQDTSIRPFKVAFDDAAIRDLKDRLKRSRSFTPPL
EGVAFEYGFNSGQLDSWLKYWANEHQFKEREKFFNQF
PQFKTNIQGLDIHFIRVTPKVPAGVEVVPLLLLHGWPGS
VREFYEAIPLITAVSKDRDFAIEVIVPSLPGYGFSDGAVR
PGLSAPHIAVVMRNLMHRLGFKQFYVQGGDWGSLIGTT
LATFFPKEVLGYHTNMGAVLSTKATLIEIIGSFYPSLIVEP
HLADRMYPMGQRYATLVEEMGYMHIQASKPDTVGVA
LTDSPAGLLAYILEKFSTWTRNEHRLKADGALTFRFTKD
QLIDNLMMYWAPSSITTSMRLYAESFNSKIFGLKLDEIPT
PVPVWVIQAKYELAYQPPCILKLKFPNLQGVTVLEDGG
HFLAFELPKEFSEDVLKAMAVFRKLSKNNVKTDL

>*T.ni*_JHEH_Q94806
MGRLLFLVPVLAIVLLPVYYLFLQGPPPLPDLDYNEWW
GPESGKQKQDTSVRPFKINFGENLVKDLKDRLKRTRPLT
PPLEGVGFEYGFNTNEINSWLKYWAEGYNFKERETFLN
QFPQFKTNIQGLDIHFIKVTPKVPAGVQVVPMLLLHGWP
GSVREFYESIPLLTAVSKDRDFALEVIVPSLPGYGFSDGA
VRPGMGAPHIGIIMRNLMNRLGYKRYFVQGGDWGSVIG
TSLATFFPEEVLGYHANIGLVLSTKAMVWQAIGSVWPS
LIMDDLSLVDRIYPLSKTLSFQVRESGYLHIQASKPDTVG
VALTDSPAGLLAYIVEKFSIWTRPELTSKPNGGLDFRFTK
DQLIDNLMMYWTSKSITTSVRLYAESFNIKVLGYQLDDI
PTPVPSWFIQGKYEIAYQPPFVLKLKYPNIVGVTVLDDG
GHFFAFELPEVFSKDVLKAVTAFRKLQKNNEKTDL

Functional Annotation study

Domain Search

The query protein sequences were submitted to HMMscan web version [18] against the PFam database and Batch CDD search for assign PFam domain information, and annotation of protein domains [15].

Gene ontology Studies

Further the protein sequences were analyzed by using Blast2GO 3.2 [4] workstation for functional annotations including functional Gene Ontology (GO), mapping terms and InterPro's signatures.

Pathway Studies

Pathway associations of JHEH in insects were identified using KEGG Automatic Annotation Server (KAAS) [13,16].

Protein Interaction Studies

String DB (version 9.1) [19,20] was used to identify and analyze the protein-protein interactions of the JHEH sequences of five major lepidopteran pests with reference to the *Bombyx mori.*

Phylogenetic analysis

Multiple sequence alignment was performed and phylogenetic tree was generated by using neighbor joining method by MEGA 6.06 tools [14,17]. The constructed tree is visualized by using fig-tree (v1.4.2).

RESULTS AND DISCUSSION

In the present study, we have analyzed the JHEH protein sequences in five major lepidopteran pests and predicted detailed structural information.

The output of Batch CDD and HMMscan search tools concluded that four *(Manduca sexta, Heliothis virescens, Spodoptera exigua and Trichoplusia ni*) of the above five JHEH protein sequences belongs to the family EHN, Super family EHN and contains specific multi domain site MhpC. Remaining one JHEH protein sequence *(Helicoverpa armigera)* belongs to family EHN, Super family EHN, and contains multi domain site abhydrolase_1. The details of Domains identified by Batch CDD and HMMscan were shown in Table 1& 2.

Primary structural analysis and physic-chemical properties of the sequences was done by PROTPARAM. The details of amino acid composition, theoretical pI, number of negatively charged residues, positively charged residues, half life, instability index, aliphatic index and grand average of hydropathicity were calculated to five lepidopteran pests and results were shown in Table: 3. The JHEH protein sequence of selected lepidopteran pests contains the instability index below 40. So the JHEH protein was stable state in all the five lepidopteran pests. The secondary structural features alpha helix, extended strand, beta turn and random coil were identified in the query protein sequences by SOPMA. The results were shown in Table: 4.

The BLAST2GO BLAST step results showed the information related to interproscan, mapping, annotation, domain associations, GO terms and function, homologues identification in JHEH sequences of five Lepidopteran pests were shown in the Table 5.

BLASTp results by USING BLAST2GO Functional analysis tools showing the species distribution among different Lepidopteran species and Hits generated against the all five JHEH query sequences were shown in Figure: 2.

Table 1 Domain and Pfam information of five JHEH sequences by using HMMscan

	Family		Clan	Description	Start	End	Domain E-values	
	Id	Accession					Ind.	Cond.
M. Sexta	**EHN**	**PF06441.9**	**CL0028**	**Epoxide hydrolase N terminus**	**52**	**161**	**8.3e-28**	**1.0e-31**
	Abhydrolase_1	**PF00561.17**	**CL0028**	**alpha/beta hydrolase fold**	**147**	**285**	**8.8e-14**	**1.1e-17**
H. virescens	EHN	PF06441.9	CL0028	Epoxide hydrolase N terminus	52	161	6.2e-30	7.6e-34
	Abhydrolase_1	PF00561.17	CL0028	alpha/beta hydrolase fold	146	266	7.6e-16	9.3e-20
S. exigua	EHN	PF06441.9	CL0028	Epoxide hydrolase N terminus	51	160	1.5e-29	1.8e-33
	Abhydrolase_1	PF00561.17	CL0028	alpha/beta hydrolase fold	146	266	3.5e-15	4.3e-19
H. armigera	EHN	PF06441.9	CL0028	Epoxide hydrolase N terminus	51	160	3.1e-28	5.8e-32
	Abhydrolase_1	PF00561.17	CL0028	alpha/beta hydrolase fold	145	270	2.6e-14	4.8e-18
	Abhydrolase_6	PF12697.4	CL0028	Alpha/beta hydrolase family	147	442	2.9e-08	5.3e-12
T.ni	EHN	PF06441.9	CL0028	Epoxide hydrolase N terminus	51	160	2.1e-28	2.6e-32
	Abhydrolase_1	PF00561.17	CL0028	alpha/beta hydrolase fold	145	285	5.8e-11	7.1e-15

Table 2 Domain and Pfam information of five JHEH sequences by using CDD BATCH server

Query	Hit type	PSSM-ID	From	To	E-Value	Bit score	Accession	Short name	Incomplete	Super family
Q#1 - >sp|Q25489|HYEP	specific	253733	53	165	8.90552e-40	137.621	pfam06441	EHN	-	cl05773
	super family	253733	53	165	8.90552e-40	137.621	cl05773	EHN super family	-	-
	multi-dom	223669	147	444	7.33896e-11	61.5675	COG0596	MhpC	-	-
Q#2 - >tr|L7R9X8|L7R9	specific	253733	53	165	3.30069e-44	149.177	pfam06441	EHN	-	cl05773
	super family	253733	53	165	3.30069e-44	149.177	cl05773	EHN super family	-	-
	multi-dom	223669	127	449	2.99515e-10	59.6415	COG0596	MhpC	-	-
Q#3 - >tr|Q1W696|Q1W6	specific	253733	52	164	1.00744e-44	150.718	pfam06441	EHN	-	cl05773
	super family	253733	52	164	1.00744e-44	150.718	cl05773	EHN super family	-	-
	multi-dom	223669	118	449	2.4587e-11	62.7231	COG0596	MhpC	-	-
Q#4 - >tr|C0KH33|C0KH	specific	253733	52	164	8.24942e-43	145.711	pfam06441	EHN	-	cl05773
	super family	253733	52	164	8.24942e-43	145.711	cl05773	EHN super family	-	-
	multi-dom	249959	179	445	3.79937e-14	70.2398	pfam00561	Abhydrolase_1	-	-
Q#5 - >tr|Q94806|Q948	specific	253733	52	164	1.46893e-43	147.637	pfam06441	EHN	-	cl05773
	super family	253733	52	164	1.46893e-43	147.637	cl05773	EHN super family	-	-
	multi-dom	223669	146	450	2.60107e-13	68.8863	COG0596	MhpC	-	-

Table 3 Comparative primary structural analysis of JHEH sequences in five lepidoepterans by PROTPARAM.

	No of Amino acids	Molecular weight	Theoretical pI	-vely charged residues (Asp +Glu)	+ely charged residues (Arg +Lys)	Estimated half-life	Instability index	Aliphatic index	Gravy
M.sexta	462	52611.80	6.47	50	47	30 hrs	35.37	95.97	-0.110
H.virescens	463	53129.42	7.23	52	52	30 hrs	33.71	81.90	-0.200
S.exigua	462	52458.74	8.64	44	48	30 hrs	30.87	88.66	-0.066
H.armigera	462	52241.75	7.17	48	48	30 hrs	37.22	95.39	0.017
T.ni	463	52365.64	7.74	46	47	30 hrs	27.24	95.51	-0.048

Table 4 Comparative analysis of secondary structural features of JHEH sequences of five lepidopterans by SOPMA tool.

Secondary structural feature	*M.sexta*	*H.virescens*	*S.exigua*	*H.armigera*	*T.ni*
Alpha helix	30.09%	41.04%	40.69%	35.50%	29.37%
Extended strand	23.38%	15.33%	17.10%	23.81%	23.54%
Beta turn	8.44%	9.29%	8.44%	9.96%	9.29%
Random coil	38.10%	34.34%	33.77%	30.74%	37.80%

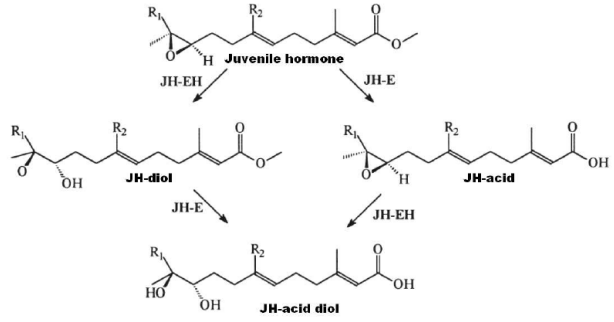


Figure 1 JH degradation pathway in insects

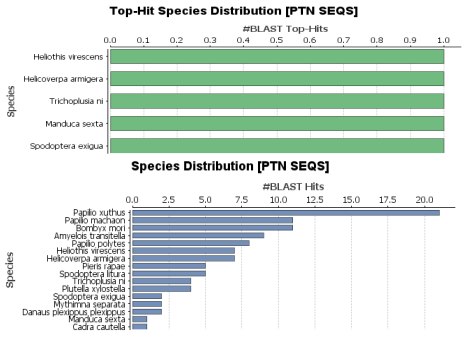
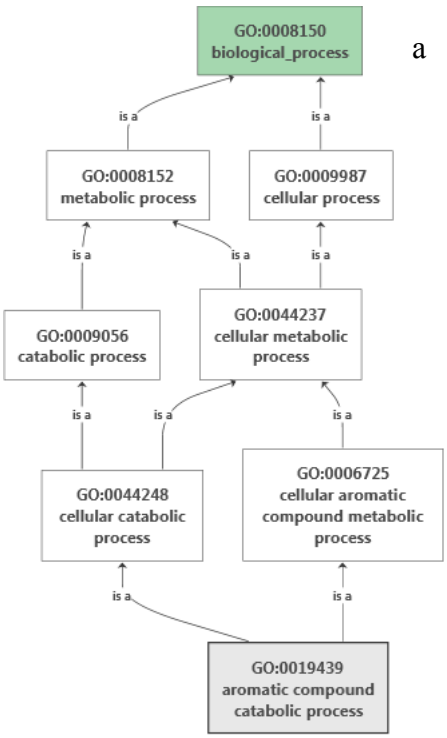


Figure 2 a& b BLASTp results by using BLAST2GO workstation

Table 5 BLAST2GO Results showing Interpro IDs, GO names list and mapping of all JHEH sequences in five lepidopterans.

S.No	Seq Name	Description	Length	#Hits & e-Value	# GO &GO IDs GO Names	Enzyme Codes & Names	InterPro IDs	InterPro GO IDs	InterPro GO Names
1.	*M.sexta_ JHEH_ Q25489*	Juvenile hormone epoxide hydrolase	462	20 & 0.0	5 & C:GO:0005789 P:GO:0019439 C:GO:0016021 F:GO:0033961 C:GO:0031090	EC:3.3.2.9 Microsomal Epoxide hydrolase	IPR000639 (PRINTS); IPR010497 (PFAM); IPR029058 (SUPERFAMILY)	F:GO:0003824 F:GO:0033961	F:catalytic activity; F:cis-stilbene-oxide hydrolase activity
2.	*H.virescens_ JHEH_ L7R9X8*	Juvenile hormone epoxide hydrolase-like	463	20 & 0.0	1 & F:GO:0033961	EC:3.3.2.9 Microsomal epoxide hydrolase	IPR000639 (PRINTS); IPR010497 (PFAM); IPR029058 (SUPERFAMILY)	F:GO:0003824 F:GO:0033961	F:catalytic activity; F:cis-stilbene-oxide hydrolase activity
3.	*S.exigua_ JHEH_ Q1W696*	Juvenile hormone epoxide hydrolase-like	462	20 & 0.0	1 & F:GO:0033961	EC:3.3.2.9 Microsomal epoxide hydrolase	IPR000639 (PRINTS); IPR010497 (PFAM); IPR029058 (SUPERFAMILY)	F:GO:0003824 F:GO:0033961	F:catalytic activity; F:cis-stilbene-oxide hydrolase activity
4.	*H.armigera_ JHEH_ C0KH33*	juvenile hormone epoxide hydrolase-like	462	20 & 0.0	5& C:GO:0005789 P:GO:0019439 C:GO:0016021 F:GO:0033961 C:GO:0031090	EC:3.3.2.9 Microsomal epoxide hydrolase	IPR000639 (PRINTS); IPR010497 (PFAM); IPR029058 (SUPERFAMILY)	F:GO:0003824 F:GO:0033961	F:catalytic activity; F:cis-stilbene-oxide hydrolase activity
5.	T.ni_JHEH_ Q94806	juvenile hormone epoxide hydrolase-like	463	20 & 0.0	5 & C:GO:0005789 P:GO:0019439 C:GO:0016021 F:GO:0033961 C:GO:0031090	EC:3.3.2.9 Microsomal epoxide hydrolase	IPR000639 (PRINTS); IPR016292 (PIRSF); IPR010497 (PFAM); IPR029058 (SUPERFAMILY)	F:GO:0003824 F:GO:0033961	F:catalytic activity; F:cis-stilbene-oxide hydrolase activity

Identified gene ontologies along with their names are represented as a hierarchical manner which was showed in Figure:3a. Figure 3b represents the combined hierarchical picture of all GO Ids, names and their functions (at Biological, Cellular and Molecular level) of JHEH sequences of five lepidopteran pests.



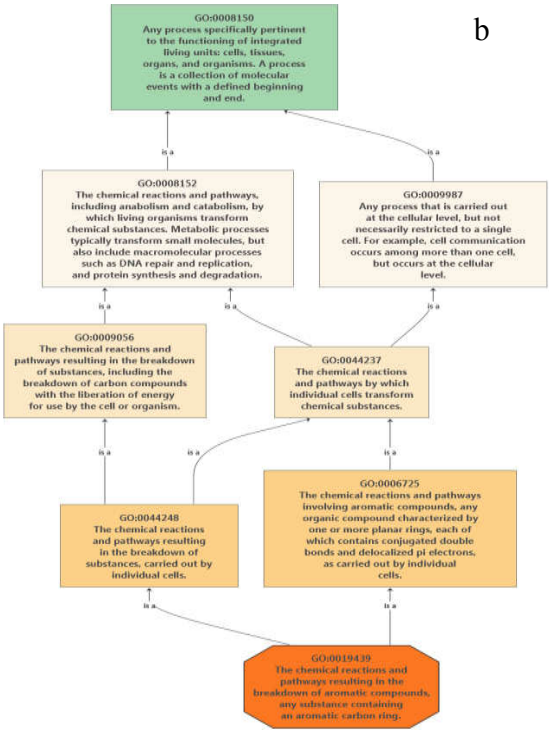


Figure 3 Graphical representation of GENE ONTOLOGY of five JHEH sequences

a) Graphical representation of the GOs along with their names
b) Functions of Identified GOs in JHEH sequences of five lepidopteron

Further Submitted the JHEH sequences to KEGG Automatic Annotation Server (KAAS). The output of KAAS showed

Insect hormonal biosynthesis pathway numbered 00981 in which JHEH is involved. The diagrammatic representation of pathway was shown in Figure:4.

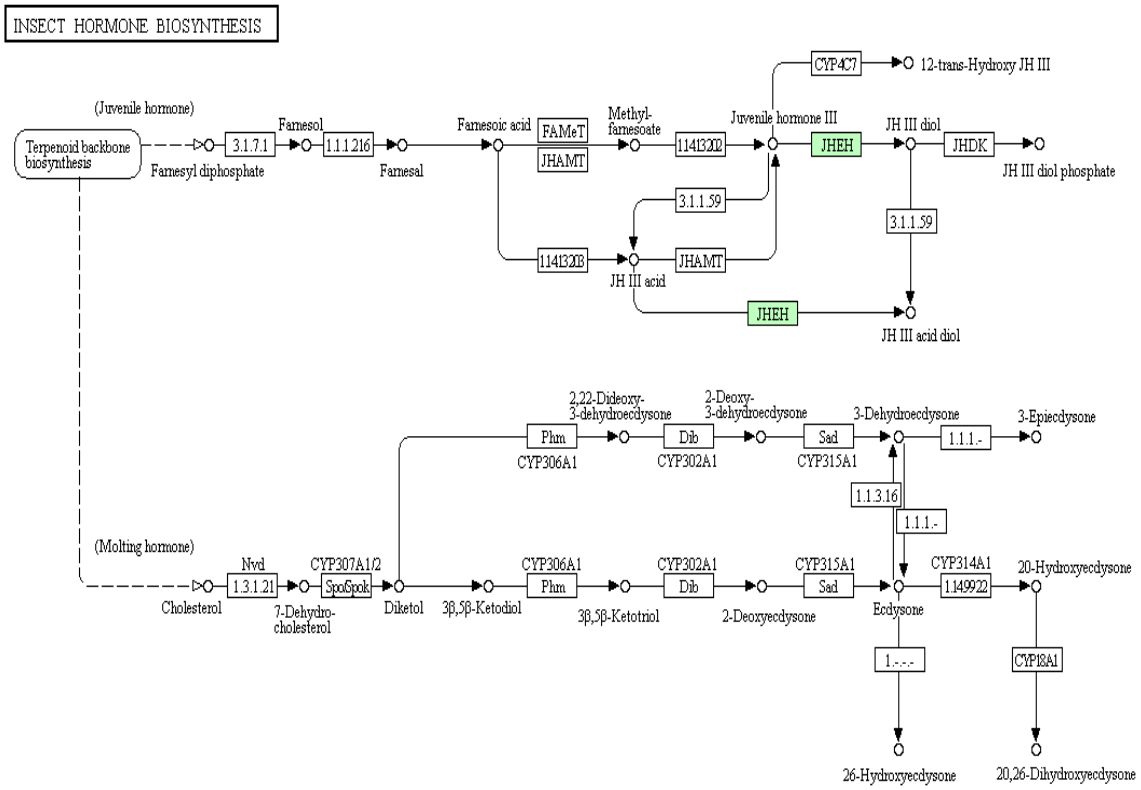


Figure 4 Pathway Insect Hormone Biosynthesis generated by KEGG (KASS)

The sequence homology information is derived by using STRING DB. *Bombyx mori* was used as a reference query to understand the conservation of gene neighborhood by using String DB. All this information signifies the importance of pathway analysis. Protein – Protein interactions of JHEH protein sequence with reference to *Bombyx mori* generated by STRINGDB was shown in Figure: 5

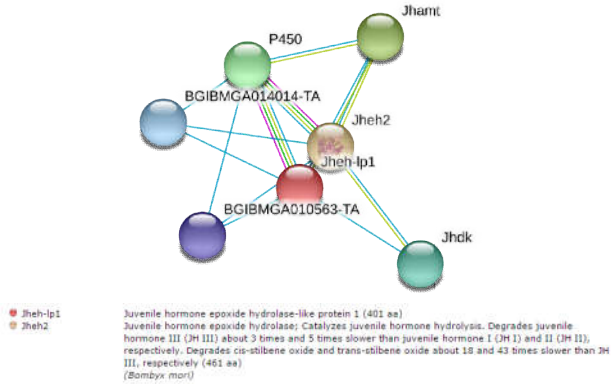


Figure 5 Protein-Protein interactions of JHEH protein sequence with reference to *Bombyx mori* generated by STRINGDB

The evolutionary relationship between all five JHEH sequences is represented by using the phylogenetic tree which showed that *T.ni* and *H.armigera* & *S.exigua* and *H.virescens* evolved from same ancestors. But *M.sexta* showed distant relation with remaining four sequences. The generated phylogenetic tree was shown in the Figure:6.

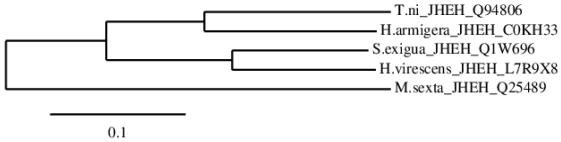


Figure 6 Phylogenetic tree generated by TreeDyn 198.3

DISCUSSION

According to Debernard *et al*., 1998, the catalytic mechanism of Manduca reata JHEH was similar to the mammalian EHS. and its enzyme activity was the highest for juvenile horomone-III[6]. As per Kamita *et al.,* [12], *Heliothis virescens* microsomal epoxide hydrolase (HvmEH1) although capable of metabolizing JH III, it is not important in the metabolism of JH in the caterpillar. The very high catalytic activity of this enzyme with some model substrates suggests that HvmEH1 may have a role in the hydration of xenobiotics in the diet or an endogenous epoxide that is yet to be identified. Hopefully, the powerful inhibitors discussed expanded the understanding of the physiological role of Hv-mEH1 in the caterpillar. With respect to *Spilosoma obliqua* JHEH (SoJHEH), one of the homolog to the above selected protein sequences, it has proven its impact in controlling the JH levels of the Insect pest which synchronized with the findings of other lepidopteran, coleopteran and dipteran insects. It also explored the dual enzyme regulating system of JH which controls varied biological activities of the insect pest and can further throw insights for developing innovative biocontrol ways. In the present study, JHEH protein sequences of five lepidopteran

pests were considered to assess their structural and functional properties in insilico. The protein sequences were retrieved from UNIPROT database as a preliminary source for the analysis. Domain analysis was done using HMMS can and Batch CDD search tools which confirmed that all JHEH sequences are having Abhydrolase, Epoxide Hydrolase and Mhpc domains and mainly belongs to Epoxide hydrolase (EHN) Super family (Table 2). Then the primary and secondary structural features of the protein sequences were analyzed and compared. The blast2GO, a universal tool for annotation, visualization and analysis in functional genomics research tool designed with the main purpose of enabling Gene Ontology (GO) [1] based data mining on sequence data, was used for Gene Ontology annotation based on similarity searches with statistical analysis and highlighted visualization on directed acyclic graphs as this tool offers a suitable platform for functional genomics research in non-model species. Figures 2 – 3 showed the Gene Ontologies for individual JHEH sequences in both perspectives based on cellular component and biological processes, mapping, annotation and GO-distribution across JHEHs of five pests respectively. Further, the JHEH protein sequences were submitted to STRINGDB [19] with reference to *Bombyx mori* and studied the interactions within (Intra) and with other (Inter) proteins. JHEH1 and JHEH2 interactions as per the relative distance were shown in Figure:5. The role of JHEH in Insect Hormone pathway by using KEGG database was showed in Figure:4. It plays a major role in mevalonate pathway in insects. Later comparative and phylogenetic analysis have been performed and studied the evolutionary relationships among considered five lepidopteran JHEHs depicted in Figure:6.. However, the structural design and functional site determinations gives more novel approaches for the development of potential targets involved in Xenobiotic metabolism. The structural and functional studies on JHEH protein sequences of lepidopteran pests furnish bright insights to produce better JH analogues that can be used for the control of lepidopteran pest menace.

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