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Tissue Location and Developmental Regulation of Pheromone Biosynthesis Activating Neuropeptide and Its Binding Proteins in *Spoladea recurvalis* (Lepidoptera: Crambidae)



Location and Distribution

INTRODUCTION

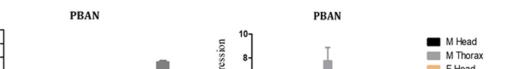
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Physiological processes and behavioral responses such as diapauses, mating, eclosion, reproduction, and metamorphosis are determined by neurohormones known as insect neuropeptides. Females produce molecules known as pheromones to entice sexual communication between male and female moths over long distances; the molecules are recognized by a distinct neural arrangement in males, and eventually processed from mating. Mostly species-specific sex pheromones are produced in female pheromone glands (PG) located on the terminal abdominal segments (ovipositors) in most moth species; when released, these pheromones attract conspecific males for mating. The Pyrokinin (PK)/pheromone biosynthesis-activating neuropeptide (PBAN) helps in regulating the sex pheromone found in insects and lepidopteran species. From the study of molecular characterization, DH-PBAN is a multigene family, encoded by five putative neuropeptides – PBAN, DH, α -, β - and γ - subesophageal ganglion. These are all well conserved C-terminal FXPRL amide motifs and are released through post-translational processing. Physiological functions such as melanization in moths, regulation of pheromone biosynthesis, and stimulation of embryonic diapause in silkworm moth are well documented. Pheromone binding proteins (PBPs) were initially discovered in Lepidoptera and later characterized in a series of insect orders including Diptera, Hymenoptera, Hemiptera and Coleoptera. PBP/dorant binding proteins (OBPs) sequences have conserved cysteine residues, and are classified based on their number and location such as "Classic", "Plus-C", "minus-C" and "duplex". However, most patterns of the cysteine residues in the amino acid sequences differ and present a signature for moth OBPs. The *Spoladea recurvalis* Fabricius beet webworm mostly inhabits tropical areas, where it is known for causing damage to vegetable crops including bean, amaranth, cucurbits, beetroot, sweet potato, and eggplant. Studies report that *S. recurvalis* can travel, causing serial crop destructi



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Use of chemical pesticides poses serious risks to the environment and human health. Developing alternative, ecofriendly control methods to interrupt mating behavior is recognized as an appropriate component for effective integrated pest management (IPM) strategies. Here, we characterized the pheromone biosynthesis activating neuropeptide and binding proteins in *S. recurvalis*, and performed the tissue-specific gene expression across developmental stages with the aim of using these proteins as primary molecular targets for designing and developing new pest management strategies.

MATERIAL AND METHODS

Bioinformatics analysis

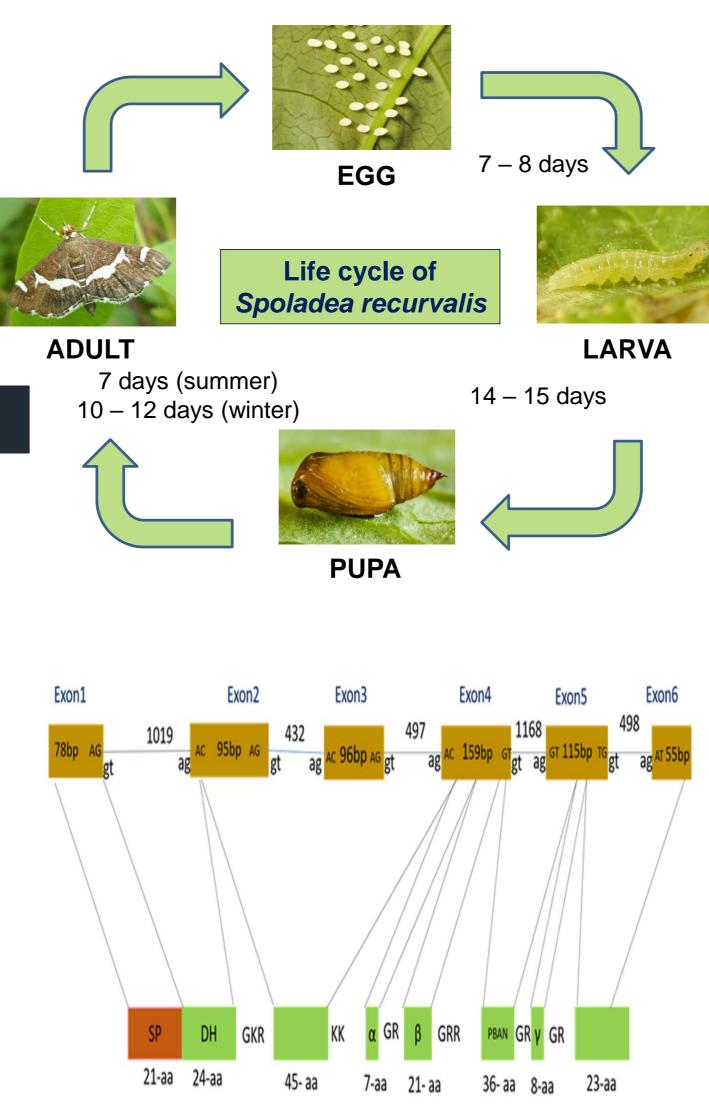
- ★ S. recurvalis transcriptome library.
- ★ Search for Pheromone binding activating neuropeptide (PBAN), Pheromone binding protein/odorant binding protein (PBP/OBP) sequence similarity search (BLASTx)
- ★ Identification of ORF and CDS.

Molecular and phylogenetic analysis

- ★ Sequence alignment, Clustal W
- ★ Signal peptide prediction by SignalP
- ★ Identification of propeptide and mature peptide prediction
- ★ Novel PBAN, PBP/OBP proteins
- ★ Neighbor-joining method MEGA6

Insect collection DNA, RNA extraction

- ★ S. recurvalis different life stages: fourth-andfifth instar larvae, pupae and male and female adults (antenna, head, thorax, abdomen)
- ★ Genomic DNA extraction, RNA extraction
- ★ 5' and 3' amplification of cDNA ends (5'-3' RACE amplification
- ★ Polymerase chain reaction (PCR)
- ★ Quantitative RealTime-PCR



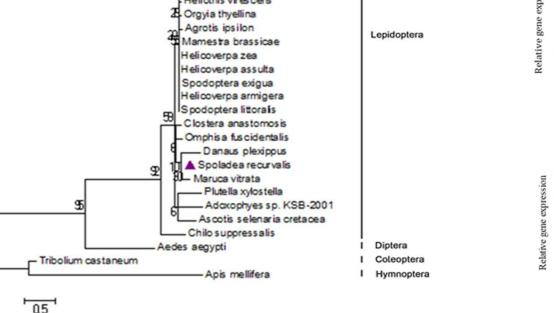


Figure 4. Phylogenetic relationship between *S. recurvalis* PBAN amino acid sequence using four insect orders determined by using MEGA 6.0 with default settings model JTT+T inferred from 1000 replicates.

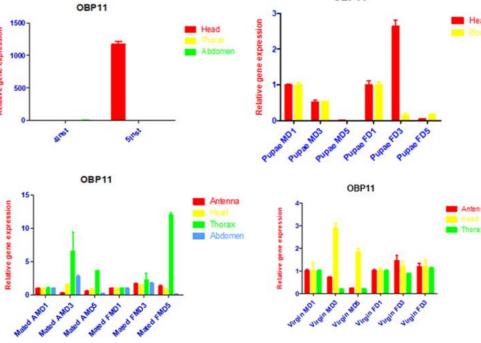
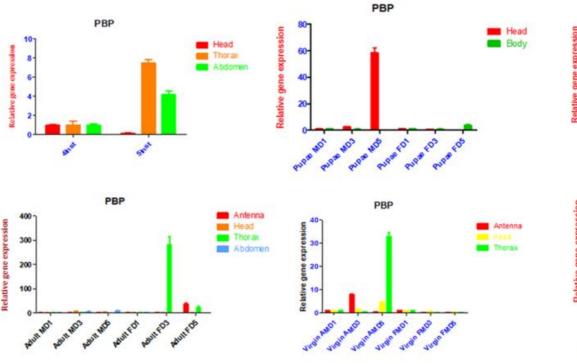


Figure 6. qRT-PCR analysis showing the expression of Spre-OBP11 relative to each stage of development.



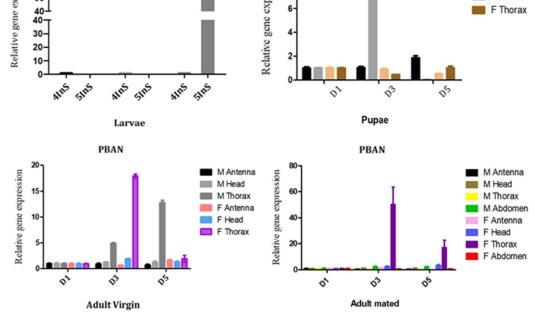


Figure 5. qPT-PCR analysis showing PBAN gene expression was found to be abundant in the 5th instar larva, higher in the thorax and head tissues of 3- and 5day-old pupae, and during day 3 and 5 from female thorax tissue in mated adults.

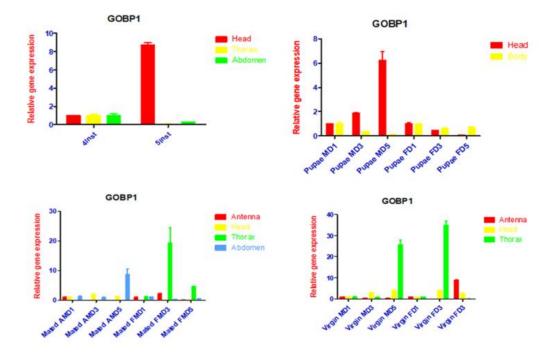
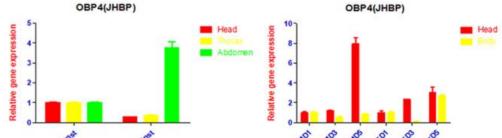


Figure 7. qRT-PCR analysis showing higher expression of Spre-GOBP1 in the head of larva and pupae, differentially expressed in mated adults.



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RESULTS

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Figure 1. *S. recurvalis* pheromone biosynthesis activating neuropeptide (DH-PBAN) gene. Five neuropeptides are highlighted in grey.

DH	a-SGNP	β-SGNP	PBAN	y-SGNP
Spoladea recurvalis VDDLKDEA - DLGA - SDRGA - LWFGPRL	VIFTPKL	SLGTVYQDKKV - YEKVEFTPRL		KINFSPRL
Maruca vitrata LDDSKDEV - DRGA - SDRGG - LWFGPRL	VVFTPKL	SIGGVFQDKK YDNVEFTPRL	- I P D A L P V T P S D - D D - V Y S F K P D S G E V D R R T S Y F N P R L	KVSFSPRL
Omphisa fuscidentalis VDDLKDEA - DRGA - SDRGT - LWFGPRL	VIFTPEL	- SMDGYSDKRT - YENVEFTPRL	- L P E K L S V T P S D S H D A V Y S F K P E M S E L D S R N N Y F S P R L	TVNFSPRL
Manduca_sexta SNDIKDEG - DRGAHSDRGA - LWFGPRL	IVIFTPEL	S L D D S T Q E K R V F Y E N F E F T P R L	- I SEDMPATP SDQEY PMYHPDPEQIDTRTRYFSPRL	THFSPRL
Antherae perma SNDIKDEG - DKGAHSDRGS - LWFGPRL	VIFTPKL	SIGDIYQEKRT - YENVEFTPRL	- L S D D M P A T P K D Q E MY H Q D P E Q V D T R T R Y F S P R L	TITFSPRL
Samia_cynthia_riam TNDVKDEG - DRGAHSDRGS - LWFGPRL	VIFTPKL	RASNAYQEKRT - YENVEFTPRL		TMTFSPRL
Bombyx mori TDMKDES - DRGAHSERGA - LWFGPRL	IIFTPKL	SVA KPQT HESLEFIPRL	- L S E DMP A T P A D Q E I Y Q P D P E E ME S R T R Y F S P R L	TMSFSPRL
Spodoptera_exigua NNNEIKDGGSDRGAHSDRAG - LWFGPRL	VIFTPKL	SLA YDDKV FENVEFTPRL	- L S D D M F A T P A D Q E L Y R P D P D Q I D S R T K Y F S P R L	TMN FSPRL
Helicovera_assulta NNNDVKDGA - ASGAHSDRLG - LWFGPRL	VIFTPKL	SLA YDDK S FENVEFTPRL	- L S D D M P A T P A D Q E MY R Q D P E Q I D S R T K Y F S P R L	TMN FSPRL
Helicopera_sea NNNDVKDGA - ASGAHSDRLG - LWFGPRL	VIFTPEL	SLA YDDK S FENVEFTPRL	- L S D D M P A T P A D Q E MY R Q D P E Q I D S R T K Y F S P R L	TMNFSPRL
Agrotis_ipalon S S N D V K D G G A D R G A H S D R G G - MWF G P R I	VIFTPEL	SLSYEDEMFDNVEFTPRL	- LADDTPATPADQE MYRPDPEQIDSRTKYFSPRL	TMNFSPRL
Orygnia_fiyellina TGNDVKDDGQDRVAHSDRGGQLWFGPRL	VIFTPKL	SLST - YEEKL YDNVEFTPRL	- L SDDMPATPPDQE YYRPDPEQIDSRTKYFSPRL	TMTFSPRL
Clostera_anastomozis TNNDNTMKDGGADRGAHSDRGG - LWFGPRL	: VVFTPKL	SMA YDDK S YENVEFTPRL	- LADDMPATPSDQE YYRQDPEQIDSRSNYFSPRL	TMTLTPRL
Ascotis_selenaria_cretacea NDLKEDG - EREANSDRQG - LWFGPRL	VIFTPKL	· · · · · · · · · · · · · · · · · · ·	QLVDDVPQRQQIEEDRLGSRTRFFSPRL	- T TMN F S P R L
Phitella_pylostella DDLKDEDIQRDA - RDRAS - MWFGPRL	VIFTPKL	NADE - DQQQ S VDFTPRL	- RLKDSGLAPPD EYR - TPELMDARAQYFSPRL	GGSMTFSPRL
Adoxophyes_sp ENFKEENFDRNIRSGRAN - VVFKFIL	VIFTPEL	SMEDPYEEKR SYDVDFTPRL	Q S E A V T S S D E Q V Y R Q D M S P V D G R L K Y F S P R L	TVKLTPRL
Danaus_plexppus S DMK D D N L D R G A H S D R G G - V W F G P R L	VIFTPKL	ALDQ - YSERM AGNIDFTPRL	- L P E R T P T T S S D - E D S I Q - D A I A A N R R P S Y F S P R L	NYNFSPRL
Ostrina_multilaks V D D L K D D V - D R G A - S D R G T - L W F G P R L	VVFTPKL	- SIDVYPEKRT - FENBEFTPRL	- L P E K V P V T P S D S H D E V Y S F K P D M E E I I S R H N Y F S P R L	TLNFSPRL
Cymindis_transversa NEIKDGA - DRGAHSDRVG - LWFGPRL	VIFTPKL	SLG YDDKA FENVEFTPRL	- LADDMPATPADQE MYRADPEQIDSRTKYFSPRL	TMN FSPRL
Spodoptera_littoralis NEIKDGGSDRGAHSDRAG - LWFGPRL	: VIFTPEL	SLA YDDKB FEMVEFTPRL	- LADDMPATPADQE LYRPDPDQIDSRTKYFSPRL	TMN FSPRL
Helicoverpa_armigera NDVKDGA - ASGAHSDRLG - LWFGPRL	VIFTPKL	SLA YDDK S FENBEFTPRL	- L S D D M P A T P A D Q E MY R Q D P E Q I D S R T K Y F S P R L	TMN FSPRL
	VYFTPKL	SMA YDDKS YEMVEFTPRL	- L P EMF A VTQ P D Q E D D K P N P E Q K D L R T K F F S P R L	- SAVSFSPRL
Aedes_aegypti A A MWF G P R L		QPQP VFYHSTTPRL		NLPFSPRL
Anopheles_gambiae A A - MWF G P RL		· · · · · · · PQP · · IFYHTTSPRL		NLPFSPRL
Solenopas genenata TSQDIA SG - MWFGPRL		QPQFTPRL		- LPWIPSPRL
Apis_mellifera T S Q D I T S G - MWF G P R L		QIT QFTPRL		- VPWTPSPRL
Tribolium_castaneum TPHESSVPNERNDDSKETYFWFGPRL		HVVNFTPRL	- ESGEEFVNNAPEDRWLONHETSGEMLYORSPPFAPRL	- H S S P F S P R L

Figure 2. Schematic representation of genomic structure and translated protein structure of *S. recurvalis*.

Figure 8. qRT-PCR analysis showing the expression of Spre-PBP was specific to thoracic region of larvae (virgin male and mated female). However, slightly higher expression was found in the head of male pupae and antenna of virgin males.

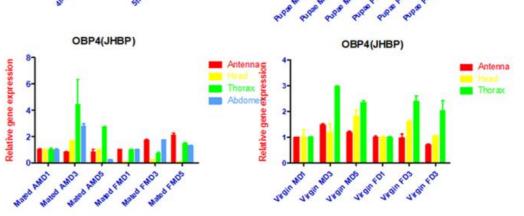


Figure 9. qRT-PCR analysis showing the transcripts level of OBP4 was expressed in higher amounts in head of pupae, differentially expressed in virgin and mated males and female months. Highly expressed in thorax and abdomen of mated adults.

CONCLUSION

- * Spre-DH-PBAN gene from this study revealed that approximately 4.2 kb contains six exons and five introns.
- * Spre-PBAN gene expression across developmental stages implied their physiological function of their role in preparation of pupation and adult development, and attracts males for mating.
- * Our results of selected PBP/GOBP/OBP exhibit some common behavior differences when compared to other lepidopterans to locate the host, recognition of host plant volatile odors, and circadian rhythm prior to oviposition.
- * Understanding molecular diversity of pheromone production and perception related genes establishes a foundation for the possible development of a semiochemical-based pest management strategy against *S. recurvalis*.

ACKNOWLEDGMENT

Figure 3. Spre-DH-PBAN shows high sequence similarities of 80% to its ortholog of Crambidae from Lepidoptera and has lowest similarities of 32% to the DH-PBAN proteins of the family Culicidae from

Diptera. Percentage of conserved amino acids are highlighted in blue.

UK aid, United States Agency for International Development (USAID),



