

草地贪夜蛾 V-ATPase 亚基 A、B、C 和 D 基因的克隆与分析^{*}

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摘要【目的】克隆获得草地贪夜蛾 Spodoptera frugiperda V-ATP 酶(Vacuolar-type proton, ATPase) V₁结构域 A、B、C和D亚基的 cDNA 序列,分析其在草地贪夜蛾不同生长阶段的表达情况,为筛选新型 农药的靶点奠定基础。【方法】利用 PCR 技术获得草地贪夜蛾 V-ATP 酶 V₁结构域 A、B、C和D亚基基 因的 ORF 区域,采用 qRT-PCR 技术检测各基因在草地贪夜蛾不同发育时期的表达水平。【结果】 A、B、C和D亚基基因均在不同鳞翅目昆虫中高度保守。A-D亚基在草地贪夜蛾各生长阶段均有表达,在幼虫 阶段和成虫阶段的表达量均较高,表明 V-ATP 酶可能影响幼虫生长发育和成虫交配繁殖。卵期和蛹期表 达量低,这与卵期和蛹期昆虫各项生命活动低有关。各亚基在各发育时期的表达量存在差异,说明 V-ATP 酶 A、B、C和D发挥不同的功能。【结论】 从草地贪夜蛾中克隆了 V-ATP 酶 V₁结构域 A、B、C和D 亚基 4 个基因在草地贪夜蛾不同发育阶段表达量具有差异,推测其对草地贪夜蛾的生长、发育和繁殖具有 重要的调控作用。

关键词 草地贪夜蛾; V-ATP 酶亚基; 克隆; qRT-PCR

Cloning and expression of *Spodoptera frugiperda* V-ATPase subunit A, B, C and D genes

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Abstract [Objectives] To clone the cDNA sequences of the A, B, C and D subunit genes of the *Spodoptera frugiperda* V-ATPase V_1 region and compare the expression of these genes in different developmental stages in order to both improve the prevention and control of *S. frugiperda* and identify new target genes for pesticides. **[Methods]** The sequences of the genes encoding the V-ATPase subunits A, B, C and D were cloned with PCR (polymerase chain reaction), and the expression of each gene was analyzed in different development stages with real-time quantitative PCR. **[Results]** The A, B, C and D subunit genes of the V_1 region are highly conserved in the Lepidoptera. All four genes were expressed during the entire developmental process but were more highly expressed in the larval and adult stages, suggesting that V-ATPase plays an important role in the growth and development of larvae and in adult reproduction. The expression of the four subunit genes differed in different development functions. **[Conclusion]** The four V-ATPase V_1 subunit A, B, C and D genes had different expression levels in different developmental stages of *S. frugiperda*, which suggests that they play an

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important role in regulating the growth, development and reproduction of this species.

Key words Spodoptera frugiperda; V-ATPase subunits; cloning; real-time quantitative PCR

V-ATP 酶在真核生物中高度保守, 是空泡膜 系统中最重要的多亚基内膜质子泵,在真核细胞 胞腔内的酸化过程中起重要作用(Kane and Stevens, 1992)。V-ATP 酶在昆虫中作为质膜的 能量来源,通过水解 ATP 产生跨膜电压驱动营 养吸收和液体分泌,促进肠道管腔环境碱化 (Harvey et al., 1998)。V-ATP 酶主要存在于内 膜(Endomembranes)和质膜(Plasma membranes) 中(Wieczorek et al., 1991)。在内膜中 V-ATP 酶酸化每个真核细胞中的特定细胞器,如核内 体、溶酶体和囊泡;而在许多动物细胞的质膜中, 它们参与了 pH 稳态和膜激活 (Kibak et al., 1992; Harvey and Wieczorek, 1997)。对于昆虫 来说, V-ATP 酶最初在马氏管中被发现, 随后也 被发现其通过与 K⁺/H⁺反载体和离子通道协同作 用,在昆虫唾液腺、唇腺、中肠和感觉器等上皮 细胞的阳离子转运中发挥重要作用(Klein, 1992; Vitavska et al., 2009; Lina et al., 2014).

目前,已经证明 V-ATP 酶对昆虫的生长、 发育和繁殖至关重要。在黑腹果蝇 Drosophila melanogaster 中, V-ATP 酶 a 亚基基因 vha100 的几种亚型在翅发育过程中发挥不同的功能, vha100-1 调节翅脉的形成, Vha100-2 的功能在 于调节角质层形成, Vha100-4 则参与了 Wg 信号 转导(Moetal., 2020)。不仅如此还发现 V-ATP 酶通过改变关键的信号通路参与调控多种生理 生化途径(Ren et al., 2018)。对果蝇的研究表 明,缺乏 V-ATP 酶功能的细胞会表现出内小体 腔室酸化受损以至于不能降解内吞性物质,以及 V-ATP 酶突变细胞使 Notch 配体无法释放并在溶 酶体中积累(Vaccari et al., 2020)。在雄性灰翅 夜蛾 Spodoptera littoralis 中, 生理时钟通过调节 输精管上皮细胞中 V-ATP 酶的水平和亚细胞分 布来产生腔静脉酸化的节律,这个过程对产生健 康可育的精子来说是不可或缺的 (Bebas et al., 2002)。基于 V-ATP 酶的重要性和保守性,目前 已有大量以V-ATP 酶部分亚基为靶标进行 RNAi的研究。对黑胸大蠊 Periplaneta fuliginosa 中编码 V-ATP 酶 B 亚基的 vha55 基因进行 RNAi,导致幼虫蜕皮缺陷,旧皮脱落不完全,生长受到抑制,胸部及腹部体节角质层弯曲起皱,这表明V-ATP 酶参与昆虫蜕皮过程(Sato et al., 2017)。

草地贪夜蛾 Spodoptera frugiperda 迁移速度 快、危害范围广、寄主种类多和繁殖能力强,自 2019年1月份侵入我国,截止2019年10月初 全国查实虫害发生已经波及了26个省(区、市), 将会对我国玉米的种植造成严重的影响(姜玉英 等, 2019; 崔志斌等, 2020)。目前有关鳞翅目 昆虫 V-ATP 酶亚基功能的研究报道很少, 尤其 草地贪夜蛾作为一种新型入侵性农业害虫,与其 相关的研究近乎空白。因此克隆草地贪夜蛾 V-ATP 酶 V1结构域 A、B、C 和 D 亚基基因对 于研究该昆虫的离子转运机制以及开发防控草 地贪夜蛾的新途径具有理论指导意义。本研究克 隆了草地贪夜蛾 V-ATP 酶 V₁结构域 A、B、C 和 D 亚基基因的开放阅读框(Open reading frame, ORF), 通过 qRT-PCR 技术检测不同亚基 在草地贪夜蛾各发育时期的表达水平,为阐明 V-ATP 酶 A、B、C 和 D 亚基对草地贪夜蛾生理 过程的影响奠定基础,为实现草地贪夜蛾防治, 进一步筛选新型农药的靶点提供依据。

1 材料与方法

1.1 供试昆虫

草地贪夜蛾幼虫饲养于温度(26±1)℃、 光照周期14L:10D、相对湿度60%-85%的人 工气候箱中。幼虫用人工幼虫饲料饲喂至化蛹, 人工幼虫饲料参考杨亚军等(2020)的配置方法。 棉球蘸取10%的蜂蜜用于成虫喂养。

1.2 试剂

T3 Super PCR Mix、TSC-C14 DH5a 感受态

细胞(擎科,北京); DNA 凝胶回收试剂盒
(Axygen 公司,美国); RNAiso reagent、
PrimeScript[™] II 1st Strand cDNA Synthesis Kit 及
TB Green[™] Premix Ex Taq[™] II (Tli RNaseH Plus)
(TaKaRa 公司,日本)。

1.3 总 RNA 提取和 cDNA 合成

收集适量草地贪夜蛾卵、1-6 龄幼虫、雌蛹、 雄蛹、雌成虫和雄成虫保存于液氮,采用 Trirol 法提取总 RNA,各时期样品均设置 3 组生物学 重复。取适量 RNA 进行琼脂糖凝胶电泳,其余 保存在 - 80 ℃。参考 PrimeScript[™] II 1st Strand cDNA Synthesis Kit 试剂盒说明书合成 cDNA 模板。

1.4 RT-PCR 反应

从 NCBI 获取 V-ATP 酶 A-D 亚基基因序列, 利用 Primer 5.0 软件设计各基因的扩增引物 (SF-VATP-F / VATP-R),利用 Primer express 3.0.1 软件设计用于 qRT-PCR 检测的引物,具体 序列见表 1。PCR 采用 25 µL 体系,各组分含量 如下: 1.1×T3 Super PCR Mix, 22 µL; 10 µmol/L 正/反向引物,1 µL; cDNA 模板,1 µL。反应条 件: 98 ℃预变性 3 min;之后为 35 个循环,条件 为 98 ℃变性 10 s,60 ℃退火 10 s,72 ℃延申 15 s;72 ℃延伸 2 min;反应产物于4 ℃保存。 参考说明书对 PCR 产物进行纯化、连接和转化, 长出单菌落的培养皿送公司测序(擎科,北京)。

表	1 引物/	序列
Table 1	Primer	sequences

名称 Name	序列 Sequences	功能 Functions
SF A-F	5'-ATGAGCAAAAGCGGTTTGAAGACAATC-3'	开放阅读框验证 ORF validation
SF A-R	5'-CTAGTCCTCGAGGTTGCGGAAGGC-3'	开放阅读框验证 ORF validation
SF B-F	5'-ATGGCAAAAACCCTTACGTC-3'	开放阅读框验证 ORF validation
SF B-R	5'-CTAGTGGCGGGAGTCGCGC-3'	开放阅读框验证 ORF validation
SF C-F	5'-ATGTCTGAATACTGGGTAATCAG-3'	开放阅读框验证 ORF validation
SF C-R	5'-TTAGGCCTTGTCCATCATGTCC-3'	开放阅读框验证 ORF validation
SF D-F	5'-ATGTCTGGAAAAGATAGGCT-3'	开放阅读框验证 ORF validation
SF D-R	5'-CTAGAACAGCAGGTCCTCGTC-3'	开放阅读框验证 ORF validation
SF QA-F	5'-ACACAGTCCATCTACATC-3'	实时荧光定量 PCR real-time qPCR
SF QA-R	5'-CCGACCTTAACATTCAAG-3'	实时荧光定量 PCR real-time qPCR
SF QB-F	5'-CTGATGACTTGCTCTACCT-3'	实时荧光定量 PCR real-time qPCR
SF QB-R	5'-TGCGGTTCTCGTAGTTAC-3'	实时荧光定量 PCR real-time qPCR
SF QC-F	5'-GGTGAGGTACTAGAAGAC-3'	实时荧光定量 PCR real-time qPCR
SF QC-R	5'-GTTAGATACGATGGTAGGT-3'	实时荧光定量 PCR real-time qPCR
SF QD-F	5'-GGTTCCGTATGATTCTGA-3'	实时荧光定量 PCR real-time qPCR
SF QD-R	5'-TTGAGCCTTAGTGACATT-3'	实时荧光定量 PCR real-time qPCR
GAPDH-F	5'-GAAGTCAAGTCCGTGGAGATG-3'	实时荧光定量 PCR real-time qPCR
GADPH-R	5'-GACCTGTGCTGTGAAGTCG-3'	实时荧光定量 PCR real-time qPCR

1.5 序列分析和系统发育树的构建

从 NCBI 数据库获取 V-ATP 酶 A-D 亚基的 基因信息,使用 Blast 程序进行同源性对比;利 用 DNAMAN 9 软件进行多序列比对;用 MEGA X 软件 Neighbor-Joining 算法(选择 P-distance, Bootstrap = 1 000)分析各亚基在不同昆虫中的 进化关系;利用 ExPASy- ProtParam tool(https:// web.expasy.org/protparam/)分析各亚基蛋白质的 理论分子量及等电点等。

1.6 qRT-PCR 分析

配置 20 µL 反应体系,各组分含量为:TB Green Premix Ex Taq II (Tli RNaseH Plus) 10 µL, 10 µmol·L⁻¹的正向和反向引物各 1 µL, cDNA 模板 1 µL, ROX Reference Dye II 0.4 µL, ddH₂O 6.6 µL。样品混匀离心后使用 QuantStudio 3 荧光定量仪扩增。反应条件: 50 ℃ 2 min, 95 ℃预变性 2 min;两步法 95 ℃ 15 s,60 ℃ 30 s,40 个循环;溶解曲线 95 ℃ 15 s,60 ℃ 1 min, 0.15 ℃/s 升温至 95 ℃持续 1 s。每个样 品设置 3 个重复,内参基因选用 GAPDH 基因 (Nascimento *et al.*, 2015)。以表达量最低的样 品为基准,利用 2^{-△△Ct}法进行相对表达量计算。

1.7 数据分析

利用 SPSS 23.0 软件进行不同发育阶段间各 基因表达量的差异比较(HSD, *P* < 0.05)。

2 结果与分析

2.1 核苷酸序列分析

通过 PCR 技术克隆得到草地贪夜蛾 V-ATP 酶 A、B、C 和 D 亚基的 ORF。A 亚基基因 (GenBank: MT707615)位于草地贪夜蛾基因 组的 10 号染色体上, ORF 全长 1 851 bp, 包含 11个外显子, 编码 616个氨基酸, 理论分子量 和等电点分别为 153.6 ku 和 4.88。A 亚基蛋白属 稳定的亲水性蛋白。A亚基包含3个保守结构域, 分别是位于 N 端的 β 筒结构域 (21-83 个氨基 酸)、N端末端的延伸区(99-220个氨基酸)和 序列中部的核苷酸结合结构域(229-454个氨基 酸)(图1)。B亚基基因(GenBank: MT707616) 位于草地贪夜蛾基因组的 14 号染色体上, ORF 全长为 1 485 bp, 包含 11 个外显子, 编码 494 个氨基酸,理论分子量和等电点分别为124.8 ku 和 4.92。B 亚基蛋白属稳定的亲水性蛋白。B 亚 基包含 2 个保守结构域,即位于 N 端的 β 筒结

构域(33-99个氨基酸)和序列中部的核苷酸结 合结构域(156-383个氨基酸)(图 2)。C亚基 基因(GenBank: MT911889)位于草地贪夜蛾 基因组的27号染色体上,ORF全长1629 bp, 包含15个外显子,编码542个氨基酸,预测分 子量和等电点分别为134.6 ku和4.90。C亚基蛋 白属稳定、亲水性蛋白。该蛋白仅有1个保守结 构域,即V-ATP酶C亚基结构域(4-372个氨基 酸)(图 3)。D亚基基因(GenBank: MT707617) 位于草地贪夜蛾基因组的10号染色体上,ORF 全长741 bp,包含6个外显子,编码246个氨基 酸,预测分子量和等电点分别为60.4 ku和5.12。 D亚基蛋白属稳定的亲水性蛋白。该蛋白包含唯 一的保守结构域,为 V-ATP 酶 D 亚基结构域 (16-207个氨基酸)(图 4)。

2.2 V-ATP 酶的氨基酸序列相似性和分子进化 分析

氨基酸序列同源性分析结果显示,草地贪夜 蛾 V-ATP 酶 A-D 亚基氨基酸序列均与斜纹夜蛾 *Spodoptera litura* 相似度最高,均高达 98%以上。 通过多重序列比对发现不同物种间 A 亚基氨基 酸序列的差异少且分散,B 亚基氨基酸序列的差 异主要集中在前 60 位,C 亚基氨基酸序列的差 异较少且主要存在于C端,D 亚基氨基酸序列的 差异同样主要存在于C端(图 1-图 4)。系统发 育进化树分析显示,草地贪夜蛾 V-ATP 酶 A-D 亚基均与斜纹夜蛾处于同一进化分支,遗传距离 最近(图 5-图 8)。以上结果表明,V-ATP 酶 A-D 亚基在鳞翅目昆虫中均高度保守。

2.3 草地贪夜蛾不同发育阶段 V-ATP 酶 A-D 亚 基表达量分析

V-ATP 酶 A 亚基在草地贪夜蛾整个发育阶段均有表达,在雄蛹中表达量最低;在雌成虫及4龄幼虫中表达量较高,分别为雄蛹的8.40倍和4.95倍,且两者之间均具有显著差异。从卵期到幼虫阶段表达量变化趋势主要呈现为先上升再下降。蛹期的表达量明显低于幼虫期及成虫期。(图9)

	β筒结构域 β-barrel domain	
草地贪夜蛾 Spodoptera frugiperda 斜纹夜蛾 Spodoptera litura 甜菜夜蛾 Spodoptera exigua 二化螟 Chilo suppressalis 烟草天蛾 Manduca sexta 相铃虫 Helicoverpa armigera 野桑蚕 Bombyx mandarina 偏瞳敵眼蝶 Bicyclus anynana 粉纹夜蛾 Trichoplusia ni		36 36 37 37 37 37 37 87
草地贪夜蛾 Spodoptera frugiperda 斜纹夜蛾 Spodoptera litura 甜菜夜蛾 Spodoptera exigua 二化曝 Chilo suppressalis 烟草天蛾 Manduca sexta 棉铃虫 Helicoverpa armigera 野養蚕 Bombyx mandarina 偏瞳蔽眼蝶 Bicyclus anynana 粉纹夜蛾 Trichoplusia ni	SVELGPGILGSIFDGIQRPLKDINELTQSIYIPKGNVPCLGDVSVERNENNVGSHITGGDLYGIVHENTLVKHKNLPPKAKGTVT SVELGPGILGSIFDGIQRPLKDINELTQSIYIPKGNVPCLGDVVWERNPLSVKVGSHITGGDLYGIVHENTLVKHKNLPPKAKGTVT SVELGPGILGSIFDGIQRPLKDINELTQSIYIPKGNVPCLGDVVWERNPLXVKVGSHITGGDLYGIVHENTLVKHKNLPPKAKGTVT SVELGPGILGSIFDGIQRPLKDINELTQSIYIPKGNVPCLABETAVERNPLXVKVGSHITGGDLYGIVHENTLVKHKNLPPKAKGTVT SVELGPGILGSIFDGIQRPLKDINELTQSIYIPKGNVPCLABETAVERNPLXVKVGSHITGGDLYGIVHENTLVKHKNLPPKAKGTVT SVELGPGILGSIFDGIQRPLKDINELTGSIYIPKGNVPSLABEVQVERNPLXVKVGSHITGGDLYGIVHENTLVKHKNLPPKAKGTVT SVELGPGILGSIFDGIQRPLKDINELTGSIYIPKGNVPSLABEVQVERNPLXVKVGSHITGGDLYGIVHENTLVKHKNLPPKAKGTVT SVELGPGILGSIFDGIQRPLKDINELTGSIYIPKGNVPSLABEVQVERNPLXVKVGSHITGGDLYGIVHENTLVKHKNLPPKAKGTVT SVELGPGILGSIFDGIQRPLKDINELTGSIYIPKGNVPSLABEVQVERNPLXVKVGSHITGGDLYGIVHENTLVKHKNLPPKAKGTVT SVELGPGILGSIFDGIQRPLKDINELTGSIYIPKGNVPSLABEVQVERNPLXVKVGSHITGGDLYGIVHENTLVKHKNLPPKAKGTVT SVELGPGILGSIFDGIQRPLKDINELTGSIYIPKGNVPSLABEVQVERNPLXVKVGSHITGGDLYGIVHENTLVKHKNLPPKAKGTVT SVELGPGILGSIFDGIQRPLKDINELTGSIYIPKGNVPSLABEVQVERNPLXVKVGSHITGGDLYGIVHENTLVKHKNLPPKAKGTVT SVELGPGILGSIFDGIQRPLKDINELTGSIYIPKGNVPSLARDVYFNLVKFGSHITGGDLYGIVHENTLVKHKNLPPKAKGTVT SVELGPGILGSIFDGIQRPLKDINELTGSIYIPKGNVPSLARDV	.76 .76 .77 .77 .77 .80 .77 .77 .77
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图 1 草地贪夜蛾与其它昆虫 V-ATP 酶 A 亚基氨基酸的多序列比对

Fig. 1 Multiple sequence alignment of V-ATPase subunit A amino acid among Spodoptera frugiperda and other insects

黑色表示高度保守的氨基酸; 横线标注出保守结构域位置。图 2, 图 3, 图 4 同。用于多重序列比对的氨基酸序列物 种来源及 GeneBank 登录号如下: 草地贪夜蛾: MT707615; 斜纹夜蛾: XP_022826560.1; 甜菜夜蛾: AQQ72785.1; 二化螟: AXF48683.1; 烟草天蛾: XP 030028570.1; 棉铃虫: XP 021181049.1; 野桑蚕: XP 028039670.1;

偏瞳蔽眼蝶: XP 023940509.1;粉纹夜蛾: XP 026731035.1。

Highly conserved amino acids are colored in black; Conservative domain positions are marked by horizontal lines.
The same as Fig. 2, Fig. 3 and Fig. 4. GeneBank accession numbers from different species are listed as followed: Spodoptera frugiperda: MT707615; Spodoptera litura: XP_022826560.1; Spodoptera exigua: AQQ72785.1; Chilo suppressalis: AXF48683.1; Manduca sexta: XP_030028570.1; Helicoverpa armigera: XP_021181049.1; Bombyx mandarina: XP_028039670.1; Bicyclus anynana: XP_023940509.1; Trichoplusia ni: XP_026731035.1.

β 筒结构域 β-barrel domain

草地贪夜蛾 Spodoptera frugiperda 斜纹夜蛾 Spodoptera litura 甜菜夜蛾 Spodoptera exigua 棉铃虫 Helicoverpa armigera 亚洲玉米螟 Ostrinia furnacalis 粉纹夜蛾 Trichoplusia ni 大蜡螟 Galleria mellonella 野桑蚕 Bombyx mandarina 夏威夷红蛱蝶 Vanessa tameamea	MAKTLTSS GATREFYL AVSRDFIS GPRLTYKT VS GVNGPLVIL DEVKFPKFSEI VGLRLADGTLRS GGVLEVS GTKAVVGVFEGTS GIDAKNTLCEF MAKTLSA AGANREFYL AVSRDFIS GPRLTYKT VS GVNGPLVIL DEVKFPKFSEI VGLRLADGTLRS GGVLEVS GTKAVVGVFEGTS GIDAKNTLCEF MAKTLSA AGANREFYL AVSRDFIS GPRLTYKT VS GVNGPLVIL DEVKFPKFSEI VGLRLADGTLRS GGVLEVS GTKAVVGVFEGTS GIDAKNTLCEF MAKTLSA SGANREFYL AVSRDFIS GPRLTYKT VS GVNGPLVIL DEVKFPKFSEI VGLRLADGTLRS GGVLEVS GTKAVVGVFEGTS GIDAKNTLCEF MAKTLSA SGANREFYL AVSRDFIS GPRLTYKT VS GVNGPLVIL DEVKFPKFSEI VGLRLADGTLRS GGVLEVS GTKAVVGVFEGTS GIDAKNTLCEF MAKTLSA SGANREFYL AVSRDFIS GPRLTYKT VS GVNGPLVIL DEVKFPKFSEI VGLRLADGTLRS GGVLEVS GSKAVVGVFEGTS GIDAKNTLCEF MAKTLSA SGANREFYL AVSRDFIS GPRLTYKT VS GVNGPLVIL DEVKFPKFSEI VGLRLADGTLRS GGVLEVS GSKAVVGVFEGTS GIDAKNTLCEF MATTAATLTS GGANREFYL AVSRDFIS GPRLTYKT VS GVNGPLVIL DEVKFPKFSEI VGLRLADGTLRS GGVLEVS GSKAVVQVFEGTS GIDAKNTLCEF MAKTI SASGANREFYL AVSRDFIS GPRLTYKT VS GVNGPLVIL DEVKFPKFSEI VGLRLADGTLRS GGVLEVS GSKAVVGVFEGTS GIDAKNTLCEF MAKTI SASGANREFYL AVSRDFIS GPRLTYKT VS GVNGPLVIL DEVKFPKFSEI VGLRLADGTLRS GGVLEVS GSKAVVGVFEGTS GIDAKNTLCEF MAKTI SASGANREFYL AVSRDFIS GPRLTYKT VS GVNGPLVIL DEVKFPKFSEI VGLRLADGTLRS GGVLEVS GSKAVVGVFEGTS GIDAKNTLCEF MAKVI SHAGAT MEFYL AVSRDFIS GPRLTYKT VS GVNGPLVIL DEVKFPKFSEI VGLRLADGTLRS GGVLEVS GSKAVVGVFEGTS GIDAKNTLCEF MAKSLS AT GANREFAL VVSRDFIS GPRLTYKT VS GVNGPLVIL DEVKFPKFSEI VGLRLADGTLRS GGVLEVS GTKAVVGVFEGTS GIDAKNTLCEF	97 97 97 97 97 100 97 97 97
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图 2 草地贪夜蛾与其它昆虫 V-ATP 酶 B 亚基氨基酸的多序列比对 Fig. 2 Multiple sequence alignment of V-ATPase subunit B amino acid among Spodoptera frugiperda and other insects

用于多重序列比对的氨基酸序列物种来源及 GeneBank 登录号如下: 草地贪夜蛾: MT707616; 斜纹夜蛾: XP_022827405.1; 甜菜夜蛾: AQQ72786.1; 棉铃虫: XP_021187618.1; 亚洲玉米螟: XP_028172197.1; 粉纹夜蛾: XP_026728890.1; 大蜡螟: XP_026754138.1; 野桑蚕: XP_028043476.1; 夏威夷红蛱蝶: XP_026490232.1。 GeneBank accession numbers from different species are listed as followed: *Spodoptera frugiperda*: MT707616; *Spodoptera litura*: XP_022827405.1; *Spodoptera exigua*: AQQ72786.1; *Helicoverpa armigera*: XP_021187618.1; *Ostrinia furnacalis*: XP_028172197.1; *Trichoplusia ni*: XP_026728890.1; *Galleria mellonella*: XP_026754138.1; *Bombyx mandarina*: XP_028043476.1; *Vanessa tameamea*: XP_026490232.1.

草地贪夜蛾 Spodoptera frugiperda 斜纹夜蛾 Spodoptera litura 棉铃虫 Helicoverpa armigera 亚洲玉米螟 Ostrinia furnacalis 夏威夷红蛱蝶 Vanessa tameamea 菜粉蝶 Pieris rapae 偏瞳蔽眼蝶 Bicyclus anynana 小斑草眼蝶 Aphantopus hyperantus	NS EYWIIS APGEKT CQCT WETL NAT KS CNLSE NYKFPI PELKVGT LEQLVGLS DELGKLETF VE GVT RKVAQYLGEVLEDQREKLHENLMANNS E NS EYWIIS APGEKT CQCT WETL NAT KS CNLSE NYKFPI PELKVGT LEQLVGLS DELGKLETF VE GVT RKVAQYLGEVLEDQREKLHENLMANNS D NS EYWIIS APGEKT CQCT WETL NAT KS CNLSE NYKFPI PELKVGT LEQLVGLS DELGKLETF VE GVT RKVAQYLGEVLEDQREKLHENLMANNS D NS EYWIIS APGEKT CQCT WETL NAT KS CNLSE NYKFPI PELKVGT LEQLVGLS DELGKLETF VE GVT RKVAQYLGEVLEDQREKLHENLMANNS D NS EYWIIS APGEKT CQCT WETL NAT KS CNLSEN NYKFPI PELKVGT LEQLVGLS DELGKLETF VE GVT RKVAQYLGEVLEDQREKLHENLMANNS D NS EYWIIS APGEKT CQCT WETL NAT KS CNLSEN NYKFPI PELKVGT LEQLVGLS DELGKLETF VE GVT RKVAQYLGEVLEDQREKLHENLMANNS D NS EYWIIS APGEKT CQCT WETL NAT KS CNLSEN NYKFPI PELKVGT LEQLVGLS DELGKLETF VE GVT RKVAQYLGEVLEDQREKLHENLMANNS D NS EYWIIS APGEKT CQCT WETL NAT KS CNLSEN NYKFPI PELKVGT LEQLVGLS DELGKLETF VE GVT RKVAQYLGEVLEDQREKLHENLMANNS D NS EYWIIS APGEKT CQCT WETL NAT KS CNLSEN NYKFPI PELKVGT LEQLVGLS DELGKLETF VE GVT RKVAQYLGEVLEDQREKLHENLMANNS D NS EYWIIS APGEKT CQCT WETL NAT KS CNLSEN NYKFPI PELKVGT LEQLVGLS DELGKLETF VE SVT RKVAQYLGEVLEDQREKLHENLMANNS D NS EYWIIS APGEKT CQCT WETL NAT KS CNLSEN NYKFPI PELKVGT LEQLVGLS DELGKLETF VE SVT RKVAQYLGEVLEDQREKLHENLMANNS D NS EYWIIS APGEKT CQCT WETL NAT KS CNLSEN NYKFPI PELKVGT LEQLVGLS DELGKLETF VE SVT RKVAQYLGEVLEDQREKLHENLMANNS D NS EYWIIS APGEKT CQCT WETL NAT KS CNLSEN NYKFPI PELKVGT LEQLVGLS DELGKLETF VE SVT RKVAQYLGEVLEDQREKLHENLMANNS D NS EYWIIS APGEKT CQCT WETL NAT KS CNLSEN NYKFPI PELKVGT LEQLVGLS DELGKLETF VE SVT RKVAQYLGEVLEDQREKLHENLMANNS D NS EYWIIS APGEKT CQCT WETL NAT KS CNLSEN NYKFPI PELKVGT LEQLVGL SE SVER KAQYLGEVLEDQREKLHENLMANNS D NS EYWIIS APGEKT CQCT WETL NAT KS CNLSEN NYKFPI PELKVGT LEQLVGL SE SVER KAQYLGEVLEDQREKLHENLMANNS D NS EYWIIS APGEKT CQCT WETL NAT KS CNLSEN NYKFPI PELKVGT LEQLVGL SE SVER KAQYLGEVLEDQREKLHENLMANNS D NS EYWIIS APGEKT CQCT WETL NAT KS CNLSEN NYKFPI PELKVGT LEDQLVGL SE SVER KS KAQYLGEVLEDQREKLHENLMANNS D NS EYWIIS APGEKT CQCT WETL NAT KS CNLSEN KFFI PELKVGT NG KS KS KS KS KS KS KS	100 100 100 100 100 100 100
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图 3 草地贪夜蛾与其它昆虫 V-ATP 酶 C 亚基氨基酸的多序列比对

Fig. 3 Multiple sequence alignment of V-ATPase subunit C amino acid among Spodoptera frugiperda and other insects

用于多重序列比对的氨基酸序列物种来源及 GeneBank 登录号如下: 草地贪夜蛾: MT911889; 斜纹夜蛾: XP_022837359.1; 棉铃虫: XP_021198264.1; 亚洲玉米螟: XP_028164657.1; 夏威夷红蛱蝶: XP_026491957.1; 菜 粉蝶: XP_022115843.1; 偏瞳蔽眼蝶: XP_023940210.1; 小斑草眼蝶: XP_034826649.1; 玉带美凤蝶: NP_001298659.1。 GeneBank accession numbers from different species are listed as followed: *Spodoptera frugiperda*: MT911889; *Spodoptera litura*: XP_022837359.1; *Helicoverpa armigera*: XP_021198264.1; *Ostrinia furnacalis*: XP_028164657.1; *Vanessa tameamea*: XP_026491957.1; *Pieris rapae*: XP_022115843.1; *Bicyclus anynana*: XP_023940210.1; *Aphantopus hyperantus*: XP_034826649.1; *Papilio polytes*: NP_001298659.1.

V-ATP 酶 B 亚基在草地贪夜蛾整个发育阶段均有表达,其中在雌蛹中表达量最低,在2龄幼虫中表达量最高,为雌蛹的12.88倍,雄性成虫次之,为雌蛹的12.38倍。B 亚基在幼虫阶段1 至4龄时表达量相对较高,5龄与6龄时开始下降,直至蛹期的表达量达到最低。(图 10)

V-ATP 酶 C 亚基在草地贪夜蛾整个发育阶 段均有表达,其中在雌蛹中表达量最低,在雄成虫 中表达量最高,为雌蛹的 8.84 倍。1-6 龄之间表 达量均无显著差异。V-ATP 酶 C 亚基在草地贪夜 蛾蛹期的表达量均低于幼虫期及成虫期(图 11)。 V-ATP 酶 D 亚基在草地贪夜蛾整个发育阶段均有表达,其中在雌蛹中表达量最低,卵及雄蛹中也较低,分别为雌蛹的 1.64 倍和 1.68 倍,三者之间均无显著差异。在雄成虫中表达量最高,为雌蛹的 31.80 倍,6 龄幼虫次之,为雌蛹的 15.47 倍,且差异均十分显著。V-ATP 酶 D 亚基在蛹期的表达量明显低于幼虫期及成虫期(图 12)。

3 讨论

V-ATP 酶在昆虫体内参与能量运转,是维持 昆虫生命体征的关键蛋白,对昆虫生长、发育和

草地贪夜蛾 Spodoptera frugiperd 斜纹夜蛾 Spodoptera litura 棉铃虫 Helicoverpa armigera 粉纹夜蛾 Trichoplusia ni 猫蚤 Ctenocephalides felis 红头松叶蜂 Neodiprion lecontei 茴香凤蝶 Papilio machaon 黄翅菜叶蜂 Athalia rosae 柑橘凤蝶 Papilio xuthus	MS GKD RLI AF FPS R GAQNLI KA RLAGAQKGHGLLKKKADAL QVRF RMILSKII ET KTLNGEVNKE AAFSLAE AKF IT GD F NQVVL (NVT KA MS GKD RLAI F PS R GAQNLI KA RLAGAQKGHGLLKKKADAL QVRF RMILSKII ET KTLNGEVNKE AAFSLAE AKF IT GD F NQVVL (NVT KA MS GKD RLAI F PS R GAQNLI KC RLAGAQKGHGLLKKKADAL QVRF RMILSKII ET KTLNGEVNKE AAFSLAE AKF IT GD F NQVVL (NVT KA MS GKD RLAI F PS R GAQNLI KC RLAGAQKGHGLLKKKADAL QVRF RMILSKII ET KTLNGEVNKE AAFSLAE AKF IT GD F NQVVL (NVT KA MS GKD RLPI F PS R GAQNLI KC RLAGAQKGHGLLKKKADAL QVRF RMILSKII ET KTLNGEVNKE AAFSLAE AKF IT GD F NQVVL (NVT KA MS GKD RLPI F PS R GAQNLI KC RLAGAQKGHGLLKKKADAL QNF RMILSKII ET KTLNGEVNKE AAFSLAE AKF IT GD F NQVVL (NVT KA MS GKD RLPI F PS R GAQNLMKS RLAGAQKGHGLLKKKADAL QNF RMILSKII ET KTLNGEVNKE AAFSLAE AKF IT GD F NQVVL (NVT KA MS GKD RLPI F PS R GAQNLMKS RLAGAQKGHGLLKKKADAL QNF RMILSKII ET KTLNGEVNKE AAFSLAE AKF IT GD F NQVVL (NVT KA MS GKD RLI F PS R GAQNLMKS RLAGAQKGHGLLKKKADAL QNF RMILSKII ET KTLNGEVNKE AAFSLAE AKF IT GD F NQVVL (NVT KA MS GKD RLI F PS R GAQNLMKS RLAGAQKGHGLLKKKADAL QNF R MILSKII ET KTLNGEVNKE AAFSLAE AKF IT GD F NQVVL (NVT KA MS GKD RLI F PS R GAQNLMKS RLAGAQKGHGLLKKKADAL QNF R MILSKII ET KTLNGEVNKE AAFSLAE AFSLAE AFF MS GKD RLI F F S R GAQNLMKS RLAGAQKGHGLLKKKADAL QNF R MILSKII ET KTLNGEVNKE AAFSLAE AFF MS GKD RLI F F S R GAQNLMS RLAGAQKGHGLLKKKADAL QNF R MILSKII ET KTLNGEVNKE AAFSLAE AFF MS GKD RLI F F S R GAQNLMS RLAGAQKGHGLLKKKADAL QNF R MILSKII ET KTLNGEVNKE AAFSLAE AFF MS GKD RLI F F S R GAQNLMS RLAGAQKGHGLLKKKADAL QNF R MILSKII ET KTLNGEVNKE AAFSLAE AFF MS GKD RLI F F S R GAQNLMS RLAGAQKGHGLLKKKADAL GVR F R MILSKII ET KTLNGEVNKE AAFSLAE AFF MS GKD RLI F F F F G F G A F F MILSKII F F K F F F F F F F F F F F F F F F
草地贪夜蛾 Spodoptera frugiperd 斜纹夜蛾 Spodoptera litura 棉铃虫 Helicoverpa armigera 粉纹夜蛾 Trichoplusia ni 猫蚤 Ctenocephalides felis 红头松叶蜂 Neodiprion lecontei 茴香凤蝶 Papilio machaon 黄翅菜叶蜂 Athalia rosae 柑橘凤蝶 Papilio xuthus	QI KI RSKKINVAGYTLET FES YTDGTDT YELAGLARGGQQLTKLKKNFGSAVKLLVELASLQTSFVTLDEVI KI TNRRVNAI EHVI I PRL 180 QI KI RSKKINVAGYTLET FES YTDGTDT YELAGLARGGQQLTKLKKNFGSAVKLLVELASLQTSFVTLDEVI KI TNRRVNAI EHVI I PRL 180 QI KI RSKKINVAGYTLET FES YTDGTDT YELAGLARGGQQLTKLKKNFGSAVKLLVELASLQTSFVTLDEVI KI TNRRVNAI EHVI I PRL 180 QI KI RSKKINVAGYTLET FES YTDGTDT YELAGLARGGQQLTKLKKNFGSAVKLLVELASLQTSFVTLDEVI KI TNRRVNAI EHVI I PRL 180 QI KI RSKKINVAGYTLET FES YTDGTDT YELAGLARGGQQLTKLKKNFGSAVKLLVELASLQTSFVTLDEVI KI TNRRVNAI EHVI I PRL 180 QI KI RSKKINVAGYTLET FES YTDGTDT YELAGLARGGQQLAKLKKNFGSAVKLLVELASLQTSFVTLDEVI KI TNRRVNAI EHVI I PRL 180 QI KI RSKKINVAGYTLET FES YDGTDT YELAGLARGGQQLSKIKKNFGSAVKLLVELASLQTSFVTLDEVI KI TNRRVNAI EHVI I PRL 180 QI KI RSKKINVAGYTLET FES YDGTDT YELAGLARGGQQLSKIKKNFGSAVKLLVELASLQTSFVTLDEVI KI TNRRVNAI EHVI I PRL 180 QI KI RSKKINVAGYTLET FES YDGTDT YELAGLARGGQQLSKIKKNFGSAVKLLVELASLQTSFVTLDEVI KI TNRRVNAI EHVI I PRL 180 QI KI RSKKINVAGYTLET FES YDGTDT YELAGLARGGQQLSKIKKNFGSAVKLLVELASLQTSFVTLDEVI KI TNRRVAI EHVI I PRL 180 QI KI RSKKINVAGYTLET FES YDGTDT YELAGLARGGQQLSKIKKNFGSAVKLLVELASLQTSFVTLDEVI KI TNRRVAI EHVI I PRL 180 QI KI RSKKINVAGYTLET FES YDGTDT YELAGLARGGQQLSKIKKNFGSAVKLLVELASLQTSFVTLDEVI KI TNRRVAI EHVI I PRL 180 QI KI RSKKINVAGYTLET FES YDGTDT YELAGLARGGQQLSKLKKNFGSAVKLLVELASLQTSFVTLDEVI KI TNRRVAI EHVI I PRL 180 QI KI RSKKINVAGYTLET FES YDGTDT YELAGLARGGQQLSKLKNFGSAVKLLVELASLQTSFVTLDEVI KI TNRRVAI EHVI I PRL 180 QI KI RSKKINVAGYTLET FES YDGTDT YELAGLARGGQQLSKLKNFGSAVKLLVELASLQTSFVTLDEVI KI TNRRVAI EHVI I PRL 180 QI KI RSKKINVAGYTLET FES YDGTDT YELAGLARGQQLSKLKNFGSAVKLLVELASLQTSFVTLDEVI KI TNRRVAI EHVI I PRL 180 QI KI RSKKINVAGYTLET FES YDGTDT YELAGLARGQQLSKLLVEN KI KNFGSAVKLLVELASLQTSFVTLDEVI KI TNRRVAI EHVI I PRL 180 QI KI RSKKINVAGYTLET FES YDGTDT YELAGLARGQQLSKLLVEN KI KNFGSAVKLLVELASLGTSFVTLDEVI KI TNRRVAI EHVI I PRL 180 QI KI RSKKINVAGYTLET FES YDGTDT YELAGLARGQQLSKL
草地贪夜蛾 Spodoptera frugiperd 斜纹夜蛾 Spodoptera litura 相铃虫 Helicoverpa armigera 粉纹夜蛾 Trichoplusia ni 猫蚤 Ctenocephalides felis 红头松叶蜂 Neodiprion lecontei 茴香凤蝶 Papilio machaon 黄翅菜叶蜂 Athalia rosae 柑橘凤蝶 Papilio xuthus	ERTLAYI I SELDELEREEFYRLKKI QDKKI I KEMABARKQAALLAG. QDLADAANLDEGDEDL 245 ERTLAYI I SELDELEREEFYRLKKI QDKKI I KDMABARKQAALLAG. QDLANAANLDEGDEDL 245 ERTLAYI I SELDELEREEFYRLKKI QDKKI I KDMABAHKQANLAAG. KDLADSANLDEGDEDL 246 ERTLAYI I SELDELEREEFYRLKKI QDKKI I KDMABAHKQANLAG. TDVRDYANLDEGDEDL 246 ERTLAYI I SELDELEREEFYRLKKI QDKKI AKATBAARLAGAHAANLAG. HDDENDANLDEGDEDL 245 ERTLAYI I SELDELEREEFYRLKKI QDKKI AKATBAARAALLAG. RDVGDI ANLDEGDEDL 244 ERTLAYI I SELDELEREEFYRLKKI QDKKI I KDMABAKQANLGG. RDVGDI ANLDEGDEDL 245 ERTLAYI I SELDELEREEFYRLKKI QDKKI I KDMABAAKQANLGG. RDVGDI ANLDEGDEDL 245 ERTLAYI I SELDELEREEFYRLKKI QDKKI I KDMABAAKQANLGG. RDVGDI ANLDEGDEDL 244
图 4	草地贪夜蛾与其它昆虫 V-ATP 酶 D 亚基氨基酸的多序列比对

Fig. 4 Multiple sequence alignment of V-ATPase subunit D amino acid among Spodoptera frugiperda and other insects

用于多重序列比对的氨基酸序列物种来源及 GeneBank 登录号如下: 草地贪夜蛾: MT707617; 斜纹夜蛾: XP_022826791.1; 棉铃虫: XP_021201538.1; 粉纹夜蛾: XP_026731198.1; 猫蚤: XP_026470160.1; 红头松叶蜂: XP_015509552.1; 茴香凤蝶: XP_014362338.1; 黄翅菜叶蜂: XP_012254421.1; 柑橘凤蝶: XP_013172759.1。 GeneBank accession numbers from different species are listed as followed: *Spodoptera frugiperda*: MT707617; *Spodoptera litura*: XP_022826791.1; *Helicoverpa armigera*: XP_021201538.1; *Trichoplusia ni*: XP_026731198.1; *Ctenocephalides felis*: XP_026470160.1; *Neodiprion lecontei*: XP_015509552.1; *Papilio machaon*: XP_014362338.1; *Athalia rosae*: XP_012254421.1; *Papilio xuthus*: XP_013172759.1.









Fig. 7 Phylogenetic tree of *Spodoptera frugiperda* V-ATPase subunit C and those from other species based on amino acid sequence



数据为平均数±标准差,柱上标有不同小写字母表示经 HSD 法检验在 P<0.05 水平差异显著。

图 10,图 11 及图 12 同。

Data are mean \pm SD, and histograms with different lowercase letters indicate significant differences at the 0.05 level by HSD test. The same as Fig. 10, Fig. 11 and Fig. 12.

繁殖至关重要(胡黎明等,2011)。V-ATP 酶 V_1 区域是一个负责 ATP 水解的 500-600 ku 的可溶 性复合物,包含 8 个不同的亚基(A、B、C、D、 E、F、G 和 H);结合在膜上的 V_0 区域是一个 250 ku 的复合物,在质子转位中起作用,包含至 少 5 个不同的亚基(a、c、c'、c"和 d 亚基 (Forgac, 2007; Cotter *et al.*, 2015)。对这些亚基进行的 生化和遗传分析已经证明了这些亚基参与核苷 酸的结合和水解、质子易位以及这些活动的耦合 (Nelson and Klionsky, 1997)。本研究从草地贪



夜蛾中成功克隆获得了 V-ATP 酶的 A、B、C 和 D 亚基基因, 多序列比对表明草地贪夜蛾与其他 物种的 V-ATP 酶 A-D 亚基具有极高的相似性, 进化分析显示草地贪夜蛾 A-D 亚基均在鳞翅目

昆虫中高度保守。

V-ATP 酶 A-D 4 个亚基在草地贪夜蛾整个生 长发育期均有表达,且在幼虫期和成虫期的表达 量明显高于蛹期。原因在于 V-ATP 酶作为维持 昆虫中肠 pH 稳态的动力源,较高的表达可以帮助幼虫大量进食,从而维持发育(Azuma et al., 1995)。有研究称,V-ATP 酶在雌性果蝇卵泡生长和卵黄发育过程中发挥作用,同时在雄性灰翅 夜蛾输精管上皮细胞中参与调节管腔酸碱环境,结合 V-ATP 酶 A-D 4 个亚基在草地贪夜蛾雌雄 成虫中均有较高表达量,可以推测 V-ATP 酶对于昆虫生殖活动有着无可取代的作用(Bebas et al., 2002; Bohrmann and Braun, 2012)。

V-ATP 酶催化核苷酸结合位点位于 A 亚基 上, A 亚基的特征是包含一个核苷酸结合的 P-loop 序列(GXXXXGKT),该序列还包含一个 紧靠在 GKT 基序之前的半胱氨酸残基,另外还 有两个保守的半胱氨酸残基与 P-loop 序列的半 胱氨酸之间形成可逆的二硫键被认为可能在体 内控制 V-ATP 酶活性方面发挥作用(Arai et al., 1987; Feng and Forgac, 1992)。A 亚基在雌性 成虫及2龄幼虫中表达量最高,可能参与幼虫唾 腺分泌及中肠消化,并在雌性成虫交配和产卵等 生理过程中起主要作用。V-ATP 酶附加的核苷酸 结合位点(非催化)位于 B 亚基上, B 亚基也含 有 ATP 结合位点,但缺少一个富含甘氨酸的 loop 序列 (Forgac, 1997)。本研究发现 B 亚基在 1 龄及雄蛾体内的表达量相对较高,6龄阶段表达 量下降可能与预蛹期取食量和活动量减少相关。 C亚基对 V-ATP 酶稳定性和活性十分重要,可以 有效地控制 V-ATP 酶复合物的可逆拆卸,但不 参与其它 V₁亚基之间的组装 (Puopolo et al., 1992; Inoue et al., 2005)。C亚基在4龄幼虫体 内的表达量明显高于其它时期。一般来说,草地 贪夜蛾幼虫4龄后食量大增,进入暴食阶段,而 V-ATP 酶在昆虫中肠中起着独特的作用,因此C 亚基可能对 V-ATP 酶在这一阶段的运转有重要 影响。D亚基位于蛋白脂环的顶部,起到连接环 和中心柄的作用(Cotter et al., 2015)。D 亚基 在幼虫期6龄幼虫中表达量明显升高,这可能与 幼虫化蛹过程相关。由此可见, V_1 结构域各个 亚基在昆虫的生长发育过程中均行使着不可取 代的功能。

综上所述, V-ATP 酶参与了各种昆虫的发育 和虫体的形成, 是研究昆虫进化和发育的良好候 选靶标。目前, 已经有大量研究阐明了 V-ATP 酶的多种调控作用, 这些调控作用的具体机制则 有待进一步研究。

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