



小菜蛾对苏云金芽胞杆菌 (Bt) 的 抗性研究进展*

尤士骏^{1,2**} 刘昭霞^{1,2} 熊磊^{1,2} 廖金英^{1,2} 何玮毅^{1,2} 尤民生^{1,2***}

(1. 福建农林大学闽台作物有害生物生态防控国家重点实验室, 福州 350002; 2. 福建农林大学应用生态研究所, 福州 350002)

摘 要 小菜蛾 *Plutella xylostella* (L.) 是为害十字花科蔬菜的一种重要的世界性害虫, 由于其分布范围广、繁殖速度快、抗性水平高, 已经成为最难防治的害虫之一。基于苏云金芽胞杆菌 *Bacillus thuringiensis* (Bt) 开发的杀虫剂在小菜蛾生物防治中发挥重要的作用, 但小菜蛾作为在田间最早对 Bt 产生抗性的害虫, 其抗性发展及抗性机理也引起了全世界的广泛关注。本文概述了小菜蛾的发生危害及其抗药性的研究动态、苏云金芽胞杆菌的起源、发展及应用, 分析了小菜蛾对 Bt 杀虫毒素产生抗药性及其抗性机理, 并从抗性发展风险、转基因油菜种植和进一步深入开展抗性机理的研究三个方面进行了展望, 以期优化小菜蛾抗药性的治理策略、提高生物农药和 Bt 作物的控害效能提供借鉴和参考。

关键词 小菜蛾, Bt 毒素, 抗药性, 抗性机理, 抗药性治理

Advances in research on the resistance of *Plutella xylostella* to *Bacillus thuringiensis* (Bt)

YOU Shi-Jun^{1,2**} LIU Zhao-Xia^{1,2} XIONG Lei^{1,2} LIAO Jin-Ying^{1,2}
HE Wei-Yi^{1,2} YOU Min-Sheng^{1,2***}

(1. State Key Laboratory of Ecological Pest Control for Fujian and Taiwan Crops, Fujian Agriculture and Forestry University, Fuzhou 350002, China; 2. Institute of Applied Ecology, Fujian Agriculture and Forestry University, Fuzhou 350002, China)

Abstract Diamondback moth (DBM), *Plutella xylostella*, is a worldwide devastating pest of Brassicaceae, and is well known as one of the most difficult pests to control, because of its wide distribution, rapid reproduction, and high resistance to agrochemicals. *Bacillus thuringiensis* (Bt)-based insecticides plays a key role of great importance in bio-control of DBM. DBM is the first pest species that evolves resistance to Bt Cry toxins in outdoor fields, and its resistance development and associated underlying mechanisms have received global attention. This paper first summarizes the global infestation of DBM and previous studies on its insecticide resistance, and elaborates the Bt story of discovery, development and application, and then further analyzes the DBM resistance to Bt toxins and resistance mechanisms. In addition, prospects on risks of resistance development, cultivation of transgenic oilseed crops, as well as further investigation on resistance mechanisms are discussed in some detail, with the hope to provide strategies for development of optimal resistance management and sustainable utility of Bt-based insecticides and Bt-transgenic crops.

Key words *Sogatella furcifera*, migration dynamics, mesoscale source areas, trajectory analysis

小菜蛾 *Plutella xylostella* (L.) 属鳞翅目菜蛾科 (Lepidoptera: Plutellidae), 是为害十字花科蔬菜的一种重要的世界性害虫, 在所有栽培十字花科蔬菜的国家和地区都有发生为害的纪录,

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**第一作者 First author, Email: sjyou9@fafu.edu.cn

***通讯作者 Corresponding author, Email: msyou@fafu.edu.cn

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被认为是分布最广泛的鳞翅目昆虫 (Talekar and Shelton, 1993; Furlong *et al.*, 2013)。由于其发生世代多, 繁殖能力强, 抗药性水平高, 给防治工作带来极大的困难, 在东南亚部分地区可造成 90% 以上的蔬菜产量损失, 全世界每年因小菜蛾造成的损失和防治费用达 40-50 亿美元 (Zalucki *et al.*, 2012; Furlong *et al.*, 2013)。

小菜蛾在我国各省区均有分布, 尤其在长江流域和南方沿海地区发生严重, 估计每年造成的损失和防治费用达到 7.7 亿美元 (Li *et al.*, 2016a)。由于受到气候条件和十字花科蔬菜种植结构的影响, 小菜蛾在我国的发生呈现明显的时空格局 (Li *et al.*, 2016b), 从南到北大致可以分为三个区: 周年繁殖区 ($<30^{\circ}\text{N}$)、越冬区 ($30^{\circ}-35^{\circ}\text{N}$) 和夏季繁殖区 ($>35^{\circ}\text{N}$) (仵均祥, 2002)。在我国南方和中部地区, 小菜蛾种群数量在炎热的夏季有所下降, 而南方的种群可迁入到北方地区并造成严重的危害 (马春森和陈瑞鹿, 1995; Li *et al.*, 2016b)。总体而言, 小菜蛾在东部和南方省份的发生面积及危害程度高于西部和北方省份 (Li *et al.*, 2016a)。

长期以来, 依赖杀虫剂的化学防治是防治小菜蛾的主要措施。杀虫剂的广泛、过量和不合理使用, 使小菜蛾长期处于较高的药剂选择压下, 从而对各种杀虫剂产生了不同程度的抗药性 (尤民生和魏辉, 2007)。Ankersmit (1953) 首次报道了印度尼西亚爪哇岛的小菜蛾对 DDT 产生抗性。此后, 菲律宾、日本、韩国、巴基斯坦、马来西亚、泰国、新加坡、印度、美国、澳大利亚、新西兰、巴西、哥斯达黎加、尼加拉瓜、南非等国家和地区也先后报道了小菜蛾的抗药性问题 (Hung and Sun, 1989; Furlong *et al.*, 2013)。在我国, Sun 等 (1978) 首次报道了台湾地区小菜蛾对二嗪磷和灭多威产生抗性。吴世昌和顾言真 (1986) 报道了我国大陆小菜蛾的抗药性问题。此后, 我国上海、福建、贵州、湖北、江苏、浙江、广东、北京、黑龙江、云南、湖南、江西、山西、四川等省 (市) 均有小菜蛾产生抗药性的报道。总体而言, 我国小菜蛾的抗药性水平南方比北方高, 小菜蛾的抗药性发展速度南方比北方

快。目前, 小菜蛾已经对有机磷、有机氯、氨基甲酸酯、拟除虫菊酯、沙蚕毒素、以及昆虫生长调节剂、阿维菌素、多杀菌素、苏云金杆菌 (Bt) 等农药产生了不同程度的抗药性, 几乎涉及到应用于小菜蛾防治的所有药剂, 给化学防治和生物防治带来了很大的困难 (尤民生和魏辉, 2007; Furlong *et al.*, 2013; Li *et al.*, 2016a)。

1 苏云金芽胞杆菌 (Bt) 及其应用

苏云金芽胞杆菌是一种普遍存在的革兰氏阳性杆状产芽胞细菌, 可以形成具有杀虫活性的伴孢晶体。有关 Bt 的起源, 最早的可以追溯到日本明治维新时期, 细菌感染对当时养蚕业的发展造成严重的威胁, 细菌学家 Ishiwata (1901) 从感染的蚕中分离出了 Bt 菌株。随后, 德国科学家 Berliner (1911) 在德国图林根州 (Thuringia) 从患有 *Schlaffsucht* 疾病的地中海粉蛾 *Anagasta ruficornis* 幼虫中发现并分离到这种产伴孢晶体的芽胞杆菌, 并正式定名为苏云金芽胞杆菌 *Bacillus thuringiensis*, 简称 Bt。从发现该菌至今已有整整 118 年的历史, 在 Web of Science 上可以发现超过 6 000 篇的研究报道 (图 1), 涉及生物学、分类命名、有效成分、杀虫机理、分子生物学、遗传学、产品化、安全性以及近年来的转基因植物等诸多方面。在 20 世纪 70 年代以前, 全世界有关 Bt 的研究报道并不多, 70 年代以后的研究工作迅猛发展, 特别是进入 21 世纪后,

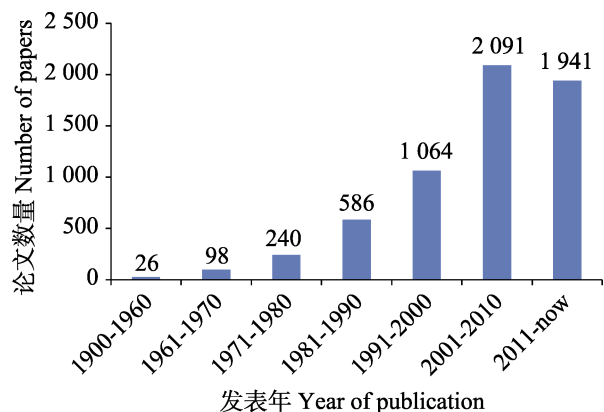


图 1 苏云金芽胞杆菌 (Bt) 研究论文的发表动态
Fig. 1 Historical trend in the research publications of *Bacillus thuringiensis* (Bt)

与 Bt 相关的生物农药的研发得到更加广泛的重视和开展。

1950 年, 美国科学家通过分离纯化蛋白得到孢子中的杀虫晶体蛋白 (Insecticidal crystal protein, 简称 IPC, 是 Bt 的活性部分), 可以用于防治鳞翅目、双翅目、鞘翅目等害虫 (Aronson *et al.*, 1986)。Bt 晶体蛋白分为 Cry 和 Cyt 两大类 (Hofte and Whiteley, 1989), 目前研究主要集中在 Cry 晶体。Cry 晶体包括三个结构域, 即 Domain I、Domain II 和 Domain III。其中 Domain I 与昆虫取食蛋白后中肠上皮细胞膜上的孔洞形成有关; Domain II 与昆虫中肠的特异性受体识别和结合有关, Domain III 可能与维持蛋白分子完整性及和受体特异性结合相关 (Galitsky *et al.*, 2001; Morse *et al.*, 2001; Boonserm *et al.*, 2005, 2006; Bravo *et al.*, 2004, 2005; Xu *et al.*, 2015; Huang *et al.*, 2016)。目前为止, Bt Cry 毒素的作用模型可归为四种 (图 2)。首先, Bt

晶体蛋白被昆虫取食后, 在昆虫中肠碱性环境中溶解, 释放出原毒素 (Protoxin), 而后被蛋白酶水解激活, 形成具有杀虫活性的毒素蛋白 (activated toxin)。经典的连续结合模型 (图 2: a) 认为 Cry1A 活化毒素首先以较低的结合力结合高丰度的 GPI 锚定蛋白 APN 或 ALP, 使毒素靠近微绒毛 (Pacheco *et al.*, 2009), 然后以较高的结合力结合钙粘蛋白, 毒素的 N 端、domain I 的 α -1 螺旋和部分 α -2a 螺旋被蛋白酶切除, 从而发生寡聚化形成预孔寡聚体结构 (Gómez *et al.*, 2002)。随后, 预孔寡聚体以更高的结合力结合 APN 和 ALP, 增加其在膜表面的浓度, 最后与处于通道打开状态的 ABCC2 蛋白发生互作, 插入肠膜, 引起膜穿孔, 导致昆虫死亡 (Bravo *et al.*, 2004; Gómez *et al.*, 2006; Gahan *et al.*, 2010)。近几年来, 大量研究也进一步证实了 ABCC2 在 Cry1A 毒素发挥毒力中所起的受体作用以及促进孔道形成的能力 (Tanaka *et al.*, 2013,

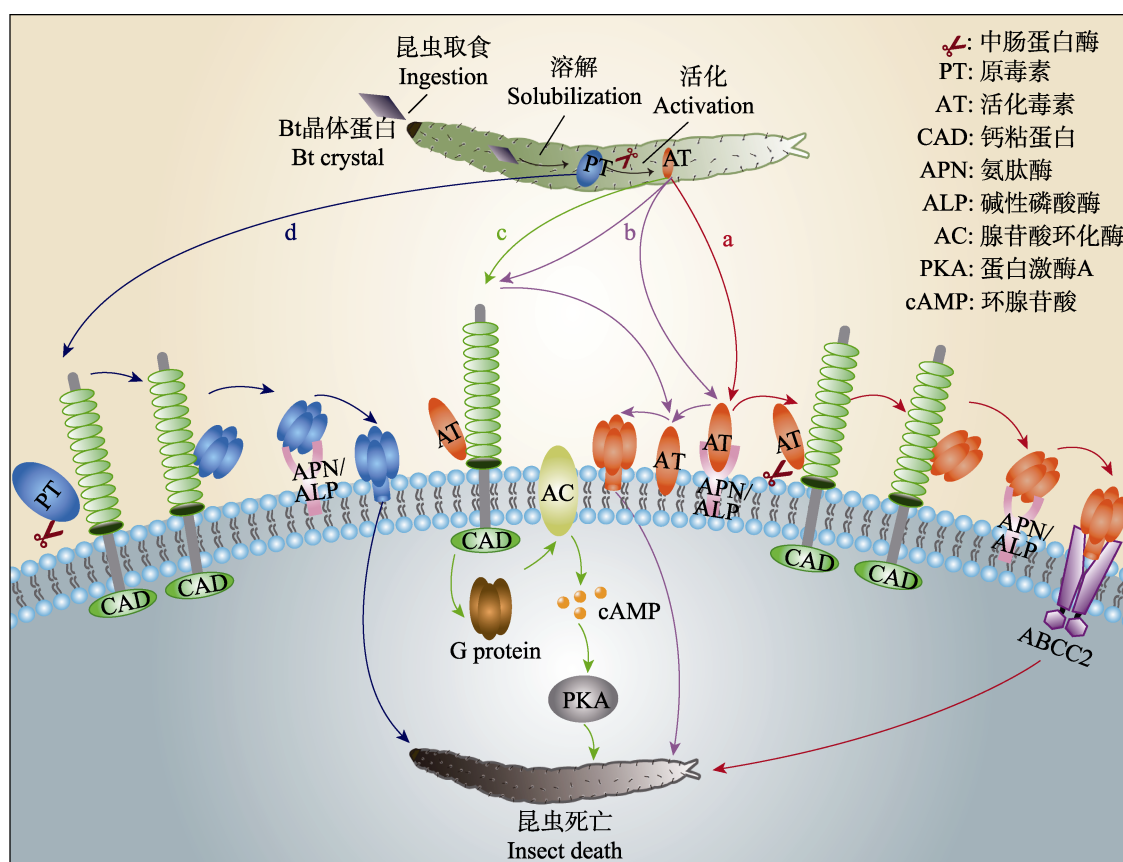


图 2 Bt Cry 毒素杀虫机理图示

Fig. 2 Illustration showing the mechanism of Bt Cry toxins against insect pests

2016 ;Bretschneider *et al.* ,2016 ;Ren *et al.* ,2016 ; Zhou *et al.* ,2016 ;Adegawa *et al.* ,2017 ;Stevens *et al.* ,2017 ;Tanaka *et al.* ,2017 ;Martinez-Solis *et al.* ,2018)。另外, Vachon 等 (2012) 认为 Cry 活化毒素结合 APN、ALP 或钙粘蛋白后,可直接插入肠膜中,在膜上发生寡聚化,形成预孔寡聚体和孔道,从而导致昆虫死亡 (图 2 : b)。除了连续结合模型, Zhang 等 (2006) 也提出了信号转导模型,认为 Cry 活化毒素与钙粘蛋白的结合激活 G 蛋白和腺苷酸环化酶,促进增加细胞内 cAMP 水平,激活蛋白激酶 A 相关的细胞内死亡信号途径 (图 2 : c)。然而,与经典作用模型不同的是,相关研究发现, Cry1A 原毒素可以直接结合钙粘蛋白,在肠道蛋白酶作用下,发生寡聚化,不同于活化毒素的是,由原毒素形成的预孔寡聚体更耐热,耐 SDS 更易插入人工囊泡中,形成孔道的能力更强 (Gómez *et al.* ,2014)。因此,学者称之为 Cry 毒素的双作用模型 (Tabashnik *et al.* ,2015 ;Soberón *et al.* ,2016),即 Cry1A 原毒素不经过蛋白酶活化也可直接结合钙粘蛋白并发生寡聚化,形成一种不同于活化蛋白的预孔寡聚体,随后与 APN 或 ALP 结合并插入肠膜,引起膜穿孔,从而导致昆虫死亡 (图 2 : d)。

美国 1928 年启动了利用 Bt 制剂防治玉米螟 *Pyrausta nubilalis* 的计划,1929 年第一次大田应用,1938 年法国第一个产品 Sporeine 面世,20 世纪 50 年代许多国家进行了商业化生产 (关雄,2006)。基于 Bt 开发的杀虫剂是全球最畅销、应用最为广泛、对人畜安全的高效昆虫病原微生物杀虫剂 (Mendelsohn *et al.* ,2003 ;Roh *et al.* ,2007),目前已登记且商业化的 Bt 制剂超过 400 种,均可直接应用于田间害虫防控 (Tabashnik *et al.* ,2011)。此外,科学工作者利用 Bt 在芽胞形成期可产生 Cry1Ab、Cry1Ac 等多种具有特异杀虫活性的毒素蛋白这一特性,通过转基因手段构建得到了转基因的大豆、玉米、棉花、油菜等转基因抗虫作物,使 Bt 的应用前景更加广阔 (Tabashnik *et al.* ,2011 ;James ,2016)。Bt 杀

虫剂在农业生产中的大量使用和转 Bt 基因作物在世界范围内种植面积的逐年增长,不仅可以有效控制许多害虫的危害 (Schnepf *et al.* ,1998 ;Musser and Shelton ,2003 ;Kleter *et al.* ,2007),减少农作物的产量损失,而且可以减少使用化学农药,降低害虫防控成本,保护生态环境,具有良好的经济、生态和社会效益 (James ,2016)。但是,长期大规模使用 Bt 制剂和转 Bt 基因作物导致靶标害虫产生选择性压力,从而产生并不断增强对 Bt 的抗性。

2 小菜蛾对 Bt 杀虫毒素的抗性

1985 年,世界上首次报道了实验室种群印度谷螟 *Plodia interpunctella* 对 Bt 产生抗性 (McGaughey ,1985)。随后,国内外多项研究陆续在实验室条件下筛选获得鳞翅目 (Tabashnik *et al.* ,1991 ;Gould *et al.* ,1995 ;Moar *et al.* ,1995) 鞘翅目 (Bauer ,1995 ;Federici and Bauer ,1998) 双翅目 (Georghiou and Wirth ,1997 ;Wirth and Georghiou ,1997) 等十余种害虫的 Bt 抗性品系 (Ferré and Van Rie ,2002)。Tabashnik 等 (1990) 在长期使用 Bt 制剂的美国夏威夷田间首次发现小菜蛾对 Bt 制剂产生抗药性。随后,陆续发现草地贪夜蛾 *Spodoptera frugiperda* (Matten *et al.* ,2008) 玉米茎叶蛾 *Busseola fusca* (Van Rensburg ,2007) 和谷实夜蛾 *Helicoverpa zea* (Luttrell *et al.* ,1999) 在田间对 Bt 制剂以及 Bt 作物产生抗性。截至目前,已经发现有 2 种昆虫在田间对 Bt 制剂产生抗性,至少有 6 种昆虫对转 Bt 基因作物产生抗性 (Tabashnik *et al.* ,2011 ,2013)。

1986 年,研究人员在长期使用 Bt 制剂的美国夏威夷田间首次发现小菜蛾对 Bt 制剂产生抗药性 (Tabashnik *et al.* ,1990)。迄今为止,全世界已有美国、巴西、中国、马来西亚、菲律宾、日本等地相继报道小菜蛾对 Bt 杀虫毒素的抗药性,这些报道涉及到的毒素晶体蛋白包括 Cry1Aa、Cry1Ab、Cry1Ac、Cry2Aa、Cry2Ab、Cry2B、Cry1Ca 和 Cry1Da (Peterson *et al.* ,2017)。小菜蛾作为首个被发现在田间对 Bt 制剂产生抗性的害虫,

表 1 小菜蛾对 Bt 毒素抗性的相关研究
Table 1 Case studies on the resistance of *Plutella xylostella* to Bt toxins

品系 Strain	国家 Country	毒素 Toxin	抗性倍数 Resistance ratio	参考文献 Reference
NO-P	USA-Hawaii	Cry1Ac	150	Tabashnik <i>et al.</i> , 1991
NO-Q	USA-Hawaii	Cry1Ac	180	Tabashnik <i>et al.</i> , 1991
NO-R	USA-Hawaii	Cry1Ac	190	Tabashnik <i>et al.</i> , 1991
No name	Philippine-Baguio	Cry1Ab	>200	Ferré <i>et al.</i> , 1991
NO-QA	USA-Hawaii	Btk	1 800-6 800	Tabashnik <i>et al.</i> , 1993
NO-QA	USA-Hawaii	Cry1F	>240	Tabashnik <i>et al.</i> , 1994
NO-QA	USA-Hawaii	Cry1Ab	>750	Tabashnik <i>et al.</i> , 1994
Resistance colony	USA-Florida	Btk	>1500	Tang <i>et al.</i> , 1996
PEN	USA- Pennsylvania	Cry1J	1000	Tabashnik <i>et al.</i> , 1997
Cry1C-Sel	USA-Massachusetts	Cry1C	12 400-63 100	Zhao <i>et al.</i> , 2000
Btk-Sel	Malaysia-Melaka	Btk	112	Sayyed <i>et al.</i> , 2000
Bta-Sel	Malaysia-Melaka	Bta	30	Sayyed <i>et al.</i> , 2000
Cry1Ab-Sel	Malaysia-Melaka	Cry1Ab	500	Sayyed <i>et al.</i> , 2000
Cry1Ac-Sel	Malaysia-Melaka	Cry1Ac	>10 500	Sayyed <i>et al.</i> , 2000
PXR	Japan	Cry1Ac	126 000	Kumaraswami <i>et al.</i> , 2001
Cry1Ac-Sel-SERD4	Malaysia	Cry1Ac	>150	Sayyed <i>et al.</i> , 2008
SZBT	China-Guangdong	Cry1Ac	1 200	Gong <i>et al.</i> , 2010
SZ-R (T2-R)	China-Shenzhen	Cry1Ac	450	Guo <i>et al.</i> , 2015a
SH-R	China-Shanghai	Btk	1 900	Guo <i>et al.</i> , 2015b
NIL-R (BC6F4)	USA-Florida	Cry1Ac	5 000	Zhu <i>et al.</i> , 2015
DBM1Ac-R	USA-Florida	Cry1Ac	5 100	Zhu <i>et al.</i> , 2015

已成为研究 Bt 抗性的最主要模式生物。从 1953 年到 2014 年间,全世界的小菜蛾已经对 91 种农药(其中包括 12 种 Bt 菌株)的活性成分产生了抗药性(IRAC, 2015; Machekano *et al.*, 2017)。相关研究表明,不同类型的 Bt 毒素筛选的小菜蛾品系,其抗性倍数明显存在差异。例如,Tabashnik 等(1991)的研究显示,小菜蛾品系 NO-P、NO-Q、NO-R 对 Cry1Ac 毒素的抗性倍数分别为 150、180、190;后来他们发现另一个小菜蛾品系 NO-QA 对 Btk 的抗性倍数在 1 800-6 800 倍之间(Tabashnik *et al.*, 1993),该品系对 Bt 毒素 Cry1F 和 Cry1Ab 的抗性倍数为 >240 和 >750(Tabashnik *et al.*, 1994)。类似的研究还有一个来源于菲律宾的小菜蛾品系也对 Cry1Ac 产

生抗性,其抗性倍数 >200 倍(Ferré *et al.*, 1991);来自美国佛罗里达州的小菜蛾抗性品系的抗性倍数为 1 500 倍(Tang *et al.*, 1996);而来自宾夕法尼亚州的小菜蛾品系 PEN 对 Bt 毒素 Cry1J 的抗性倍数为 1 000 (Tabashnik *et al.*, 1997)。根据 Zhao 等(2000)的研究,最初来自美国马萨诸塞州的小菜蛾品系 Cry1C-Sel,在 Cry1C 毒素筛选后,其初孵幼虫和 2 龄幼虫存在不同的抗性倍数,分别为 12 400 倍和 63 100 倍。同年, Sayyed 等(2000)的研究也显示,在实验室内,用 Bt 毒素 Btk、Bta、Cry1Ab 和 Cry1Ac 筛选之后,相对于已经在实验室保存了 150 代的敏感品系 ROTH,小菜蛾的抗性倍数分别为 112 倍、30 倍、500 倍和 10 500 倍。另一个来自日本的抗性

品系 PXR, 对 Bt 毒素 Cry1Ac 的抗性倍数高达 126 000 倍 (Kumaraswami *et al.*, 2001)。在中国广东省深圳市的大白菜田中采集的小菜蛾 SZ 品系, 用活化的 Cry1Ac 对 SZ 品系进行 20 代筛选, 得到抗性品系 SZBT, 与敏感品系 ROTH 相比, SZBT 品系对 Cry1Ac 的抗性为 1 200 倍 (Gong *et al.*, 2010)。Guo 等 (2015b) 在实验室中用 Cry1Ac 毒素连续选择产生的 SZ-R 品系小菜蛾, 以及 Btk 毒素筛选之后的 SH-R 品系小菜蛾, 最终的生测结果表明, 相比于敏感品系, 他们的抗性倍数达到了 450 倍和 1 900 倍。在 Bt 毒素 Cry1Ac 筛选后, 小菜蛾品系 DBM1Ac 的幼虫的抗性倍数为 5 100 倍, 而回交 6 次自交 4 次的小菜蛾品系 NIL-R (BC6F4) 的抗性倍数几乎和前者相同, 为 5 000 倍 (Zhu *et al.*, 2015)。Liu 等 (2017) 的报道表明, 来自马来西亚的小菜蛾品系 Cry1Ac-Sel-SERD 4 对 Bt 毒素的抗性倍数为 >150。

3 小菜蛾对 Bt 杀虫毒素抗性的机制

Pardo-Lopez 等 (2013) 总结了 Bt Cry 毒素在鳞翅目昆虫中产生抗药性的多种机理, 包括 Cry 毒素活化的改变; 糖脂类或酯酶对毒素的隔离作用; 昆虫免疫反应的提升; Cry 毒素受体 (如钙粘蛋白 CAD、碱性磷酸酶 ALP 等) 的改变等。因此, 昆虫对 Bt 产生抗性的机制可能发生在 Bt 毒素蛋白发挥作用的每一个环节。特别值得注意的是, 一种昆虫可能形成一套抗性机制对同种或是不同种 Cry 毒素都产生抗性。

自 1990 年 Tabashnik 等人首次报道小菜蛾在田间产生 Bt 抗性以来, 众多学者一直致力于 Bt 抗性基因筛选与抗性机理解析等研究 (Gahan *et al.*, 2001, 2010; Morin *et al.*, 2003; Baxter *et al.*, 2011; Tiewisiri and Wang, 2011; Tabashnik *et al.*, 2013; Park *et al.*, 2014; Adang *et al.*, 2014; Stevens *et al.*, 2017; Peterson *et al.*, 2017)。Peterson 等 (2017) 概述了 20 多年来不同国家关于小菜蛾对 Cry 毒素抗性的研究结果, 发现主要的抗性机制有毒素活化不完全、毒素被隔离或凝结、结合位点改变或缺失、生理适应性、ABC

转运蛋白突变 (表 2)。

Lei 等 (2014) 利用 RNA 测序比较了小菜蛾 Bt 抗性品系与敏感品系的转录组, 筛选获得 2 925 个差异表达基因; 随后, Ayra-Pardo 等 (2015) 利用抑制性消减杂交法比较了小菜蛾 Bt 抗性品系与敏感品系幼虫的转录本, 筛选获得上百个可能的 Bt 抗性基因, 但上述基因功能与互作通路仍未被解析。在分子机理的研究中, 发现小菜蛾对 Cry 毒素的抗性品系 NO-QAGE 无法合成完整的 ABCC2, 即 *abcc2* 基因第 20 个外显子缺失了 30 bp, 导致 ABCC2 蛋白的第 12 个跨膜区被移除, 使得羧基端暴露在胞外区 (Baxter *et al.*, 2011)。Guo 等 (2015a) 提出了反式调控机制, 认为丝裂原活化蛋白激酶 (Mitogen-activated protein kinases, MAPK) 信号途径反式调控中肠 *alp*、*abcc2* 和 *abcc3* 基因的表达, 从而导致小菜蛾 Bt 抗性的产生。与此同时, MAPK 信号途径的激活还有可能导致中肠 *abcg1* 基因表达量显著下降从而进一步导致小菜蛾对 Bt Cry 毒素产生高抗性 (Guo *et al.*, 2015a)。但 MAPK 信号通路中有哪些成员参与了抗性调节, 以及各成员之间的相互作用仍有待进一步研究 (Crickmore, 2016)。

总体来看, 小菜蛾的 Bt 抗性机理与鳞翅目昆虫较一致, 但也有其特异性。例如, 在对受体基因突变介导的 Bt 抗性研究中发现, 小菜蛾对 Cry 毒素的抗性与中肠钙粘蛋白基因的改变并无关联 (Baxter *et al.*, 2005; Guo *et al.*, 2015a), *alp* 和 *apn* 基因也未发生顺式突变 (Baxter *et al.*, 2008)。因此, 小菜蛾 Bt 抗性涉及一个复杂的分子调控网络, 多种不同类型的中肠蛋白都有可能参与其中 (Xia *et al.*, 2016)。

4 小结与展望

小菜蛾是为害十字花科蔬菜最为严重的鳞翅目害虫, 对全世界的十字花科蔬菜的生产造成严重的威胁和损失 (Furlong *et al.*, 2013; Li *et al.*, 2016a)。作为世界性害虫, 小菜蛾对杀虫剂的抗性发展迅速, 几乎对市场所有可用的杀虫剂产生了抗性, 而且是最早在田间对生物农药苏云金

表 2 小菜蛾对 Cry 毒素抗性机制 (Peterson *et al.*, 2017)
Table 2 Mechanisms of Cry toxin resistance in *Plutella xylostella* reported from laboratory, greenhouse, and field studies

小菜蛾品系 Strain	国家 Country	毒素 Toxin	抗性机制 Mechanism of resistance
343	美国 USA	<i>Btk</i> ^c	结合位点改变或缺失 (未确定) Altered/loss of binding site (not yet identified)
Dpl-r	美国 USA	<i>Btk</i> ^c	结合位点改变或缺失 (未确定) Altered/loss of binding site (not yet identified)
Multiple	巴西 Brazil	<i>Bta</i> ^o ; <i>Btk</i> ^c	生理适应性 Physiological adaptation
SZ-R(T2-R)	中国 China	Cry1Ac	ABC 转运蛋白突变 Mutation in ABC transporter protein
SH-R	中国 China	Cry1Ac	ABC 转运蛋白突变 Mutation in ABC transporter protein
<i>Btk</i> -Sel	马来西亚 Malaysia	<i>Btk</i> ^c	结合位点改变或缺失 (未确定) Altered/loss of binding site (not yet identified)
<i>Bta</i> -Sel	马来西亚 Malaysia	<i>Bta</i> ^o	结合位点改变或缺失 (未确定) Altered/loss of binding site (not yet identified)
Cry1Ab-Sel	马来西亚 Malaysia	Cry1Ab	结合位点改变或缺失 (未确定) Altered/loss of binding site (not yet identified)
Cry1Ac-Sel	马来西亚 Malaysia	Cry1Ac	毒素活化不完全; 毒素被隔离或凝结; 结合位点改变或缺失 (未确定) Toxin activation defects; toxin sequestration/coagulation; altered/loss of binding site (not yet identified)
No name	菲律宾 Philippines	Cry1Ab	结合位点改变或缺失 (未确定) Altered/loss of binding site (not yet identified)
PHI	菲律宾 Philippines	Cry1Aa, Cry1Ab, Cry1Ac	结合位点改变或缺失 (未确定) Altered/loss of binding site (not yet identified)
Cry1Ac-R (DBM1Ac)	美国 USA	Cry1Ac	结合位点改变或缺失 (未确定); 钙黏蛋白 (CDH)、氨肽酶 N (APN) 和碱性磷酸酶 (ALP) 转录改变、ABC 转运蛋白突变 Altered/loss of binding site (not yet identified); mutation in CDH; altered transcription of APN; altered transcription of ALP; mutation in ABC transporter protein
NIL-R (BC6F4)	美国 USA	Cry1Ac	结合位点改变或缺失 (未确定) Altered/loss of binding site (not yet identified)
NO-95C	美国 USA	Cry1C	推测: 毒素激活缺陷; 蛋白酶活性改变 Putative: toxin activation defects; altered proteinase activity
NO-P	美国 USA	Cry1Ac	结合位点改变或缺失 (未确定) Altered/loss of binding site (not yet identified)
NO-Q	美国 USA	Cry1Ac	结合位点改变或缺失 (未确定) Altered/loss of binding site (not yet identified)
NO-QA	美国 USA	Cry1Aa, Cry1Ab, Cry1Ac	结合位点改变或缺失 (未确定) Altered/loss of binding site (not yet identified)
NO-QAGE	美国 USA	Cry1Ac	ABC 转运蛋白突变 Mutation in ABC transporter protein
NO-R	美国 USA	Cry1Ac	结合位点改变或缺失 (未确定) Altered/loss of binding site (not yet identified)
NO-U	美国 USA	Cry1Ac	结合位点改变或缺失 (未确定) Altered/loss of binding site (not yet identified)
PEN	美国 USA	Cry1Aa, Cry1Ab, Cry1Ac	结合位点改变或缺失 (未确定); 非特异性毒素结合增加 Altered/loss of binding site (not yet identified); increased nonspecific toxin-binding
PXR	日本 Japan	Cry1Ac	醯脂类水平降低 Reduced glycolipid levels
SZBT	中国 China	Cry1Ac	结合位点改变或缺失 (未确定) Altered/loss of binding site (not yet identified)

c: Cry1Aa, Cry1Ab, Cry1Ac, Cry2Aa, Cry2Ab, Cry2B; o: Cry1Aa, Cry1Ab, Cry1Ca, Cry1Da.

芽胞杆菌(Bt)产生抗性的昆虫(Tabashnik *et al.*, 1990)。小菜蛾的抗药性问题一直备受世界各国专家学者们的关注,中国学者对小菜蛾抗药性的研究尤为重视。例如,根据2015年节肢动物抗药性数据库的统计数字,中国发表的小菜蛾抗药性的研究论文居世界第一(IRAC, 2015; Machekano *et al.*, 2017)。虽然在许多地区,苏云金芽胞杆菌对小菜蛾的防控还有一定的效果,但小菜蛾对Bt杀虫毒素产生抗性的风险不容忽视(Xia *et al.*, 2014)。

据统计,全世界26个国家2016年种植了1.851亿 hm^2 转基因作物,比2015年的增加了540万 hm^2 ,比1996年增加了100倍(James 2016)。主要包括转基因大豆、棉花、玉米和油菜四种主要作物,这些转基因作物的种植面积所占的比例分别为78%、64%、33%和24%,其中转Bt油菜的大量种植,将大大增加小菜蛾对Bt产生抗性的风险。我国2016年转基因作物的种植面积是280万 hm^2 ,在全世界排名第八,主要包括棉花、白杨、木瓜3种作物(国际农业生物技术应用服务组织, 2017)。虽然目前我国还没有开始种植转基因油菜,但有必要加强小菜蛾对转基因油菜的抗性风险评估和抗性机理研究,以便为大面积种植转基因油菜提供科学依据。

关于小菜蛾对Bt的抗性机理研究开始于20世纪90年代初(Ferré *et al.*, 1991)。众多研究表明,Bt抗性与许多因素有关,可能在Cry毒素发挥作用过程中的任何一个环节产生抗性(Vachon *et al.*, 2012; Peralta and Palma, 2017; Peterson *et al.*, 2017)。值得我们特别注意的是,一种昆虫可能形成一套抗性机制而对同种或是不同种Cry毒素都产生抗性(Peterson *et al.*, 2017)。目前已有多项涉及Bt抗性机理的研究报道:毒素蛋白在中肠蛋白酶水解的过程发生改变(Ferré and Van Rie, 2002);受体基因发生突变,导致毒素无法结合受体(Crickmore, 2016; Coates, 2016);膜转运蛋白发生突变(Gahan *et al.*, 2010; Heckel, 2012);信号通路介导的反式调控机制调节受体基因的表达(Tiewsi and Wang, 2011; Guo *et al.*, 2015a; Melo *et al.*, 2016)等。但是,

每个抗性机理的通路尚不完整,缺乏与之相关的完整蛋白质组信息以及用于后续功能验证的抗体资源,导致无法深入挖掘和验证Bt抗性相关的蛋白。目前,已获得注释的小菜蛾中肠蛋白总共有6764个(Zhu *et al.*, 2016),但与小菜蛾中肠表达的基因总数(1.2万个)(You *et al.*, 2013)相比,仍有大约一半的蛋白未被鉴定,这说明现有的数据还不能满足蛋白质组研究所需的覆盖度,而且缺乏用于后续蛋白互作网络功能研究的完整抗体资源,因此,完整注释中肠蛋白质组对阐明小菜蛾Bt抗性的分子机制具有重要意义。单克隆抗体组作为近年来新兴的一项高通量技术(Uhlén *et al.*, 2015),因其具有高质量、高通量和低成本的优势,日益受到研究人员的关注。基于单克隆抗体组技术的蛋白质组学可为Bt抗性研究提供高通量数据,有助于筛选更多新的Bt抗性基因,为深入探讨害虫对Bt产生抗性的分子机理提供重要基础;同时,探明害虫对Bt的抗性机理可为制定和改进害虫抗性治理的策略、提高生物农药和Bt作物的控害效能提供科学依据。

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