

靶标害虫对 Bt 玉米的抗性发展和治理策略*

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摘 要 全生育期有效表达 Bt 杀虫蛋白的转基因抗虫玉米为靶标害虫的防治提供了新途径。但是, 靶标害虫抗性种群的发展严重威胁了转基因抗虫玉米的可持续应用。截止到 2018 年, 已经有 13 例报道表明靶标害虫对转基因抗虫玉米产生了田间抗性; 5 例监测结果表明靶标种群没有降低对 Bt 玉米的敏感性, 其中包括转 *vip3Aa* 玉米。抗性治理策略成功的关键主要包括: Bt 杀虫蛋白的高剂量表达、靶标害虫的隐性遗传、初始抗性等位基因频率较低、不完全抗性、适合度代价等。当抗性为非隐性遗传时, 可以通过增加庇护所的种植面积达到延缓抗性发展的目的。

关键词 转基因抗虫玉米; 靶标害虫; 田间抗性; 抗性治理

Evolution of resistance to transgenic *Bacillus thuringiensis* maize in pest insects and a strategy for managing this

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Abstract Transgenic *Bacillus thuringiensis* maize expressing Bt toxins is a new tool for controlling target pest insects. However, the evolution of resistance within target pest populations threatens the sustainable utilization of Bt maize. By 2018 about 13 cases of resistance to Bt maize have been reported. In 5 other cases there was no significant decrease in susceptibility to Bt maize, including transgenic maize that produces the Vip3Aa protein. Factors that favor a successful resistance management strategy that can delay resistance include high-dosages, the recessive inheritance of resistance, low frequency of the alleles that confer resistance, incomplete resistance and fitness costs. When the inheritance of resistance is not recessive, increasing the size of refugia can still significantly delay the development of resistance.

Key words transgenic *Bacillus thuringiensis* maize; target pests; field resistance; resistance management

玉米作为重要的粮食作物、优质的饲料作物和高产的经济作物, 在世界粮食中占有举足轻重的地位。害虫为害每年都造成严重的产量损失, 对玉米生产和农业安全造成严重威胁。农药抗性的产生导致化学防治在生产中的应用受到一定的限制。利用苏云金芽孢杆菌 (*Bacillus thuringiensis*, Bt) 杀虫晶体蛋白的植物基因工程研究为玉米害虫的防治提供了新途径。Bt 在芽孢形成的过程中产生的杀虫晶体蛋白 (Insecticidal

crystal protein, ICP) 对鳞翅目、鞘翅目、双翅目等多种害虫具有特异的杀虫作用, 是目前研究最多、应用最广泛的生物制剂 (Alyokhin, 2011; Sanahuja *et al.*, 2011; Pardo-López *et al.*, 2013; ISAAA, 2017)。Bt 在营养生长对数中期分泌的胞外蛋白——营养期杀虫蛋白 (Vegetative insecticidal proteins, VIP) 对鞘翅目和鳞翅目害虫具有特异性 (Ruiz *et al.*, 2014)。另外, Vip 蛋白和 Cry 蛋白不存在序列同源性、作用机理不

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同、受体位点不同,同多种 Cry 蛋白具有协同增效作用 (Estruch *et al.*, 1996; Lee *et al.*, 2003; Hernández-Martínez *et al.*, 2013)。1996 年,美国率先批准了转 *cry1Ab* 玉米 (Bt176) 的商业化种植。2003 年,美国开始商业化种植转 *cry1F* 玉米 (TC1507) 和转 *cry3Bb1* 玉米 (MON863), 分别防治欧洲玉米螟 *Ostrinia nubilalis* 和玉米根萤叶甲 *Diabrotica virgifera virgifera*; 但是, 由于靶标害虫抗性种群的产生, 2010 年取消了 MON863 的商业化种植。同年, 菲律宾相关部门批准了转 *cry1Ab* 玉米 (Bt11) 的商业化种植, 用于防治亚洲玉米螟 *Ostrinia furnacalis*。巴西分别于 2008 年和 2009 年批准了转 *cry1Ab* 玉米 (MON810) 和转 *cry1F* 玉米 (TC1507) 的商业化种植, 用于防治草地贪夜蛾 *Spodoptera frugiperda*。2010 年, 阿根廷商业化种植了表达 Cry1A.105 和 Cry2Ab2 杀虫蛋白的转基因抗虫玉米 (MON89034) 防治小蔗螟 *Diatraea saccharalis*。另外, 美国环境保护署 (Environmental Protection Agency, EPA) 相继注册了转 *cry34/35Ab1* 玉米 (591227) 转 *mCry3A* 玉米 (MIR604) 转 *eCry3.1Ab* 玉米 (5307) 转 *Cry3Bb1* 玉米 (MON87411), 注册年限分别为 2005 年、2006 年、2012 年和 2015 年 (<https://archive.epa.gov/pesticides/reregistration/web/html/current-previously-registered-section-3-plant-incorporated.html>)。田间试验表明: 转 *cry1Ab* 玉米 (MON810 和 Bt11) 对亚洲玉米螟 (He *et al.*, 2003; 何康来等, 2004; 王冬妍等, 2004) 以及棉铃虫 *Helicoverpa armigera* (常雪艳等, 2006) 甜菜夜蛾 *Spodoptera exigua* (王振营等, 2005) 和黏虫 *Mythimna separata* (王振营等, 2005; 常雪等, 2007) 初孵幼虫具有很好的杀虫效果。虽然中国政府还没有批准转基因玉米的商业化种植, 但是科研工作者已经研发出一批性状良好的转基因玉米品种, 如转 *cry1Ie* 玉米 (IE034) (Zhang *et al.*, 2013) 转 *cry1Ac* 玉米 (BT799) (王月琴等, 2014) 转 *cry1Ah* 玉米 (HGK60) (宋苗等, 2016) 转 *cry1Ab/cry2Aj* 和 *G10evo* (EPSPS) (双

抗 12-5) (王江等, 2016) 转 *cry1Ah/cry1Ie* 玉米 (杨召军等, 2012) 转 *cry1Ac/cry1Ie* 玉米 (Jiang *et al.*, 2016), 均对亚洲玉米螟表现良好的抗虫性。

转基因抗虫玉米不仅能够有效防治靶标害虫, 增加作物产量, 还能大幅度降低化学农药的使用量, 保护环境和生物多样性 (He *et al.*, 2003; Duvick, 2005; Pereira *et al.*, 2008; Carpenter, 2010; Hutchison *et al.*, 2010; Li and Romeis, 2010; Osteen and Fernandez-Cornejo, 2013; Shi *et al.*, 2013)。

1996 年, 美国首次批准了转 *cry1Ab* 基因玉米 (BT176) 的商业化种植, 在随后的 21 年里, 转基因玉米在全球范围内得到了迅速的推广和应用, 为全球种植者带来 637 亿美元的经济收入 (ISAAA, 2017)。2017 年, 全球玉米种植总面积为 1.88 亿 hm^2 , 转基因玉米种植面积高达 5 970 万 hm^2 , 其中包括转基因抗虫玉米 530 万 hm^2 (Insect resistance, IR) 转基因耐除草剂玉米 630 万 hm^2 (Herbicide tolerant, HT) 复合性状转基因/耐除草剂玉米 4 810 万 hm^2 (Insect resistance/Herbicide tolerant, IR/HT)。大面积的连续种植转基因抗虫玉米, 使靶标害虫长期处于一定的筛选压下, 携带抗性等位基因的个体不断富集, 抗性等位基因频率不断增加, 削弱了转基因抗虫玉米的优势, 导致一些转基因抗虫玉米推广的失败甚至被强制撤回 (Van Rensburg, 2007; Storer *et al.*, 2010, 2012; Velez *et al.*, 2013; Farias *et al.*, 2014; Gassmann, 2016)。因此, 有必要实施抗性治理策略。

为了更加客观合理的分析田间抗性监测数据, 明确田间抗性发展水平, 实施抗性治理策略, 本文重新归纳了“田间抗性”的分类标准; 总结了现阶段靶标害虫对转基因抗虫玉米抗性发展的现状; 讨论了延缓抗性产生的主要策略; 最后, 分析了我国转基因抗虫玉米的研发现状和展望了种植前景。

1 田间抗性的分类

关于田间抗性的定义和标准一直存在争议,

IRAC (Insecticide Resistance Action Committee) 认为: 种植者严格按照相关说明种植转基因作物, 并期望达到防治靶标害虫的目的; 但是, 靶标害虫长期处于转基因抗虫作物的环境中, 敏感个体被淘汰, 抗性个体存活、繁衍、逐渐发展成为无法控制的抗性种群规模, 田间抗性产生, 导致转基因抗虫作物应用失败 (<http://www.irac-online.org/about/resistance/>)。Tabashnik 等认为: 田间抗性是指在田间条件下, 靶标害虫种群长期暴露在 Bt 杀虫蛋白选择压力之下, 引起基因水平上的变异, 最终导致该种群对 Bt 杀虫蛋白的敏感性降低 (Tabashnik *et al.*, 2009, 2013, 2014; Tabashnik and Carrière, 2017)。Tabashnik 和 Carrière (2017) 重新将田间抗性分为三类, 主要包括:

(1) 实质抗性 (Practical resistance)。田间抗性个体的数量 > 50%, 显著降低了 Bt 作物的防治效果, 且不能实际控制害虫抗性种群的发展。抗性个体的鉴定主要通过以下三种方式: 测定 Bt 杀虫蛋白对靶标害虫室内敏感品系的毒力, 取 LC_{99} 值 95% 置信区间的上限作为诊断剂量浓度, 在诊断剂量浓度下存活的个体即为抗性个体。该诊断剂量浓度能够杀死全部或者几乎全部的敏感个体 (Marçon *et al.*, 2000; Tabashnik *et al.*, 2013)。在转基因作物上能够完成一个世代, 且能产生可育后代的个体即为抗性个体 (Siegfried *et al.*, 2007; Tabashnik *et al.*, 2013, 2014)。若 Bt 杀虫蛋白对靶标害虫的毒力 LC_{50} 值大幅度增加, 则认为该种群 > 50% 的个体为抗性个体 (Tabashnik *et al.*, 2014)。

(2) 敏感水平 (No decrease in susceptibility)。田间害虫种群长期暴露在 Bt 杀虫蛋白下, 但是对 Bt 杀虫蛋白的敏感性没有显著下降 (Tabashnik *et al.*, 2013)。

(3) 早期预警抗性 (Early warning resistance)。害虫种群显著降低了对 Bt 杀虫蛋白的敏感性, 但是没有相关报道说明该转基因抗虫事件降低了防治效果 (Zhang *et al.*, 2011; Tabashnik and Carrière, 2017)。

2 靶标害虫对 Bt 玉米的抗性发展现状

2012 年, 仅有 3 例相关报道证实靶标害虫对转基因抗虫玉米产生了实质抗性。在波多黎各转 *cry1F* 基因玉米 TC1507 种植区监测到高水平抗性的草地贪夜蛾种群, 抗性种群的发展显著削弱了 TC1507 的防治效果, 导致该转基因事件被强制召回, 这是迄今为止因为抗性种群的产生导致转基因品种被强制召回的第一个事件 (Store *et al.*, 2010, 2012); 同时也是靶标害虫对 Bt 作物产生抗性历经时间最短的事件 (Gould *et al.*, 1997; Roush, 1997)。干旱的气候条件、转基因作物的大面积推广、庇护所的不合理设置等, 同抗性种群的产生密切相关 (Kruger *et al.*, 2009; Store *et al.*, 2010)。2007 年, 在南非表达 Cry1Ab 的 MON810 种植区采集的玉米蛀茎夜蛾 *Busseola fusca* 滞育种群, 其后代能够在 MON810 上存活, 对 Cry1Ab 产生了一定水平的抗性 (Van Rensburg, 2007)。Bt 作物对靶标害虫田间防治效果的降低先于抗性产生的生测记录, 同早期不积极采取措施进行抗性监测有关 (Kruger *et al.*, 2011, 2012; Store *et al.*, 2010, 2012)。2003 年, 美国注册并且批准了转 *cry3Bb1* 玉米 (MON863) 的商业化种植; 2009 年, 田间监测到玉米根萤叶甲抗性种群。Cry3Bb1 杀虫蛋白的非高剂量表达和适合度代价同抗性种群的产生密切相关 (Gassmann *et al.*, 2011, 2012; Gassmann, 2012, 2016)。

截止 2018 年, 13 例报道证实靶标害虫对表达不同杀虫蛋白的转基因抗虫玉米产生了实质抗性, 主要包括 7 种昆虫: 玉米蛀茎夜蛾 (对 Cry1Ab 杀虫蛋白) (Van Rensburg, 2007)、小蔗螟 (对 Cry1A.105 杀虫蛋白) (Blanco *et al.*, 2016; Grimi *et al.*, 2018)、玉米根萤叶甲 (对 Cry3Bb, Cry34/35Ab, mCry3A, eCry3.1Ab 杀虫蛋白) (Gassmann *et al.*, 2011, 2014, 2016; Andow *et al.*, 2015; Jakka *et al.*, 2016; Zukoff *et al.*, 2016)、草地贪夜蛾 (对 Cry1Ab、Cry1F 杀虫蛋白) (Storer *et al.*, 2010; Huang *et al.*, 2014;

Farias *et al.*, 2016; Omoto *et al.*, 2016) 西部豆夜蛾 *Striacosta albicosta* (对 Cry1F 杀虫蛋白) (Eichenseer *et al.*, 2008; Ostrem *et al.*, 2016) 美洲棉铃虫 *Helicoverpa zea* (对 Cry1Ab, Cry1A.105 杀虫蛋白) (Storer *et al.*, 2001; Dively *et al.*, 2016)。另外, 美国的小蔗螟 (对 Cry1Ab 杀虫蛋白) (Ghimire *et al.*, 2011; Huang *et al.*, 2012) 和菲律宾的亚洲玉米螟 (对 Cry1Ab 杀虫蛋白) (Alcantara *et al.*, 2011) 对转基因抗虫玉米处于早期预警抗性水平。2018 年在西班牙首次从田间种群中检测到地中海玉米蛀茎夜蛾 *Sesamia nonagrioides* 对 Cry1Ab 的抗性等位基因, 抗性等位基因频率高达 0.003 6 (Camargo *et al.*, 2018)。田间抗性种群的发展, 减少了有效防治害虫的 Bt 蛋白种类。因此, 有必要筛选新的杀虫基因和构建高毒力的工程菌。

全球抗性监测数据表明: 西南玉米秆草螟 *Diatraea grandiosella* 对 Cry1Ab 杀虫蛋白 (Huang *et al.*, 2010) 欧洲玉米螟对 Cry1Ab 及 Cry1F 杀虫蛋白 (Crespo *et al.*, 2010; Farinós *et al.*, 2010; Siegfried *et al.*, 2014; Thieme *et al.*, 2018) 以及草地贪夜蛾对 Vip3Aa20 杀虫蛋白 (Bernardi *et al.*, 2015) 的抗性依然处于敏感水平。

从转基因抗虫玉米的首次商业化种植到田间抗性种群的产生, 所需要的周期越来越短, 其中, 交互抗性是加速抗性发展的关键因素 (Tabashnik, 2016)。Cry3Bb 和 eCry3.1Ab 存在交互抗性。虽然表达 eCry3.1Ab 杀虫蛋白的转基因玉米还没有商业化种植, 但是, 田间已经监测到对 eCry3.1Ab 产生抗性的玉米根萤叶甲种群。这一案例直接说明交互抗性的存在能够加速田间抗性的发展 (Jakka *et al.*, 2016; Zukoff *et al.*, 2016)。巴西商业化种植 *cry1Ab* 玉米 (MON810) 和 *cry1F* 玉米 (TC1507) 两年后, 转基因抗虫玉米对草地贪夜蛾的防治效果明显下降。这一现象的发生, 同 Cry1Ab 和 Cry1F 杀虫蛋白之间存在交互抗性密切相关 (Farias *et al.*, 2014; Monnerat *et al.*, 2015; Omoto *et al.*, 2016)。

靶标害虫对 Bt 作物适应性的增强、庇护所种植面积减少、转基因作物种植面积的增加都

促进了田间抗性的发展。另外, 田间抗性产生的周期与昆虫发生代数呈负相关 (ISAAA, 2017; Tabashnik and Carrière, 2017)。

3 抗性治理策略

为了让 Bt 作物的优势得到最大程度的发挥, 更好的为人类服务, 科研工作者和监管部门一直致力于抗性治理策略 (Insect resistance management, IRM) 的研究。抗性治理策略, 就是采取一定的策略和方案, 阻止或者延缓靶标害虫抗性种群的发展, 实现转基因作物的可持续应用。目前, 应用最为广泛的抗性治理策略主要包括“庇护所”策略和“多基因”策略。

3.1 “庇护所”策略

2002 年以前, “庇护所”策略是应用最为广泛的田间抗性治理策略。在美国和澳大利亚等国家, 该策略均由政府部门强制执行 (Tabashnik *et al.*, 2008; Hutchison *et al.*, 2010)。该策略要求:

(1) 庇护所。在种植 Bt 抗虫作物的同时, 在其周边种植一定面积的非 Bt 作物, 以提供足够多的敏感个体, 与 Bt 作物区存活的少量抗性纯合子个体进行自由交配。产生的杂合子后代能够被高剂量表达的抗虫作物毒杀 (Liu and Tabashnik, 1997; Roush, 1997)。

(2) 高剂量表达 (隐性遗传)。转基因抗虫作物必须表达足够的杀虫蛋白剂量, 使靶标害虫种群内所有的抗性基因都为功能隐性遗传。杂合子后代不能在抗虫作物上存活, 从而达到延缓抗性发展的目的。因此, “庇护所”策略, 又称为“高剂量/庇护所”策略 (Tabashnik *et al.*, 2003)。

一般情况下, 通过评估抗性个体、敏感个体和抗性杂合子个体在抗虫作物上的存活情况, 判断该转基因事件是否为高剂量表达 (Tabashnik *et al.*, 2013)。生物参数为显性度 (h), 取值范围为 0 (完全隐性) - 1 (完全显性), 当 $h < 0.05$ 时, 即为高剂量表达 (Liu and Tabashnik, 1997; Tabashnik *et al.*, 2013)。EPA 规定: 当转基因抗虫作物杀虫蛋白表达剂量能够杀死至少 99.99% 的敏感个体时, 即为高剂量表达。如果敏感个体

的存活率 $>0.01\%$,该剂量浓度下杂合子个体的存活率会更高,抗性表现为不完全隐性遗传,反而会促进抗性的发展 (Tabashnik *et al.*, 2013)。数学模型表明:当抗性为不完全隐性遗传时,增加庇护所的种植面积,依然能够达到延缓抗性产生的目的。比如:当初始抗性等位基因频率为 0.001,且抗性为单基因调控的完全隐性遗传 ($h=0$) 时,仅需 5%的庇护所就能达到延缓 20 年产生抗性的目的;但是,当抗性为部分显性遗传时 ($h \geq 0.04$),需要 50%的庇护所才能达到相同的效果 (Tabashnik *et al.*, 2008)。

(3) 初始抗性等位基因频率要低。抗性等位基因频率要足够低 (一般 <0.001),才能最大限度的使敏感个体和抗性个体进行交配,抗性基因主要以抗性杂合子的形式存在。杂合子个体不能在高剂量浓度下存活,从而达到稀释抗性基因的目的。数学模型表明:当存在适合度代价时,在抗性等位基因频率较高的情况下,“庇护所”策略依然能够有效发挥作用 (Georghiou and Taylor, 1977; Carrière and Tabashnik, 2001; Pittendrigh *et al.*, 2004; Gould *et al.*, 2006)。比如:当抗性为隐性遗传、存在适合度代价、存在合理面积的庇护区域,“庇护所”策略有效发挥作用的抗性等位基因频率高达 0.3 (Carrière and Tabashnik, 2001)。

(4) 适合度代价。靶标害虫对 Bt 蛋白产生抗性是典型的生物进化现象,是抗性基因被选择的结果。因此,处于 Bt 杀虫蛋白筛选压下的抗性基因型适合度一般比敏感品系要好,但是,在不接触 Bt 杀虫蛋白的情况下,抗性个体的适合度一般较敏感个体低,抗性基因型的这种劣势,被称为“适合度代价”。如此,庇护所区域更有利于敏感个体的生存 (Carrière and Tabashnik, 2001; Gassmann *et al.*, 2009)。

(5) 不完全抗性。如果抗性个体在转基因抗虫作物上的适合度低于常规作物,则这种抗性为不完全抗性 (Carrière and Tabashnik, 2001; Carrière *et al.*, 2010)。

另外,成功实施该防治策略的难点主要表现在两个方面:1. 如何保证庇护所区域敏感个体

的数量,最大限度的实现敏感个体与 Bt 区存活的少量抗性个体的自由交配;2. 如何将庇护所区域的害虫为害降到最低,避免损失过量,最大限度的提高种植者的经济效益。如果庇护所区域的害虫为害造成巨大的经济损失,可能再次导致化学合成药剂的大量使用 (Roush, 1998; Shelton *et al.*, 2000)。

3.2 “多基因”策略

2003 年起,美国和澳大利亚率先商业化种植转 *cry1Ac+cry2Ab2* 基因棉花 (Bollgard II) 防治美洲棉铃虫和棉铃虫 (Brévault *et al.*, 2013)。“多基因”策略就是在同一株植物中同时导入两个或多个基因,用于防治同一种靶标害虫。主要达到:延缓或阻止抗性的产生与发展、提高转基因作物的防治效果、扩大杀虫谱等目的 (Zhao *et al.*, 2005; Carrière *et al.*, 2015, 2016)。该策略要求:

(1) 不同 Bt 杀虫蛋白之间不存在交互抗性。交互抗性是指当害虫对一种选择制剂产生抗性后,对其他从未接触过的制剂同样产生抗性的现象 (Tabashnik *et al.*, 2014)。只有导入的 Bt 杀虫蛋白不存在交互抗性,才能保证当靶标害虫对一种 Bt 杀虫蛋白产生抗性后,其他的 Bt 杀虫蛋白能够继续发挥作用,达到杀死抗性个体的目的 (Zhao *et al.*, 2005; Brévault *et al.*, 2013)。高水平的交互抗性显著降低多价作物的超杀能力;如果害虫种群对 Bt 杀虫蛋白的固有敏感性较低,低水平的交互抗性也会促进抗性的发展 (Carrière *et al.*, 2010, 2015; Brévault *et al.*, 2013; Welch *et al.*, 2015)。了解抗性机理和交互抗性水平,有利于为工程菌的构建和转基因抗虫作物的研发提供优良的杀虫基因组合 (Carrière *et al.*, 2015; Welch *et al.*, 2015)。

(2) 高剂量表达。数学理论模型认为:每种 Bt 杀虫蛋白的剂量表达至少杀死 95%的敏感个体,转双价基因作物才能更好的发挥作用 (Roush, 1998)。当敏感个体的存活率增加时,显著降低多价作物的超杀效果 (Carrière *et al.*, 2015)。

(3) 庇护所。种植一定面积的非 Bt 作物, 为目标害虫的敏感个体提供存活场所。相比“庇护所”策略, 该策略需要的庇护所面积相对较小, 能够大幅度提高种植者的经济效益 (Roush, 1998)。

(4) 避免同时种植转相同或者相似基因的单价基因作物。数学模型、室内试验和温室实验表明: 若同时种植转双价基因作物和相同或者相似基因的单价作物, 会促进抗性的发展, 大大削弱了多基因作物的有效性和持久性 (Zhao *et al.*, 2005; Onstad and Meinke, 2010; Santos-Amaya *et al.*, 2015)。比如: 在田间对转 *cry1F* 玉米产生抗性的草地贪夜蛾种群, 能够很快对转 *cry1A.105 + cry2Ab* 玉米产生抗性 (Santos-Amaya *et al.*, 2015)。

无论是第一代转单价基因作物, 还是第二代转多价基因作物, 庇护所都发挥着至关重要的作用。但是, 如何优化庇护所的空间结构, 一直存在争议。自 1996 年首次商业化种植转基因作物以来, 广泛采用“结构庇护所”模式, 即非 Bt 作物以块状模式种植在 Bt 作物区内或周边 (Carrière *et al.*, 2015, 2016)。从 2010 年开始使用种子混合法 (Refuge-in-a-bag) 延缓靶标害虫对转多价基因抗虫玉米产生抗性, 该方法降低了农民必须合理安排块状庇护所区域的要求 (Head *et al.*, 2014)。但是, 如果幼虫能够在 Bt 植株和非 Bt 植株间自由移动, 降低了敏感个体的存活率和庇护所的有效面积, 提高了杂合子个体的存活率和适合度, 反而会促进抗性的发展 (Heuberger *et al.*, 2011; Ives *et al.*, 2011; Head *et al.*, 2014; Brévault *et al.*, 2015)。

4 我国转基因抗虫玉米的研发和靶标害虫抗性演化与抗性机理研究现状

作为优质的生物燃料和动物饲料, 玉米是我国畜牧养殖业的支柱。病虫害是制约我国玉米生产的主要因素, 其中, 亚洲玉米螟是我国玉米生产中最重要害虫, 在大发生年份, 可导致玉米损失 30% 以上甚至是绝收 (王振营等, 2000)。

亚洲玉米螟为害亦能够加剧玉米镰孢穗腐病的发生, 导致玉米籽粒真菌毒素增加, 降低产品质量, 影响食用安全 (宋立秋等, 2009)。20 世纪 80 年代, 我国就开始了转基因抗虫玉米的研发工作。在国家转基因重大课题的支持下, 已经克隆了一批具有自主知识产权的功能基因、构建了成熟的转基因玉米技术体系、研发出一批性状良好的转基因玉米品种。*cry1Ah* 和 *cry1Ie* 是分别从菌株 BT8 和 Btc007 中分离克隆的、具有自主知识产权的新型杀虫蛋白基因, 其编码的 Cry1Ah 和 Cry1Ie 杀虫蛋白对亚洲玉米螟具有很高的毒力 (Wang *et al.*, 2017; Shabbir *et al.*, 2018)。田间抗性评价结果表明: 转 *cry1Ah* 玉米 (HGK60) 对亚洲玉米螟和棉铃虫具有很强的杀虫活性, 达到高抗级别; 对黏虫有一定的杀虫活性, 表现为抗性级别 (宋苗等, 2016)。转 *cry1Ie* 玉米 (IE034) 不仅能够有效防治亚洲玉米螟的危害, 同时对抗 Cry1Ac 的棉铃虫具有良好的杀虫作用 (Zhang *et al.*, 2013)。转 *cry1Ac* 玉米对亚洲玉米螟有很高的杀虫作用和良好的田间抗螟性 (王培等, 2013; 王月琴等, 2014)。转 *cry1Ac/cry1Ie* 双价抗虫基因玉米对亚洲玉米螟敏感品系、抗 Cry1Ac 品系和抗 Cry1Ie 品系均表现为良好的田间抗螟性 (Jiang *et al.*, 2016)。另外, 开展了多个有关亚洲玉米螟抗性演化和抗性机理的研究工作, 主要包括:

(1) 筛选和保持多个亚洲玉米螟抗性品系。以亚洲玉米螟敏感品系 (ACB-BtS) 为起始虫源, 采用人工饲料混合法进行幼虫全生育期汰选。经过多年的工作, 得到多个抗性品系: 抗 Cry1Ab 品系 ACB-AbR (Resistance ratio, RR > 190) (Zhang *et al.*, 2017) 抗 Cry1Ac 品系 ACB-AcR (RR > 3 000) (Zhang *et al.*, 2017) 抗 Cry1F 品系 ACB-FR (RR > 1754) (Wang *et al.*, 2016) 抗 Cry1Ie 品系 ACB-IeR (RR > 854.5) (Wang *et al.*, 2017) 抗 Cry1Ah 品系 ACB-AhR (RR > 193.7) (Shabbir *et al.*, 2018)。

(2) 评价了亚洲玉米螟抗性品系对其他 Bt 杀虫蛋白的交互抗性。Cry1Ab 和 Cry1Ac 之间存在高水平的交互抗性, 与两种蛋白氨基酸序列的

相似性 (>85%) 密切相关 (Hofte and Whiteley, 1989; Chamber *et al.*, 1991)。CryIIe 同其他 Bt 杀虫蛋白不存在交互抗性 (韩海亮等, 2009; 贺明霞等, 2013; Wang *et al.*, 2017)。CryIAb、CryIAc 同 CryIAh 和 CryIF 存在低水平的交互抗性 (Xu *et al.*, 2010; Wang *et al.*, 2016; Shabbir *et al.*, 2018)。

(3) 探讨了亚洲玉米螟对不同 Bt 杀虫蛋白的抗性遗传规律和抗性机理。ACB-AbR、ACB-FR、ACB-IeR、ACB-AhR 对汰选蛋白的抗性均为常染色体调控、显性度随着浓度的增加而降低, 且为多基因调控 (Zhang *et al.*, 2014; Wang *et al.*, 2016, 2017; Shabbir *et al.*, 2018)。当抗性水平较低时, ACB-AcR 对 CryIAc 的抗性为单基因调控; 当抗性水平增加时, 变为多基因调控 (Zhang *et al.*, 2014)。V-ATPase 亚基 A、热激蛋白 70、碱性磷酸酯酶被鉴定为亚洲玉米螟中肠上的受体蛋白 (徐丽娜, 2010; Jin *et al.*, 2016); ACB-AbR 对 CryIAb 杀虫蛋白的抗性水平上升与杀虫蛋白的降解作用以及细胞本身的修复作用增强有关 (Xu *et al.*, 2015); 亚洲玉米螟类钙黏蛋白的表达水平、MPR 区域突变尤其是缺失突变可能是对 CryIAc 杀虫蛋白产生抗性的原因 (Jin *et al.*, 2014)。

(4) 监测了亚洲玉米螟对 Bt 杀虫蛋白敏感性的时空变化。测定了多个亚洲玉米螟田间种群对 CryIAb、CryIAh、CryIIe 杀虫蛋白的敏感性, 虽然不同地理种群对杀虫蛋白的敏感性存在差异, 但是这种差异在自然差异范围之内, 依然处于敏感水平。以上相关研究为转基因抗虫玉米的商业化种植奠定了较为雄厚的基础。虽然中国政府还没有批准转基因抗虫玉米的商业化种植, 但是, 部分转基因事件已经进入生物安全评价或环境释放安全评价阶段。

总之, 转基因抗虫玉米长时间在世界范围内大面积的推广, 一方面实现了经济效益、环境效益和社会效益的统一, 另一方面也引发了靶标害虫抗性种群的产生。为了延缓抗性的产生, 有必要明确田间抗性发展水平, 积极开展抗性监测和抗性治理措施, 实现转基因玉米的可持续应用。

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