生物安全学报 2014, 23(4): 238-247
JOURNAL OF BIOSAFETY http://www.jbscn.org

DOI: 10.3969/j.issn.2095-1787.2014.04.003

RNAi 生物技术作物环境风险评估研究进展

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摘要: RNA 干涉(RNAi)在昆虫遗传和功能基因研究方面广泛应用。近年来,RNAi 被认为是具有应用潜力的害虫防治新方法。具有良好抗虫性状的 RNAi 生物技术作物已研究成功,预示其商业化应用成为可能。因此,有关 RNAi 作物的生态风险是商业化应用前人们所关心的问题。要建立 RNAi 生物技术作物环境安全评价准则,监管者、受益各方及风险评估者必须要了解 RNAi 理论及其在生物技术领域的应用。科学分析并准确提出 RNAi 作物的生态风险问题,如非期望的基因沉默、靶外结合或脱靶效应、靶标害虫的抗性、小干涉 RNA (siRNA)的环境持久性和不确定性等,并通过研究获得科学数据,将为政府依法监管提供依据。RNAi 生物技术作物的环境风险评估主要包括功能基因及其表达特征(如 dsRNA 序列、长度、表达浓度及沉默效果的持续性等)、杀虫谱及对非靶标生物的影响、环境中的残留问题、功能性状的持续稳定性等。现行的生物技术作物环境风险评估方法和内容需要进一步修改完善,以适应今后 RNAi 生物技术作物的发展和应用。

关键词: RNAi; 生物技术作物; 害虫防治; 环境风险评估

Progress for environmental risk assessments of RNAi-based crops

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Abstract: RNA interference (RNAi) has been widely used for genetic research in insects. Recently, RNAi via ingestion was considered as a potential tool for insect control. Several studies demonstrated that targeted insects can be effectively by the ingestion of RNAi-based insect resistant biotech crops with obvious commercial implications. In the meantime, the environmental risks should be carefully considered before the commercial use of RNAi-based crops. To set up an environmental risk assessment framework for RNAi-based biotech crops, the regulatory agencies and stakeholders as well as risk analysts need to be familiar with the science of RNAi and its application to plant biotechnology. Scientific questions need to be answered about unintended gene silencing (including non-target effects), off-target binding (off-target effects), target resistance, stability and persistence of small interference RNA (siRNA) in various ecosystems, uncertainties, etc. In comparison of information required in an application to a regulatory agency for a transgenic *Bt* crop, data collection for RNAi-based insect resistance biotech crops should include the molecular characterization and expression of dsRNA (i.e. nucleotide sequence, length, the concentration of dsRNA for an optimal silencing and the persistence of the silencing effect), the potential activity spectrum and the impacts on non-targets, the environmental persistence of small RNA molecules, the persistence of the silencing effect and therefore the efficiency of RNAi as an insect control technique. The accepted environmental risk assessment process will need to be adaptable for analysis of RNAi-based biotech crops, which could prompt the development and production. Filling the numerous knowledge gaps surrounding these risks will improve this predictability.

Key words: RNAi; biotech crop; insect pest control; environmental risk assessment

转苏云金芽孢杆菌 *Bacillus thuringiensis* (*Bt*) 杀虫蛋白基因抗虫作物已在世界许多国家大面积应用,以防治鳞翅目和鞘翅目害虫(James, 2014; Vaughn *et al.*, 2005)。*Bt* 杀虫蛋白被靶标昆虫摄入后在中肠酶的作用下,经一系列构型变化,插入中肠细胞的原生质膜,从而形成孔洞,最终杀死害虫

收稿日期(Received): 2014-09-30 **接受日期(Accepted)**: 2014-11-05
基金项目: 转基因作物新品种培育重大专项(2014ZX08011-003)
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(Rajamohan *et al.*,1998)。然而,室内和田间的大量研究结果表明,大面积使用单一和具有相同作用模式的 *Bt* 杀虫蛋白,会导致靶标害虫产生抗性,从而使转 *Bt* 基因抗虫作物防治害虫的功能失效(Tabashnik *et al.*,2008)。因此,害虫防治策略中包含不同杀虫作用机理的转基因作物,对于预防和治理害虫抗性,使转基因抗虫作物在害虫防治中得以可持续利用具有重要意义。

RNA 干涉(RNA interference,RNAi)在基因功能研究方面显示了重要作用(Bucher *et al.*,2002),同时在临床医学(Huvenn & Smagghe,2010)和害虫防治领域(Gatehouse & Price,2011)有巨大的潜力。由于 RNAi 的杀虫机理与 *Bt* 蛋白完全不同,其可作为害虫 *Bt* 抗性治理的又一潜在新途径。有研究表明,基于 RNAi 的转基因抗虫作物可用于害虫防治(Baum *et al.*,2007; Mao *et al.*,2007)。但是,有关 RNAi 转基因抗虫作物的环境安全性也受到科学家的关注。因此,本文就目前 RNAi 生物技术作物及其环境风险评价研究进行简要综述。

1　RNAi 生物技术作物研发进展

1.1　dsRNA 施用方法的研究

在真核生物(包括昆虫)中普遍存在由双链 RNA(double-stranded RNA,dsRNA)引发特异序列的基因沉默现象(Hannon,2002)。在植物中被称之为转录后基因沉默(post-transcriptional gene silencing)(Baulcombe,2004),在动物中则被称之为 RNAi(Hannon,2002)。通过取食或注射大量 dsRNA 引发重要基因沉默,可导致昆虫停止取食,进而死亡。在传统的遗传模式生物中应用 RNAi 开展基因功能研究已有十多年的历史。在昆虫方面也有许多报道,如通过微量注射沉默基因以探索基因功能(Amdam *et al.*,2003; Brown *et al.*,2009; Bucher *et al.*,2002; Suazo *et al.*,2009; Tomoyasu & Denell,2004)。由于基因沉默仅发生于"感染"了 dsRNA 的细胞,选择适宜的施用方法是 RNAi 成功的重要一步。已报道的方法包括注射法(Bettencourt *et al.*,2002; Quan *et al.*,2002)、摄食法(Turner *et al.*,2006)、浸泡法(Aronstein *et al.*,2006; Eaton *et al.*,2002; Rajagopal *et al.*,2002; Timmons & Fire,1998)和基因枪法(Yuen *et al.*,2008)等。与此同时,科学家在积极探讨和研发应用 RNAi 防治害虫的新方法。例如,直接喷雾 dsRNA 后,亚洲玉米螟 *Ostrinia furnacalis*(Guenée)幼虫的死亡率可达到 50%,同时喷雾处理幼虫和人工饲料,死亡率可达 73% ~ 100%(Wang *et al.*,2011);点滴法施用丙酮稀释的 *AaeIAP*1 dsRNA 能杀死埃及伊蚊 *Aedes aegypti*(L.)雌蚊(Pridgeon *et al.*,2008);将几丁聚糖基因 AgCHS1 dsRNA 制成纳米微胶囊喂食非洲疟蚊 *Anopheles gambiae*(Zhang *et al.*,2010)及以表达 dsRNA 的转基因工程菌喂食实蝇等都能取得一定的杀虫效果(Gura,2000; Li *et al.*,2011; Timmons & Fire,1998)。在实验室,喂食 dsRNA 实现 RNAi 具有简单、无需特殊设备(注射法需要精密的微量注射仪)、易操作、经济、省时、无入侵性伤口(相比注射法)等特点,且是客观自然性的摄入,因此实用性强;同时,符合田间应用技术的开发,如 dsRNA 制剂、RNAi 生物技术作物等。

1.2　RNAi 生物技术作物的研发

随着转 *Bt* 基因抗虫作物的应用,主要靶标鳞翅目害虫得到了有效控制(Wu *et al.*,2008),而一些次要害虫如盲蝽、蚜虫、飞虱等刺吸式害虫上升为主要害虫(Lu *et al.*,2010),目前还未发现对这类昆虫有杀虫活性的 *Bt* 杀虫蛋白。因此,RNAi 生物技术作物将为包括这类昆虫在内的害虫防治提供了新途径(Gatehouse & Price,2011)。此外,由于 *Bt* 作物的大面积应用,某些靶标害虫已在一些地区对 *Bt* 作物产生了抗性(Ali *et al.*,2006; Li *et al.*,2004、2007; Matten *et al.*,2008; Tabashnik *et al.*,2008; van Rensburg,2007),使 *Bt* 作物的持续应用受到严重的威胁。研发 RNAi 生物技术作物对害虫 *Bt* 抗性治理具有重要意义。如有研究表明,喂食 dsRNA 沉默小菜蛾 *Plutella xylostella* L.氯菊酯抗性品系过表达的细胞色素 P450 基因 *CYP*6*BG*1,可显著提高其对杀虫剂氯菊酯的敏感性(Bautista *et al.*,2009)。

早期的研究表明,经口摄食的 dsRNA 对不同害虫的作用效果不一致。如对斜纹夜蛾 *Spodoptera litura*(Fabricius)幼虫注射 dsRNA 能引发 RNAi 反应,而摄食 dsRNA 不能引起 RNAi 反应(Rajagopal *et al.*,2002);而苹浅褐卷蛾 *Epiphyas postvittana*(Walker)摄食大量的 dsRNA 能引发目标基因转录水平(mRNA)下降,但未引起死亡(Turner *et al.*,2006)。近年来,许多研究表明,添加了 dsRNA 的人工饲料或表达 dsRNA 的转基因植物均能成功杀

死靶标害虫(Whyard *et al.*,2009),如鞘翅目昆虫玉米根叶甲(WCR) *Diabrotica virgifera virgifera* LeConte、南方玉米根叶甲(SCR) *Diabrotica undecimpunctata howardi* Barber 和马铃薯甲虫 *Leptinotarsa decemlineata* (Say)(Baum *et al.*,2007),鳞翅目昆虫棉铃虫 *Helicoverpa armigera* Hübner(Mao *et al.*,2007)和甜菜夜蛾 *Spodoptera exigua* Hübner(Zhu *et al.*,2012),半翅目昆虫褐飞虱 *Nilaparvata lugens* (Stal)(Zha *et al.*,2011),疾病媒介昆虫采采蝇 *Glossina* 和埃及伊蚊(Coy *et al.*,2012; Walshe *et al.*,2009),以及筑巢昆虫白蚁 *Reticulitermes flavipes* (Kollar)(Zhou *et al.*,2008)等。

1.3 dsRNA 作用机理的研究

dsRNA 随昆虫摄食进入中肠,被中肠细胞"吞食",在 RNase Ⅲ核酶家族的 Dicer 作用下加工成小干涉(si)RNA(21 bp+每条链 3′ 2 个延长碱基)而启动 RNAi 信号通路,即 siRNA 结合到一个被称作 RNA 诱导沉默复合体(RISC)的蛋白复合体上,由 RISC 启动降解特异性的目标 mRNA(Fire *et al.*,1998)。RNA 依赖的 RNA 聚合酶(RdRp)利用 siRNA 为引物、目标基因为模版,合成新的 dsRNA,进而引起 RNAi 效应在虫体内扩散(Price & Gatehouse,2008)。如果目标 mRNA 在昆虫体内编码的是一个基本功能性蛋白,其表达受阻将导致昆虫死亡。在昆虫方面,经口摄食 dsRNA 的 RNAi 系统作用机理已有报道(Bolognesi *et al.*,2012),其整个作用时序过程一般包括:dsRNA 摄食;1 d 后,靶标 mRNA 在中肠和体组织显著下降,此时目标蛋白水平还未受到影响,没有出现死亡;3 d 后,靶标 mRNA 在中肠和体组织持续减少,并在组织中持续蔓延;5 d 后靶标蛋白显著降低;随分子水平靶标基因表达的时序性降低及系统性扩散,生物测定的幼虫表现为生长受到抑制,继而死亡。

在人工饲料中添加目标 dsRNA 饲喂靶标昆虫的致死(或生长显著受抑制)时间在 12 d 以上,125 种 dsRNA 在 52 ng · cm^{-2}剂量下有显著的杀虫活性,其中 14 种 dsRNA 对 WCR 的 $LC_{50} \leqslant 5.20$ ng · cm^{-2},杀虫效果最好的 LC_{50}达到 0.57 ng · cm^{-2}(Baum *et al.*,2007)。以能沉默 WCR β-微管蛋白、V-ATPase A 亚基和 V-ATPase E 亚基同源基因的 dsRNA 饲喂 SCR,具有显著的杀虫活性;以能沉默 WCR V-ATPase A 亚基和 V-ATPase E 亚基同源基因的 dsRNA 饲喂马铃薯甲虫,具有显著的杀虫活性;表达 WCR V-ATPase A 亚基等目标基因的 dsRNA 的转基因玉米能显著降低根的受害(Baum *et al.*,2007)。

2 RNAi 生物技术作物的环境风险

与转 *Bt* 基因生物技术作物相似,RNAi 生物技术作物的环境风险主要包括遗传稳定性、生存竞争能力与杂草性、花粉介导的基因飘逸、潜在的接触途径和生态残留、对非靶标节肢动物(包括天敌及有益昆虫)的影响。基于 RNAi 生物技术作物的特异性,环境风险尤其要考察以下几个方面。

2.1 非期望的基因沉默(unintended gene silencing)

基于 RNAi 的生物技术作物对生活和栖息在其植株、组织器官或残留秸秆等中的非靶标生物可能存在风险,即 RNAi 有时错误沉默了其他生物的基因。据报道,取食了 siRNA 的昆虫由于液泡膜上 ATP 酶看家基因(housekeeping gene)的 mRNA 被切割而使其生长受抑制或死亡(Baum *et al.*,2007)。如果靶标害虫的看家基因和其他非靶标如有益昆虫等的同源性足够高,就有可能引起非期望的基因沉默负效应(Bachman *et al.*,2013)。基因组数据库和科学设计的室内喂食生测试验为确定非期望基因沉默的影响提供了依据。但是,目前许多非靶标生物基因组数据相对短缺。

可遗传的基因突变(即碱基替换、删除、插入)发生在包括作物及其害虫等在内的所有生物中。同时,在种群内个体间存在遗传多态性(即 DNA 序列的微小变异)(Gordon & Waterhouse, 2007; Whangbo & Hunter,2008)。因此,非靶标生物如发生突变,就可能对 RNAi 生物技术作物产生敏感。

2.2 靶外结合(off-target binding)

大量文献报道了 siRNA 引发的各种基因沉默现象(Hammond *et al.*,2001)。由于 RNAi 可能沉默完全错误的基因(Jackson *et al.*, 2003; Jarosch & Moritz,2012; Scacheri *et al.*,2004),siRNA 介导的基因沉默的特异性是一个在 RNAi 应用中必须要考虑的关键因素。研究表明,由于靶标位点单核苷酸错配和 G∶U 间的摇摆配对,可能引起潜在的靶外结合(Saxena *et al.*,2003),导致非靶标内源基因表达的沉默。RNAi 生物技术作物也可能发生变异,改变 siRNA 分子的碱基序列和基因沉默模式,进而产

生靶外结合效应。有学者认为,siRNA 介导的基因沉默的特异性是 siRNA 特异,而不是靶标基因特异,即使 siRNA 和 mRNA 之间的部分互补(仅 11 个连续的核苷酸)可改变非特异性 mRNA 的转录水平,siRNA 也可能交互沉默序列或相似度较低的非靶标 mRNA (Birmingham *et al.*, 2006; Haley & Zamore,2004; Jackson *et al.*,2003)。靶外结合沉默能引起明显表型效应(Fedorov *et al.*,2006; Lin *et al.*,2005),且其主要是由于 siRNA 的 RISC-entering strand 存在一个 4 个碱基的基序 UGCC (Fedorov *et al.*,2006)。

为避免靶外结合引起的沉默效应,简单的预防方法是比对生物基因组数据库是否存在与 siRNA 靶标基因同源的基因。此外,通过化学修饰 siRNA,特别是先导链第 2 位进行 2′-O-甲基核糖取代(Jackson *et al.*,2006),可降低或消除非期望的靶外结合沉默效应。

2.3　靶标害虫的抗性

由于害虫种群内某些个体靶标 mRNA 突变和多态性可能引起其对某一特定的 dsRNA 序列的基因沉默产生抗性,从而导致 RNAi 生物技术作物防治效果下降。目前,有关靶标害虫对于 RNAi 产生抗性的模式缺乏研究。如果这一问题发生,可选择或针对同一基因的其他部位或某一新基因设计一种新的 dsRNA 来治理(Yu *et al.*,2013)。

2.4　siRNA 的环境持久性

实验室土壤微环境中 *Bt* 棉花和 *Bt* 玉米表达的 Cry1Ac 和 Cry1Ab 杀虫蛋白会很快降解,半衰期为 16 d 或更短(Badea *et al.*, 2010; Sims & Holden, 1996; Sims & Ream,1997);在连续种植 Cry1Ac 棉或 Cry1Ab 玉米多年的农田土壤中也未检测到相应 *Bt* 杀虫蛋白的残留或富集(Head *et al.*,2002)。据此,美国环保署发布,连续种植 *Bt* 作物,在土壤中不会出现 *Bt* 杀虫蛋白富集现象(Kough & Edelstein,2012)。有关植物组织或胞外 DNA 在微生态土壤环境中的降解动态已有报道。如转基因和非转基因大豆冷冻干燥叶子 DNA 在土壤中的半衰期仅 1.4 d(Levy-Booth *et al.*,2008);转基因和非转基因玉米和大豆的 DNA 于室温下在土壤渗滤液中的半衰期分别不到 2 和 4 h(Gulden *et al.*,2005)。在一项土壤中添加干燥的 DNA 或 RNA,通过分析氮的释放情况衡量核酸的降解,结果表明,RNA 和 DNA 在各种土壤中的降解速度相同(Keown *et al.*, 2004)。体外转录的纯 WCR 的空泡排序蛋白基因 *DvSnf*7 的 dsRNA 或来自抗 WCR 的转基因玉米残体的 *DvSnf*7 dsRNA 在不同土壤中(包括不同土壤结构、pH、黏性等)的半衰期为 15~28 h,2 d 内检测不到生物活性(Dubelman *et al.*,2014)。由此可见,裸露的核酸会在土壤中很快分解,为界定基于 RNAi 的农业生物技术产品在环境中残留的潜在生态风险提供了依据。

2.5　不确定性

在任何生态风险评估中,认知的不确定性是固有的,评估者应了解不确定性的范围(Suter,2007)。基于蛋白的生物技术作物已商业化种植 20 年,对其风险评估的研究时间可能更长。这不仅明确了外源基因表达的蛋白尤其是 *Bt* 蛋白的杀虫作用模式,而且回答了许多生态风险问题(Conner *et al.*, 2003; Sanvido *et al.*, 2007)。von Krauss *et al.* (2008)对不确定性进行了评估,认为在田间多变的条件下和随时间推移基因沉默表现原理不确定,专家之间对于基因沉默的因果关系看法不尽相同。在风险评估基础上做决策时,监管者和利益相关方需要找到风险和不确定性相关的风险评估之间的平衡点。RNAi 技术整体上呈现低环境风险,但如果了解到这些低风险的高度不确定性,在商业化之前就必须进行大量的试验和管理方法的研究。

3　RNAi 生物技术作物的环境风险评估

RNAi 和 *Bt* 生物技术作物都是通过作物系统表达能启动 RNAi 的 dsRNA 或具有杀虫作用的 *Bt* 杀虫蛋白,而栖息于作物田的生物种类不会因此而改变。因此,暴露于二者的生物种类理论上相同或相似;为使作物整个生育期都能受到保护,目的基因在整个生育期都能表达。但是,RNAi 生物技术作物的靶标对象更多,杀虫剂量阈值较高,致死或生长显著受抑制的时间较长,需要特殊的生测方法测定致死效应。因此,RNAi 与转 *Bt* 基因等生物技术作物的环境风险评估既有相同的框架,亦有不同的内容。

3.1　功能基因及其表达特征

Bt 作物外源目的基因编码 *Bt* 杀虫蛋白,与害虫中肠缘膜上的受体结合使细胞溶解,作用位点在

昆虫中肠上(Bravo *et al.*,2007)。目的基因表达的稳定性通过特定的免疫试纸条进行定性检测,ELISA 进行蛋白定量检测。RNAi 作物外源目的基因编码 20~24 nt siRNA,siRNA 与昆虫体内蛋白形成 RISC,导致靶标 mRNA 降解,引起目的基因沉默(Fire *et al.*,1998; Ghildiyal & Zamore,2009; Obbard *et al.*,2009; Price & Gatehouse,2008)。虽然在模式植物拟南芥 *Arabidopsis* 中已明确多种作用模式,但在多数农作物中还缺乏相应的数据。影响 RNAi 效果的因素主要有以下几个方面。

(1)dsRNA 序列。Terenius *et al.*(2011)系统分析总结了鳞翅目中不同种类昆虫和功能基因与 RNAi 效果的关系。除靶标基因沉默效果外,还与靶外结合和非期望的非靶标生物影响有关。Whyard *et al.*(2009)认为,dsRNA 可作为种特异性的"胃毒"杀虫剂,如利用种特异性的 dsRNA 可分别沉默果蝇 *Drosophila melanogaster* Meigen、赤拟谷盗 *Tribolium castaneum* (Herbst)、桃蚜 *Acyrthosiphon pisum* (Harris)和烟草天蛾 *Manduca sexta* (L.) 4 种害虫的 V-ATP 酶基因,针对靶标 γ 微管蛋白基因 3′ UTR 区(不存在 19~21 nt 序列片段的匹配)设计短(<40 nt)的 dsRNA 也可选择性杀死果蝇属 *Drosophila* 的 4 种果蝇。

(2) dsRNA 长度。不同长度的 dsRNA 引起 RNAi 的效果不同(Whyard *et al.*,2009)。WCR 和马铃薯甲虫的 *DvSnf*7 dsRNA 与目标基因最少有 21 nt 的连续匹配才能有显著的 RNAi 生物活性;对于相似种,*DvSnf*7 同源序列上最少有 3 个 21 nt 的匹配才能在甄别高剂量下产生显著的活性(Bachman *et al.*,2013)。多数喂食试验中获得较好效果的 dsRNA 长度为 300~600 nt。dsRNA 长度影响昆虫细胞系(Saleh *et al.*,2006)以及昆虫(Mao *et al.*,2007)的摄取和沉默效果。研究表明,较长 dsRNA 的沉默效果较好,可能与其在环境中存留时间更长有关(Baum *et al.*,2007)。

(3)dsRNA 浓度。取得最佳沉默效果的 dsRNA 浓度因靶标基因和生物种类而异,并不是 dsRNA 的浓度越高越好(Meyering-Vos & Muller,2007; Shakesby *et al.*,2009)。

(4)沉默效果的持续性。给橘小实蝇 *Bactrocera dorsalis* (Hendel)分别喂食沉默核糖体蛋白 Rpl19、V-ATPaseD 亚基、脂肪酸碳链延长酶 Noa 和一种小型 GTP 酶 Rab11 基因的 4 种 dsRNA *ds-rpl*19、*ds-v-ATP-d*、*ds-noa*、*ds-rab*11 溶液和表达 dsRNA 的转基因大肠杆菌(*Escherichia coli* strain HT115),可使目标基因沉默,引起 20%虫体死亡或雌蝇产卵量下降,然而,当持续饲喂 14 d 后,目标基因的表达反而上调(Li *et al.*,2011)。

(5)靶标生物的发育时期(虫态)。如以唾液腺 nitrophorin 2 的 dsRNA 处理吸血椿象 *Rhodnius prolixus* Stål 2 龄若虫,可达到 42%的基因沉默效果,但处理 4 龄若虫则没有效果(Araujo *et al.*,2006);神经肽类激素(咽侧体抑制素和促咽侧体素)基因 *Spofr/Manse*-AS 和 *Spofr*-AT 2 对秋粘虫 *Spodoptera frugiperda* (Smith)末龄幼虫的沉默效果显著高于雌成虫(Griebler *et al.*,2008)。

3.2 杀虫谱及非靶标生物的影响

RNAi 生物技术作物的作用方式是通过摄食进入生物体内。因此,应该建立农业生态系统中生物多样性数据,明确接触 RNAi 生物技术作物的种类,且建立这些种类的基因组数据库,清楚与 siRNA 具有同源性序列基因的表现型,就能确定 RNAi 生物技术作物的杀虫活性谱。

有关 *Bt* 作物对非靶标生物的影响评价,各国要求有异,根据 *Bt* 蛋白杀虫作用谱的特异性,通常在各生态功能团内选择一定的相关指示性生物进行生物测定和分级启动测试(Romeis *et al.*,2008)。

通过靶外结合和非期望的基因沉默的毒理基因组学分析 RNAi 生物技术作物防治害虫的靶标,可能是分类学上特异性的一种(Whyard *et al.*,2009)。靶标害虫分类学上高度特异性的优点在于仅有靶标害虫的近似种受到 dsRNA 的影响(Romies *et al.*,2008)。因此,要确定某一 dsRNA 的杀虫谱,必须评估其对与靶标害虫亲缘相近种类的潜在影响。

3.3 环境残留

明确生物技术作物外源性状表达产物(无论是杀虫蛋白还是 dsRNA)在环境中的时空分布是生态安全评价的重要内容之一,同时可界定环境中非靶标生物接触的客观途径和剂量。因此,应加强对 sRNA 在环境中的持久性和位移趋势,如基因飘逸、sRNA 在土壤和水体中的存留时间、被非靶标生物摄入的可能性,以及发生系统性基因沉默后的表型

特征等方面的研究。生态毒理学模式生物的基因文库比对和 DNA 芯片法是开展该项评价的重要方法(Robbens *et al.*,2007)。

3.4　功能性状的持续稳定性

在评价 RNAi 生物技术作物功能性状的遗传稳定性时,编码 siRNA 基因比编码蛋白基因的变异率高(Obbard *et al.*,2009)。因此,应分析害虫的抗性发展,评估其发生时间、影响规模(局部的、区域的或全国性的),以及严重程度,并建立预测模型。此外,只有建立统一的 sRNA 活性标准,才能开展可比性评价(von Krauss *et al.*,2008)。

4　结束语

具有植物保护性状的 RNAi 作物是继 *Bt* 作物之后推动害虫防治技术提升的又一重要成就。同时,为研发对重大害虫,如蚜虫(Mutti *et al.*,2006; Whyard *et al.*,2009)、飞虱(Chen *et al.*,2010; Upadhyay *et al.*,2011)、蜻(Araujo *et al.*,2006)具有杀虫谱专一(Alsford *et al.*,2011; Haas & Zody,2010)的新一代生物技术作物提供了广阔的前景。此外,dsRNA 制剂的研发和应用将极大地丰富农作物病虫害防治技术(Burand & Hunter,2013; Huvenne & Smagghe,2010)。如喷施 dsRNA 粗提液可有效控制植物病毒病的发生,且目的 dsRNA 可在叶面存留数天(Tenllado *et al.*,2004)。纳米包裹(Zhang *et al.*,2010)、土壤处理和拌种等方法可用于防治地下害虫。工程菌是大量生产 dsRNA 的重要途径之一(Timmons & Fire,1998)。

现行的转基因生物环境风险评估程序需要进一步完善,以适应新的 RNAi 生物技术作物研发和应用的需求。建立专家意见库,进而准确提出潜在风险问题,以确保收集有价值的科学数据,甄别风险评估中的不确定性;确立科学合理的室内和田间可控条件下的评价试验设计和实施方案;提供有关遗传稳定性、靶外结合(或脱靶效应)、非期望的基因沉默(包括非靶标的影响),以及 sRNA 在各种生境中残留时间的科学评估数据。

生物信息学方法将是风险评估的最基本的方法。因此,需要解决环境监测中 sRNA 的提取和鉴定方法;建立标准化的生态毒理学模式生物的基因文库和 DNA 芯片(Robbens *et al.*,2007);研究 RNAi 作物身份验证、监测等的定量和定性检测方法。从科学的角度来看,具有作物保护性状的 RNAi 作物的环境风险是可预测的,且在科学管理条件下是可避免或可控的。

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