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RNAi与转*Bt*基因技术协同抗虫研究进展

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摘 要： 转基因抗虫作物降低了昆虫的危害并减少了农药的使用，但是转*Bt*（*Bacillus thuringiensis*）基因抗虫作物的大面积连续种植使得靶标害虫对转*Bt*基因植物产生抗性进化，导致其长期抗虫效果受到挑战。为延缓靶标害虫对Bt蛋白的抗性进化，提高转基因作物的抗虫能力，RNA干扰（RNA interference，RNAi）技术与转*Bt*基因技术协同应用于农业害虫防治。本文综述了RNAi技术应用于农业害虫防控的进展，以及与转基因抗虫技术联合防治农业害虫的研究进展，分析其面临的挑战和技术难题，以期支撑RNAi与转基因技术发展成一种绿色环保和可持续发展的害虫综合防控技术。

关键词： 双链RNA；RNA干扰；转基因作物；害虫防治
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Review on the Synergistic Insect-resistant Application of RNAi and *Bt*-transgenic Technologies

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Abstract: The cultivation of transgenic insect-resistance crops has decreased the damage of insects on plants and the application of chemical pesticides in the field. However, the long-term continuous cultivation of *Bt*-transgenic crops worldwide has increased the resistance evolution of target insects to *Bt*-transgenic plants in the field, leading its long-term insect resistance to be in challenge. To delay the resistance evolution of target insects to Bt proteins and to improve the insect-resistance of transgenic crops, RNAi and *Bt*-transgenic biotechnologies have been synergistically applied to control agricultural pests. Here, we review the progresses on RNAi technology applied in agricultural pest-control and on the synergistic application of *Bt*-transgene technology in agricultural pest-control, and discuss the challenges and technological difficulties, aiming to promote the combined application of RNAi and transgenic technologies to be environment-friendly and sustainable in controlling agricultural pests.

Key words: dsRNA; RNA interference; genetically modified crops; pest control

喷施化学农药降低了害虫对经济作物的危害，但同时也带来了环境污染问题[1]。转基因抗虫作物的商业化种植降低了化学农药的使用[2]。现今，转*Bt*基因棉花（*Gossypium hirsutum*）、油菜（*Brassica napus*）和玉米（*Zea mays*）等已在美国、加拿大等国家广泛种植[3]。我国也从1997年开始广泛种植了抗虫转*Bt*基因棉花[3]。随着转*Bt*基因植物在田间大面积的连续种植，使得靶标害虫对Bt毒素产生抗性进化，影响了转*Bt*基因植物的抗虫效果[4]。因此，为延缓靶标害虫的抗性进化，在转基因植物周边种植非转基因植物来建立“庇护所”，或者构建多个基因的转基因抗虫植物[4-5]。此外，也有研究将RNAi技术与转*Bt*基因抗虫技术相结合，在大田喷施双链RNA（double stranded RNA，dsRNA）杀虫剂或者构

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建双价转 dsRNA+*Bt* 基因植物，延缓靶标害虫的抗性进化，同时协同提高作物的抗虫能力[6-7]。因此，本文将首先介绍 RNAi 干扰机制及其在农业害虫防控方面的应用，然后综述了 RNAi 干扰技术协同转基因抗虫技术的研究进展。

1　RNAi 干扰机制

1998 年，Fire 等[8]首次在秀丽隐杆线虫（*Caenorhabditis elegans*）发现 dsRNA 是引发转录后基因沉默的主要原因，并将这种现象定义为 RNAi。RNAi 即当外源 dsRNA 进入宿主细胞后，细胞内的核酸内切酶 Dicer 识别并加工 dsRNA 为 21–25 bp 的 siRNA（small interference RNA，siRNA），siRNA 会与细胞内特异性的酶结合形成 RNA 诱导的沉默复合物（RNA-induced silencing complex，RISC），RISC 与宿主细胞中特定基因的 mRNA 结合，引发目标基因 mRNA 的转录后基因沉默[9-10]。在昆虫中，dsRNA 介导昆虫基因沉默还需要 dsRNA 摄取和系统扩散的蛋白参与[11]。昆虫主要通过两种方式吸收和传递 dsRNA，第一种是跨膜蛋白 SID-1 蛋白（系统 RNAi 缺陷性蛋白）介导的 dsRNA 的细胞间转运[12]；第二种是通过受体介导的细胞内吞作用传递 dsRNA[13-14]。

2　RNAi 技术应用于农业害虫防控

RNAi 技术不仅用于昆虫尤其是非模式昆虫的生殖生长、发育调控、免疫调节和环境应答等过程中关键基因的功能分析，也广泛用于害虫防治的应用研究中。昆虫生长发育中的一些功能基因的干扰常引发昆虫的生长发育的异常，甚至死亡，例如昆虫的几丁质酶基因[15]、*v-ATPase* 基因[16]、蛹期特异性基因[17]、电子传递链蛋白[18-19]和昆虫激素相关基因[6]。这些基因常作为 RNAi 害虫防治的重要靶标。RNAi 已用于多种农业害虫的防治，例如鳞翅目[15]、半翅目[20]、双翅目[21]、鞘翅目[16]、膜翅目和蜚蠊目[22]等昆虫，以及线虫[23]、蜱、螨[24]等的防控研究。使用方法主要有两种：田间直接喷施 dsRNA 杀虫剂和植物介导的 RNAi 抗虫技术。

2.1　喷施dsRNA杀虫剂

将 dsRNA 作为生物农药直接喷施或灌溉常应用于大田害虫的综合治理[25-26]。例如将靶向亚洲玉米螟（*Ostrinia furnacalis*）幼虫发育相关基因的 dsRNA 溶液喷洒给幼虫，喷洒的 dsRNA 能够穿透亚洲玉米螟幼虫的体壁，并在昆虫体腔内循环，致使玉米螟幼虫发育迟缓[26]。San 等[27]在马铃薯（*Solanum tuberosum*）叶片上喷施靶向马铃薯甲虫（*Leptinotarsa decemlineata*）*Actin* 基因的 dsRNA，显著提高了马铃薯甲虫的死亡率。除了将 dsRNA 直接喷洒给昆虫幼虫和植物叶片引发死亡，dsRNA 也可被植物根系吸收，再由植物维管系统运输到各个组织中，引发摄食植物的昆虫死亡[28]。用荧光标记的 ds*EYFP* 溶液浸泡水稻（*Oryza sativa*）根系 24 h 后，水稻鞘和茎部以及取食水稻的稻飞虱（*Nilaparvata lugens*）体内均可检测到 ds*EYFP*[28]。用 ds*Actin* 溶液灌溉水稻或玉米能干扰昆虫 *Actin* 基因表达，显著提高了取食水稻或玉米的稻飞虱和亚洲玉米螟的死亡率[28]。

然而，大田喷施的 dsRNA 能快速降解，制约了 dsRNA 的抗虫效率。Dubelman 等[29]在土壤中施用 ds*DvSnf7* 后发现，dsRNA 会在施用后约 2 d 内降解，并无法检测到生物活性，dsRNA 并未在土壤环境中持续存在或积累。Fischer 等[30]对释放到水环境中的 ds*DvSnf7* 残留时间分析，表明 dsRNA 在淡水环境中也能迅速降解。

为了提高在大田喷施 dsRNA 的抗虫效率，采用微生物传递、脂质体修饰和纳米材料包埋等释放 dsRNA 的新方法[31]。利用微生物传递 dsRNA 时，常将基因工程改造后稳定表达 dsRNA 的微生物施用给昆虫[32]。将干扰东方黏虫（*Mythimna separata*）几丁质酶基因（*Chitinase*，*MseChi*）的 dsRNA 转入大肠杆菌中表达，施用该大肠杆菌能有效防控东方粘虫[15]。利用脂质体包埋也可高效传递 dsRNA，如 Huang 等[33]用脂质体载体包埋的微管蛋白 dsRNA 能有效防控德国小蠊（*Blattella germanica*）。此外，DNA 纳米结构也成为 dsRNA 高效传递的载体[34]。

2.2　转dsRNA植物介导的害虫防治

转 dsRNA 植物介导的害虫防治指利用转基因技术在植物中表达的特异性 dsRNA，并通过昆虫摄食植物引发昆虫相关基因的转录后基因沉默即宿主诱导的基因沉默（host inducing gene silence，HIGS），干扰害虫新陈代谢和生长发育的关键基因[35]。拟

南芥（*Arabidopsis thaliana*）[36]、烟草（*Nicotiana tobacum*）[37]、马铃薯[38]、玉米[39]、小麦（*Triticum aestivum*）[40]、棉花[19]和大豆（*Glycine max*）[39]等多种作物均培育了转 dsRNA 基因抗虫品种。

Mao 等[41]最先通过构建转 dsRNA 基因的拟南芥和烟草植株，干扰棉铃虫 P450 单加氧酶基因：*CYP6AE14* 基因的表达，削弱棉铃虫对棉酚的耐受性，调控棉铃虫幼虫的生长发育。从 290 个候选基因中鉴定出干扰基因 *v-ATPase* 表达最具调控玉米根叶甲（*Diabrotica virgifera virgifera*）幼虫生长的潜力，Baum 等[42]将 ds*v-ATPase A* 基因转入玉米中，能有效防控玉米根叶甲。

植物介导的 RNAi 已成功应用于半翅目蚜科害虫的防治[10, 43]。例如在拟南芥中表达靶向桃蚜（*Myzus persicae*）丝氨酸蛋白酶基因（serine protease，*MySP*）的 dsRNA，显著降低了桃蚜 *MySP* 基因的表达量[44]。将玉米根叶甲 *v-ATPase C* 基因的 dsRNA 转入玉米中，Li 等[45]发现与短链 RNA 相比，长链 dsRNA 能高干扰玉米根叶甲 *v-ATPase C* 基因表达。将靶向棉铃虫几丁质酶基因（*HaCHI*）的 dsRNA 转入烟草和番茄（*Lycopersicon esculentum*）中，致使棉铃虫幼虫发育畸形[46]。以番茄潜叶蛾（*Tuta absoluta*）*v-ATPase A* 和精氨酸激酶（*Arginine kinase*，*AK*）基因为干扰靶点，将针对 *v-ATPase A* 和 *AK* 的 dsRNA 转入番茄叶片中，番茄潜叶蛾幼虫死亡率增加，减少了昆虫取食对番茄叶片的损伤[47]。将针对根结线虫（*Meloidogyne incognita*）的 *16D10* 转入葡萄（*Vitis vinifera*）根系，抑制了根结线虫对葡萄根系的侵染[23]。

转 dsRNA 植物介导的 RNAi 技术优化了 dsRNA 的传递方式，但 dsRNA 易被植物细胞质中的 Dicer 酶降解为小片段，影响长链 dsRNA 的完整性[48]。而且昆虫肠道中的 RNase 也会降解昆虫摄食的 dsRNA，降低 dsRNA 的干扰效率，影响杀虫效果[49]。Bally 等[50-52]提出将 dsRNA 在植物细胞器—叶绿体中表达的方法，保护了转基因植物中 dsRNA 的完整性，提高了 dsRNA 从植物到昆虫的传递效率。

3 靶标害虫对转 *Bt* 基因植物的抗性进化

随着转 *Bt* 基因植物的连续种植，靶标害虫对转 *Bt* 基因植物进化产生抗性[4, 53]。靶标害虫对 Bt 蛋白产生抗性进化的分子机制是：靶标害虫中肠环境中蛋白酶[54-55]的改变和中肠刷状缘膜囊上 Bt 蛋白特异性受体蛋白如类钙黏蛋白[56]、碱性磷酸酶、氨肽酶 N[57]与 ABC 转运蛋白[58]等多种蛋白的突变常引起 Bt 蛋白抗性[59-60]。为减缓靶标害虫对转 *Bt* 基因作物产生抗性进化，主要有以下几种方法：（1）通过串联叠加多种抗虫基因，扩大转基因植物的杀虫谱，例如转 *Bt* 和豇豆胰蛋白酶抑制剂（*Cowpea trypsin inhibitor*，*CPT1*）基因（*Cry1Ac+CPT1*）水稻[61]、转 *Cry1Ah+Cry1Ie* 基因玉米[62]和转融合 *Bt* 与苏云金芽胞杆菌营养期杀虫蛋白（*Vegetative insecticidal protein*，*Vip*）基因（*Cry1Ab+Vip3A*）水稻[63]。然而，由于多种抗虫蛋白存在交互抗性现象，这种串联叠加多种抗虫基因减缓靶标害虫抗性进化还有待深入研究[64]；（2）高剂量表达 Bt 蛋白提高转基因作物对靶标害虫的毒性，使得抗性等位基因不能遗传给下一代[65-66]，如果抗性表现为隐性遗传，高剂量表达能有效延缓靶标害虫的抗性进化。然而，高剂量策略对其他抗性类型的昆虫带来高选择压力，加速其抗性进化；（3）此外，构建非转基因植物“庇护所”的方法能有效延缓害虫抗性进化[4]。

4 RNAi 与转 *Bt* 基因技术协同抗虫

将 RNAi 和转 *Bt* 基因抗虫技术结合研发双价转基因抗虫作物，增加昆虫对 Bt 蛋白的敏感性以提高杀虫效率，成为延缓靶标害虫对 Bt 蛋白产生抗性进化的有效途径之一。例如，孟山都公司研究发现转 ds*DvSnf7* 玉米对抗玉米根叶甲的抑制作用[67]，并培育出转 ds*DvSnf7* 和 *Cry3Bb1* 基因的抗虫转基因玉米的新品种 MON87411 并在市场中推广种植[7, 68]。

首先，RNAi 和 Bt 蛋白能否联合协同抗虫需要找到昆虫生长发育和新陈代谢中的关键基因。胰凝乳蛋白酶（chymotrypsins，CTP）作为鳞翅目昆虫的一种主要的蛋白水解消化酶类，在昆虫体内降解活化的 Bt 毒素、降低 Bt 毒性、诱导抗虫性方面发挥重要作用。通过 RNAi 干扰亚洲玉米螟幼虫中的 7 个 *CTP* 基因，显著提高了 Cry1Ab 蛋白对亚洲玉米螟幼虫的死亡率[69]。有研究表明，抑制甜菜夜蛾几丁质合酶 B 基因（*Chitin synthase B*，*CHS-B*）的表达，

可显著增加其幼虫的死亡率，饲喂 ds*CHS-B* 也提高了 Cry1Ac 和 Cry1Ca 蛋白对甜菜夜蛾幼虫的毒性[70]。影响昆虫激素代谢的关键酶类也成为 RNAi 与转 *Bt* 基因协同抗虫的作用靶标。通过 RNAi 抑制昆虫保幼激素（juvenile hormone，JH）代谢中的保幼激素酸甲基转移酶（*JH acid methyltransferase*，*JHAMT*）和保幼激素结合蛋白（*JH-binding protein*，*JHBP*）基因，培育转 *Bt* 基因和 dsRNA 的双价转基因抗虫棉花显著增加靶标害虫致死率，延缓了靶标害虫对 Bt 蛋白的抗性进化[6]。v-ATPase 是质子泵在昆虫中为新陈代谢供能，RNAi 抑制棉铃虫中 *v-ATPase A* 基因的表达，提高了棉铃虫幼虫对活化的 Cry1Ac 蛋白的敏感性[71]。因此，RNAi 干扰昆虫关键功能基因的表达，提高害虫对 Bt 蛋白的敏感性，提升 Bt 毒素的杀虫效果成为减缓昆虫对 Bt 蛋白抗性的有效方法[6, 69]。

其次，RNAi 和转 *Bt* 基因抗虫技术能否协同抗虫，需要研究 RNAi 干扰昆虫的功能基因是否影响 Bt 毒蛋白对靶标害虫的杀虫效果。向甜菜夜蛾（*Spodoptera exigua*）幼虫体内注射抑制抗菌肽 gloverin—*Seglv* 的 dsRNA，提高了甜菜夜蛾对 Bt 蛋白的敏感性[72]。Ochoa-Campuzano 等[73]在马铃薯甲虫中也有类似发现，抑制马铃薯甲虫幼虫中与 Bt 蛋白互作的肠膜蛋白—prohibitin-1 蛋白表达，幼虫对 Cry3Aa 蛋白的敏感性增强，使得昆虫在培养的第 5 天死亡率达到 100%。由于 *Sl102* 基因在海灰翅夜蛾（*Spodoptera littoralis*）血细胞中高表达，参与细胞的免疫过程，通过大肠杆菌介导的 RNAi 抑制 *Sl102* 基因的表达后，增加了海灰翅夜蛾对 Cry1Ca 蛋白的敏感性[74-75]。通过小菜蛾（*Plutella xylostella*）Bt 蛋白抗性种群和敏感种群进行中肠转录本的表达差异分析，发现 3 个基因：*PxSDF2L1*、*PxCDKAL1* 和 *PxHEL-1* 在抗性品系中显著上调表达，用 RNAi 抑制这 3 种基因的表达，对 Bt 蛋白抗性和敏感小菜蛾幼虫的死亡率都增加，与敏感系相比抗性群体的死亡率上升更为显著[60]。由此可见，由 RNAi 介导的抑制昆虫功能基因可以协同 Bt 蛋白介导的转基因技术用于害虫防治（表 1）。

表 1　RNAi 与转 *Bt* 基因技术协同抗虫

Table 1　Synergistic application of RNAi and *Bt*-transgenic technologies in controlling pests

物种 Species	目标基因 Target gene	*Bt* 蛋白基因 *Bt* gene	联合作效率 Synergistic efficiency	参考文献 Reference
马铃薯甲虫 *Leptinotarsa decemlineata*	*prohibitin-1*	*Cry3Aa*	死亡 Mortality	[56]
海灰翅夜蛾 *Spodoptera littoralis*	*Sl102*	*Cry1Ca*	免疫力下降 Down-regulation of immunocompetence	[57]
亚洲玉米螟 *Ostrinia furnacalis*	*chymotrypsin-like genes*	*Cry1Ab*	死亡率显著升高 Significantly increased mortalities	[60]
甜菜夜蛾 *Spodoptera exigua*	*CHS-B*	*Cry1Ac* 和 *Cry1Ca*	死亡 Mortality	[61]
棉铃虫 *Helicoverpa armigera*	*JHAMT* 和 *JHBP*	*Cry1Ac*	死亡 Mortality	[6]
棉铃虫 *Helicoverpa armigera*	*v-ATPase-A*	*Cry1Ac*	体重抑制率显著升高，对 Cry1Ac 更敏感 Higher weight inhibition，more sensitive to Cry1Ac	[62]

5　展望

RNAi 干扰与转 *Bt* 基因抗虫技术结合不仅有望延缓靶标害虫对 Bt 蛋白的抗性进化，同时 RNAi 抗虫技术具有序列特异性和种属特异性的优势，可防治特定的害虫种类（表 2）。两种生物抗虫技术的结合有望成为一种绿色环保和可持续发展的害虫综合防控技术。

然而，RNAi 干扰与转 *Bt* 基因抗虫技术协同抗虫面临一些挑战。首先，RNAi 技术在应用于抗虫的过程中受到一些因素的制约：（1）RNAi 的干扰效率

表 2 RNAi 技术应用于农业害虫防控

Table 2 RNAi technology applied in controlling agricultural pests

干扰途径 RNAi approach	植物 Plant	昆虫 Insect	目标基因 Target gene	参考文献 Reference
宿主诱导的基因沉默 HIGS	玉米和大豆 *Zea mays* and *Glycine max*	绿盲蝽 *Apolygus lucorum*	*V-ATPase-E*	[39]
	本氏烟 *Nicotiana benthamiana*	桃蚜 *Myzus persicae*	*MpC002 and Rack-1*	[36]
	拟南芥 *Arabidopsis thaliana*	桃蚜 *Myzus persicae*	*MySP*	[44]
	烟草 *Nicotiana tabacum*	烟粉虱 *Bemisia tabaci*	*acetylcholinesterase*（*AChE*） *ecdysone receptor*（*EcR*）	[37]
	小麦 *Triticum aestivum*	麦长管蚜 *Sitobion avenae*	*zinc finger*（*SaZFP*）	[40]
	马铃薯 *Solanum tuberosum*	马铃薯甲虫 *Leptinotarsa decemlineata*	*EcR*	[38]
	拟南芥 *Arabidopsis thaliana* 烟草 *Nicotiana tabacum*	棉铃虫 *Helicoverpa armigera*	*CYP6AE14*	[41]
	拟南芥 *Arabidopsis thaliana*	棉铃虫 *Helicoverpa armigera*	*mitochondrial complex I*（*NDUFV2*）	[19]
饲喂法 Dietary feeding		豌豆蚜 *Acyrthosiphon pisum*	*ApAQP1*	[76]
显微注射法 Microinjection		黄曲条跳甲 *Phyllotreta striolata*	*odorant receptor*（*PsOr1*）	[77]
		德国小蠊 *Blattella germanica*	*RXR*	[22]
微生物介导 Bacterial expression and oral delivery		粘虫 *Mythimna separata*	*MseChi*	[15]
		亚洲玉米螟 *Ostrinia furnacalis*	*Glutathione-S-Transferase*	[78]

受到昆虫对 dsRNA 的传递吸收效率、剂量效应、目的基因组织特异性的影响，同时农业害虫的生命周期和摄食行为也是干扰 RNAi 效率的重要因素[79-81]；（2）dsRNA 不但引发靶基因的转录后基因沉默，还可能引发非靶标基因的转录后基因沉默而产生脱靶效应[82]；（3）针对靶标害虫特异性的 dsRNA 可能影响非靶标害虫的种群动态[83]。

其次，将 RNAi 技术与转 *Bt* 基因抗虫技术联合用于大田面临一些技术难题。例如昆虫的一些重要功能基因是否都可用 RNAi 技术实现高效基因沉默？害虫摄取的 RNAi 是否会被其序列多态性所规避[84]？由于害虫对 Bt 蛋白存在着抗性进化，转 dsRNA 和 *Bt* 基因的双价转基因抗虫植物是否也会在田间连续种植栽培中对 dsRNA 产生抗性而降低其抗虫效果[85]？在 RNAi 和转 *Bt* 基因联合作用时，dsRNA 与 Bt 蛋白二者之间作用的独立效应已得到证明，但两种方式的独立作用是否与 RNAi 选择的靶基因存在交互影响还有待深入研究[7]。

此外，基于转 dsRNA 和 *Bt* 基因双价转基因植物释放的环境安全性和食品安全性的评估目前仍不充分，缺乏对双价或多价转基因植物的评估标准。

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