

Potential resistance management for the sustainable use of insect-resistant genetically modified corn and rice in China

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Many lines of insect-resistant genetically modified (IRGM) corn and rice containing *Bacillus thuringiensis* (Bt) insecticidal genes have been developed and undergone different environmental biosafety assessments stages in China, showing robust application prospects. The potential of targeted pests to develop resistance to Bt crops is widespread, which threatens the sustainable utility of IRGM corn and rice. In this study, the potential risks of target pest complexes developing resistance to IRGM corn and rice are evaluated. Theoretical and empirical studies implementing precautionary insect resistance management (IRM) strategies to delay resistance evolution are summarized and challenges to IRM are discussed. Additionally, solutions facing these challenges are proposed. Finally, directions for future studies in developing IRGM corn and rice and IRM plans are discussed.

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Introduction

Corn and rice are important crops in China. Their planting areas are 35.0 and 30.1 million hectares, respectively [1]. Nonetheless, due to the increasing size of the Chinese population, grain yields need to be augmented to meet the increased demand. Insect pests are an important constraint on crop production [2,3]. Traditionally, intensive chemical control methods in China have been used to control pests, but this approach has repeatedly led to the evolution of pesticide resistance [4]. Insect-resistant gene modified (IRGM) crops containing *Bacillus thuringiensis* (Bt) insecticidal proteins have been introduced as an alternative for controlling target pests [5]. In China, many transgenic corn and rice lines have been transformed with

Bt genes, which are highly effective against rice and corn pest complexes [6–8] and have shown robust prospects for commercial applications. However, the potential risk of targeted pests developing resistance is a key consideration influencing IRGM corn and rice regulation and sustainable use after widespread adoption. Therefore, predicting the likelihood of resistance development and implementing precautionary insect resistance management (IRM) strategies for the sustainable use of IRGM corn and rice are critical. Robust IRM strategies are expected to delay insect resistance evolution, prolong IRGM crop life, and benefit the public [9]. During practical use, IRM implementation depends on several factors and faces many challenges. In this paper, we review the development of IRGM corn and rice, explore potential resistance risks of target pest complexes and IRM strategies, discuss challenges to IRM, and propose future research directions.

Development of IRGM corn and rice and control efficacy against target pest complexes

In the main corn-growing areas of China, *Ostrinia furnacalis*, *Helicoverpa armigera* and *Mythimna separata* are the major corn pests and usually cause over 10–20% yield loss per year [6]. Four major lepidopteran pests, *Chilo suppressalis*, *Scirpophaga incertulas*, *Sesamia inferens* and *Cnaphalocrocis medinalis*, are widely distributed in the main rice-planting areas of China and cause serious damage to rice resulting in economic losses of approximately US\$ 992 million annually [10]. More than six IRGM corn and eight IRGM rice lines containing Bt insecticidal genes have been developed in China to control these pests [11–17,18**]. Among these IRGM corn lines, some contain single *Bt* genes, such as *cryIIe* in event IE09S034, *cryIAh* in G186 and codon-optimized *cryIAc* in Bt-799, which exhibit high efficacy for controlling *O. furnacalis* and *H. armigera* under laboratory and field conditions [15–17]. The combinations of *cryIIe* and *cryIAh* or *cryIAb* and *cry2Aj* genes in transgenic corn have been suggested to confer full protection against these pests [12–14]. The use of multiple Bt genes stacked with the *epsps* or the *G10evo-epsps* genes in G6H or in Shuangkang 12-5 events provides not only higher insect-resistance to rice or corn pest complexes but also higher herbicide-tolerance to glyphosate [14,19]. IRGM rice lines transformed with a single insect-resistance gene (KMD, Huahui1, Bt-Shanyou 63, T1C-19, and mfb-MH 86) show a broad spectrum of

insect-resistance to rice pest complexes [7,20–23]. Combinations of *cry1Ac* and *CpTI* (cowpea trypsin inhibitor) or *cry1Ab* and *vip3H* not only provide high resistance to rice pests but also delay resistance evolution [19,24,25]. The *cry2A* gene is also a candidate for use in stacked rice lines combined with *cry1A*, *cry1C* or *cry9C* genes, although lines transformed with *cry2A* exhibit only medium resistance to rice pests [26].

Of the developed IRGM corn and rice lines, only Huahui 1 and *Bt* Shanyou 63 rice lines were approved for commercial use in Hubei Province in 2009 (<http://www.isaaa.org/gmapprovaldatabase/commercialtrait/default.asp?TraitTypeID=2&Trait=Insect%20Resistance>). Other transgenic lines have entered different phases of environmental safety assessments, with robust prospects for commercial use.

Resistance risk assessment and resistance mechanism of target pests to IRGM corn and rice

Though GM corn and rice are not commercially used in China, the baseline susceptibilities of target pests to Bt proteins produced by these crops have been surveyed. The Cry1Ab LC50 values of 10 populations of *O. furnacalis* from major corn-growing regions in China ranged from 0.10 to 0.81 µg/g (Cry1Ab protein/diet), showing only small variations in the susceptibility of *O. furnacalis* to Cry1Ab across its range in China [27]. Surveys conducted in 2002 and 2010 of over 10 distinct field populations across major rice-growing areas demonstrated significant variations in the susceptibility of *C. suppressalis* to both Cry1Ab and Cry1Ac [28*,29*,30**]. Similarly, interpopulation variation in susceptibility to Cry1Ac has been observed in the Fuzhou *C. suppressalis* population [24]. Moreover, 10 different *C. medinalis* populations exhibited a wide range of susceptibility, with the relative susceptibility ratio between the most susceptible and tolerant populations being 50-fold for Cry1Ac and 30-fold for Cry1Ab [31]. These interpopulation differences in susceptibility support the existence of the genetic diversity necessary for evolution of resistance.

Furthermore, populations of *S. inferens* were observed to display higher tolerance to Cry1A proteins compared with *C. suppressalis*, inferring that *S. inferens* may have a high potential to evolve resistance to Cry1A toxins [29*,30**].

Laboratory selection data also support the potential for developing resistance in corn and rice pests. In the case of *O. furnacalis*, laboratory selection of colonies for over 71 generations increased the LC50 values for Cry1Ab and Cry1Ac by as much as 39.7- and 78.8-fold, respectively [32,33*]. For rice stem borers, *C. suppressalis* developed 34-fold resistance to Cry1Ab and 28.3-fold resistance to Cry1Ca after continuous selection for 58 and 19 generations, respectively (Han *et al.*, unpublished). After

21 generations of selection in *C. suppressalis* and 8 generations in *S. inferens*, the LC50 of Cry1Ac increased by 8.4- and 4.4-fold, respectively, and the estimated realized heritability (h^2) for Cry1Ac tolerance was 0.11 for *C. suppressalis* and 0.292 for *S. inferens* [29*]. In Cry1Ab-resistant *O. furnacalis*, there were high levels of cross-resistance to Cry1Ac (36-fold) and Cry1Ah (131-fold), and minor cross-resistance to Cry1Fa (6-fold). However, no cross-resistance was observed between Cry1Ie and Cry1Ab or Cry1Ac proteins [32,33*,34**]. In agreement with this resistance phenotype, and alternative Cry1Ac-selected strain of *C. suppressalis* exhibited cross-resistance to Cry1Ab but no cross-resistance to Cry1Ca or Cry2Aa (Han *et al.*, unpublished). These patterns showed that Cry1Ie (for control of *O. furnacalis*) and both Cry1Ca and Cry2Aa (for control of rice stem borers) are ideal candidate genes for the pyramided IRGM corn or rice with *cry1A* gene [32,33*,34**].

The mechanisms of resistance and the mode of action of Bt proteins in target pest complexes have been studied to design IRM strategies. Resistance to Cry1A is associated with the transcriptional down-regulation of a cadherin-like protein gene in *O. furnacalis* [35], which has been confirmed in subsequent transcriptome analyses comparing Cry1Ab-resistant and susceptible *O. furnacalis* strains [36**]. Several micro RNAs (miRNAs) targeting aminopeptidase N and a cadherin-like protein, both potential Bt receptor genes, showed significant differential expression between susceptible and Cry1Ab-resistant *O. furnacalis* strains [37**]. Data from proteomic analyses, heterologous protein expression in insect cell cultures, ligand binding and RNA inference [38–40] have suggested functional roles for aminopeptidase N and cadherin-like protein in the action of Cry1A in *C. suppressalis* and *M. separata*. Additionally, the lower susceptibility of *S. inferens* to Cry1A proteins relative to *C. suppressalis* was confirmed to be associated with reduced Cry1A-binding as a result of decreased binding site concentration [30**]. In conclusion, reduced binding of Cry proteins to membrane target sites has been identified as a primary resistance mechanism in these lepidopteran insects and thus toxins that do not share binding sites, such as Cry1Ie or Cry1Ca with Cry1A toxins, are optimal candidates for gene pyramiding in IRM plans.

Insect resistance management (IRM) and challenges to IRM

The high-dose plus refuge strategy is currently the most widely used approach for Bt crop IRM [9]. The optimal size and form of refuges and their distance to Bt fields depend on population size, feeding habits and the dispersal abilities of pests. These factors are critical for the successful implementation of IRM [41,42]. 120 For instance, successful practical experience of IRM for *Ostrinia nubilalis* in the USA showed that 20% non-Bt corn refuge must be planted in the US Corn Belt, and 50% of these

refuges must be planted within 2 miles of cotton-growing areas (≤ 0.25 miles is preferred) to mitigate insect resistance [43]. In addition, IRGM corn crops expressing high doses of insecticidal protein are preferred for release. For example, due to the differential susceptibility of corn pest complexes, IRGM corn may not provide high dose against tolerant *Helicoverpa zea*. Therefore, management options, such as increasing refuge size, increasing the use of alternative hosts, and limiting the total acreage, are crucial for effective IRM in these cases [43]. The management tactics used for *O. nubilalis* can be used as a reference for IRM in *O. furnacalis* in China due to the similar biology of the two species. However, the refuge strategy may be difficult to implement in China because of challenges associated with coordinating small farms. As discussed above, no cross-resistance or sharing of receptors have been observed between Cry1Ie and Cry1Ab or Cry1Ac proteins in *O. furnacalis*, supporting their pyramided use in IRGM corn. Natural refuges may be a good option for IRM of *M. separata* since it is a migrant and polyphagous pest of graminaceous crops such as sorghum and millet [44].

Based on our current knowledge of rice stem borer biology, maintaining a separate non-Bt field within 1 km of a Bt rice field would provide a suitable form of refuge [45]. Considering a 500:1 ratio of susceptible-to-resistant insects as a suitable mating proportion in the field [46], our cage tests in Bt and non-Bt fields suggest that the optimal percentages of refuge without pesticide spray are 10% for IRM of *C. suppressalis* and 2% for *C. medinalis* (Han *et al.*, unpublished). Similar to the IRM for corn pest complexes, IRGM rice may not provide a high dose against *S. inferens* with low susceptibility to Bt protein [29^{*},30^{**}]. Therefore, suitable management options for refuges are required to ensure the effective implementation of IRM tactics [43]. Based on previous studies, pyramiding of *cry1A* and *cry1Ca* or *cry2Aa* genes in IRGM rice is an optimal strategy for increasing control of *S. inferens* and reducing resistance evolution [30^{**}]. Moreover, Manchurian wildrice (*Zizania latifolia*) and chufa (*Cyperus esculentus*), which are non-rice host plants, could also be used as a natural refuge for rice stem borer [47,48].

IRM strategies for IRGM corn or rice will inevitably face many challenges because the successful implementation of IRM depends on many factors. For instance, the high-dose/refuge strategy is especially effective in delaying resistance evolution when the initial resistance allele frequency in the pest population is low. Therefore, the initial resistance frequency in field populations of target pests should be evaluated before IRGM corn or rice is commercially used. Furthermore, as an added measure to provide valuable and early warning information for the government and farmers, effective resistance monitoring programs should be performed, as soon as IRGM corn or rice is commercially cultivated. An additional issue for

IRGM crops is the exposure of polyphagous pests to multiple toxins in multiple crops. For instance, *H. armigera* infests both corn and cotton. If IRGM corn with the *cry1A* gene is used commercially, a key refuge for *H. armigera* will be lost and resistance to commercially planted Bt cotton (*cry1Ac* gene) may evolve more rapidly, which severely challenges the development of IRGM corn and corn pest management strategies [49]. Rice stem borers can feed on *Z. latifolia* and chufa in addition to rice [47], yet planting areas for these crops are small in China, and alternative host plants as natural refuges are limited in most rice-growing regions [48], which also influences the efficiency of IRM. Notably, single-gene and pyramided IRGM corn and rice lines have been simultaneously developed in China and have entered environmental biosafety assessment, which may result in the concurrent use of these IRGM lines in fields. Previous reports have shown that the concurrent use of transgenic plants expressing both one and two Bt genes will select for resistance to two-gene plants more rapidly than the use of only plants expressing two Bt proteins [50].

There are four major target pests of rice and three main target pests of corn. These pests are biologically diverse, have different peak outbreak periods and display differential susceptibilities to Bt proteins in IRGM lines. These factors may further challenge Chinese IRM strategies for IRGM rice and corn. To address these challenges, the following measures should be taken to promote the sustainable use of IRGM corn and rice. First, the regulation of IRGM corn and rice planting should be strengthened and varieties lacking production of high doses of insecticidal protein should not be released. The timely development and application of pyramided IRGM corn and rice and certification for their commercial use should be approved by planned staging. Additionally, the implementation of resistance monitoring programs would provide early warning information for governments and farmers. Further, researches on mechanisms and genes involved in insect resistance are crucial to advance monitoring programs. Finally, the development of educational programs related to agricultural biotechnology that provide a basic understanding of IPM and IRM to corn and rice farmers will contribute substantially to achieving the sustainable use of IRGM crops.

Future directions and research needs

Other than rice stem borers, planthoppers are also major rice pests in Chinese rice ecosystems; the damage caused by planthoppers is equal or even higher than that caused by rice stem borers [51]. Because the majority of first-generation IRGM rice lines targeted stem borers, increased attention should be given to identifying new insecticidal genes with different modes of action to target planthoppers [51]. Emphasis should also be placed on the development of IRGM corn or rice lines with stacked traits to ameliorate the constraints on corn and rice yields

in the field. In addition, the development of novel IRM tactics and techniques for IRGM corn or rice with stacked new traits is important for their sustainable use.

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