Review

Development of Bt Rice and Bt Maize in China and Their Efficacy in Target Pest Control

Qingsong Liu 1, Eric Hallerman 2, Yufa Peng 1 and Yunhe Li 1,∗

1 State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China; qingsongliu00@gmail.com (Q.L.); yfpeng@ippcaas.cn (Y.P.)
2 Department of Fish and Wildlife Conservation, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0321, USA; ehallerm@vt.edu
∗ Correspondence: liyunhe@caas.cn; Tel.: +86-10-6281-5947; Fax: +86-10-6289-6114

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Abstract: Rice and maize are important cereal crops that serve as staple foods, feed, and industrial material in China. Multiple factors constrain the production of both crops, among which insect pests are an important one. Lepidopteran pests cause enormous yield losses for the crops annually. In order to control these pests, China plays an active role in development and application of genetic engineering (GE) to crops, and dozens of GE rice and GE maize lines expressing insecticidal proteins from the soil bacterium Bacillus thuringiensis (Bt) have been developed. Many lines have entered environmental release, field testing, and preproduction testing, and laboratory and field experiments have shown that most of the Bt rice and Bt maize lines developed in China exhibited effective control of major target lepidopteran pests on rice (Chilo suppressalis, Scirpophaga incertulas, and Cnaphalocrocis medinalis) and maize (Ostrinia furnacalis), demonstrating bright prospects for application. However, none of these Bt lines has yet been commercially planted through this writing in 2016. Challenges and perspectives for development and application of Bt rice and maize in China are discussed. This article provides a general context for colleagues to learn about research and development of Bt crops in China, and may shed light on future work in this field.

Keywords: Bacillus thuringiensis; Cry proteins; target insects; commercialization; ELISA

1. Introduction

Rice (Oryza sativa L.) and maize (corn) (Zea mays L.) are important cereal crops in China. Rice serves as a staple food for more than half of the world’s people [1], and maize serves as food, feed, and industrial material [2,3]. The yields of rice and maize have increased significantly since the adoption of high-yielding selectively bred and hybrid varieties [4], and have reached nearly 208 and 215 million tons in 2014, respectively, in China [5]. However, with the growth of China’s population and the steady decrease in the amount of arable land, the yield of the two crops must increase to meet the increasing demand [1,6]. Multiple factors constrain the production of rice and maize, among which insect pests are an important one. The major insect pests on the two crops are lepidopterans. On rice, four major lepidopteran pests—rice striped stem borer Chilo suppressalis (Family Crambidae), yellow stem borer Scirpophaga incertulas (Family Crambidae), pink stem borer Sesamia inferens (Family Noctuidae) and rice leaf roller Cnaphalocrocis medinalis (Family Crambidae)—cause severe yield losses annually [7]. It has been estimated that the rice stem borers cause an annual 3.1% loss of yield nationally, equivalent to an economic loss of $US 1.9 billion each year in China [8]. The major lepidopteran pests on maize are Ostrinia furnacalis (Family Crambidae), Mythimna separata (Family Noctuidae), and Helicoverpa armigera (Family Noctuidae), causing 10% of yield loss in spring maize, 20%–30% in summer maize,
and over 30% with heavy infestations, resulting in a huge economic loss every year [3,9]. Aside from the direct yield losses, maize infestation by lepidopteran pests may result in the production of fumonisins, mycotoxins that lower the quality of maize and may pose negative effects on livestock [3]. Multiple strategies have been developed to control rice and maize pests, with chemical insecticide application as the main measure [9,10]. However, the application of chemical insecticides has brought a series of problems, such as air, water, and soil pollution, food contamination, the resurgence of resistant herbivores, and reduction of populations of natural enemies of the crop pests.

Genetic engineering (GE) technology provides a powerful and clean tool for insect pest control. Since the first commercialization in the United States in 1996, GE crops have been widely and rapidly adopted worldwide [11]. Among the GE crops in commercial production, those expressing insecticidal proteins from the soil bacterium Bacillus thuringiensis (Bt), generally called Bt crops, are an important subset. To improve agricultural productivity, China has played an active role in the development and application of GE crops since the 1980s [3,12,13]. A huge research project, called the National GMO New Variety Breeding Program, was initiated in China in 2008, which is expected to invest $US 3.5 billion through 2020 [12,13]. With the massive financial support, great progress has been made in research and development of GE crops, and a large number of GE crop events and varieties have been obtained in China, with traits including herbicide tolerance, insect resistance, drought resistance, stress tolerance, heightened quality and high yield [1,3,14]. In particular, dozens of Bt rice and Bt maize lines have been developed, and many have entered environmental release field testing, and preproduction testing [13]. Critically, two Bt rice lines, Huahui 1 and Bt Shanyou 63, have obtained biosafety certificates for commercial production, although they have not been grown commercially to date.

In the current article, we summarize the development of Bt rice and Bt maize and analyze the expression levels of Cry protein and their efficacy in target pest control. Meanwhile, the prospects of commercialization of Bt rice and Bt maize in China are discussed, with the objective of providing a general sense of research and development of Bt crops in China.

2. Bt Rice

2.1. Bt Rice Lines Developed

The first insect-resistant genetically engineered (IRGE) rice line expressing a Bt delta-endotoxin gene driven by the CaMV 35S promoter was developed in 1989 [15], and so far dozens of Bt rice lines have been produced in China. Most of the Bt rice lines were developed by public-sector scientists from Huazhong Agricultural University, Fujian Academy of Agricultural Sciences, Chinese Academy of Sciences, Chinese Academy of Agricultural Sciences, and Zhejiang University. These Bt rice lines can be divided into three categories, namely: (i) lines containing a single Bt gene, such as cry1Ab in the Kemingdao (KMD) and mfb-MH86 lines; cry1Ac in AC-1, E10, and E54; cry1C in T1C-19, and C-54; cry2A in T2A-1, T2A-2, T2A-3, and T2A-4; and cry9C in 9C-1, 9C-2, 9C-3, 9C-4, and 9C-5; (ii) containing a fusion Bt gene, such as the cry1Ab/1Ac fusion gene in TT51-1 (Huahui 1), TT9-3, and Bt Shanyou 63; and the cry1Ab/vip3H gene in G6H-1, G6H-2, G6H-3, G6H-4, G6H-5, and G6H-6; and (iii) containing stacked insecticidal genes such as cry1Ac and modified CpTI (cowpea trypsin inhibitor) in MSA, MSB, and Kefeng6 (Table 1). In addition, some Bt rice lines were stacked with other types of transgenes, such as bar for herbicide tolerance, and Xa21 for disease resistance (Table 1). In the development of Bt rice lines, China made great efforts for independent innovation, and also took an active part in international cooperation. For example, KMD was developed by Zhejiang University in collaboration with the University of Ottawa, and Huahui 1 and Bt Shanyou 63 were developed by Huazhong Agricultural University in collaboration with the International Rice Research Institute [16]. Agrobacterium- and gene gun-mediated transformations are commonly used for Bt rice development, and the promoters used for driving the expression of Bt genes include ubiquitin, rice rbcS (small subunit of ribulose-1, 5-bisphosphate carboxylase/oxygenase) promoter, and Actin1 (Table 1).
Table 1. Insect-resistant Bt rice lines and their efficacy on target lepidopteran pests in China.

| Insecticidal Proteins | Plant Lines | Promoter; Method of Transformation | Parental Line or Cultivar | Expression Level of Bt Protein | % Efficacy on Target Pests | References |
|-----------------------|-------------|------------------------------------|---------------------------|-------------------------------|-----------------------------|------------|
| **Cry1Ab**            | KMD1        | Ubiquitin; Agrobacterium-mediated  | Xiushui 11 (japonica)     | 3.74–7.50 µg/g in stems FW; 3.78–9.13 µg/g in leaves FW; 12.78 µg/g in pollen DW | 100% for 1st- or 3rd-instar larvae of 8 lepidopteran species; 78% (4th-instar), and 68% (5th-instar) for C. medinalis | 100% for C. suppressalis, S. incertulas and C. medinalis [17–21] |
|                       | KMD2        | Ubiquitin; Agrobacterium-mediated  | Xiushui 11 (japonica)     | 4.32–8.84 µg/g in stems FW; 3.97–8.29 µg/g in leaves FW; 31.37 µg/g in pollen DW | 100% for C. suppressalis | 100% for C. suppressalis [17,18,21] |
|                       | mfb-MH86    | Ubiquitin; Agrobacterium-mediated  | Minghui 86 (indica)       | 9.71–34.09 µg/g in leaves DW; 7.66–18.51 µg/g in stems DW; 1.95–13.40 µg/g in roots DW | 100% for C. suppressalis | - [22] |
|                       | T1Ab-10     | Ubiquitin; Agrobacterium-mediated  | Minghui 63 (indica)       | 7.54 µg/g in leaves FW | - | 100% for C. medinalis, 98.2%–100% for C. suppressalis, 98.9%–100% for S. incertulas [23] |
|                       | DL (hybrid) | -                                  | Zhejing22 (japonica)      | 1.66–3.31 µg/g in leaves FW; 0.11–0.17 µg/g in seeds FW | - | 91.7%–100% for C. suppressalis; 97.5%–100% for C. suppressalis [25,26] |
|                       | -           | Actin1; Gene gun-mediated          | Zhongguo 91 (japonica)    | - | 100% for C. suppressalis | - [27] |
|                       | -           | Ubiquitin; Agrobacterium-mediated  | Zhongguo 91 (japonica)    | - | - | >99% for C. suppressalis [28,29] |
|                       | Ac-1, Ac-2  | Ubiquitin; Agrobacterium-mediated  | Xiushui 11 (japonica)     | 11.09 (Ac-1), and 14.48 (Ac-2) µg/g in leaves FW | 100% for C. suppressalis, S. incertulas, C. medinalis, and Psara licarisalis | - [30] |
|                       | P6, H7      | Ubiquitin; Agrobacterium-mediated  | Guanglingxiangjing (japonica) | 0.025%–0.10% in leaves | 100% for 2nd-instar C. suppressalis and C. medinalis | 100% for C. medinalis [32] |
|                       | E10, E19    | Ubiquitin; Agrobacterium-mediated  | Wuxiangjing9 (japonica)   | 0.025%–0.10% in leaves | 100% for 2nd-instar C. suppressalis and C. medinalis | 100% for C. medinalis [32] |
| Insecticidal Proteins | Plant Lines | Promoter; Method of Transformation | Parental Line or Cultivar | Expression Level of Bt Protein* | % Efficacy on Target Pests |
|-----------------------|-------------|-----------------------------------|-------------------------|--------------------------------|--------------------------|
|                       |             |                                   |                         | In Laboratory | In Field                | References               |
| Cry1C                 | T1C-19      | Ubiquitin; Agrobacterium-mediated | Minghui 63 (indica)     | Up to 3.65 µg/g in leaves DW | 85%–100% for C. suppressalis | 94.8%–100% for C. medinalis; 99.98%–100% for C. suppressalis | [33–37] |
| RJ-5                 |             | Rice rbcS promoter; Agrobacterium-mediated | Zhonghua 11 (japonica) | 0.87 µg/g in leaves FW; 0.0026 µg/g in endosperm FW | - | 97.9% for stem borers, and 99.4% for leaf folders | [38] |
| C-6                  |             | Ubiquitin; Agrobacterium-mediated | Hanhui 3 (indica)       | 0.46–2.11 µg/g in leaves FW | - | 100% for C. medinalis | [39] |
| C-S4                 |             | Rice rbcS promoter; Agrobacterium-mediated | Jili 518 (japonica)     | 2.27 µg/g in leaves FW | - | 97.1% for C. suppressalis | [40] |
| Cry2A                 | T2A-1, T2A-2, T2A-3, T2A-4 | Ubiquitin; Agrobacterium-mediated | Minghui 63 (indica)     | 9.65–12.11 µg/g in leaves FW | 100% for S. incertulas | 92.5%–94.6% for S. incertulas; 95.8%–99.0% for C. medinalis | [41] |
| T2A1                 |             | Ubiquitin; Agrobacterium-mediated | Minghui 63 (indica)     | Up to 87.25 µg/g in leaves DW; 33.5 µg/g in pollen DW | 55.6%–100% for C. suppressalis; 64.68% (1st-instar), and 64.92% (3rd-instar) for C. medinalis | 95.7%–100% for C. medinalis; 99.9%–100% for C. suppressalis | [34,35,37,42] |
| 2A-1, 2A-2, 2A-3     |             | Ubiquitin; Agrobacterium-mediated | Minghui 63 (indica)     | 109.35–138.75 µg/g in leaves FW | 100% for S. incertulas | 84.6%–91.7% for C. suppressalis | [31] |
| B2A68                |             | Ubiquitin; Agrobacterium-mediated | D68 (indica)            | 10.45–26.84 µg/g in leaves FW | 100% for C. suppressalis | - | [43] |
| Cry9C                | 9C-1, 9C-2, 9C-3, 9C-4, 9C-5 | Ubiquitin; Agrobacterium-mediated | Minghui 63 (indica)     | 655.46, 324.55, 166.63, 365.07, and 182.61 µg/g in leaves FW, respectively | 100% for S. incertulas | 100%, 100%, 91.3%, 96.2%, and 91.7% for C. suppressalis, respectively | [31] |
| T51-1 (Huahui 1)     |             | Actin1; Gene gun-mediated | Minghui 63 (indica)     | 20 µg/g soluble protein in leaves; 1.39 µg/g in leaves FW; 0.78 µg/g in stems FW; 0.87 µg/g in roots FW; Up to 8.02 µg/g in roots DW | 91.7%–100% for C. suppressalis; 100% for S. incertulas | 84.8%–100% for C. medinalis; 91.4%–95.7% for S. incertulas | [34,44,45] |
| Cry1Ab/1Ac           | T9-3, T9-4 | Actin1; Gene gun-mediated | IR72 (indica)           | Up to 0.01% in leaves | - | >90% for S. inferens, C. suppressalis, S. incertulas, C. medinalis, and N. Anescens | [46,47] |
| Shanyou 63 (hybrid)  |             | - | - | Up to 7.55 µg/g in leaves FW; 1.11 µg/g in stems FW; 0.84 µg/g in roots FW | 67.9% for C. suppressalis (3rd-instar); 100% (1st- and 3rd-instar), and 85% (5th-instar) for S. inferens | 92.5%–100% for C. suppressalis; 88%–100% for C. medinalis; 98.9%–99.62% for S. incertulas | [26,37,44,45,48,49] |
Table 1. Cont.

| Insecticidal Proteins | Plant Lines     | Promoter; Method of Transformation | Parental Line or Cultivar | Expression Level of Bt Protein * | % Efficacy on Target Pests References |
|-----------------------|-----------------|-----------------------------------|---------------------------|----------------------------------|----------------------------------------|
|                       |                 |                                   |                           |                                  |                                        |
| Cry1Ab/Vip3H          | G6H1, G6H2,    | Ubiquitin; Agrobacterium-mediated | Xiushui 110 (japonica)    |                                 |                                        |
|                       | G6H3, G6H4,    |                                   |                           |                                 |                                        |
|                       | G6H5, G6H6     |                                   |                           |                                 |                                        |
|                       |                 |                                   |                           |                                 |                                        |
| Cry1Ac/Cry1I-like S21 | pGreen;        | Xiushui 134 (japonica)            |                           |                                 |                                        |
|                       | Agrobacterium-mediated |                       |                           |                                 |                                        |
|                       |                 |                                   |                           |                                 |                                        |
| MSA                   | Actin1;        | Minghui 86 (indica)               |                           |                                 |                                        |
|                       | Gene gun-mediated |                       |                           |                                 |                                        |
|                       |                 |                                   |                           |                                 |                                        |
| MSB                   | Actin1;        | Minghui 86 (indica)               |                           |                                 |                                        |
|                       | Gene gun-mediated |                       |                           |                                 |                                        |
|                       |                 |                                   |                           |                                 |                                        |
| Kefeng6 (KF6)         | Actin1;        | Minghui 86 (indica)               |                           |                                 |                                        |
|                       | Gene gun-mediated |                       |                           |                                 |                                        |
|                       |                 |                                   |                           |                                 |                                        |
| IYouKF6 (hybrid)      |                 |                                   |                           |                                 |                                        |
|                       |                 |                                   |                           |                                 |                                        |

* % of total soluble protein or µg/g tissue fresh weight (FW) or dry weight (DW); “-” denotes “unclear”; * Chilo suppressalis, Scirpophaga incertulas, Cnaphalocrocis medinalis, Herpillogona licarisalis, Sesamia inferens, Naranga anescens, Mycalesis gotama, and Parnara guttata.
2.2. Bt Protein Expression in Rice Plants

Efficient stable expression of Bt proteins in plants is the basis for high efficacy in controlling target pests, and also is important for delaying the development of Bt resistance of target pests [22,26,64]. At an early stage of Bt crop development, unmodified Bt genes were directly introduced into plants, resulting in low levels of protein expression, which were not sufficient for efficiently controlling target pests [65]. Subsequently, cry1A(b) and cry1A(c) genes were modified before being introduced into cotton [66], tobacco, and tomato [67], leading to increased expression of Cry proteins and thus improved resistance to target insect pests, and led to realization of commercial use of Bt crops.

To detect the expression of Bt proteins in GE plants, multiple immunological methods were developed, including Bradford’s method, Western blotting, immunohistochemical staining, lateral flow strips, and enzyme-linked immunosorbent assay (ELISA) [26]. Among these methods, ELISA is a relatively efficient detection method, offering simple, fast, and reliable protein determination, and it has been widely used for qualitative and quantitative analyses of Cry proteins in Bt plants [22,26].

Using the ELISA method, various studies have been conducted to measure the levels of Cry proteins in Bt rice plants (Table 1). In general, the levels of Cry protein in different plant tissues varied significantly, with the highest level in leaves, followed by stems, roots and seeds. An exception was found in Bt rice lines (G6H-1, G6H-2, G6H-3, G6H-4, G6H-5, and G6H-6), in which the content of Cry1Ab was higher in stems than in leaves. Throughout the growing season, different Bt rice lines display different expression dynamics of Cry proteins. To sum up, expression dynamics can be classified into three patterns: (i) declining expression, with high expression at an early stage (seedling stage) with gradually decreased levels at later stages; this is the most frequent pattern not only in Bt rice, but also in Bt cotton, soybean and maize [22,25,26,34,38,56,62,68]; (ii) increasing expression, namely low expression at an early stage with increased expression levels at later stages [17,69]; and (iii) relatively consistent expression [26,34,70]. Interestingly, relatively high Cry protein expression levels were detected in lines expressing cry2Aa or cry9C genes among the Bt rice lines. The actual mechanism for this phenomenon is unclear, although it was inferred that it might be associated with the high contents of the bases G and C in these genes, since it was found that under the same conditions (the identical promoter, terminator, binary expression vector, recipient variety, and similar selection criteria), genes containing higher G and C content could be more highly expressed in plants [31].

In Bt rice, constitutive promoters such as ubiquitin, and Actin1 are widely used to express Bt genes, resulting in Bt proteins being produced in the whole plant (Table 1). However, in order to reduce the potential risk of Bt resistance developed by target insect pests and due to consumer safety concerns, tissue-specific promoters have started to be used for Bt rice development. For example, the green tissue-specific promoters rice rbcS and pGreen have been used in the rice lines RJ-5 and S21, respectively, resulting in the content of Cry proteins being only 0.0026 µg/g in endosperm [38] and 0–0.0076 µg/g [51] in seeds. It seems that such tissue-specific promotors have prospects for wider application.

2.3. Target Pest Control

Laboratory and field experiments have been extensively conducted to test the efficacy of the Bt rice lines against target pests in China. Under laboratory conditions, bioassays were conducted in which the stems or leaves were cut from rice plants and fed to different instars of target pests, and the mortalities of the tested pests were taken as an indicator of the resistance of Bt rice plants to target pests. However, under field conditions, the percentage of plants with rolled leaves (mainly for C. medinalis), dead-heart (mainly for C. suppressalis), and white-head (mainly for S. incertulas) were taken as indicators of the species-specific damage characteristics caused by different caterpillars.

Multiple lepidopteran pests were tested for their susceptibility to Bt rice, with the focus on C. suppressalis, S. incertulas, and C. medinalis due to their severity in rice fields (Table 1). Laboratory bioassays indicated that Bt rice lines showed high resistance to young (<2nd-instar) caterpillars, and the efficacy of resistance decreased significantly with increasing age of the insects. For example, the
corrected mortalities of 1st- to 6th-instar C. suppressalis in 7-day bioassays feeding on rice expressing cry1Ac + CpTI genes were 89.6%, 87.1%, 72.37%, 50.0%, 26.6%, and 0%, respectively [59]. Since the expression of Bt proteins in rice normally shows a declining tendency with the growth of rice plants, the resistance to target pests generally declined over the growth stages of rice plants; at mature stage most Bt rice lines showed relatively poor anti-pest efficacy [34,55,56,71]. However, Bt rice lines such as Bt-DL, mfb-MH86, and Huahui 1 showed high and consistent pest resistance throughout the growing season due to the stable expression of Bt protein in rice plants [22,26,34].

Field trials also showed that many Bt rice lines exhibited high resistance to target pests, providing 90%–100% control of stem borers and 80%–100% control of leaf-folders (Table 1). Similar to laboratory results, most Bt rice lines performed better in target insect pest control during the early growing season, and poorer control later in the growing season, with the exception of Bt rice lines Bt-DL, mfb-MH86, and Huahui 1, which exhibited excellent control of target pests throughout the rice-growing season.

As mentioned above, the efficacy of Bt rice lines for controlling target pests is positively correlated with the level of expression of Cry proteins in plant tissues [22,26,56,62]. However, high Cry protein expression does not always exert high insect resistance, since resistance is also related to the types of Bt proteins produced in rice plants; different Bt proteins showed significantly different toxicity to different target species. For example, although some rice lines contained Cry9C or Cry2A proteins at much higher levels than Cry1C and Cry1A proteins in some other rice lines, these lines showed equivalent or even lower resistance to target pests than the cry1C or cry1A rice lines [31]. This phenomenon can be explained by the results of Jiao et al. (2016) [72], showing susceptibility of C. suppressalis larvae to five Cry proteins in the order Cry1Ca > Cry1Ab > Cry1Ac > Cry2Aa > Cry1Fa. By comparison, Cry1Ab, Cry1Ac and Cry1C proteins seem to be ideal insecticidal proteins for incorporation into rice to control lepidopteran rice pests. Further, these three Bt proteins have relatively low toxicity to silkworm Bombyx mori (Lepidoptera: Bombycidae) larvae, which we are trying to protect [72].

Various studies have shown that Bt rice could provide effective control of major lepidopteran pests. However, both laboratory and field studies have pointed out that these rice lines show relatively low resistance to S. inferens especially at late growth stages of rice [46,50,55,62]. Laboratory study also confirmed that S. inferens exhibited significantly lower susceptibility to Cry1A proteins than C. suppressalis, which suggests that S. inferens is likely to develop resistance to Bt rice after commercial planting [73]. Therefore, more attention should be paid to those species in development of insect resistance management strategies for Bt rice.

3. Bt Maize

3.1. Bt Maize Lines Developed

Development of Bt maize started in the late 1980s in China, but moved relatively slowly during the initial stage. Greater progress was achieved in the past decade, especially after the initiation of the National GMO New Variety Breeding Program in 2008. To date, over a dozen Bt maize lines have been obtained (Table 2). Most were developed by public-sector scientists from the Chinese Academy of Agricultural Sciences, Zhejiang University, China Agricultural University, and Shandong University. In recent years, several agricultural biotechnology companies became involved in Bt maize development, for example the DBN Sci-tech Group, China National Seed Group Co., Ltd., (Beijing, China) and Beijing Origin Seed Technology, Inc. (Beijing, China) Similar to Bt rice, all Bt maize lines developed in China express cry1 and/or cry2 genes targeting lepidopteran pests. Most of the Bt maize lines contain a single Bt gene, such as cry1Ac in the BT-799 and Zhengdan958K lines, cry1le in IE095034, and cry1Ah in G186 (Table 2). Some Bt maize lines contain a fusion Bt gene, such as cry1Ab/cry2Aj in Shuangkang 12-5, and cry1Ah/cry1le in HIF21 (Table 2). In addition, there were several Bt maize lines stacked with the epsps, bar, or G10eso-epsps genes, thereby exhibiting both pest resistance and herbicide tolerance (Table 2). Agrobacterium-, gene gun- and pollen tube-mediated techniques were commonly used for Bt maize transformation. The promoters used in Bt maize mainly include pZmUbi-1 (Zea mays polyubiquitin-1), P35S, and CaMV 35S (Table 2).
Table 2. Insect-resistant *Bt* maize lines and their efficacy on target lepidopteran pests in China.

| Insecticidal Proteins | Plant Lines | Promoter; Method of Transformation | Recipient Cultivar | Expression Level of *Bt* Protein | Efficacy on Target Lepidopteran Pests | References |
|-----------------------|-------------|------------------------------------|-------------------|-------------------------------|--------------------------------------|------------|
| **Modified Cry1Ab**    | **-**       | pZmLb1-1, Agrobacterium-mediated   | HII               | 0.30-0.47 µg/g in leaves FW   | 78% of leaves for *O. furnacalis* in 5-day bioassays | 0.14 survivors, 2.43 tunnels/plant, 3.64 cm tunnel length/plant | [74] |
| BST-799               | mCry1Ac     | CaMV 35S; Gene gun-mediated        | Zheng 58          | 0.77 µg/g in leaves FW; 0.23 µg/g in silks DW; 0.30 µg/g in husks DW; 0.15 µg/g in young kernels DW; 0.059 µg/g in pollen DW | -                                    | Leaf damage ratings (LDR) below 2 for *O. furnacalis* | [75–77] |
| Zhengdan958K          |             | -                                 | Zhengdan 958      | -                             | 100% of whorl leaves, 83.3% of silk, 97.2% of husk, and 63.5% of young kernel for *O. furnacalis* | -         | [75] |
| **BT-40**             | Cry1Ac      | CaMV 35S; -                        | HiII × H99        | 0.087-0.23 µg/g in whorl leaves FW; 0.044 µg/g in silks FW | 84.7%-97.2% of whorl leaves for *O. furnacalis*; LDR was 1.15 for *O. furnacalis* | -         | [78] |
| BT-38                 |             | Zheng 58                           | 0.44 µg/g in whorl leaves FW | 98.6% of whorl leaves for *O. furnacalis* | -                                    | [78] |
| BT-181                |             | Zheng 58                           | 0.42 µg/g in whorl leaves FW | 97.2% of whorl leaves for *O. furnacalis* | -                                    | [78] |
| BT-105                |             | Chang 7-2                          | 0.42 µg/g in whorl leaves FW | 100% of whorl leaves for *O. furnacalis* | -                                    | [78] |
| **Q1, Q2, Q3**        | Cry1AcM     | pZmLb1-1, Agrobacterium-mediated   | Chang 7-2         | -                             | LDR was below 2.0, >80% of kernels, and >90% of husks for *O. furnacalis*; LDR was below 1.91, >80% of kernels, and >90% of husks for *O. furnacalis* | [79] |
| Z1, Z2, Z3            |             | Zheng 58                           | -                 | LDR was below 2.0, >80% of kernels, and >90% of husks for *O. furnacalis*; LDR was below 2.07, >80% of kernels, and >90% of husks for *O. furnacalis* | -                                    | [79] |
| Q1, Q2, Q3            |             | pZmLb1-1, Agrobacterium-mediated   | Qi 319            | -                             | LDR was below 2.0, >80% of kernels for *O. furnacalis*; LDR was below 1.11, >90% of husks for *O. furnacalis* | [80] |
| L1, L2, L3            |             | pZmLb1-1, Agrobacterium-mediated   | 9801              | -                             | LDR was below 2.0, >80% of kernels for *O. furnacalis*; LDR was below 1.15, >90% of husks for *O. furnacalis* | [80] |
| **HKG60**             | Cry1Ah      | Ubiquitin; Agrobacterium-mediated  | Z 31              | 2.88, and 3.50 µg/g in leaves FW at 6-leaf stage, and heading stage, 3.62, and 9.98 µg/g in tassels FW at heading stage and filling stage | 100% of leaves for *O. furnacalis*, >80% for *H. armigera* in 3-day bioassay | LDR was 1.29, and 2.47 for *O. furnacalis*, and *M. separata*, high resistant of kernel to *H. armigera* | [81] |
| Q11, X8               |             | Ubiquitin; Agrobacterium-mediated  | Q31 × Z3          | Up to 0.05% in leaves | -                                    | LDR was 2.4 (Q11), and 3.4 (X8) for *O. furnacalis* | [82] |
| **G186**              |             | Ubiquitin; Agrobacterium-mediated  | Z31               | Up to 1 µg/g in leaves FW | 100% of leaves for *O. furnacalis* | LDR was 1.3 for *O. furnacalis* | [83] |
Table 2. Cont.

| Insecticidal Proteins | Plant Lines | Promoter; Method of Transformation | Recipient Cultivar | Expression Level of Bt Protein * | Efficacy on Target Lepidopteran Pests | References |
|-----------------------|-------------|-----------------------------------|-------------------|----------------------------------|---------------------------------------|------------|
|                       |             |                                   |                   | In Laboratory | In Field b |                                    |            |
| Cry1C                 | ZmKc-2-3    | Ubiquitin; Gene gun-mediated      | HiII              | 3.43, 2.71, 0.99, 0.79, 0.65, 0.66, 0.19, and 0.09 µg/g in leaves, tassel handles, stems, filaments, tassels, female ear tips, pollen, and grains FW, respectively | - | 100% for O. furnacalis | [9]  |
| Cry1E                 | IE09S034    | Ubiquitin; Agrobacterium-mediated | Z31               | - | 85.42%–90.62% for O. furnacalis; 50% for for H. armigera | LDR was below 2.5 for O. furnacalis | [84]  |
|                       |             |                                   |                   | 96% of whorl leaves, tassels, silks, and point of spikes, and 88% of grains for O. furnacalis in 7-day bioassays | 100% for O. furnacalis | [77,85]  |
| Cry1Ab/2Aj            | N10, N20, N30, N40, N50 | Ubiquitin; Agrobacterium-mediated | ZhengDan 958 | 22.80 µg/g in pollen DW | | 93.2%–100% of whorl leaves, tassels, husks, silks and kernels for O. furnacalis | [86]  |
|                       | N30         | Ubiquitin; Agrobacterium-mediated | Hind-II           | 14.31–22.67 µg/g in whorl leaves DW, 20.93–49.33 µg/g in silks DW | 100% of whorl leaves, tassels, husks, silks and kernels for O. furnacalis | LDR was 1.0–1.50 for O. furnacalis | [87]  |
|                       | V3          | Ubiquitin; Agrobacterium-mediated | Hind-II           | 4.51–9.72 µg/g in whorl leaves, tassels, husks, silks and kernels FW | 100% of whorl leaves, tassels, husks, silks and kernels for O. furnacalis | LDR was 1.0, 100% for O. furnacalis | [87]  |

a % soluble protein (w/w) or µg/g tissues fresh weight (FW) or dry weight (DW); "-" denotes "unclear". b Leaf damage ratings (LDR) followed the criteria described by He et al. [89], in which 1.0 = rare or sporadic pin-holes on a few leaves; 2.0 = intermediate pin-holes on a few leaves; 3.0 = many pin-holes on several leaves; 4.0 = rare or sporadic match-head-sized holes on a few leaves; 5.0 = intermediate match-head-sized holes on a few leaves; 6.0 = many match-head-sized holes on several leaves; 7.0 = rare or sporadic holes larger than match-head-sized holes on a few leaves; 8.0 = intermediate holes larger than a match-head on a few leaves, and 9.0 = many holes larger than a match-head on several leaves. The resistance-level classifications were as follow: 1.0–2.09 (highly resistant); 2.1–4.09 (resistant); 4.1–6.09 (moderately resistant); 6.1–8.09 (susceptible); and 8.1–9.0 (highly susceptible).
3.2. Bt Protein Expression in Maize Tissues

The same as for Bt rice, the highest Bt protein level in maize normally was detected in leaves, followed by stems and roots, and the least in seeds [78,83,90–92]. There also was high content of Cry protein detected in husks, kernels, tassels, and silks of Bt maize [75,86,87]. In general, it was reported that Cry protein concentrations in Bt maize tissues decreased with maize growth [90,91]. However, there also were cases when Cry protein content in maize tissues increased with age. For example, the contents of mCry1Ac protein in the leaves, stems and roots of BT-799 increased with the growth of the plants, and reached a peak at the seed maturation stage [92]. The expression dynamics of Cry protein were investigated in several Bt maize lines. For example, the content of Cry1Ah in the leaves, stems and roots of BT-799 increased through early stages, reaching the peak at the heading stage, but then decreased at later stages [83]. The content of CryFLIa (modified Cry1Ab) protein in leaves of HiII showed an increasing trend before the 8-leaf stage, and decreased at the heading stage, but increased again at the filling stage [74]. The expression of Bt proteins in plants is a complicated process which can be affected by the genetic background of different recipient cultivars, promoters, transformed genes, transformation methods, growth environment, physiological conditions, and the plant’s energy resources.

3.3. Target Pest Control

Studies were conducted to test pest-control efficacy of Bt maize, mainly focusing on the target lepidopteran pest O. furnacalis. In laboratory studies, the mortality of O. furnacalis larvae was evaluated when fed leaves, tassels, husks, silks, spikes, and kernels from Bt maize as compared to non-Bt control maize. The results showed that the majority of the currently developed Bt maize lines caused over 85% mortality of O. furnacalis larvae; a few Bt maize lines caused 100% mortality when O. furnacalis neonates were fed Bt maize leaves (Table 2). It seems that Bt maize has poorer control of H. armigera than O. furnacalis. For example, the Bt maize line IE09S034 caused over 85% mortality of O. furnacalis larvae, but only 50% mortality of H. armigera (Table 2). In field trials, Bt and non-Bt control maize plants were artificially infested with O. furnacalis neonates, and a few days later, leaf damage ratings (LDR) were assessed (Table 2). According to the criteria described by He et al. (2000) [89] (for details see the footnotes under Table 2), the LDRs by O. furnacalis on all test Bt maize lines were below 3, and the resistance levels were characterized as “highly resistant” or “resistant”. Several Bt maize lines, such as BT-X, Shuangkang 12-5, V3, HGK60, G186, and N30, exhibited excellent control of O. furnacalis, with LDRs less than 1.5. In general, the Bt maize lines expressing Cry1Ab or Cry1Ac protein performed better in controlling O. furnacalis larvae, suggesting that both cry1Ab and cry1Ac genes are ideal for maize transformation for controlling lepidopteran pests.

4. Conclusions and Future Perspectives

Both laboratory and field studies showed that multiple Bt rice and Bt maize lines developed in China expressed effective control of target lepidopteran pests (Tables 1 and 2). In addition, many studies have been conducted to assess the ecological and food safety of Bt rice and Bt maize. While we do not address these issues in the current review, the results indicate that the currently developed Bt rice and Bt maize pose negligible risk to the environment and human health [1,93]. Thus, we conclude that compared to conventional pesticide-treated crop production, planting of Bt rice and Bt maize should be safer to the consumer and more environmentally friendly. However, as mentioned above, some lepidopteran pests, such as S. inferens on rice and H. armigera on maize cannot be efficiently controlled by the current Bt rice and Bt maize lines, and scientific insect resistance management strategies should be developed prior to commercial cultivation of these Bt plants [64].

The currently developed Bt rice and Bt maize lines are all for controlling lepidopteran pests. However, other insects, such as planthoppers on rice and aphids and spider mites on maize, also cause considerable economic losses annually. Unfortunately, there are as yet no optimal genes for use to control such piercing and sucking insects. Investigation of such genes is an urgent issue.
Once such genes are identified, they should be stacked with Lepidopteran-resistance genes for rice and maize transformation.

Although excellent Bt rice and Bt maize lines have been obtained, no Bt crops have yet been commercially planted in China. An important milestone for Bt rice came in 2009 when the Ministry of Agriculture of China issued biosafety certificates for commercial production of the Bt rice lines Huahui 1 and Bt Shanyou 63 in Hubei province, and in 2014 when the biosafety certificates were renewed. The delay in commercial use of Bt rice is largely caused by low public acceptance due to extreme concerns about the food safety of GE crops and the low scientific literacy of the public about GE crops more generally [1]. This situation is not particular to China, and occurs in many countries, for example in European Union countries, in which GE crop products are even less accepted. To change this condition, more work should be done by government and non-governmental organizations, such as developing targeted and well-funded educational programs and increasing public dialog on GE crops [1,94,95].

Public dialog and risk communication of GMOs can be accomplished through television, the Internet using media such as Weibo (the Chinese version of Twitter) and WeChat (the most universal communication applications recently in China), newspapers, and periodicals [95,96]. In addition, it is important for agricultural oversight agencies to enhance their ability to supervise and regulate GMO biosafety, since any potential incidents associated with GMO biosafety may impair public confidence in the biosafety on GMOs [1].

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