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MINI-REVIEW

Expression of *cry* genes in *Bacillus thuringiensis* biotechnology

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Abstract

Bacillus thuringiensis is a gram-positive, spore-forming bacterium that produces insecticidal crystal proteins during sporulation. The production of these crystals results primarily from the expression of *cry* genes. In this review, we focus on the expression and application of *cry* genes directed by both *cry* gene promoters and non-*cry* gene promoters in different hosts. However, not all *cry* genes and niches are compatible with *B. thuringiensis*. New delivery systems offsetting the current limitations in *B. thuringiensis* application are needed to improve Cry production, niche fitness, and persistence. This review examines currently available research and highlights areas in need of further research and development for more effective production and utilization of Cry insecticidal proteins.

Keywords *cry* gene promoters · Non-*cry* gene promoters · Bacterial insecticide · *Bacillus thuringiensis* biotechnology

Introduction

Bacillus thuringiensis (Bt) is a gram-positive, spore-forming bacterium that produces insecticidal crystal proteins (ICPs) during sporulation. ICPs are mainly classified into Cry and Cyt, and their crystals have various forms: bipyramidal (Cry1), cuboidal (Cry2), flat rectangular (Cry3A), irregular (Cry3B), spherical (Cry4A and Cry4B), and bar shape (Cry11A). As an insect pathogen, Bt insecticidal activity is attributed to parasporal crystals, which are toxic to a wide variety of insect species among the orders Lepidoptera, Coleoptera, Hymenoptera, Diptera, Homoptera, Orthoptera, and Mallophaga, and against nematodes, mites, and protozoa (Schnepf et al. 1998). As of October 2018, approximately 846 *cry* and *cyt* genes have been discovered (http://www.lifesci.sussex.ac.uk/home/Neil_Crickmore/Bt/). *cry* genes are expressed during the stationary phase, and their products generally accumulate in mother cell compartments to form a crystal inclusion that can account for 20 to 30 % of the dry weight of sporulating cells (Schnepf et al. 1998).

Several *cry* gene promoters have been identified, and their transcription has been extensively reviewed (Agaisse and Lereclus 1995; Baum and Malvar 1995; Deng et al. 2014; Komano et al. 2000). Here, we primarily focus on the expression and application of *cry* genes directed by *cry* gene promoters and non-*cry* gene promoters in different hosts, which include Bt microorganisms, non-Bt microorganisms, and transgenic crops in order to provide an overview of current knowledge and to highlight areas that would benefit from further research. As Bt has been considered the most successful bioinsecticide of the last century (Jouzani et al. 2017), an overview of known expression mechanisms and methods to increase application and effectiveness will help to provide possible direction for further research.

Expression of *cry* genes directed by *cry* gene promoters

Transcriptional regulation mechanisms of *cry* genes have been classified into two types: sporulation-dependent promoters are controlled by sporulation-specific sigma factors SigK and/or SigE, and sporulation-independent promoters are under the control of the vegetative SigA factor. Accessory factors also contribute to the transcriptional regulation of *cry* gene expression such as Spo0A, ORF2, and CcpA (Deng et al. 2014). Recently, Peng et al. (2018) reported that expression of *cry5Ba* was silenced when YBT-1518 strain was outside of the host; however, when ingested by *Caenorhabditis elegans*, Cry5Ba was synthesized in vivo by

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YBT-1518. Cry5Ba silencing was due to sRNA BtsR1, which bind to the ribosomal binding site of the *cry5Ba* transcript via direct base pairing and inhibited *cry5Ba* expression. Identification of these *cry* gene promoters and clarification of their regulation mechanisms provide expression elements for *cry* gene expression.

The expression of many *cry* genes is under the control of *cry* gene promoters in Bt used for studying novel gene activity, genetic insecticidal mechanisms, construction of engineered strains, and other applications (Table 1). The transcription of *cry1A* promoter is controlled by both SigE and SigK (Aceves-Diez et al. 2007; Bravo et al. 1996; Buasri and Panbangred 2012; Perez-Garcia et al. 2010; Sedlak et al. 2000; Walter and Aronson 1999; Yang et al. 2012) and is most commonly used for the expression of *cry* genes, including *cry1Ac* (Roh et al. 2004; Sun et al. 2016; Xia et al. 2009a; Xia et al. 2005; Xia et al. 2009b; Yan et al. 2014), *cry2Ab27* (Somwatcharajit et al. 2014), *cry11A* (Wu and Federici 1995), *cry8* (Amadio et al. 2013), *cry64Ba*, and *cry64Ca* (Liu et al. 2018). Co-expression of *cry1Ac* and Av3 produced a neurotoxin of *Anemonia viridis* that improved insecticidal toxicity in the Bt acrystalliferous strain Cry$^-$B (Yan et al. 2014). Gomez et al. (2014) used Bt to synthesize Cry1Ab, Cry1Ab F371A, or mutant Cry1AbMod proteins to study the mechanism of pore-forming toxins directed by *cry1A* promoter (Pacheco et al. 2009). Another study used *cry1Ac* promoter to direct co-expression of *cry64Ba* and *cry64Ca* genes in acrystalliferous Bt strain HD73$^-$, which resulted in high insecticidal activity against two important Hemipteran rice pests, *Laodelphax striatellus* and *Sogatella furcifera* (Liu et al. 2018).

The transcription of *cry3* gene is initiated during vegetative growth, activated at the end of the exponential phase, and continues for several hours during the stationary phase (Agaisse and Lereclus 1994b; Agaisse and Lereclus 1995; Salamitou et al. 1996). Production of Cry1AbMod and Cry1AcMod (Garcia-Gomez et al. 2013), Cry1Ac (Chaoyin et al. 2007), Cry1C (Sanchis et al. 1996), Cry3A (de Souza et al. 1993), and Cry8 (Amadio et al. 2013) were found to be directed by the *cry3A* promoter. pSTK is an *E.coli*-Bt shuttle vector, used to produce Cry8Ga1 (Jia et al. 2014) and Cry69Aa1 (Guan et al. 2014), that carries *cry3A* promoter. Cry1AbMod and Cry1AcMod-encoded genes were cloned efficiently under the regulation of the *cry3A* promoter region to drive their expression in Bt but demonstrated no expression

Table 1 Expression of *cry* genes directed by *cry* gene promoters in Bt

Cry protein	Promoters	Vectors	Bt strain	Ref.
Cry1Ab	*cry1A* promoter	pHT315	4Q7$^-$	(Gomez et al. 2014; Pacheco et al. 2009)
Cry1Ac5	*cry1Ac* promoter	pHT315	Cry$^-$B	(Sun et al. 2016)
Cry1Ac-av3	*cry1Ac* promoter	pHT315	Cry$^-$B	(Yan et al. 2014)
Cry1Ac	*cry1Ac* promoter	pHT3101	4Q7$^-$	(Roh et al. 2004; Xia et al. 2005)
Cry1AbMod/Cry1AcMod	*cry3A* promoter	pHT315	407$^-$	(Garcia-Gomez et al. 2013)
Cry1Ac	*cry1Ac* promoter	pHT315	Cry$^-$B	(Xia et al. 2009b)
Cry1Ac	*cry1Ac* promoter	pHT304	XBU001	(Xia et al. 2009a)
Cry1Ac	*cry3A* promoter	pBMB31-304	BMB171	(Chaoyin et al. 2007)
Cry1Ba	*cry8E* promoter	pHT315	HD73$^-$	(Zhou et al. 2014)
Cry1C	*cry3A* promoter	pHT304-18Z	407$^-$	(Sanchis et al. 1996)
Cry2Ab27	*cry1Ac* promoter	pHT304-18Z	SP41 and 407$^-$	(Somwatcharajit et al. 2014)
Cry2Ab	*cry2Aa* promoter	pHT3101	4Q7$^-$	(Jain et al. 2006)
Cry3A	*cry3A* promoter	pHT304	HD1$^-$	(de Souza et al. 1993)
Cry4B/Cry4A	*cry4B* promoter	pHT3101/pHT315	4Q2-71	(Delecluse et al. 1993; Rodriguez-Almazan et al. 2012)
Cry5B	*cry5B* promoter	pHT304	BMB171	(Sajid et al. 2018)
Cry6A	*cry6A* promoter	pHT304	BMB171	(Dementiev et al. 2016)
Cry8	*cry1Ac* /*cry3Aa* promoters	pHT3101	4Q7$^-$	(Amadio et al. 2013)
Cry8Ga1	*cry3A* promoter	pSTK	BIOT185	(Jia et al. 2014)
Cry8Kb3/Cry8Pa3	*cry8Kb3/cry8Pa3* promoters	pHT3101	4Q7$^-$	(Navas et al. 2014)
Cry11A	*cry1Ac* promoter	pHT3101	4Q7$^-$	(Wu and Federici 1995)
Cry26Aa/Cry28Aa	*cry1Ca* promoter	pHT304	YBT-020	(Ji et al. 2009)
Cry64Ba/Cry64Ca	*cry1Ac* promoter	pHT315	HD73$^-$	(Liu et al. 2018)
Cry69Aa1	*cry3A* promoter	pSTK	HD73$^-$	(Guan et al. 2014)

in *E. coli* cells (Garcia-Gomez et al. 2013). Nevertheless, the Cry1AMod proteins produced from the *cry3A* promoter in Bt were not soluble and showed low toxicity against *Plutella xylostella* larvae. Construction of site-directed mutagenesis of cysteine residues in the p3A-Cry1AbMod and p3A-Cry1AcMod protoxin gene reestablished solubility and toxicity to *P. xylostella*. This suggested that the combination of the *cry3A* promoter expression system with single cysteine mutations is a useful system for efficient expression of Cry1AMod toxins in Bt (Garcia-Gomez et al. 2013).

Expression of other *cry* genes is directed by their own promoters, including *cry4B* and *cry4A* (Delecluse et al. 1993), *cry5B* (Sajid et al. 2018), *cry8Kb3* and *cry8Pa3* (Navas et al. 2014), and *cry2Ab* (Jain et al. 2006). Dementiev et al. (2016) used Cry6Aa to determine the Cry6Aa structure in protoxin and trypsin-activated forms as well as the pore-forming mechanism of action, which was under the control of its own promoter in Bt strain BMB171. Other studies have used plasmid pHT618 to produce Cry4Ba and Cry11Aa crystals by their own promoters in an acrystalliferous Bt strain to compare the cadherin-binding affinity of Cry4Ba and Cry11Aa (Delecluse et al. 1993; Rodriguez-Almazan et al. 2012). Zhou et al. (2014) compared the transcriptional activity of *cry1Ac*, *cry3A*, *cry4A*, and *cry8E* promoters and found that *cry8E* promoter showed the highest transcriptional activity among these promoters in Bt. The researchers constructed a novel *E. coli*-Bt shuttle vector, pHT315-8E21b, for *cry* gene expression using the *cry8E* promoter and multiple cloning sites from vector pET21b (based on vector pHT315), then produced Cry1Ba in the *sigK* mutant against *Ostrinia furnacalis* and *Plutella xylostella*. The findings suggested that *cry8E* promoter can be an efficient transcriptional element for *cry* gene expression and utilized for construction of a genetically engineered strain.

Expression of *cry* genes directed by non-*cry* gene promoters

Non-cry gene promoters are usually used to direct the expression of *cry* genes (Table 2). The most commonly used promoter is P*cyt1A*, which is also a SigE- and SigK-controlled promoter (Sakano et al. 2017). It has been used to direct many Cry protein production including Cry1C-t (Park et al. 2000), Cry2A and Cry2B (Crickmore et al. 1994), Cry2A and Cry11A (Park et al. 1999), Cry3A (Park et al. 1998), Cry9Ec1 (Wasano et al. 2005), Cry11Aa and Cry11Ba (Sun et al. 2014), Cry11B (Park et al. 2001), Cry19A (Barboza-Corona et al. 2012), Cry20Aa (Lee and Gill 1997), Cry27A (Saitoh et al. 2000), Cry30Ca, Cry60Aa, Cry60Ba (Sun et al. 2013), and Cry41Aa (Krishnan et al. 2017). One study constructed vectors that expressed *cry3A* with (pPFT3As) and without (pPFT3A) in the STAB-SD sequence, using *cytA* promoters to drive expression. They found that the volume of Cry3A crystals produced with *cyt1Aa* promoters and the STAB-SD sequence was 1.3-fold that of typical bipyramidal Cry1 crystals toxic to Lepidopteran insects (Park et al. 1998). The dual-promoter/STAB-SD system offers an additional method for potentially improving the efficacy of insecticides based on Bt. Krishnan et al. (2017) used *cyt1Aa* promoter to direct the expression of *cry41Aa* to study the action mechanism of certain protein toxins from the normally insecticidal bacterium Bt in targeted human cell lines. An amylase promoter fragment, *amyE*, was fused into the promoter region to induce the expression of *cry1Ac* (Yang et al. 2003) and *cry1C* (Chak et al. 1994) in the early log phase instead of the Bt *cry* replicon, which was promoted only at the sporulation stage. A recent report showed that there are also some highly active non-*cry* gene promoters for expression of *cry* genes. P*exsY*, a strong activity promoter of the exosporium basal layer structural gene *exsY* in the late sporulation phase, was used to express *cry1Ac* genes in Bt in order to discover new elements for *cry* gene expression (Zheng et al. 2014). A SigE-dependent strong promoter of a non-*cry* gene (HD73_5014) was used to direct strong *cry1Ac* gene expression in Bt HD73 (Zhang et al. 2018). The expression of the *cry1Ac* gene directed by the HD73_5014 gene promoter was the same level as that directed by the previous strongest known *cry* promoter, P*cry8E*. The expression of crystal proteins initiated by these high-activation promoters in Bt could be used to develop safe high-efficiency biological pesticides.

Expression of *cry* genes in non-Bt microorganisms

E. coli expression systems are commonly used for the expression of *cry* genes, especially in terms of novel gene activity and genetic insecticidal mechanisms. *T7* and *tac* promoters are most commonly used to direct the expression of *cry* genes carried on pET or pGEX series vectors with His or GST tags. For example, evaluation of Cry1A (Azizoglu et al. 2016; Huang et al. 2004; Khasdan et al. 2007; Reddy et al. 2013), Cry1Ea11 (Huang et al. 2018), Cry2 (Ogunjimi et al. 2002; Pan et al. 2014; Reyaz and Arulselvi 2016; Reyaz et al. 2017; Saleem and Shakoori 2017; Yilmaz et al. 2017), Cry4 (Boonserm et al. 2004; Zhang et al. 2014), Cry6A (Wang et al. 2017), Cry11A (AP et al. 2016), Cry21 (Iatsenko et al. 2014), Cry46Ab (Hayakawa et al. 2017), and Cry78Aa toxic activity (Wang et al. 2018a), as well as studying the insecticidal mechanisms of Cry1A (Adegawa et al. 2017; Martinez-Solis et al. 2018; Tanaka et al. 2016) and Cry2 (Shu et al. 2017; Xu et al. 2016). Another study used pET21b vector to express Cry9Aa and Vip3Aa toxins to study the specific interaction between two Bt toxins creating insecticidal synergism and unraveling the molecular basis of this interaction

Table 2 Expression of *cry* genes directed by non-cry gene promoters in Bt

Cry gene	Promoters	Vectors	Bt strain	Ref.
Cry1C-t	*cyt1A* promoter	pHT3101	4Q7^{-}	(Park et al. 2000)
Cry2A/Cry2B	*cry2A*/*cytA* promoter	pSV2	An acrystalliferous Bt strain	(Crickmore et al. 1994)
Cry2A/Cry11A	*cyt1A* promoter	pHT3101	4Q7^{-}	(Park et al. 1999)
Cry3A	*cyt1Aa* promoter	pHT3101	4Q7^{-}	(Park et al. 1998)
Cry9Ec1	*cyt1A2* promoter	pHT3101	BFR1	(Wasano et al. 2005)
Cry11Aa/ Cry11Ba	*cyt1A* promoter	pHT304	4Q7^{-}	(Sun et al. 2014)
Cry11B	*cyt1A* promoter	pHT3101	Bt subsp. israelensis strain	(Park et al. 2001)
Cry19A	*cyt1A* promoter	pHT3101	4Q7^{-}	(Barboza-Corona et al. 2012)
Cry20Aa	*cyt1Aa* promoter	pHT304	YG1 or LG101	(Lee and Gill 1997)
Cry27A	*cyt1A* promoter	pHT3101	BFR1	(Saitoh et al. 2000)
Cry30Ca/Cry60Aa/Cry60Ba	*cyt1A* promoter	pHT315	4Q7^{-}	(Sun et al. 2013)
Cry41Aa	*cyt1Aa* promoter	pSVP27a	4D7	(Krishnan et al. 2017)
Cry1Ac	*amyE* promoter	pHY300PLK	Tt14	(Yang et al. 2003)
Cry1C	*amyE* promoter	pSB909.4	Cry^{-}B	(Chak et al. 1994)
Cry1Ac	PexsY	pHT315	HD73^{-}	(Zheng et al. 2014)
Cry1Ac	HD73_5014 promoter	pHT315	HD73^{-} and HDsigK^{-}	(Zhang et al. 2018)

(Wang et al. 2018b). Herrero et al. (2004) used pBD150 plasmid to produce wild-type Cry1Ca and mutant protoxins in *E.coli* to determine toxin-binding parameters for specific receptors in brush border membrane vesicles of *Spodoptera exigua*.

Other microorganisms have also been used to express *cry* genes. Expressions of *cry1Aa* and *cry1Ia* under the control of the *lac* promoter in *Photorhabdus temperata* strain K122 against *Prays oleae* (Tounsi et al. 2006) resulted in a clear improvement in oral toxicity. This demonstrates that the heterologous expression of Bt *cry* genes in *P. temperata* can be used to improve and broaden the host range for insect control. Additionally, baculoviruses have been genetically modified to express *cry1Ab* under polyhedrin promoters in order to accelerate their killing speed (El-Menofy et al. 2014). Durmaz et al. (2015) used a strong constitutive promoter (P6 promoter) to express *cry5B* in *Lactococcus lactis* for use as an anthelminthic, while co-expression of mosquitocidal toxins *cyt1Aa* and *cry11Aa* from Bt subsp. *Israelensis* under the control of *Asticcacaulis excentricus tac* promoter enhanced toxicity to the third instar larvae of *Culex quinquefasciatus* expressing only *cry11Aa* (Zheng et al. 2007). Cry34Ab1 and Cry35Ab1 binary insecticidal proteins were produced in recombinant *Pseudomonas fluorescens* to provide large quantities of protein for safety-assessment studies associated with the registration of transgenic corn plants (Huang et al. 2007). Alberghini et al. (2005) also used *Pseudomonas* as a host for expression of *cry9Aa*. Agaisse and Lereclus (1994a) used *Bacillus subtilis* for *cry3A* expression to study the transcriptional regulation of the *cry3A* gene.

Bt is a large family of recognized entomopathogens found in various habitats (Jouzani et al. 2017). Many Bt-targeted insect pests inhabit niches that Bt cannot survive or stabilize in, such as the plant rhizosphere. Many Bt toxins have shown insecticidal activity against underground pests including nematodes and white grubs (Bi et al. 2015; Ruan et al. 2015). However, the LC50 of Cry proteins/spores for nematodes in most reports was quite low (Jouzani et al. 2017). This limits the application of Bt products that can kill underground pests. Thus, new stable microorganisms are required for *cry* gene expression as an alternative delivery system to develop new-generation biopesticides with improved persistence.

Expression of *cry* genes in Bt transgenic crops

Bt toxin proteins have been extensively used in plant genetic engineering to deter pests. Bt application has allowed agriculture to cater to human interest, for example, food growth free of chemical pesticides and reduced environmental damage due to avoidance of excessive chemical application by using various promoters to produce toxin proteins (Fig. 1).

Cauliflower mosaic virus (CaMV) promoter 35S has been shown to be active in most plant organs and is considered constitutively expressed throughout plant development; it is also considered a common strong promoter in activating Bt toxin genes. The c*ry3A* genes driven by 35S expressed in potatoes were toxic to the first-instar, and the more resistant third-instar Colorado potato beetle larvae (Adang et al. 1993); in transgenic Norway spruce (*Picea abies*), c*ry3A* genes

showed toxicity against the spruce bark beetle (Briza et al. 2013). Diamondback moth (*Plutella xylostella* L.), cabbage looper (*Trichoplusia ni* Hübner), and corn earworm (*Helicoverpa zea* Boddie) were completely controlled in transgenic canola containing *cry1Ac* (Stewart et al. 1996). Cry1Ab transgenic rice showed 100 % feeding mortality rates for the yellow stem borer (*Scirpophaga incertulas*) and the striped stem borer (*Chilo suppressalis*), and feeding inhibition of *Cnaphalocrocis medinalis* and *Marasmia patnalis* (Wunn et al. 1996). Cry9C transgenic maize delayed the development and increased mortality rates of *Plodia interpunctella* (Giles et al. 2000). Cry6A toxin protein produced by 35S can confer tomato resistance to an endoparasitic nematode (*Meloidogyne incognita*) (Li et al. 2007). *cyt2Ca1* driven by 35S expressed in citrus roots not only resulted in regular growth but also protected the roots from larval *Diaprepes abbreviatus* (Mahmoud et al. 2017). Transgenic Pigeon pea containing Cry2Aa gained resistance to gram pod borer (*Helicoverpa armigera*) (Singh et al. 2018). Double CaMV 35S promoter expressed *cry1Ac* against *Dendrolimus punctatus* (Walker) and *Crypyothelea formosicola* (Staud) in transgenic Loblolly pine (*Pinus taeda* L.) (Tang and Tian 2003). 35S promoter and alfalfa mosaic virus translational enhancer (AMV coat protein 5′ untranslated leader sequence) were used to express *cry3A* and *cry1Ab* in tobacco against potato beetle larvae (Sutton et al. 1992), tobacco hornworm (*Manduca sexta*), and tobacco budworm (*Heliothis virescens*) (Carozzi et al. 1992) respectively.

Additionally, there is a non-constitutive promoter reported to be stronger than 35S when expressing Bt toxin genes. Chrysanthemum ribulose-1, 5-bisphosphate carboxylase/oxygenase small subunit (Rubisco SSU) promoter expressed *cry1Ca* in shallot and provided resistance against beet armyworm (Zheng et al. 2005). *cry1Ac* driven by *Arabidopsis thaliana* Rubisco small subunit *ats1A* promoter with its associated transit peptide showed 10- to 20-fold mRNA and protein expression compared to expression driven by the 35S promoter (Wong et al. 1992).

Rice *actin1* promoter and maize *ubiquitin1* promoter are common strong plant-derived promoters in Bt transgenic rice, both enable high expression of *cry* genes and high insect resistance. Cry1Ac under the control of maize *ubiquitin1* promoter was confirmed as highly toxic to yellow stem borer larvae (Khanna and Raina 2002; Nayak et al. 1997). Under the control of rice *actin1* promoter, transgenic elite rice lines producing a Cry1Ab/Cry1Ac fusion protein showed high protection against leaffolder and yellow stem borer without reduced yield (Tu et al. 2000), while producing Cry1Ac protein exhibited resistance to striped stem borer (*Chilo suppressalis* (Walker) in the laboratory (Liu et al. 2016).

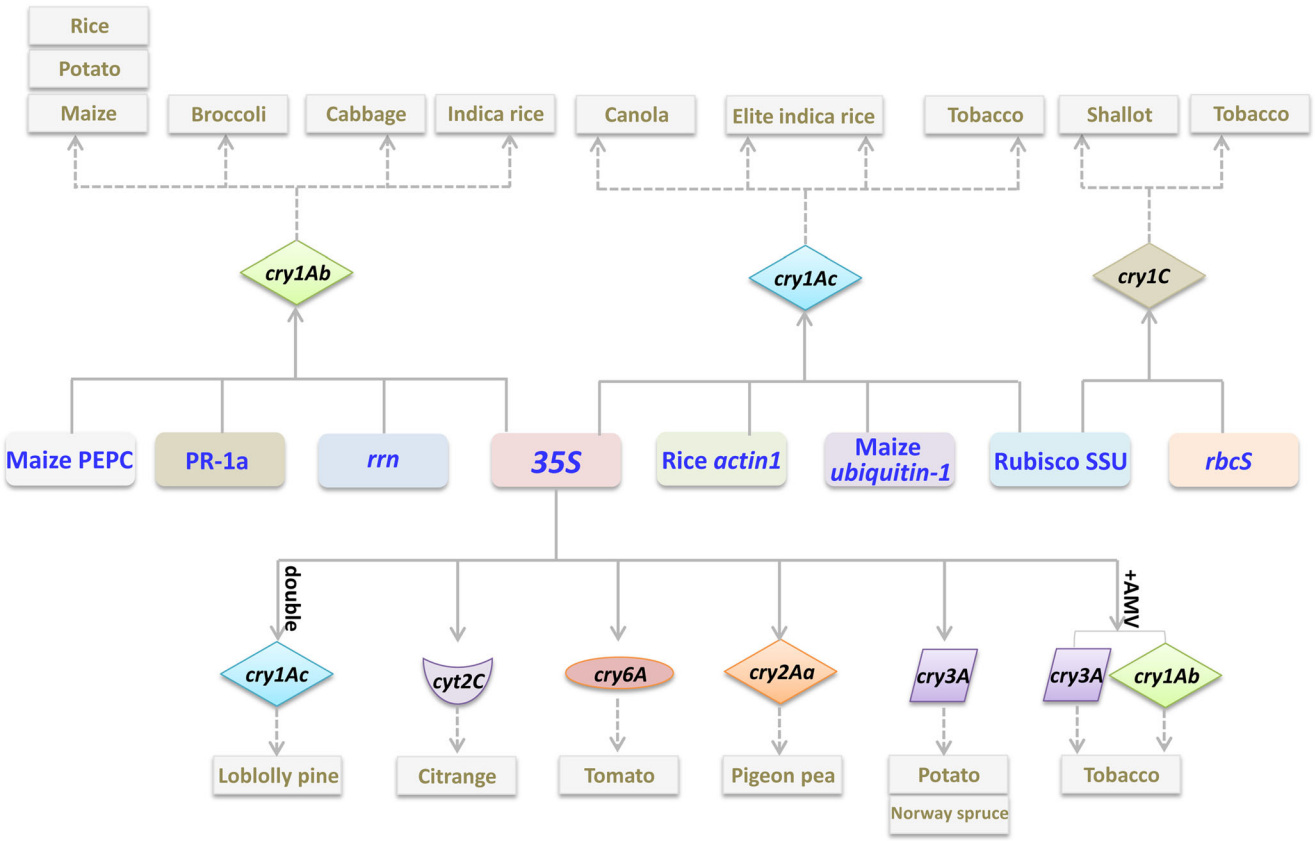

Fig 1 Expression of *cry* genes in Bt transgenic crops

Tissue-specific promoters can control Bt gene expression in a tissue-dependent manner; Bt toxin genes driven by tissue-specific promoters will be expressed only in tissues where insect resistance is desired, leaving the rest of the plant tissues unmodified. Green-tissue-specific *rbcS* promoter from *Nicotiana plumbaginifolia* is a strong promoter; by expressing *cry1C* primarily in leaf and green tissues, transgenic haploid tobacco gained resistance to *Spodoptera litura* (Christov et al. 1999). Maize phosphoenolpyruvate carboxylase gene (PEPC) promoter expressed a hybrid *cry1Ab/1Ac* gene in *Jatropha curcas* that then displayed insecticidal activity to *Archips micaceanus* (Gu et al. 2014). *cry1Ab* driven by maize PEPC promoter expressed in maize (Koziel et al. 1993), rice (Datta et al. 1998), and potato (Hagh et al. 2009) showed toxicity against European corn borer, larvae of the yellow stem borer, and tuber moth (*Phthorimaea operculella* (Zeller) respectively.

There is also an inducible promoter for Bt transgenic plants. The PR-1a promoter is inducible by chemicals including salicylic acid, 2,6-dichloro-iso-nicotinic acid (INA), and 1,2,3-benzothiadiazole-7-carbothioic acid *S*-methyl ester (BTH), which has been registered as an antifungal chemical for field application. *cry1Ab* driven by PR-1a promoter in transgenic broccoli can control diamondback moth (*Plutella xylostella* L.) from damage under chemical regulation (Cao et al. 2006).

Conclusion and perspectives

In microorganisms, the Bt system is still very efficient for *cry* gene expression. Many available strong promoters can direct most *cry* gene expression in acrystalliferous Bt strains. However, not all *cry* genes and niches are compatible with Bt. New delivery systems including Bt subspecies with genetically different backgrounds, other microorganisms, and even strong promoters can improve Cry production, niche fitness, and persistence.

In transgenic plants including potato, tomato, tobacco, rice, maize, and broccoli, *cry* genes have been used extensively. Constitutive promoters, such as 35S, have been frequently utilized for expressing *cry* genes to protect transgenic plants from pests. However, pests often damage the plants in special tissues during growth phases. Thus, more efficient tissue-specific and inducible promoters need to be investigated to express *cry* genes in plants suffering from pest infestation.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval No human or animal studies were performed for this review.

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