



Review

Genetic engineering of crops for insect resistance: An overview

ASHWINI TALAKAYALA, SUMALATHA KATTA and MALLIKARJUNA GARLADINNE* 

Plant Molecular Biology laboratory, Agri Biotech Foundation, Rajendranagar,
Hyderabad 500 030, India

*Corresponding author (Email, garladinnemarjun@gmail.com)

Phytophagous insect incidence is a serious threat for reduction of crop productivity globally. There is an estimation of one fourth of crop is being destroyed by insects annually. Indeed, the development of insect-resistant crops is a great milestone in agriculture to increase crop yield and reduce pesticide dependency. Genetic engineering facilitates development of insect resistant crops by expressing bacterial δ -endotoxins and vegetative insecticidal proteins and other plant genes like lectins, protease inhibitors, etc. In addition, RNA interference and genome editing through CRISPR Cas9 also provides new solutions for the development of insect-resistant crops. The resultant genetically modified crops showed resistance against lepidopteran, dipteran, homopteran and coleopteran insects. The insect-resistant crops have made a significant economic impact worldwide in terms of higher yield and low pesticide usage. In this review, we focus on different strategies for developing transgenics against insect pest control by expressing different insecticidal proteins in crops.

Keywords. *Bacillus thuringiensis*; CRISPR/Cas9; crystalline proteins; fusion proteins; insect resistance; RNA interference; transgenic crops; vegetative insecticidal proteins

1. Introduction

Insect pests are the major biotic stress factor that causes considerable crop losses globally by direct feeding and transmission of plant diseases (Douglas 2018). Novel strategies to control a broad spectrum of phytophagous insect pests have been the mainstay of crop protection and thereby increasing the crop yield. Chewing and sap-sucking pests are the major insects, which cause significant damage to crop productivity (Vanti *et al.* 2018). Recent advances to understand the molecular basis of insect–plant interactions and biotechnological applications are providing solutions to meet the challenges. Genetic engineering plays a pivotal role in conferring resistance against insects (Birkett and Pickett 2014). This tool is being exploited to introduce specific DNA sequences or genes into crop plants through *Agrobacterium*-mediated transformation or

particle bombardment for insect control (Juturu *et al.* 2015).

B. thuringiensis (*Bt*) is a gram-positive soil bacterium which forms parasporal crystals which contain insecticidal proteins ICPs (Panwar *et al.* 2018). ICPs are endotoxins that are highly toxic to certain classes of insects. They kill the insects by acting on epithelium tissue of midgut of the larvae of Lepidopteran, Coleopteran, Dipteran insects (Paul and Das 2020) (figure 1). The ICPs lyse the midgut epithelial cells of the target insect by forming pores in the membrane of the cells which cause ion leakage and insect mortality (figure 1). ICPs, vegetative insecticidal proteins (VIPs), lectins, fusion proteins, and pathogen-related protein genes have been expressed in plant species by different public and private funded laboratories worldwide (Paul and Das 2020). The first transgenic cotton was developed by Monsanto and Delta and Pine companies, USA (Little *et al.* 2017). Later, the research has been extended by incorporating several insecticidal genes in different combinations in different crops by genetic engineering approaches. In this review, we summarize

This article is part of the Topical Collection: Genetic Intervention in Plants: Mechanisms and Benefits.

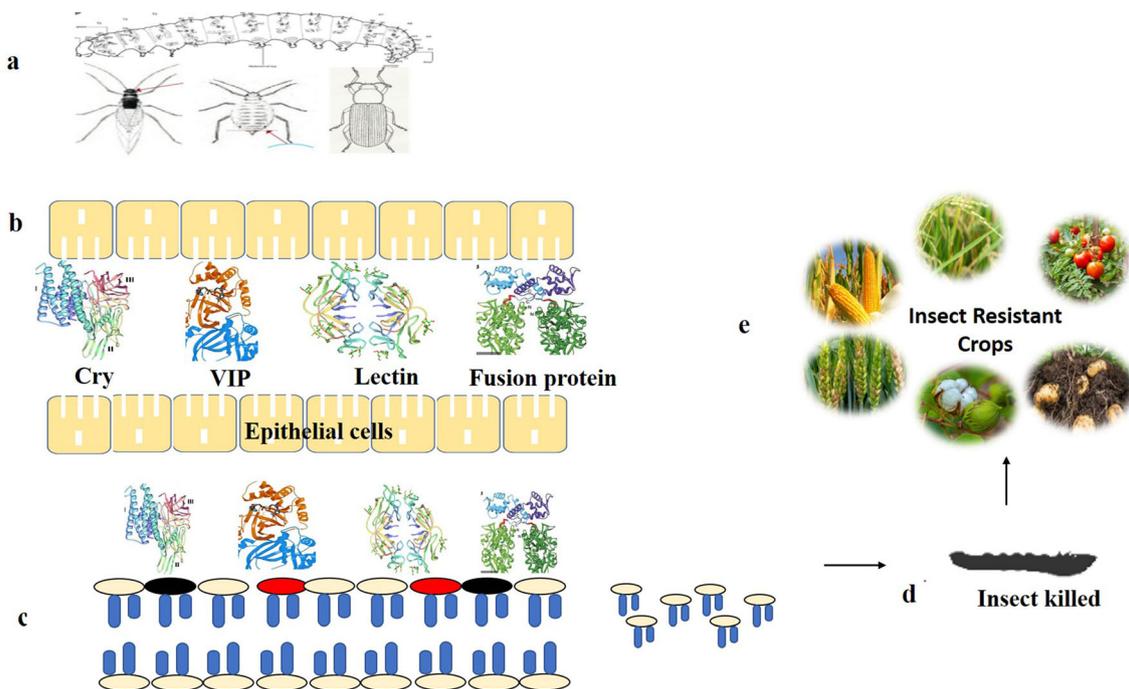


Figure 1. Schematic representation of the mode of action of the insecticidal proteins such as Cry proteins, VIP proteins, lectins and other fusion proteins in the intestine of lepidopterans, aphids and in other insects. (a) Different insects that feeds on major crops, (b) mode of action of different proteins in the gut epithelial cells, (c) cell lysis by ICP on epithelial cells, (d) death of the insect, (e) insect-resistant crops.

the present status of the development of insect-resistant crops.

2. Genetic engineering of *Cry* genes for insect resistance

Transgenic plants producing ICP have made a tremendous impact on the successful development of insect resistance. The *B. thuringiensis* (*Bt*) produces the ICPs which are synthesized during the process of sporulation. *Bt* toxins, ICPs, δ -endotoxins or crystalline proteins can be solubilized and activated by proteinases in the midgut region of an insect. The toxin binds to a specific receptor, creating a pore in the epithelial cells of the insect gut and finally kills the insect pest (Whalon and Wingerd 2003) (figure 1). Resistance to insect pests was shown mainly when *Bt* toxin producing ICP genes under the control of tissue-specific or constitutive promoters and introduced in different crop species, including maize, sweet potato, cotton and tomato (Chang et al. 2017; Zhong et al. 2019; Katta et al. 2020; Soliman et al. 2017)

The first-generation *Bt* cotton (Bollgard I – BG I) expressing *Cry1Ac* was commercialized and released in 2002 in India. Second-generation pyramided trait

(Bollgard II) expressing *Cry1Ac* and *Cry2Ab2* (MON15985 event) was approved in 2006, which currently occupies 95% of the cotton growing area in India. It is evidenced that the pyramided trait expressing *Cry1Ac* and *Cry2Ab* toxins showed superior phenotype than the cotton expressing *Cry1Ac* alone (Carrière et al. 2015). Introduction of *Bt* cotton controlled the major bollworms, including *Helicoverpa armigera*, *Earias vittella* and *Pectinophora gossypiella* in cotton growing regions and it ultimately improved the crop yields and also farmer's income. Then, wide-strike cotton containing *Cry1Ac*+*Cry1F* by Dow agro Sciences was introduced in USA in the year 2004. Both BG II and wide-strike cotton expressing multiple toxins have higher potential in controlling the broad range of caterpillar insects than BG I. Apart from cotton, four insect-resistant *Bt* brinjal varieties were approved and commercialized in Bangladesh in the year 2014 (Koch et al. 2015). Transgenic soybean expressing *Cry1Ac*+*Cry1Ab* was approved for commercial use in Latin America (Koch et al. 2015). The transgenic cotton expressing *cry1Ac* gene under the regulation of a strong constitutive Figwort Mosaic Virus (FMV) promoter showed elevated expression of *Cry1Ac* toxin that was lethal to lepidopteran insects, especially *H. armigera* (Singh et al. 2016). A combination of constitutive

and tissue-specific promoters would be a great advantage to express the genes continuously and for targeted organ-specific expression. Gao *et al* (2009) reported that *H. armigera* developed cross resistance between Cry1Ac and Cry2Ab in cotton cultivating regions in China. Hence, the transgenic plants expressing ICPs with different combinations are essential to curb the broad spectrum of harmful insect pest populations globally.

Transgenic tomato carrying a synthetic *cry1Ab* gene was highly effective and exhibited 100% larval mortality against tomato leaf miner i.e., *Tuta absoluta* at T₀ generation (Soliman *et al.* 2017). In another study, modified *cry1Ac* expression in tomato resulted in larval mortality to *T. absoluta* ranging from 38–100% (Selale *et al.* 2017). An efficient transformation protocol developed in chickpea by transferring *cry2IIAa* gene with Mendelian segregation at 15% transformation efficiency and showed resistance against pod borer (Sawardekar *et al.* 2017). Another synthetic *cry1Ab* gene has also been transferred into tomato which conferred resistance against *T. absoluta* with insect mortality of 100% within 4–5 days (Soliman *et al.* 2017). A triple gene construct containing *cry2Ab*, *cry1F* and *cry1Ac* genes driven by three different promoters, α -globulin, nodulin and double CaMV 35S respectively, was transferred to an elite cotton variety Narasimha. The transgenic plants showed expression of all the three ICP genes and the respective proteins. The transcripts of all ICP genes accumulated and showed the highest insect mortality to *H. armigera* and *S. litura* at T₂ generation (Katta *et al.* 2020).

Rice lines (*var. Bg94-1*) expressing insecticidal protein Cry2A conferred 80% mortality against rice leaf folder (RLF). About 19 transformed rice lines showed constant expression of Cry2A protein at T₁ generation with significant insecticidal activity (Gunasekara *et al.* 2017). Ghosh *et al* (2017) developed an independent transgenic pigeon pea lines by transferring *cry1Ac* and *cry2Aa* constructs. These transgenes in transgenic lines were inherited over three generations and showed resistance against *H. armigera* causing larval mortality of about 80–100%. The transgenic cotton expressing *Cry10Aa* under the control of cotton ubiquitination related promoter, i.e., *uceA1.7* resulted in strong resistance to cotton boll weevil (*Anthonomus grandis*) and bioassay indicated that 100% mortality to CBW was observed when the larvae fed on leaves of cotton transgenics at T₁ generation (Ribeiro *et al.* 2017).

A tissue-culture-independent *Agrobacterium*-mediated transformation protocol was used for the

development of pigeonpea transgenics by transferring *cry2Aa* gene and generated 17 putative transformants showing protein expression levels ranging from 0.04 μ g/g to 1.45 μ g/g in fresh leaf tissue. The weight and length of the larvae were reduced significantly after *H. armigera* fed on detached leaves of transgenic plants (Baburao and Sumangala 2018). In another study, transgenic pigeonpea developed through an *in-planta* transformation method using *cry2Aa* gene with 0.8% of transformation frequency at T₁ generation. The Cry2Aa protein accumulated in these transgenic plants ranging from 25–80 μ g/g FW, which showed 80–100% larval mortality to pod borer, i.e., *H. armigera* (Singh *et al.* 2018). Expression of *cry1Aa* gene in sweet potato conferred resistance against lepidoptera insect, i.e., *Spodoptera litura* (Zhong *et al.* 2019). The amount of Cry1Aa protein accumulated in transgenic lines significantly induced larval mortality and reduction of growth rate by disturbing the integrity of the midgut of *S. litura*. Anatomical data revealed that the columnar epithelium cells were seriously damaged thus improved the plant resistance to *S. litura* and enhanced yield (Zhong *et al.* 2019).

A combination of *cry1Ac* and *cry2Ab* cloned in the same T-DNA was used to generate transgenic cotton by *Agrobacterium*-mediated transformation. The multiple *Cry* gene expressing plants were shown to exhibit resistance against *S. litura* with 93% larval mortality (Siddiqui *et al.* 2019). Expression of *Cry8* like gene from *B. thuringensis* in soybean conferred resistance to *Coleopteran* pest, i.e., *Holotrichia parallela* and the protein was expressed largely at root stage where insects' feeds on roots (Qin *et al.* 2019). Muddanuru *et al* (2019) developed an industrially important non-edible castor by expressing the *cry1Aa* gene through *Agrobacterium* transformation technique, which implies resistance against two lepidopteran pests, i.e., *Achaea Janata* (semi-looper) and *S. litura*. About 8 transgenic events were generated and their protein was accumulated as 0.16–2.76 ng/mg and insect bioassay results indicated 20–80% insect mortality. The transgenic cotton MNH93 carrying *cry1Ab* showed 0.26% of transformation frequency with 40–60% larval mortality against *H. armigera* in T₁ progeny (Khan *et al.* 2011). Khan *et al* (2013) used cotton embryo as explant for transferring *Cry1Ab* against *Heliothis* larvae by producing pure line germplasm throughout five generations in MNH3 cotton cultivar. Expression of *cry2AX* gene in T₀ generation of the cotton event CH12 showed 88% mortality in *H. armigera* with 1.0 μ g/g protein concentration in fresh leaf tissue (Sakthi *et al.* 2015). Transgenic cotton CH12 carrying *cry2AX1*

showed stable integration and expressed protein constantly for about 90 days after sowing (DAS) with 1.055–1.5 µg/g in fresh leaf with 90% mortality of *H. armigera* in T₃ plants (Jadhav et al. 2020). Expression of cry genes in different crop species is depicted (table 1)

3. Insect resistance through VIP genes

B. thuringiensis harbours another kind of insecticidal proteins such as vegetative insecticidal proteins (VIP) formed during vegetative stage of the growth cycle. VIP causes swelling and disruption of the midgut

epithelial cells by osmotic lysis. The effect of VIP gene has been studied on cotton bollworm (*H. armigera*) and tobacco budworm (*Heliothis virescens*) (table 2). The cotton lines expressing *VIP3A* alone and another pyramided *VIP3A* and *cry1Ab* (*VipCot*) exhibited superior resistance to both the insects, *H. zea* and *H. virescens*. However, non-Bt cotton fruits were severely damaged by the insects (Bommireddy et al. 2011). A fusion protein *Vip3AcAaa* (derivative of *Vip3Aa1* and *Vip3Ac1*) expressing cotton lines showed broader insect resistance and it is indicating that the chimeric *VIP3AaAc1* protein is an excellent option for insect pest management (Chen et al. 2018). A fusion gene *Vip3Ba1* has been transferred to cowpea and resultant

Table 1. Transgenic crops carrying *Bt* genes for insect resistance

S. No.	Target Crop	Transgene	Target Insect	References
1	Cotton	<i>cry2AX1</i>	<i>H.armigera</i>	Jadhav et al. (2020)
2	Cotton	<i>cry2Ab</i> <i>cry1F</i> <i>cry1AC</i>	Lepidopteran pest <i>H. armigera</i> , <i>S. litura</i>	Katta et al. (2020)
3	Sweet Potato	<i>cry1Aa</i>	<i>S. litura</i>	Zhong et al. (2019)
4	Cotton	<i>cry1AC</i> <i>cry2Ab</i>	<i>S. litura</i>	Siddiqui et al. (2019)
5	Soyabean	<i>cry 8 like</i>	Coleopteran- <i>Holtrichia panallele</i>	Qin et al. (2019)
6	Castor	<i>cry1AC</i>	Lepidoteran- <i>Achaea janata</i> <i>S. litura</i>	Muddanuru et al. (2019)
7	Cotton	<i>cry2AX</i>	<i>H. armigera</i>	Sakthi et al. (2015)
8	Pigeon pea	<i>cry2Aa</i>	Pod borer- <i>H. armigera</i>	Singh et al. (2018)
9	Tomato	<i>cry1Ab</i>	<i>T. absoluta</i>	Soliman et al. (2017)
10	Tomato	<i>cry1Ac</i>	<i>Tuta Absoluta -tomato leaf miner</i>	Selale et al. (2017)
11	Chickpea	<i>CryIIAa</i>	Pod borer	Sawardekar et al. (2017)
12	Rice	<i>cry2A</i>	Leaf folder	Gunasekara et al. (2017)
13	Pigeon pea	<i>cry1AC</i> <i>cry2Aa</i>	<i>H. armigera</i>	Ghosh et al. (2017)
14	Cotton	<i>cry1Aa</i>	<i>Anthamous grandis</i>	Ribeiro et al. (2017)
15	Pigeon pea	<i>cry2Aa</i>	<i>H.armigera</i>	Baburao and Sumangala (2018)
16	Cotton	<i>cry1Ab</i>	<i>Heliothis</i>	Khan et al. (2013)
17	Cotton	<i>cry1Ab+NptII</i>	<i>H.armigera</i>	Khan et al. (2011)

Table 2. Expression of VIP genes for insect resistance

S. No.	Target Crop	Transgene	Target Insect	References
1	Sugarcane	<i>Vip3A</i>	Sugar cane stem borer (<i>Chilo infuscatellus</i>)	Riaz et al. (2020)
2	Cotton	<i>Vip3AcAaa</i> (<i>Vip3Aa1+Vip3Ac1</i>)	Lepidopteran	Chen et al. (2017)
3	Cowpea	<i>Vip3Ba1</i>	Legume pod borer (<i>Maruca vitrata</i>)	Bett et al. (2017)
4	Cotton	<i>Vip3A+Cry1Ab</i>	<i>Heliothis. zea</i> and <i>H. virescens</i>	Bommireddy et al. (2011)

plants showed resistance to legume lepidopteran pest, pod borer (*Maruca vitrata*). Cowpea expressing *Vip3Ba1* had strong inhibition of larval growth and showed further resistance against pod borer (Bett *et al.* 2017). Transgenic sugarcane with single copy insertion of *VIP3A* toxin under the regulation of polyubiquitin promoter had greater resistance against sugar cane stem borer (*Chilo infuscatellus*) with 100% mortality and 5.35–8.89 µg/ml of protein was accumulated in transgenic plants (Riaz *et al.* 2020).

4. Insect resistance through lectins

Plant lectins are carbohydrate-binding proteins, which have high binding affinity for specific sugar element of glycoproteins and glycolipids in cell membrane. Transgenic rice expressing *Allium sativum* leaf agglutinin (ASAL) and *Galanthus nivalis* lectin (GNA), showed insect resistance against major sap sucking pests including brown planthopper (BPH), white backed planthopper (WBPH) and green leafhopper. The transgenic rice expressing mannose-specific GNA under the regulation of phloem-specific promoter conferred partial resistance to brown planthopper (*Nilaparvata lugens*) and other hemipteran pest (Bharathi *et al.* 2011). Expression of recombinant fusion protein (Hv1a/GNA containing spider venom toxin ω-ACTX-Hv1a linked to snowdrop lectin) in *Arabidopsis*

showed effectivity to peach potato aphids and grain aphids (Nakasu *et al.* 2014).

Expression of *GNA* gene in potato conferred resistance to aphids and in comparison to non-transgenic plants (Mi *et al.* 2017). Lectins are novel defense genes including lentil lectin (LL) and Chickpea protease inhibitor (CPPI) genes, which were transformed into *Brassica juncea* with transformation efficiency of 30–40% and resulted plants that showed enhanced resistance to sap sucking pest, i.e., aphids (Rani *et al.* 2017b). The transgenic cotton lines expressing insect gut binding lectin from *Sclerotium rolfsii* showed resistance to sucking and chewing insects at T₁ generation. Moreover, around 69% of aphid population was reduced and 100% mortality of *S. litura* was observed within 96 h (Vanti *et al.* 2018). Mannose binding lectin i.e., Pinellia pedatisecta agglutinin (PPA) was over-expressed through *Agrobacterium*-mediated transformation, which conferred resistance to seven homozygous lines against wheat aphid in BE104 (Duan *et al.* 2018). Transgenic *B. juncea* expressing a novel lectin gene, i.e., *Colocasia esculenta* tuber agglutinin (CEA) and, a GNA showed higher expression against *Lipaphis erysimi* (Mustard aphid) at T₁ and T₂ generations. The transgenic mustard expressing CEA conferred enhanced insect mortality of about 70–81% compared to control plants, thus CEA was considered as an efficient insecticidal and non-allergenic protein (Das *et al.* 2018). Transgenic tomato expressing two

Table 3. Expression of lectin genes for insect resistance

S. No.	Target crop	Transgene	Target insect	References
1	Cowpea	Arcl on APA locus from <i>Phaeselous vulgaris</i>	Bruchids	Grazziotin <i>et al.</i> (2020)
2	Tomato	Remusatia vivipara (<i>rvi 1</i>) and <i>Sclerotium rolfsii</i> (<i>srl 1</i>)	Root knot nematode (<i>Meloidogyne incognita</i>)	Bhagat <i>et al.</i> (2019)
3	Brassica juncea-Mustard	<i>Colocasia esculenta</i> tuber agglutinin (CEA)+GNA	Mustard aphid (<i>Lipaphis erysimi</i>)	Das <i>et al.</i> (2018)
4	Wheat	Pinellia pedatisecta agglutinin (PPA)	Aphids	Duan <i>et al.</i> (2018)
5	Cotton	Insect gut binding lectin from <i>Sclerotium rolfsii</i>	Chewing, sucking pest	Vanti <i>et al.</i> (2018)
6	Brassica juncea-Mustard	Lentil Lectin-LL CPPI	Aphids	Rani <i>et al.</i> (2017b)
7	Potato	<i>Galanthus nivalis</i> agglutinin (GNA)	Aphids	Mi <i>et al.</i> (2017)
8	Potato	Hv1a/GNA	peach potato aphids and grain aphids	Nakasu <i>et al.</i> (2014)
9	Rice	Mannose specific GNA	BPH (<i>Nilaparvata lugens</i>) and hemipteran pest	Bharathi <i>et al.</i> (2011)

lectin genes such as *Remusatia vivipara* (*rv11*) and *Sclerotium rolfsii* (*srl 1*) showed 4.59% transformation efficiency in homozygous events. These plants exhibited enhanced resistance against root knot nematode, i.e., *Meloidogyne incognita* (Bhagat et al. 2019). Expression of *Arcelin 1* gene from *Phaseolus vulgaris* L. showed enhanced resistance against bruchids such as *Zabrotes subfasciatus* and *Callosobruchus maculatus* in cow pea. Number of eggs, emerging insects and loss of grain mass were significantly lesser in transgenic cowpea than in control against both bruchid insects (Grazziotin et al. 2020). Expression of lectin genes showed resistance to sap sucking insects in different crop plants (table 3).

5. Insect resistance through fusion proteins

Domains from different insecticidal proteins were joined together to generate fusion proteins. These proteins would transcribe and translate as a single polypeptide unit in the host plant system. The fusion proteins are more effective and augment efficacy against phytophagous insects. Transgenic rice expressing *cry2AX1* (derivative of *cry2Aa* and *cry2Ac*) gene showed enhanced resistance to lepidopteran pests (Chakraborty et al. 2016). Other fusions such as, *cyt2Aa* confers enhanced aphid mortality (Chougule et al. 2013) and *cry51Aa2*, against lygus species in cotton (Gowda et al. 2016).

A fusion protein derived from lectin and protease inhibitor was transferred into *B. juncea* by

Agrobacterium-mediated transformation using cotyledonary node explants (Rani et al. 2017a). The resultant plants showed resistance to phytophagous aphids with 40% survivability upon feeding on transgenics. This fusion protein accumulated 2.8-fold higher than that in the control plants. (Rani et al. 2017b). *Bt* maize expressing *cry1Ab/cry2Aj* fusion protein in kernel showed resistance against lepidopteran pest like *S. exigua* and *Harmonia axyridis* (Chang et al. 2017). The transgenic cotton lines expressing fusion protein, i.e., *cry1Be+cry1Fa* exhibited enhanced resistance to *S. litura* and *O. nubilalis* insects. These events were released by Dow Agrosiences (Meade et al. 2017). In another study, a hybrid fusion protein, i.e., *cry1Ac+ASAL* expressed in rice for enhanced level and durable resistance to major insects like YSB (Yellow stem borer), LF (Leaf folder) and BPH (brown plant hopper). Moreover, transgenic plants showed higher insect mortality in comparison to control plants. It indicated that this construct showed a remarkable entomotoxic effect against lepidopteran and hemipteran insects (Boddupally et al. 2018). Another combination of fusion protein, i.e., *cry1Ab+vip3A*, was constructed and transferred to rice, which showed resistance against rice pests, Asiatic rice borer and rice leaf folder (Xu et al. 2018). Pyramiding of nine transgenes, including seven plant defense genes (*Chi*, *Glu*, *Ace-AMP1*, *Tlp*, *Rs-AFP2*, *ZmPROPEP1* and *Pti4* and two anti-apoptosis genes (*lap* and *p35*), were implicated in resistance against necrotrophic pathogens, *Rhizoctonia* and *Bipolaris maydis*. Significant level of protein expression was observed in maize that aided the disease

Table 4. Fusion proteins for insect resistance in crop plants

S. No.	Target crop	Transgene	Target insect	References
1	Sugarcane	<i>cry2Aa+ cry1Ca</i> <i>cry1Ab+cry1Ac</i>	Shoot borer	Koerniati et al. (2020)
2	Rice	<i>cry1AC+ASAL</i>	Yellow stem borer Leaf folder BPH	Boddupally et al. (2018)
3	Rice	<i>cry1Ab+vip3A</i>	Asian stem borer, rice leaf folder	Xu et al. (2018)
4	Rice	<i>cry2Aa+cry1Ca</i>	<i>Chilo suppressalis</i>	Qiu et al. (2019)
5	Rice	<i>cry2AX1</i>	Rice leaf folder	Rajadurai et al. (2018)
6	Brassica juncea- mustard	<i>Lectin protease protein</i>	Aphid	Rani et al. (2017b)
7	Maize	<i>cry1Ab/cry2Aj</i>	<i>S. exigua</i> , <i>Harmonia axyridis</i>	Chang et al. (2017)
8	Cotton	<i>cry1Be+cry1Fa</i>	<i>S. litura</i> <i>O. nubilalis</i>	Meade et al. (2017)
9	Rice	<i>cry2AX1</i> (<i>cry2Aa+cry2Ac</i>)	Lepidopteran	Chakraborty et al. (2016)
10	Cotton	<i>cry51Aa2</i>	<i>Lygus species</i>	Gowda et al. (2016)

management (Zhu *et al.* 2018). Qiu *et al.* (2019) generated rice lines expressing *cry2Aa+cry1Ca* protein lethal to *Chilo suppressalis* insect pest. A synthetic gene of *cry1Ab-cry1Ac* fusion protein was transferred into sugarcane (*Bulu Lawang*) showed resistance against shoot borer (Koerniati *et al.* 2020). Marker-free transgenic rice lines were developed through the expression of *cry2AX1* gene driven by specific RbcS promoter and the resultant plants showed resistance against two major pests of rice, rice leaf folder (*C. medinalis*) and rice yellow stem borer (*S. incertulas*) and about 45–55% larval mortality was observed at T₂ generation (Rajadurai *et al.* 2018). Pyramiding of *cry* genes in combination with *VIP* genes is a sustainable strategy for achieving good management of lepidopteran insects (table 4). These innovations could pave the way for development of crops resistant to lepidopteran, coleopteran and hemipteran pests.

6. RNA interference (RNAi) approach

RNAi is the process of sequence-specific suppression of gene expression. RNAi-based insecticidal strategy offers new dimensions for environment-friendly insect pest management in plants (Mamta and Rajam 2017). Double-stranded RNA (dsRNA) specific to an essential gene of an insect pest processed by RNAase III enzyme Dicer to small interfering RNAs (siRNAs), guide the Argonaut pro-teins of RNA-induced silencing complex (RISC) to degrade complementary mRNA (Scott *et al.* 2013). dsRNAs are commonly used for interference of specific

gene silencing through genetic modifications in plants for developing pathogen resistance. Application and spraying of dsRNAs in maize, triggered the RNAi mechanism to initiate gene knockdown in piercing, sucking and stem borer insects and enhanced insect mortality rates (Li *et al.* 2015). Rice knockdown lines with two aminopeptidase N genes (*APN1* and *APN2*) decreased susceptibility to *C. suppressalis*, which is a major destructive pest in the rice and conferred resistance to two *Bt* varieties, TT51 (*cryAb* and *cry1Ac*) and T1C-19 (*cry1Ca*). These are the major receptor for knockdown to develop a resistance variety (Qiu *et al.* 2017). Silencing of *dvvgr* and *dvbol* genes in maize resulted in reduction of insect fecundity, minimal larval feeding and reduction in insect reproduction of western corn rootworm (*D. virgifera*) (Niu *et al.* 2017). Expression of dsRNA encoding *SpbP0* in soybean resulted in reduction of soybean pod borer larval mortality rate even at T₃ generation (Meng *et al.* 2017). A combination of Bt toxin along with RNAi (dsRNA) was pyramided and transgenic cotton lines were produced which interfere with Juvenile hormone methyl transferase (*JHMT*) gene in *H. armigera* (Ni *et al.* 2017). Transgenic potato expressing hairpin RNAi construct of molting-associated *EcR* gene (Ecdysone receptor) exhibited an enhanced resistance against Colorado potato beetle (CPB) with an insect mortality of 15–80% and larval weight was reduced (Hussain *et al.* 2019). Kola *et al.* (2019) generated rice lines by knocking down of Acetylcholinesterase gene (*AChE*) and showed resistance against yellow stem borer with reduced larval length and weight within 15 days. dsRNA/Nano carrier formulation was employed for

Table 5. Transgenic crops for insect resistance through RNA interference

S. No.	Target Crop	Silenced gene	Target insect	References
1	Soyabean	<i>TREH, ATPD, ATPE, CHSI</i>	<i>Aphid</i>	Yan <i>et al.</i> (2020)
2	Potato	<i>ECR</i> gene	Colorado potato beetle	Hussain <i>et al.</i> (2019)
3	Rice	<i>AchE</i> -Acetylcholine esterase	Yellow stem borer	Kola <i>et al.</i> (2019)
4	Rice	Aminopeptidase N genes <i>APN1+APN2</i>	<i>C. suppressalis</i>	Qiu <i>et al.</i> (2017)
5	Maize	<i>dvvgr</i> <i>dvbol</i>	Corn root worm <i>D. virgifera</i>	Niu <i>et al.</i> (2017)
6	Soyabean	<i>SpbP0</i> -dsRNA	Lepidopteran-soya bean pod borer	Meng <i>et al.</i> (2017)
7	Cotton	Juvenile hormone methyl transferase (<i>JHMT</i>)	<i>H. armigera</i>	Ni <i>et al.</i> (2016)
8	Tomato Tobacco	Chitinase gene- <i>HaCHI</i>	<i>H. armigera</i>	Mamta <i>et al.</i> (2016)
9	Maize	dsRNA-Spray	Lepidopteran	Li <i>et al.</i> (2015)

Table 6. Insect engineered for pest management using CRISPR/Cas9

S. No.	Target insect	Target gene	Character	References
1	<i>Rhopalosiphum padi</i>	β -1-3glucanase in maize	Reduction of callose deposition in maize sieve tubes	Kim et al. (2020)
2	<i>H. armigera</i>	α -6- nicotinic acetylcholine receptor (<i>nAChR</i>)	Resistance to an insecticide, spinosyn	Zuo et al. (2020)
3	<i>Ostrinia furnacalis</i>	<i>ABCC2</i>	Higher-level resistance to <i>Bt</i> cry1Fa toxin	Wang et al. (2020a, b)
4	<i>Plutella xylostella</i> (lepidopteran)	<i>PxABCC2</i> <i>PxABCC3</i>	Resistance to cry1Ac protoxin	Guo et al. (2019)
5	<i>H. armigera</i>	<i>CYP6AE</i>	Reduced survival rate of insects exposed to insecticides and phytochemicals	Wang et al. (2018)
6	<i>H. armigera</i>	<i>tetraspamin</i>	Dominant resistance to <i>Bt</i> toxin cry1Ac	Jin et al. (2018)
7	<i>H. armigera</i> <i>H. punctigera</i>	<i>HaABCA2</i> at 2bp deletion of exon 2 <i>HaABCA2</i> a 5bp deletion of exon 18	Resistance to both cry2Aa and cry2Ab	Wang et al. (2017)

targeting *TREH*, *ATPD*, *ATPE* and *CHSI* genes that induced high percentage of soybean aphid (*Aphis glycines*) mortality in soybean (Yan et al. 2020). Recently, it has been shown that silencing of chitinase gene (*HaCHI*) in *H. armigera* to develop insect resistance in tobacco and tomato through HI-RNAi caused down-regulation of the target gene transcripts induced mortality and developmental deformities at larval, pupal and adult stage (Mamta et al. 2016; Mamta and Rajam 2017). These results indicated that the RNAi approach is one of effective approaches to develop insect-resistant plants (table 5).

7. CRISPR-Cas-mediated genome editing for insect control

Genome editing tool is rapidly expanding its possibilities and opportunities for introducing resistance traits in crop plants. Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR associated 9 (CRISPR/Cas9) is a bacterial defense mechanism against invading bacteriophages. Cas9 and customizable single guide RNA (sgRNA) are two main important components required for CRISPR-Cas9-mediated genome editing. Cas9 is a monomeric RNA guided DNA endonuclease that contains two domains such as RuvC and HNH nucleases, which cleaves non-complementary and complementary DNA strands respectively, leading

to formation of blunted in target DNA and subsequently disrupts function of a gene through formation of frameshift in the targeted region (Doudna and Charpentier 2014). The CRISPR/Cas9 induced mutation in two β -1-3glucanase genes of golden promise cultivar of barley led to reduction of callose deposition in sieve tubes. Thus, the aphid, *Rhopalosiphum padi*, failed to access the phloem sap and it negatively affected the aphid growth and diminished the host preference in barely (Kim et al. 2020).

On the other hand, CRISPR/Cas9 tool has been employed for knockout of many insect genes including *H. armigera*, *S. exigua* etc. Knockout mutations in α -6-nicotinic acetylcholine receptor (*nAChR*) through CRISPR/Cas9 showed resistance to an insecticide, spinosyn, both in *H. armigera* and *S. exigua* (Zuo et al. 2020; Wang et al. 2020a, b). Knockout of two ABC transporters, *PxABCC2* and *PxABCC3* in lepidopteran pest *Plutella xylostella* through CRISPR/Cas9 tool, resulted higher level of resistance to cry1Ac protoxin compared to susceptible strains (Guo et al. 2019). The CRISPR/Cas9-mediated knockout of *ABCC2* gene in *Ostrinia furnacalis* confers a higher level resistance to *Bt* cry1Fa toxin (Wang et al. 2020a, b). In another study, knockout of ABC transporter gene, *HaABCA2* through CRISPR/Cas9 conferred resistance to both cry2Aa and cry2Ab in *H. armigera* (Wang et al. 2017). CRISPR/Cas9-mediated editing of *CYP6AE* gene cluster (P450 genes) in *H.*

armigera revealed that there was reduced survival rate of insects when exposed to insecticides and phytochemicals (Wang *et al.* 2018). A point mutation in *te-traspainin* gene through CRISPR technology, conferred dominant resistance to Bt toxin cry1Ac in cotton bollworm *H. armigera*. Further the mutations were analysed through genome wide association and genetic mapping by comparing resistant and susceptible strain (Jin *et al.* 2018). These results indicate that the CRISPRCas9 approach is one of effective approaches to develop resistance to insects. Several other genes have also been edited in lepidopteran pest using CRISPR tool (table 6).

8. Conclusions

Phytophagous insects particularly lepidopteran, coleopteran and hemipterans diminish crop yield either by feeding or by the transmission of viruses. Novel plant resistance traits have been developed by exploitation of biotechnological approaches, including the expression of Bt toxins (Cry, VIP), fusion proteins, lectins and other pathogen-related genes. The genetic engineering of crop species by the introduction of insecticidal genes offered tremendous protection against invasive and destructive crop pests in cotton and maize. As a result, the area of GM crops cultivation increased from 1.7 million to 190 mha between 1997 and 2017 globally. Despite the deployment of novel technologies in crops to achieve resistance, some of the agriculture pests often develop resistance to insecticidal toxins and devastate the crop production. The research groups should take up the challenges of understanding plant insect interactions. The durability of resistant traits in plants can be augmented and complemented by new technologies that offer more effective solutions against evolving insect pests. Advances like RNAi and CRISPR approaches can be used to silence/edit susceptible or negative regulatory alleles of plant immunity genes for developing insect resistance in plants.

References

- Baburao TM and Sumangala B 2018 Development and molecular characterization of transgenic Pigeon pea carrying *cry2Aa* for pod borer resistance. *J. Pharm. Phytochem.* **75** 1581–1585
- Bett B, Gollasch S, Moore A, James W, Armstrong J, Walsh T, Harding R and Higgins TJ 2017 Transgenic cowpeas (*Vigna unguiculata* L. Walp) expressing *Bacillus thuringiensis* Vip3Ba protein are protected against the Maruca pod borer (*Maruca vitrata*). *Plant Cell Tiss. Org. Cult.* **131** 335–345
- Bharathi Y, Kumar SV, Pasalu IC, Balachandran SM, Reddy VD and Rao KV 2011 Pyramided rice lines harbouring *Allium sativum* (asal) and *Galanthus nivalis* (gna) lectin genes impart enhanced resistance against major sap-sucking pests. *J. Biotechnol.* **152** 63–71
- Bhagat YS, Bhat RS, Kolekar RM, Patil AC, Lingaraju S, Patil RV and Udikeri SS 2019 *Remusatia vivipara* lectin and *Sclerotium rolfsii* lectin interfere with the development and gall formation activity of *Meloidogyne incognita* in transgenic tomato. *Transgenic Res.* **28** 299–315
- Birkett MA and Pickett JA 2014 Prospects of genetic engineering for robust insect resistance. *Curr. Opin. Plant Biol.* **19** 59–67
- Boddupally D, Tamirisa S, Gundra SR, Vudem DR and Khareedu VR 2018 Expression of hybrid fusion protein (Cry1Ac: ASAL) in transgenic rice plants imparts resistance against multiple insect pests. *Sci. Rep.* **8** 1–10
- Bommireddy PL, Leonard BR, Temple J, Price P, Emfinger K, Cook D and Hardke JT 2011 Field performance and seasonal efficacy profiles of transgenic cotton lines expressing Vip3A and VipCot against *Helicoverpa zea* (Boddie) and *Heliothis virescens* (F.). *J. Cotton Sci.* **15** 251–259
- Carrière Y, Crickmore N and Tabashnik BE 2015 Optimizing pyramided transgenic *Bt* crops for sustainable pest management. *Nat. Biotechnol.* **33** 161
- Chakraborty M, Reddy PS, Mustafa G, Rajesh G, Narasu VL, Udayasuriyan V and Rana D 2016 Transgenic rice expressing the *cry2AX1* gene confers resistance to multiple lepidopteran pests. *Transgenic Res.* **25** 665–78
- Chang X, Lu Z, Shen Z, Peng Y and Ye G 2017 Bitrophic and tritrophic effects of transgenic *cry1Ab/cry2Aj* maize on the beneficial, nontarget *Harmonia axyridis* (Coleoptera: Coccinellidae). *Envir. Entomol.* **46** 1171–6
- Chen W, Liu C, Lu G, Cheng H, Shen Z and Wu K 2018 Effects of Vip3AcAa+ Cry1Ac cotton on midgut tissue in *Helicoverpa armigera* (Lepidoptera: Noctuidae). *J. Insect Sci.* **18** 13
- Chougule NP, Li H, Liu S, Linz LB, Narva KE, Meade T and Bonning BC 2013 Retargeting of the *Bacillus thuringiensis* toxin Cyt2Aa against hemipteran insect pests. *Proc. Natl. Acad. Sci. USA* **110** 8465–70
- Das A, Ghosh P and Das S 2018 Expression of *Colocasia esculenta* tuber agglutinin in Indian mustard provides resistance against *Lipaphis erysimi* and the expressed protein is non-allergenic. *Plant Cell Rep.* **37** 849–63
- Doudna JA and Charpentier E 2014 The new frontier of genome engineering with CRISPR-Cas9. *Science* **346** 6213
- Douglas AE 2018. Strategies for enhanced crop resistance to insect pests. *Annu. Rev. Plant Biol.* **69** 637–660

- Duan X, Hou Q, Liu G, Pang X, Niu Z, Wang X, Zhang Y, Li B and Liang R 2018 Expression of *Pinellia pedatisecta* lectin gene in transgenic wheat enhances resistance to wheat aphids. *Molecules* **23** 748
- Gao Y, Wu K, Gould F and Shen Z 2009 Cry2Ab tolerance response of *Helicoverpa armigera* (Lepidoptera: Noctuidae) populations from Cry1Ac cotton planting region. *J. Econ. Entomol.* **102** 1217–23
- Ghosh G, Ganguly S, Purohit A, Chaudhuri RK, Das S and Chakraborti D 2017 Transgenic pigeonpea events expressing Cry1Ac and Cry2Aa exhibit resistance to *Helicoverpa armigera*. *Plant Cell Rep.* **36** 1037–1051
- Gowda A, Rydel TJ, Wollacott AM, Brown RS, Akbar W, Clark TL, Flasiniski S, Nageotte JR, Read AC, Shi X and Werner BJ 2016 A transgenic approach for controlling Lygus in cotton. *Nat. Commun.* **7** 12213
- Grazziotin MA, Cabral GB, Ibrahim AB, Machado RB and Aragão FJ 2020 Expression of the Arcelin 1 gene from *Phaseolus vulgaris* L. in cowpea seeds (*Vigna unguiculata* L.) confers bruchid resistance. *Ann. Appl. Biol.* **176** 268–274
- Guo Z, Sun D, Kang S, Zhou J, Gong L and Qin J 2019 CRISPR/Cas9-mediated knockout of both the *PxABCC2* and *PxABCC3* genes confers high-level resistance to *Bacillus thuringiensis* Cry1Ac toxin in the diamondback moth, *Plutella xylostella* (L.). *Insect Biochem. Mol. Biol.* **107** 31–38
- Gunasekara JM, Jayasekera GA, Perera KL and Wickramasuriya AM 2017 Development of a Sri Lankan rice variety Bg 94–1 harbouring *Cry2A* gene of *Bacillus thuringiensis* resistant to rice leaffolder [*Cnaphalocrocis medinalis* (Guenée)]. *J. Natl. Sci. Found Sri Lanka* **45** 2
- Hussain T, Aksoy E, Çalişkan ME and Bakhsh A 2019 Transgenic potato lines expressing hairpin RNAi construct of molting-associated EcR gene exhibit enhanced resistance against Colorado potato beetle (*Leptinotarsa decemlineata*, Say). *Transgenic Res.* **28** 151–64
- Jadhav MS, Rathnasamy SA, Natarajan B, Duraiyalagaraja S and Varatharajulu U 2020 Study of expression of indigenous Bt cry2AX1 gene in T3 progeny of cotton and its efficacy against *Helicoverpa armigera* (Hubner). *Braz. Arch. Biol. Technol.* **63** <https://doi.org/10.1590/1678-4324-2020180428>
- Jin L, Wang J, Guan F, Zhang J, Yu S, Liu S and Yang Y 2018 Dominant point mutation in a *tetraspainin* gene associated with field-evolved resistance of cotton bollworm to transgenic Bt cotton. *Proc. Natl. Acad. Sci. USA* **115** 11760–11765
- Juturu VN, Mekala GK and Kirti PB 2015 Current status of tissue culture and genetic transformation research in cotton (*Gossypium spp.*). *Plant Cell Tiss. Org. Cult.* **120** 813–839
- Katta S, Talakayala A, Reddy MK, Addepally U and Garladinne M 2020 Development of transgenic cotton (Narasimha) using triple gene *cry2Ab-cry1F-cry1Ac* construct conferring resistance to lepidopteran pests. *J. Biosci.* **45** 31
- Kim SY, Bengtsson T, Olsson N, Hot V, Zhu LH and Ahman I 2020 Mutations in Two Aphid-Regulated β -1, 3-Glucanase Genes by CRISPR/Cas9 Do Not Increase Barley Resistance to *Rhopalosiphum padi* L. *Front. Plant Sci.* **11** 1043
- Khan GA, Bakhsh A, Ghazanfar M, Riazuddin S and Husnain T 2013 Development of transgenic cotton lines harboring a pesticidal gene (Cry1Ab). *Emirates J. Food Agric.* **25** 434–442
- Khan GA, Bakhsha A, Riazuddin S and Husnain T 2011 Introduction of Cry1Ab gene into cotton (*Gossypium hirsutum*) enhances resistance against Lepidopteran pest (*Helicoverpa armigera*). *Spanish J. Agric. Res.* **1** 296–302
- Koch MS, Ward JM, Levine SL, Baum JA, Vicini JL and Hammond BG 2015 The food and environmental safety of Bt crops. *Front. Plant Sci.* **6** 283
- Koerniati S, Sukmadjaja D and Samudra IM 2020 C synthetic gene of *Cry1Ab-Cry1Ac* fusion to generate resistant sugarcane to shoot or stem borer. In IOP Conference Series: *Earth Environ. Sci.* **418** 012069
- Kola VS, Pichili R, Padmakumari AP, Mangrauthia SK, Balachandran SM and Madhav MS 2019 Knockdown of *acetylcholinesterase* (AChE) gene in rice yellow stem borer, *Scirpophaga incertulas* (Walker) through RNA interference. *Agri. Gene* **11** 100081
- Li H, Guan R, Guo H and Miao X 2015 New insights into an RNAi approach for plant defence against piercing, sucking and stem-borer insect pests. *Plant Cell Environ.* **38** 2277–2285
- Little NS, Catchot AL, Allen KC, Gore J, Musser FR, Cook DR and Luttrell RG 2017 Supplemental Control with Diamides for *Heliothis* in Bt Cotton. *Southwestern Entomol.* **42** 15–26
- Mamta B and Rajam MV 2017 RNAi technology: a new platform for crop pest control. *Physiol. Mol. Biol. Plants* **23** 487–501
- Mamta B, Reddy KR and Rajam MV 2016 Targeting chitinase gene of *Helicoverpa armigera* by host-induced RNA interference confers insect resistance in tobacco and tomato. *Plant Mol. Biol.* **9** 281–292
- Meade T, Narva K, Storer NP, Sheets JJ, Burton SL, Woosley AT, inventors, and Dow AgroSciences LLC, assignee 2017 Insect resistance management with combinations of Cry1Be and Cry1F proteins. *US Patent* **9** 556 453
- Meng F, Li Y, Zang Z, Li N, Ran R, Cao Y, Li T, Zhou Q and Li W 2017 Expression of the double-stranded RNA of the soybean pod borer *Leguminivora glycinivorella* (Lepidoptera: *Tortricidae*) ribosomal protein P0 gene enhances the resistance of transgenic soybean plants. *Pest Manag. Sci.* **73** 2447–2455
- Mi X, Liu X, Yan H, Liang L, Zhou X, Yang J, Si H and Zhang N 2017 Expression of the *Galanthus nivalis*

- agglutinin (GNA) gene in transgenic potato plants confers resistance to aphids. *Comptes Rendus Biol.* **340** 7–12
- Muddanuru T, Polumetla AK, Maddukuri L and Mulpuri S 2019 Development and evaluation of transgenic castor (*Ricinus communis* L.) expressing the insecticidal protein Cry1Aa of *Bacillus thuringiensis* against lepidopteran insect pests. *Crop Protec.* **119** 113–25
- Nakasu EY, Edwards MG, Fitches E, Gatehouse JA and Gatehouse AM 2014 Transgenic plants expressing ω -ACTX-Hv1a and snowdrop lectin (GNA) fusion protein show enhanced resistance to aphids. *Front. Plant Sci.* **28** 673
- Ni M, Ma W, Wang X, Gao M, Dai Y, Wei X, Zhang L, Peng Y, Chen S, Ding L and Tian Y 2017 Next-generation transgenic cotton: pyramiding RNAi and *Bt* counters insect resistance. *Plant Biotechnol. J.* **15** 1204–1213
- Niu X, Kassa A, Hu X, Robeson J, McMahan M, Richtman NM, Steimel JP, Kernodle BM, Crane VC, Sandahl G and Ritland JL 2017 Control of western corn rootworm (*Diabrotica virgifera*) reproduction through plant-mediated RNA interference. *Sci. Rep.* **7** 1–3
- Paul S and Das S 2020 Natural insecticidal proteins, the promising bio-control compounds for future crop protection. *Nucleus* **69** <https://doi.org/10.1007/s13237-020-00316-1>
- Panwar BS, Ram C, Narula RK and Kaur S 2018 Pool deconvolution approach for high-throughput gene mining from *Bacillus thuringiensis*. *Appl. Microbiol. Biotechnol.* **102** 1467–1482
- Qin D, Liu XY, Miceli C, Zhang Q and Wang PW 2019 Soybean plants expressing the *Bacillus thuringiensis* cry8-like gene show resistance to *Holotrichia parallela*. *BMC Biotechnol.* **19** 66
- Qiu L, Sun Y, Jiang Z, Yang P, Liu H, Zhou H, Wang X, Zhang W, Lin Y and Ma W 2019 The midgut V-ATPase subunit A gene is associated with toxicity to crystal 2Aa and crystal 1Ca-expressing transgenic rice in *Chilo suppressalis*. *Insect Mol. Biol.* **28** 520–527
- Qiu L, Fan J, Zhang B, Liu L, Wang X, Lei C, Lin Y and Ma W 2017 RNA interference knockdown of aminopeptidase N genes decrease the susceptibility of *Chilo suppressalis* larvae to *Cry1Ab/Cry1Ac* and *Cry1Ca*-expressing transgenic rice. *J. Invert. Pathol.* **145** 9–12
- Rajadurai G, Kalaivani A, Varanavasiyappan S, Balakrishnan N, Udayasuriyan V, Sudhakar D and Natarajan N 2018 Generation of insect resistant marker-free transgenic rice with a novel *cry2AX1* gene. *Ele. J. Plant Breed.* **9** 723–32
- Rani S, Sharma V, Hada A and Koundal KR 2017a Efficient Genetic Transformation of Brassica juncea with Lectin Using Cotyledon Explants. *Int. J. Adv. Biotechnol. Res.* **7** 1–12
- Rani S, Sharma V, Hada A, Bhattacharya RC and Koundal KR 2017b Fusion gene construct preparation with lectin and protease inhibitor genes against aphids and efficient genetic transformation of *Brassica juncea* using cotyledon explants. *Acta Physiol. Plant.* **39** 115
- Riaz S, Nasir IA, Bhatti MU, Adeyinka OS, Toufiq N, Yousaf I and Tabassum B 2020 Resistance to *Chilo infuscatellus* (Lepidoptera: Pyraloidea) in transgenic lines of sugarcane expressing *Bacillus thuringiensis* derived Vip3A protein. *Mol. Biol. Rep.* **47** 1–10
- Ribeiro TP, Arraes FB, Lourenço-Tessutti IT, Silva MS, Lisei-de-Sá ME, Lucena WA, Macedo LL, Lima JN, Santos Amorim RM, Artico S and Alves-Ferreira M 2017 Transgenic cotton expressing Cry10Aa toxin confers high resistance to the cotton boll weevil. *Plant Biotechnol. J.* **15** 997–1009
- Sakthi AR, Naveenkumar A, Deepikha PS, Balakrishnan N, Kumar KK, Devi EK, Balasubramani V, Arul L, Singh PK, Sudhakar D and Udayasuriyan V 2015 Expression and inheritance of chimeric *cry2AX1* gene in transgenic cotton plants generated through somatic embryogenesis. *In Vitro Cell Dev. Biol. Plant* **51** 379–389
- Sawardekar SV, Katageri IS, Salimath PM, Kumar PA and Kelkar VG 2017 Standardization of in-vitro genetic transformation technique in chickpea (*Cicer arietinum* L.) for pod-borer resistance. *Adv. Agric. Res. Technol. J.* **1** 2
- Scott JG, Michel K, Bartholomay LC, Siegfried BD, Hunter WB, Smaghe G, Zhu KY and Douglas AE 2013 Towards the elements of successful insect RNAi. *J. Insect Physiol.* **59** 1212–1221
- Selale H, Dağlı F, Mutlu N, Doğanlar S and Frary A 2017 Cry1Ac-mediated resistance to tomato leaf miner (*Tuta absoluta*) in tomato. *Plant Cell Tiss. Org. Cult.* **131** 65–73
- Siddiqui HA, Asif M, Asad S, Naqvi RZ, Ajaz S, Umer N, Anjum N, Rauf I, Sarwar M, Arshad M and Amin I 2019 Development and evaluation of double gene transgenic cotton lines expressing Cry toxins for protection against chewing insect pests. *Sci. Rep.* **9** 1–7
- Singh AK, Paritosh K, Kant U, Burma PK and Pental D 2016 High expression of Cry1Ac protein in cotton (*Gossypium hirsutum*) by combining independent transgenic events that target the protein to cytoplasm and plastids. *PLoS One* **11** 158603
- Singh S, Kumar NR, Maniraj R, Lakshmikanth R, Rao KY, Muralimohan N, Arulprakash T, Karthik K, Shashibhushan NB, Vinutha T, Pattanayak D, Kumar PA and Sreevathsa R 2018 Expression of Cry2Aa, a *Bacillus thuringiensis* insecticidal protein in transgenic pigeon pea confers resistance to gram pod borer, *Helicoverpa armigera*. *Sci. Rep.* **8** 1–2
- Soliman HI, Abo-El-Hasan FM, El-Seedy AS and Mabrouk YM 2017 Agrobacterium-mediated transformation of tomato (*Lycopersicon esculentum* mill.) using a synthetic cry1ab gene for enhanced resistance against *Tuta absoluta* (Meyrick). *J. Microbiol. Biotech. Food Sci.* **7**(1) 67–74
- Vanti GL, Katageri IS, Inamdar SR, Hiremathada V and Swamy BM 2018 Potent insect gut binding lectin from

- Sclerotium rolfsii* impart resistance to sucking and chewing type insects in cotton. *J. Biotechnol.* **278** 20–27
- Wang H, Shi Y, Wang L, Liu S, Wu S, Yang Y and Wu Y 2018 *CYP6AE* gene cluster knockout in *Helicoverpa armigera* reveals role in detoxification of phytochemicals and insecticides. *Nat. Commun.* **9** 1–8
- Wang J, Ma H, Zuo Y, Yang Y and Wu Y 2020a CRISPR-mediated gene knockout reveals nicotinic acetylcholine receptor (nAChR) subunit $\alpha 6$ as a target of spinosyns in *Helicoverpa armigera*. *Pest Manag. Sci.* **76** <https://doi.org/10.1002/ps.5889>
- Wang X, Xu Y, Huang J, Jin W, Yang Y and Wu Y 2020b CRISPR-Mediated Knockout of the *ABCC2* Gene in *Ostrinia furnacalis* Confers High-Level Resistance to the *Bacillus thuringiensis* Cry1Fa Toxin. *Toxins* **12** 246
- Wang J, Wang H, Liu S, Liu L, Tay WT, Walsh TK and Wu Y 2017 CRISPR/Cas9 mediated genome editing of *Helicoverpa armigera* with mutations of an ABC transporter gene *HaABCA2* confers resistance to *Bacillus thuringiensis* Cry2A toxins. *Insect. Biochem. Mol. Biol.* **87** 147–153
- Whalon ME and Wingerd BA 2003 Bt: mode of action and use. *Archives of Insect Biochemistry and Physiology. Entol. Soc. America* **54** 200–211
- Xu C, Cheng J, Lin H, Lin C, Gao J and Shen Z 2018 Characterization of transgenic rice expressing fusion protein Cry1Ab/Vip3A for insect resistance. *Sci. Rep.* **8** 1–8
- Yan S, Qian J, Cai C, Ma Z, Li J, Yin M, Ren B and Shen J 2020 Spray method application of transdermal dsRNA delivery system for efficient gene silencing and pest control on soybean aphid *Aphis glycines*. *J. Pest Sci.* **93** 449–459
- Zhong Y, Ahmed S, Deng G, Fan W, Zhang P and Wang H 2019 Improved insect resistance against *Spodoptera litura* in transgenic sweet potato by overexpressing Cry1Aa toxin. *Plant Cell Rep.* **38** 1439–1448
- Zhu X, Zhao J, Abbas HM, Liu Y, Cheng M, Huang J, Cheng W, Wang B, Bai C, Wang G and Dong W 2018 Pyramiding of nine transgenes in maize generates high-level resistance against necrotrophic maize pathogens. *Theor. Appl. Genet.* **131** 2145–2156
- Zuo Y, Xue Y, Lu W, Ma H, Chen M, Wu Y and Hu Z 2020 Functional validation of nicotinic acetylcholine receptor (nAChR) $\alpha 6$ as a target of spinosyns in *Spodoptera exigua* utilizing the CRISPR/Cas9 system. *Pest Manag. Sci.* **76** 2415–2422