Different Generations of Genetically Modified Crops for Insect Resistance

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Abstract

Crops are exposed to a variety of insect pests throughout their lifetime. Insect pests cause significant damage to crop plants by feeding on their tissues or sap. Besides the conventional methods which are based on using chemicals, the genetic transformation of plants with insecticidal toxin genes such as *Bt* has been widely applied to control insect pests. In addition to *Bt* genes, other toxin genes from different sources were also transferred to plants. Transgenic plants have been on the market for over two decades and have had remarkable achievements so far. However, current restrictions on these products, as well as public concern make scientists explore new approaches. The advent of RNA interference technology and later the CRISPR/Cas genome editing tool has opened up a promising new avenue in the development of next-generation biotech crops. These new approaches allow scientists to introduce new plant genotypes resistant to pests and diseases without transferring toxin genes, and all it takes is to edit target regions in the genome or apply modifications to the host transcriptome content. In this chapter, we will review different generations of biotech crops developed for insect resistance.

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11.1 Introduction

Sustainable crop production is one of the biggest challenges we face to ensure the availability of adequate nutrition for the world's growing population. According to the United Nations report, the world population which is currently around 8 billion in 2023, will reach 9.7 billion in 2050. The increase in food demand by 59–98% during this time renders traditional agricultural practices insufficient to secure the food supply (Valin et al. 2014). In addition, unfortunately, global climate change, biotic and abiotic stress factors cause serious problems in agricultural production. According to a research report, global crop losses caused by pests and diseases have been calculated as up to 37%, with 13% of losses due to insects (Gatehouse et al. 1992). Insect pests damage different parts of plants including roots, stems, leaves, and fruits either by chewing these parts or sucking the plant sap. Moreover, these pests may cause indirect damage to the host plant as a vector for viral, bacterial, and fungal pathogens (Mahmood-ur-Rahman et al. 2021). In order to protect the crops against biotic factors, farmers traditionally adopt a variety of synthetic insecticides, however, the increasing use of chemicals has been proven to be harmful to the environment and public health (Curry et al. 2002). The advent of recombinant DNA technology has opened up a horizon of promise to reduce chemical use concerns. The successful transformation of tobacco plants with *Cry* gene from *Bacillus thuringiensis* (Vaeck et al. 1987) made the genetic modification of crops a novel approach to reducing insecticide use. Together with tobacco, transgenic cotton plant was also produced in 1987 (Umbeck et al. 1987). Since the introduction of the first biotech crop to the market in 1996, the production ratio of these crops has increased more than 100-fold, with 190.4 million hectares grown in 29 countries in 2019 (ISAAA 2019).

So far, numerous plant transformation studies with *Bt* genes have taken important steps forward. However, based on laboratory selection and data collected from the field, the resistance conferred by *Bt* genes proved fragile as some species developed resistance to Cry toxins (Tabashnik 1994; Ferré et al. 1995). Moreover, the genetic modification of crops has been questioned and criticized by the public and scientists (Godfrey 2000). Thus, despite the successes achieved by Bt crops, they turned out to be insufficient on their own to be considered as a guaranteed long-term alternative approach to agricultural production. Using resistance genes isolated from plants such as agglutinin lectin genes (*GNA*, *ASAL*, *ACA, WGA*), Potato inhibitor II genes, and the gene stacking strategy was then carried out to improve the utility of these crops (Bakhsh et al. 2015). Besides, the employment of genetic modification technologies such as RNAi and CRISPR/Cas system pave the way to novel insect pest management studies. This chapter reviewsdifferent generations of genetically modified

crops including Bt-, RNAi- and CRISPR/Cas-based developed crops resistant to insect pests.

11.2 Transformation of Crop Plants with Resistance Genes

11.2.1 Bt Crops

The genetic transformation of plants with insecticidal toxin genes such as Cry toxins has been widely used to control insect pests. *Bacillus thuringiensis* is the source of different insecticidal agents, including *Cry* toxins, and has been deployed in pest management strategies. *B.thuringiensis* is a gram-positive soil-dwelling spore-producing bacterium that has been used as a biological control agent for nearly a century. *B. thuringiensis* is safe for humans and is the most environmentally compatible microbial insecticide worldwide (Ibrahim et al. 2010). This unique bacterium is the source of insecticidal toxin genes, mainly *Vip*, *Sip*, *Cry* (Crystal), and *Cyt* (Cytolytic) genes, which are produced throughout the bacterium's life cycle (Santos et al. 2022). *B. thuringiensis* can colonize inside the insect gut, therefore, it is an appropriate insecticidal agent for pest management strategies (Deist et al. 2014).

According to the classification by Crickmore et al. (1998), *Cry* genes are divided into 51 groups and subgroups, and Cry toxins based on the insect host specifications are classified into six main groups including group 1 lepidoptera (*Cry1*, *Cry9* and *Cry15*); group 2 lepidoptera and diptera (*Cry2*); group 3coleoptera (*Cry3*, *Cry7* and *Cry8*); group 4 diptera (*Cry4*, *Cry10*, *Cry11*, *Cry16*, *Cry17*, *Cry19* and *Cry20*); group 5 lepidoptera and coleoptera (*Cry1I*); and group 6 nematodes (*Cry6*) (reviewed in Ibrahim et al. 2010).

Using the advantage of recombinant DNA technology in the late 1980s, the first *Bt* gene was transferred to tobacco and cotton plants (Vaeck et al. 1987; Umbeck et al. 1987) and commercialization of transgenic crops expressing the *Bt* gene started in the mid-1990s and by 1999 different transgenic Bt crops such as potato, cotton, and corn were also introduced (Tabashnik et al. 2013). To date, different *Cry* genes have been transferred to agricultural crops to confer resistance to different pest species of lepidoptera, coleoptera, diptera (reviewed in Bakhsh et al. 2015). The introduction of Bt crops has reduced the use of chemical pesticides in the fields and their subsequent harmful side effects. Most Bt strains are harmful to lepidopterans; however, some are also lethal to coleopterans (McPherson et al. 1988) or dipterans (Yamamoto and McLaughlin 1981). It has been determined that Bt proteins do not show any toxicity to beneficial insects, other animals, or humans (Klausner 1984). Modification of *Bt* genes for improved expression in plants was a critical step toward achieving insect resistance in plants (Perlak et al. 1991). Codon-optimized genes conferring protection against insects of coleoptera and lepidoptera were respectively transferred to potato and cotton at first (Perlak et al. 1991). After the first reports of insect resistance, many successful studies were carried out to confer resistance against insect pests (Table 11.1).

Table 11.1 List of the toxin genes transferred to some of the crop plants

Crop	Toxin genes	Targeted insect order	Reference
Alfalfa	*Cry3a*	Coleoptera	Tohidfar et al. (2013)
Canola	*Cry1Ac*	Lepidoptera	Tabashnik et al. (1993) Stewart Jr et al. (1996) Ramachandran et al. (1998) Halfhill et al. (2001) Wang et al. (2014a, b) Rahnama and Sheykhhasan (2016)
Chickpea	*Cry1A (c)* *Cry2Aa* *Cry1Ac* + *Cry1Ab* *ASAL* *Vip3Aa*	Lepidoptera Hemiptera	Sanyal et al. (2005) Indurker et al. 2010 Chakraborti et al. (2009) Acharjee et al. (2010) Mehrotra et al. (2011) Singh et al. (2022)
Cotton	*Cry1Aa* *Cry1Ab* *Cry1Ac* *Cry2A* *Cry1EC* *Cry2Ab* *Cry3Bb1* *Cry3* *Cry11* *Cry1h* *Cry1Ia12* *potato proteinase inhibitor* *GNA* *ACA* *ASAL*	Lepidoptera Hemiptera	Perlak et al. (1990) Majeed (2005) Wu et al. (2006) Tohidfar et al. (2008) Khan et al. (2011) Pushpa et al. (2013) Vajhala et al. (2013) Anayol et al. (2016) Bakhsh et al. (2016) Khabbazi et al. (2018) Siddiqui et al. (2019) Zafar et al. (2022) Razzaq et al. (2023) Tariq et al. (2022)
Maize	*Cry3Bb1* *Cry1Ab* *Cry1Ab* (MON810) *Cry19c* *GNA*	Lepidoptera Hemiptera	Koziel et al. (1993) Vaughn et al. (2005) Wang et al. (2005) Gassmann et al. (2011)
Potato/sweet potato	*Cry3A* *Cry3Aa* *Cry1Ac* *Cry1Ab* Cowpea trypsin inhibitor *Cry1Ba1* *Cry1Ca5* *Cry9Aa2* *GNA* *ConA*	Coleoptera Lepidoptera Hemiptera	Peferoen et al. (1990) Cheng et al. (1992) Adang et al. (1993) Perlak et al. (1993) Newell et al. (1995) Morán et al. (1998) Gatehouse et al. (1999) Meiyalaghan et al. (2006) Jacobs et al. (2009) Mi et al. (2015) Salehian et al. (2021)
Rice	*Cry1A(b)* *Cry1A(c)*	Lepidoptera Hemiptera	Fujimoto et al. (1993) Wünn et al. (1996)

(continued)

Table 11.1 (continued)

Crop	Toxin genes	Targeted insect order	Reference
	PinII *Cry1C* *Cry2AX1* SBK + SCK *GNA* *ASAL* *DB1/ G95A-mALS*		Cheng et al. (1998) Bashir et al. (2005) Tang et al. (2006) Zhang et al. (2013) Ramesh et al. (2004) Yoshimura et al. (2012) Chandrasekhar et al. (2014) Chakraborty et al. (2016) Boddupally et al. (2018) Liu et al. (2022)
Soybean	*Cry1Ab* *Cry1Ac* *Cry8-like* *eCry1Gb.1Ig*	Lepidoptera	Parrott et al. (1994) Dufourmantel et al. (2005) Dang and Wei (2007) Qin et al. (2019) Je et al. (2022) Chae et al. (2022)
Tomato	*Cry1Ac* *Cry1Ab*	Lepidoptera	Mandaokar et al. (2000) Kumar and Kumar (2004) Koul et al. (2014)

Most of the transgenic crops are harboring constitutive promoters in particular the 35S CaMV promoter driving the foreign genes which providethe strong production of toxin protein in whole plant tissues and organs including root, stem, flowers, pollens, etc. Hence to restrict the unnecessary production of toxins in plants, foreign genes can be expressed by inducible promoters. For instance, to restrict the production of Crytoxin to insect-biting sites in plants toxin genes were expressed under the control of the wound-inducible promoter (AoPR1) isolated from *Asparagus officinalis* (Özcan et al. 1993; Bakhsh et al. 2016; Anayol et al. 2016; Khabbazi et al. 2018). The use of AoPR1 promoter confines the accumulation of Bt toxin to the wounding part of the plant, therefore, it is a valuable approach in insect pest management considering the public concerns regarding transgenic Bt crops.

11.2.2 Transgenics Harboring Plant-Derived Insect Resistance Genes

Cultivation of Bt crops increased crop production and reduced the use of chemical insecticides in the field (Toenniessen et al. 2003). Therefore, it has had an important contribution to global food security and poverty reduction. Reports indicate that this technology is beneficial for farming communities and consumers (Qaim 2009). To date, many *Cry* resistance genes have been transferred to crops to cope with damaging insects. While most of these have had a satisfactory outcome at first, the efficacy of resistance genes has been compromised by the widespread cultivation of transgenic crops. Based on reports some of the pest species have evolved resistance

against Cry proteins which indicates that the toxic effect of these genes has diminished (Calles-Torrez et al. 2019; Smith et al. 2019; Tabashnik and Carrière 2019). For example, the excessive use of *Cry1Ac* has led to the development of resistance in insect pests. This resistance is due to mutations occurring in the midgut receptors like cadherin. Development of crops harboring the codon-optimized *Bt* genes is an efficient method to combat the field-evolved resistance to Bt toxins (Tabashnik and Carrière 2017; Benowitz et al. 2022; Siddiqui et al. 2023). In addition, the investigation of new insecticidal genes and approaches is a necessity for sustainable pest management strategies. Using plant-derived toxin genes alone or in combination with *Cry* genes could be another approach to this goal (Khabbazi et al. 2018; Boddupally et al. 2018). Different lectin genes are toxic to members of coleoptera, lepidoptera (Czapla and Lang 1990), and diptera (Eisemann et al. 1994). Lectins stimulate endocytosis and possibly other toxic metabolites in the midgut, resulting in the inhibition of nutrient absorption or disruption of midgut cells (Czapla and Lang 1990). Plant lectin genes are toxic to sap-sucking insects of hemiptera and have an inhibitory effect on their growth and fecundity (Wang et al. 2005; Chakraborti et al. 2009; Khabbazi et al. 2018).

Transformation of crops with *Galanthus nivalis* agglutinin lectin gene (*GNA*) isolated from the snowdrop plant confers resistance to *Aphis gossypii, Rhopalosiphum maidis, Sitobion avenae* and other sap-sucking members of hemiptera (Khabbazi et al. 2018; Wang et al. 2005; Stoger et al. 1999). This gene as well as other agglutinin lectin genes derived from garlic (*ASAL*), onion (*ACA*), wheat (*WGA*) etc. has no harmful effect on the mammalian oral system (Peumans and van Damme 1996; Khabbazi et al. 2016) and have been transferred to some of the important crops such as cotton, maize, chickpea and rice (Table 11.1) and resulted in increased resistance to different sap-sucking insects including aphids, jassids, planthoppers and whiteflies (Bakhsh et al. 2015). Along with the transformation of plants with resistance genes, RNAi and CRISPR/Cas-based methods are other relatively new approaches contributing to sustainable pest management strategies in agriculture.

11.3 RNA Interference-Mediated Modifications in Plants

11.3.1 What Is RNAi and How Does It Work?

RNA silencing is a process that causes the downregulation of a target gene expression.This technology is a worthy reverse genetics tool to study gene function (Harmon et al. 2000). It is divided into transcriptional gene silencing (TGS) and post-transcriptional gene silencing (PTGS). PTGS also known as RNA interference (RNAi) is a highly specific homology-based gene silencing tool that is frequently used to downregulate the expression of target genes via mRNA degradation and hence is also called a knockdown process (Small 2007; Tang and Galili 2004). RNAi is triggered by the introduction of double-stranded RNA (dsRNA) molecules microRNAs (miRNAs) and small interfering RNAs (siRNAs) are the two main

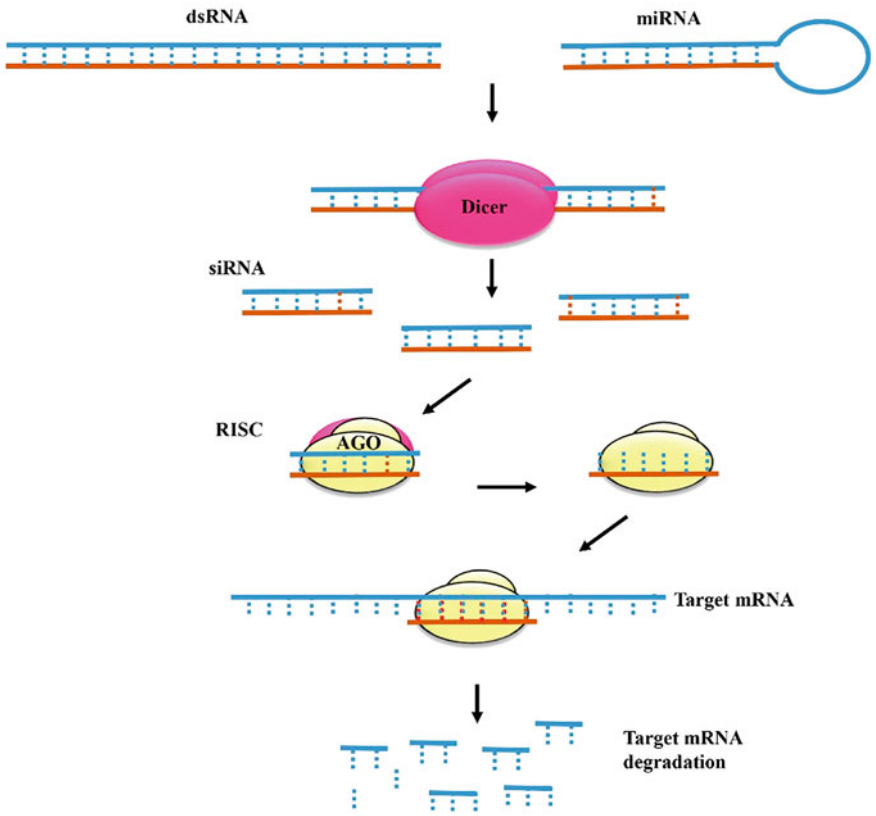

Fig. 11.1 Mechanism of the RNAi pathway. Long dsRNA or miRNA molecules are cleaved by the Dicer enzyme into short duplexes of 21–25 nucleotide RNAs. Small RNAs bind to the RISC complex and single-stranded short RNAs are produced and directed to the target mRNA, causing degradation of the mRNA

classes of small non-coding RNAs that initiate gene silencing in plants (Axtell 2013). Small interfering RNA constructs are short duplexes of 21 to 25 nucleotides produced after long dsRNA precursors are cleaved by the ribonuclease III Dicer-like (DCL) enzyme (Zamore et al. 2000; Bernstein et al. 2001). Afterward, miRNAs or siRNAs incorporated into RNA-induced silencing complex (RISC), argonaute, and other effector proteins bind to complementary mRNA molecules and subsequently degrade the mRNA and causing downregulation of the target gene (Fig. 11.1) (Bosher and Labouesse 2000; Kim and Rossi 2007; Mittal et al. 2011).

11.3.2 RNAi-Based Gene Regulation for Insect Resistance in Plants

The RNAi process is conserved in higher eukaryotes and naturally protects the host from viruses in plants, but is currently used in a variety of ways for different purposes, including insect-plant interaction studies (Khabbazi et al. 2020). RNAi has opened a new avenue in insect pest management strategies. This technology is particularly effective in controlling insects of the order coleoptera, whereas insects of lepidoptera and hemiptera are recalcitrant in response to RNAi which may be due to the biological barriers limiting the use of RNAi in these species (Terenius et al. 2011; Baum and Roberts 2014).

dsRNAs are either expressed by host plants or applied by methods like microinjection, feeding and spraying to control the insect pest damage on plants. Host-induced gene silencing (HIGS)-mediated RNAi has been successfully used in a variety of crop species to manage different agricultural insect pests including sap-sucking and chewing species (Table 11.1). In this approach, plant genetic background is engineered to produce dsRNAs targeting the essential genes in insect pests. After insects are fed with transgenic plants, dsRNAs are transported to the insect salivary glands or gut, and adsorbing cells subsequently activate the insect RNAi machinery and silence the targeted genes that interfere with insect vital metabolism. The utilization of plant-mediated RNAi provides a promising tool in crop protection without the use of chemicals and has the potential to target an unlimited number of genes in insects (Zhang et al. 2017).

Aphids are the members of the order hemiptera that damage crop plants by phloem-feeding and transmitting viral diseases. In HIGS-mediated RNAi studies in aphids, the focus has been on studying the management of *Myzus persicae* and *Sitobion avenae* aphid species in transgenic host plants including *Arabidopsis thaliana*, *Nicotiana tabacum*, *N. benthamiana*, and *Solanum lycopersicum* and *Triticum aestivum* (reviewed in Zhang et al. 2022). RNAi-mediated knocking down of the salivary effectors (MpC002, MpPIntO1, MpPIntO2, Mp55), Receptor of Activated Kinase C (Rack1), CuticularproteinMyCP, Acetylcholinesterase 1 (Ace1), Dynein heavy chain 64C (MpDhc64C), Chitin synthase 1(CHS1), Zinc finger protein (SaZFP), Carboxylesterase(CbE E4) and Lipase maturation factor 2-like gene adversely affected aphid fecundity and survival.

dsRNA-mediated downregulation of the *Sucrose non-fermenting 7* (*DvSnf7*) gene coding for an essential protein in vacuolar sorting in transgenic maize plant (*Zea mays*) conferred resistance to the western corn rootworm, *Diabrotica virgifera* (Coleoptera: Chrysomelidae) (Baum et al. 2007). *Snf7* dsRNA alone takes a long time to kill WCR larvae, so the RNAi pathway is accompanied by *Cry* genes from *B. thuringiensis* to accelerate the killing action. Further, combining the *Bt* and RNAi mechanisms reduces the occurrence of insect resistance to Bt crops. Maize plant expressing three different *Cry* genes plus dsRNA constructs for the *DvSnf7* gene, event MON87411, was approved for commercialization and release by The Canadian Food Inspection Agency (CFIA) in 2016 (Head et al. 2017). Later, in 2017, The United States Environmental Protection Agency (US-EPA) also granted permission for the commercial planting of MON87411 (Zotti et al. 2018).

Cotton bollworm, *Helicoverpa armigera*, is another devastating agricultural insect pest belonging to the order Lepidoptera. This pest has a wide host range and causes millions of dollars in losses each year (Sharma 2001). Cotton contains a polyphenolic compound called gossypol to protect itself from herbivorous insects, however, *H. armigera* can tolerate its moderate concentrations owing to the P450 monooxygenase gene, *CYP6AE14,* as this enzyme detoxifies the gossypol content. Feeding *H. armigera* larvae on leaves of transgenic Arabidopsis, tobacco, and cotton plants expressing dsRNA for *CYP6AE14*, resulted in suppression of the P450 monooxygenase gene in *H. armigera* and retarded larvae growth and enhanced host resistance to cotton bollworms (Mao et al. 2007, 2011).

Later Kumar et al. (2014) described how *Manduca sexta* larvae feeding on native *Nicotiana attenuata* can tolerate high concentrations of nicotine, a neurotoxin produced by tobacco species. Wolf spiders (*Camptocosa parallela*) avoid nicotine-fed larvae, therefore, *M. sexta* larvae deter its predator by exhaling nicotine through the spiracles as an anti-spider signal. Transformation of *N. attenuata* with constructs producing dsRNA to target the *M. sexta CYP6B46* gene and feeding the larvae with transgenic plants silenced the *CYP6B46* gene. Subsequently, insect vulnerability to spider predation was increased because of the less nicotine exhaled.

Another approach to managing insect pest damage is to interfere with chitin metabolism. Chitinase hydrolyzes chitin and, therefore, its function is vital for insect molting andmetamorphosis (Agrawal et al. 2013). Transgenic tobacco and tomato plants expressing RNAi constructs for the chitinase (*HaCHI*) gene significantly reduced chitinase production and adversely affected the overall growth and survival of *H. armigera* after continuous feeding with leaves of transgenic HaCHI-RNAi lines (Mamta and Rajam 2016).

In another study, tobacco plants were transformed to produce dsRNA targeting the *Sl102* gene in *Spodoptera littoralis*. *Sl102* is a gene involved in the immune cellular responses of *S.littoralis*, which was knocked down to increase the susceptibility of the insect pest against the pathogenicity of *B. thuringiensis*-based insecticides. Experimental larvae reared on transgenic leaves showed low transcript levels for the *Sl102* genewhich was positively associated with food consumption in the larvae (Di Lelio et al. 2022).

11.4 CRISPR/Cas System

11.4.1 Origin, Classification, and Efficiency

Genome editing of plants has achieved remarkable success since the advent of sequence-specific nucleases (Shelake et al. 2019). Zinc finger nucleases (ZNFs) and transcription activator-like effector nucleases (TALENs) were the pioneer editing tools. However, these tools are technically complex and cumbersome, with low efficiency, and therefore are not used any further (Kumar et al. 2018). In contrast, the discovery of the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas system paved the way for a simple and precise method to

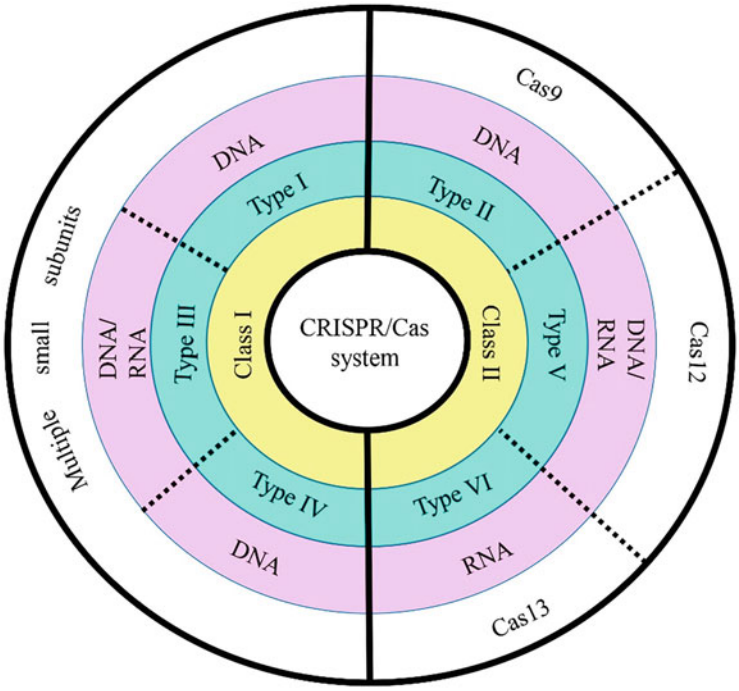

Fig. 11.2 The conventional classification of CRISPR/Cas system

modify several targets in the genome at the same time. CRISPR/Cas system was first discovered in the sequences of DNA from *Escherichia coli* (Ishino et al. 1987). Archaea and bacteria naturally use this system to protect themselves against viral invasions (Bhaya et al. 2011). After genetic elements such as phages invade the host cell, small nucleic acid fragments of invading pathogens are inserted into the host's CRISPR loci (spacers) and stored there for later encounters (Amitai and Sorek 2016). When the host cell faces a new invasion, spacer sequences are transcribed, and individual CRISPR RNAs (crRNAs) lead the Cas nuclease to the cognate nucleic acid sequences of the pathogen and cleave them (Barrangou et al. 2007). Depending on the nature of the interfering molecules, CRISPR/Cas system is divided into two classes (Fig. 11.2). Class 1 includes types I, III, and IV multiprotein effector modules that target DNA, DNA/RNA, and DNA molecules, respectively. Class 2 includes types II, V, and VI effector modules that associate with DNA, DNA/RNA, and RNA molecules, respectively. Unlike class 1, members of class 2 are single protein effector modules and the most notable examples of this class are Cas9, Cas12, and Cas13 (reviewed in Gostimskaya 2022). Type II CRISPR/Cas9 has been isolated from *Streptococcus pyogenes* and is based on RNA-guided interference with DNA and has the most contribution to genome editing studies in plants (Khabbazi et al. 2021). This system consists of a Cas9 nuclease and a single guide RNA (sgRNA) molecule. A twenty-nucleotide at the 5'end of the sgRNA

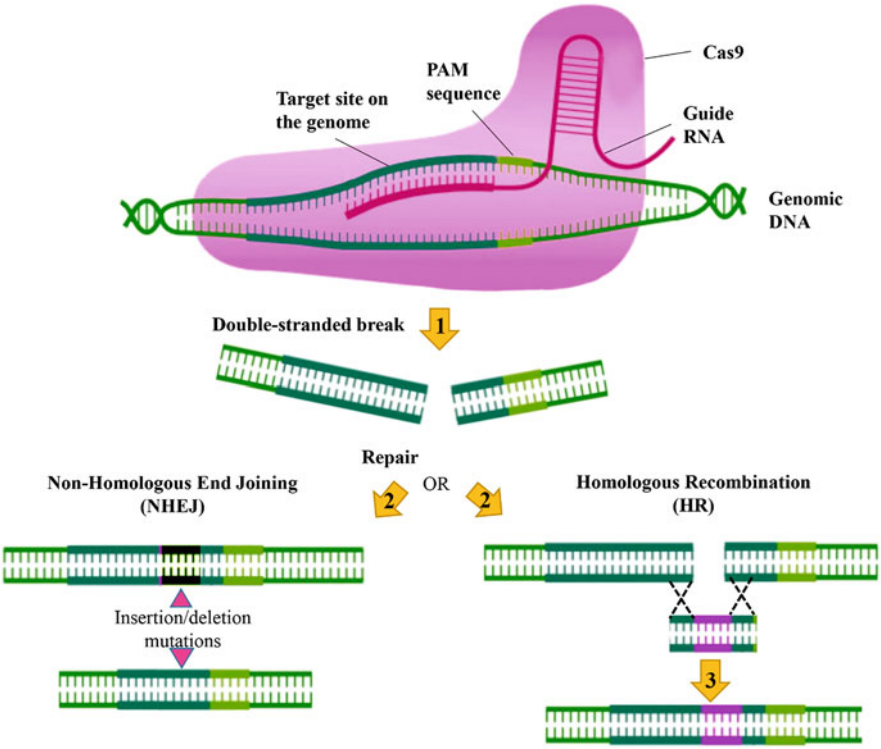

Fig. 11.3 The mechanism of CRISPR/Cas9 genome editing. Guide RNA molecule directs the Cas9 nuclease to the target site in the genome. The presence of PAM near the matching sequence in the genome is critical in finding the target site.After double-strand breaks are made, the broken ends are repaired via the NHEJ or HR processes

directs the CRISPR/Cas9 complex to the complementary sequence in the genome. The presence of conserved protospacer-adjacent motifs (PAM) near target sites in the genome plays a critical role in the in-target function of this complex. Following double-stranded DNA breaks by the Cas9 enzyme, it is subjected to cell repair machinery, which can be error-prone non-homologous end joining (NHEJ) repair or precise homology-directed repair (HDR) (Fig. 11.3). The targeted insertion or modification of desired sequences into the genome makes the HDR approach an outstanding tool for the genetic engineering of plants (Voytas and Gao 2014).

The importance of the breakthrough CRISPR/Cas technology is particularly emphasized as it causes heritable targeted modifications and also contributes to the development of transgene-free plants (Wang et al. 2014a, b; Pan et al. 2016). The first genome editing study in plants was reported by Feng et al. (2013), however, the same year there were other works that reported the successful use of the CRISPR system in genome modification of plants such as Arabidopsis, tobacco, wheat, and rice (Upadhyay et al. 2013; Jiang et al. 2013; Feng et al. 2013). Afterward, numerous studies have been conducted to apply desired modifications to a variety of plants

including maize, soybean, potato, cotton, grapes, tomato, cucumber, Cacao tree, sweet orange, Grapefruit, apple, etc. (Khabbazi et al. 2021).

11.4.2 CRISPR/Cas-Based Genome Editing of Plants for Insect Resistance

Insects can damage crops by directly feeding on plant tissues or indirectly transmitting various diseases, thereby significantly reducing crop production and yield. The application of extensive chemicals has caused serious harm to human and animal health as well as the environment. After the successful contribution of transgenic crops for example Bt crops in reducing the usage of chemicals yet the existence of political, ethical, and societal resistance to these crops is a serious issue in many countries. The possibility of employing CRISPR/Cas technology in genome editing of plants towards insect resistance has already been discussed (Douglas 2018). Employing the CRISPR tool provides the opportunity to tackle the concerns in two ways; creating de novo resistance in case there is no convenient R-gene available, and controlling the insect pest population dynamics by breaking insecticide resistance, killing or causing sterility in insects. In such situations, CRISPR technology has the potential to develop designer plants for generating superior traits or to initiate a gene drive to selectively propagate mutations that lead to reduced fecundity or female death in the target insect population (Bisht et al. 2019).

Elucidation of molecular mechanisms of plant defense systems is a prerequisite for developing a new strategy to generate insect-resistant crops. Plants have developed a complex defense mechanism under millions of years of selection pressure from insects (Erb and Reymond 2019). Species of different orders show a strong spatio temporal variation in the expression of metabolites involved in defense against insects (Barton and Boege 2017). The expression level of immunity-associated genes in Arabidopsis plants is correlated with the duration of the vegetative stage (Davila Olivas et al. 2017; Glander et al. 2018), illustrating the relationship between flowering and resistance to insects.

Plant Calcium ion (Ca^{2+}) signals are involved in a wide variety of signaling pathways in the cell. Ca^{2+}enacts an important role in the circadian regulation of photoperiod-controlled flowering in the common morning glory (*Ipomoea purpurea*) (Dodd et al. 2010). Calcium-dependent protein kinase (*CDPK*) is one of the main receptors in the calcium signaling pathway and transduces the signal by phosphorylation (Harmon et al. 2000). In Arabidopsis, the loss of function of the *CPK33* causes late flowering (Kawamoto et al. 2015). $Ca^{2+}$is also involved in early defense signaling in plants (Yan et al. 2018), after insect feeding, there is a striking Ca^{2+}influx limited to a few cell layers lining the injured site (Maffei et al. 2007). In Arabidopsis plants, *CPK3* and *CPK13* activate the herbivore-induced network by increasing the transcription levels of plant defensin gene *PDF1.2* (Kanchiswamy et al. 2010). In another study, the knockdown of *NaCDPK4* and *NaCDPK5* genes in Coyote Tobacco (*Nicotiana attenuata*) up-regulated jasmonic acid accumulation and increased resistance to *Manduca sexta* (Yang et al. 2012).

CRISPR/Cas9 mediated knockout of *CPK* gene (*GmCDPK38*) in soybean resulted in late flowering time in *gmcdpk38* mutants regardless of the photoperiodic conditions. In addition to delayed flowering time, *gmcdpk38* mutants also exhibited enhanced resistance to *Spodoptera litura* (Li et al. 2022). This revealed the dual role of *GmCDPK38* in regulating photoperiod-induced flowering in soybean and resistance to *Spodoptera litura*, suggesting a possible link between flowering and insect resistance.

Resistance to insects is multifaceted with highly complex regulation in both insects and the host plant itself. Phytohormones such as jasmonic acid (JA), salicylic acid (SA), abscisic acid (ABA) and ethylene can affect plant response to insect pests. Deficiency of ABA in plants increases their susceptibility to insect pests (Thaler and Bostock 2004; Dinh et al. 2013) whereas exogenous application of ABA can increase plant resistance to brown planthopper (BPH) by promoting callose formation (Liu et al. 2017). In a recent study, overexpression of the 9-cis-epoxycarotenoid dioxygenase-3 (NCED3) enzyme in rice plants increased ABA biosynthesis and subsequent resistance to BPH (Sun et al. 2022).

Responses of plants to insect pests are also correlated with the feeding manner and the degree of damage at the feeding site. Therefore, the molecular response of plants induced by sap-sucking insects is different from chewing pests. One study demonstrated the role of serotonin regulation as part of the defense mechanism against insect pests in plants (Lu et al. 2018). In rice plants, the cytochrome P450 gene *CYP71A1* encodes tryptamine 5-hydroxylase, which catalyzes the conversion of tryptamine to serotonin (Fujiwara et al. 2010). Sap-sucking insects cause only a little damage to plant tissue therefore salicylic acid (SA) signaling pathway has the main role in insect infestation (Li et al. 2017). Serotonin biosynthesis is induced by insect infestation in rice, and its suppression confers resistance to BPH and striped stem borers (SSB). CRISPR-mediated *CYP71A1* gene knockout inhibits serotonin production resulting in higher salicylic acid levels and thus resistance to BPH and SSB in rice (Lu et al. 2018). However, *cyp71a1* mutant individuals showed increased resistance to rice blast, *Magnaporthe grisea* (Ueno et al. 2008) and susceptibility to rice brown spot disease *Bipolaris oryzae* (Ishihara et al. 2008).

11.5 Conclusion

Until the beginning of the current century, different approaches such as classical plant breeding methods and the application of chemicals in the field contributed to enhancing crop yield and production. The requirement of sufficient agricultural production for the increasing world population and ensuring global food security have led plant scientists to explore more efficient strategies, especially in terms of pest management. Existing criticism of traditional pest control approaches, such as environmental and health concerns and the development of resistance to insecticides, has rendered these methods inadequate on their own. The advent of recombinant DNA technology and the introduction of genetically modified crops expressing the *Bt* toxin gene was a new era in agriculture. Later advances in molecular biology

discoveries such as RNAi and CRISPR/Cas technologies soon opened up a new avenue in the production of biotech crops. The next generation of GM crops has the potential to address concerns about transgenic crops and is of great importance for developing sustainable and environmentally friendly methods for crop improvements.

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