Chapter

RNAi-Mediated Control of Lepidopteran Pests of Important Crop Plants

Shipra Saxena, Sneha Yogindran, Manmohan Arya, Yogita Sharma and Chandra Pal Singh

Abstract

Insects as pests destroy annually an estimated 18–20% of the crop production worldwide. Caterpillars, the larval stage of moths, are the major pests of agricultural products owing to their voracious feeding habits. In the past few decades, the potent methods of insect control, such as insecticides and Bt toxins, have been constrained as a result of health hazards, environmental issues, and development of resistance, after their prolonged application. Thus, there is need to find alternative options to improve plant protection strategies. Recently, RNA interference (RNAi), the post-transcriptional gene-silencing mechanism, has emerged as one of such a novel, sustainable, and environment friendly approaches for insect management and crop protection. RNAi technology relies on selection of a vital insect pest target gene and its expression as a double stranded RNA or stem-loop RNA molecule, which is recognized by the host RNAi machinery and processed into small interfering RNAs (siRNAs) or microRNAs (miRNAs). The siRNA/miRNA along with the RNA-induced silencing complex (RISC) binds to the complimentary mRNA and induce gene silencing at post-transcriptional level. With effective target-gene selection and transgenic plants expressing these precursor RNA molecules, insect pests of various crops have been efficiently managed. In this chapter, we discuss the basic mechanism of RNAi and its application in controlling lepidopteran pests of important crop plants.

Keywords: RNAi, lepidopteran pests, crop protection, plant-mediated RNAi, insect resistant transgenics

1. Introduction

The year 2020 has been declared as the International Year of Plant Health by the United Nations General Assembly, to contemplate over the issue of feeding 10 billion people by 2050 and raise global awareness about the challenge modern agriculture is going to face too in a profitable, efficient, and sustainable way. The challenge would be hardened by additional factors like climate change, decrease in arable land due to degradation, and urban expansion, as well as need for more nutritious food [1]. A major hindrance to crop production is loss by insect pests right from the seedling stage to the post harvesting stage of the product. These losses lead to reduced yields, decreased quality, and thus food insecurity resulting in the deaths of millions of people throughout the world and impacting trade and

economy of many developing countries. Annually, 20–40% of global crop production is lost due to pests valued more than US$ 70 billion [2]. Moreover, in the coming years with the increasing global temperatures, plant scientists expect a 10 to 25% increase in crop damage due to insect pests, majorly in the temperate regions [3]. In the class Insecta, the order Lepidoptera, represents the second largest order, with 180,000 species in 128 families and 47 superfamilies. Amongst these, more than 160,000 species are moths [4]. Moths are known for their economic values as the silkworm *Bombyx mori*, as well as a food product like larvae of *Gonimbrasia belina* and *Usta Terpsichore* [5]. The larval stage of moths are major pests of agricultural and forest products pests in most parts of the world [6–8].

The most common method of crop protection from insect pests is calendar-based spraying of insecticides. However, these chemicals cause an increased cost of production, residual toxicity, resistance issues, outbreaks of secondary pests, and potential health hazards on humans and environmental threats [9]. Considering these issues, genetic engineering has emerged as an effective way to control the pest population. Use of Bt toxins from soil bacterium *Bacillus thuringiensis,* has shown great potential in controlling the devastating insect pest population. The bacteria produce insecticidal crystal proteins (ICP), such as Cry and/or Cyt proteins called δ-endotoxins that interact with receptors present in the insect midgut cells. This interaction activates the host proteases and results in oligomeric pore formation, which leads to ionic imbalance in the cell ultimately killing the insects [10–12]. Bt based bio-insecticides have been successfully employed against lepidopteran, dipteran and coleopteran larvae [13, 14]. However, topical application does not last long due to the degradation by UV light, weather and certain proteases [15]. This problem was addressed by introducing the Cry genes into the plants through genetic engineering [16–18]. Genetic transformation of plants to express Bt toxins resulted into enhanced tolerance towards the pests and helped the farmers to control the infestation. Apart from Bt proteins, other insecticidal proteins such as vegetative insecticidal proteins, chitinases, α-amylase inhibitors, protease inhibitors etc., have been shown also to control the pest population [19]. The use of Bt toxins and other proteins to generate transgenic crops has been reviewed by [19, 20]. However, various recent studies have demonstrated that insects have gained resistance towards the Bt proteins in the field [21]. Thus, finding alternative options to improve plant protection strategies is critical to secure global food production for the next decades.

In the past few decades, RNA interference (RNAi), a natural defense mechanism by sequence specific down regulation of cognate mRNA, has emerged as a reverse genetics tool for functional genomics along with various practical applications in areas of therapeutics, agriculture etc. RNAi as a technology has shown immense potential in the area of crop improvement traits like introduction of male sterility, enhancement of nutritional contents, reduction of amount of food allergens and toxic compounds, disease and pest resistance, resistance against various abiotic stresses and enhanced production of secondary metabolites. Down-regulation of insect genes through RNAi has been efficiently used to control insect pests in various crop plants [22–24]. The present chapter focuses on the basic RNAi mechanism in insects and the application of this natural defense machinery in controlling the pest population of some important crop plants and widely consumed vegetable crops.

2. Basics of RNAi mechanism

RNAi is a natural phenomenon of gene regulation that occurs at the post-transcriptional level [25]. Though the discovery of RNAi was demonstrated through

exogenous delivery of dsRNA against unc22 gene into *Caenorhabditis elegans* [26], now it is clear that RNAi was operational from long back in plants against RNA viruses, known as Virus-induced gene silencing [27]. Most of the higher eukaryotes including animals, plants and insects, possess RNAi mechanism for silencing the genes in a sequence-specific manner [28]. In RNAi-governed gene silencing, the two classes of small non-coding RNAs play key role, which are small interfering RNAs (siRNAs) and microRNAs (miRNAs). SiRNAs and miRNAs are generated from the double-stranded RNA (dsRNA) and stem-loop RNA precursors, respectively [28]. The precursors of miRNAs are abundantly present in cell endogenously, while major sources of dsRNAs are exogenous. [28, 29]. Average size of mature siRNA and miRNA fall in the range of 21–23 nucleotides. The basic process of RNAi consists of several steps and requires the involvement of RNase enzymes and RNA-binding proteins [26–29]. The Dicer, an RNaseIII type endonuclease, plays a crucial role in processing the dsRNA into 20–25 bp long siRNAs in the cytoplasm (**Figure 1**) [27–31]. Whereas the production of mature miRNA duplex requires multiple processing by the Dicer and other co-factors in the nucleus. First primary-miRNA is processed into a single stem-loop bearing structure referred to as precursor-miRNA (pre-miRNA). Subsequently, pre-miRNA is cleaved and mature miRNA duplex is produced. In plants, multiple Dicer enzymes have been identified and distinctively referred to as Dicer-like proteins (DCL) [31]. The produced mature duplexes of siRNA and miRNA contain a two-nucleotide long overhang at both the 3′ ends [28, 32]. Each duplex of siRNA and miRNA initiates the formation of the RNA-induced silencing complex (RISC) [28, 33]. However, mature RISC is a multi-protein complex that possesses Argonaute protein (Ago) as the core effector molecule in both siRNA and miRNA pathways [28, 33]. Upon incorporation into the RISC, siRNA/miRNA duplex loses one of the strands known as the passenger (sense) strand [28, 33]. While the other strand, referred to as the guide strand (antisense), remains loaded on the RISC and further directs the complex to search for the cognate target mRNA. SiRNA/miRNA finds the specific targets based on the complementarity between siRNA/miRNA and mRNA target sequences [28, 29, 33]. In most of the instances in plants, the perfect complementary base-pairing between siRNA/miRNA and mRNA target induces the endonuclease activity of Ago resulting in cleavage of the target

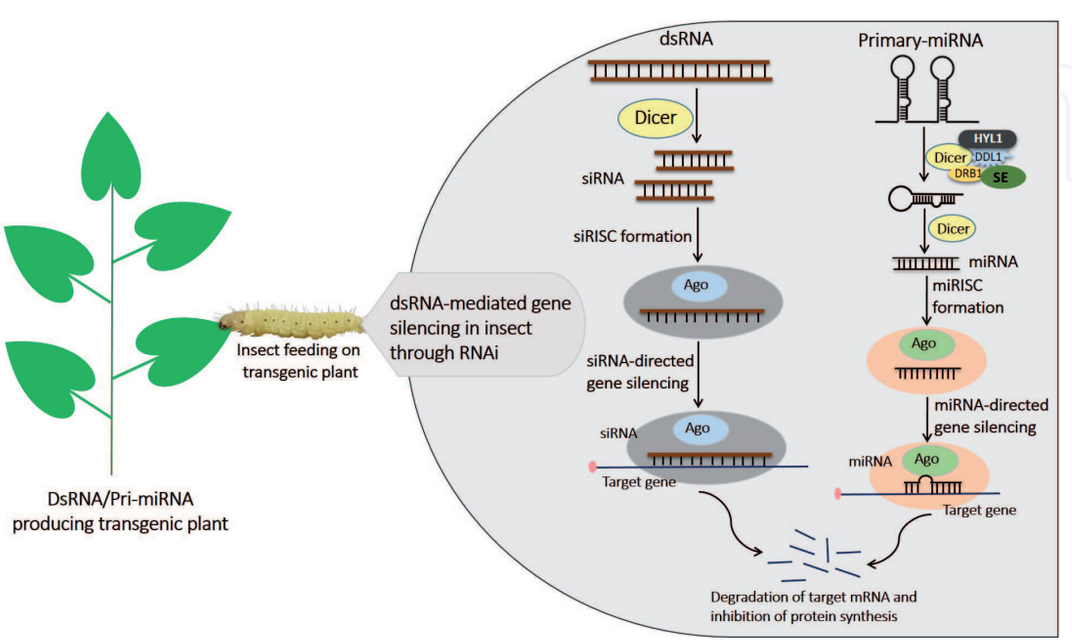

Figure 1.
An overview of dsRNA-mediated knockdown for insect genes through RNAi mechanism in transgenic plants.

mRNA and suppression of the translation [28, 29, 33]. While in insects the mostly miRNAs bind to the cognate mRNA target via partial complementary and leads to the translation repression.

3. RNAi as a pest control technology

The potential of specific gene targeting made RNAi an important method to be applied for plant protection against insect pests [32, 34, 35]. This novel approach provides an opportunity to target any essential gene of the insect. The dsRNA against specific insect gene is expressed from a construct harboring the sense and antisense RNA in the form of DNA. The cloned fragment can be transferred into the plant via agrobacterium-mediated transformation method or in vitro produced dsRNAs/siRNAs can be directly applied to the plants or dsRNA-expressing bacteria are spread on plants as insecticides [36]. Feeding of insects on these dsRNA/siRNAs leads to induction of RNAi-mediated gene silencing (**Figure 1**) [37]. Insects receive either dsRNA or siRNA from these plants wherein siRNAs are straightly incorporated into the RISC, the dsRNA first gets processed into several siRNA molecules in the insect's gut by DCL, then turns to RISC loading [30]. Generated siRNA molecules target the specific insect gene against which they were originally designed based on the sequence complementary between siRNA and target mRNA (**Figure 1**) [32, 34, 35, 37]. This results in suppressed insect growth or mortality [32, 34, 35, 37]. However, miRNA-based gene silencing is achieved through expressing stem-loop bearing primary or precursor miRNA in the plants. Which subsequently get processed by the miRNA-pathway components to give rise mature miRNA duplex and regulates the expression of specific mRNA target.

As a crop protection method, RNAi-based strategies offer the following advantages over the other conventional methods such as chemical insecticides, biological control, or protein-coding transgenes [38]:

1. Highly specific- targets only the intended pest – minimal or null impact on non-target organisms (pollinators, parasitoids, predators and vertebrates)

2. Biodegradable – environment friendly and minimal risk to human health

3. Non-toxic- a natural product as dsRNA is either produced enzymatically in vitro or in vivo through engineered bacteria or host plant

4. No protein production involved

5. It can act individually as well as synergistically with conventional approaches like insecticides and Bt.

Depending upon the method of production of dsRNA and its subsequent delivery to the target pest, there are two major approaches for RNAi-mediated crop protection, topical application of dsRNA (non-transformative) through spraying/injection/root drenching, etc. and generation of transgenic plants expressing dsRNA. Topical application of dsRNA products has been demonstrated through foliar application [39], trunk-injection [39, 40], irrigation [39, 41] and microbe-based [42–45]. Recently, a biotechnology company called RNAagri reported mass making of the encapsulated ready-to-spray dsRNA, APSE RNA Containers (ARCs) by engineered *Escherichia coli* [46]. These non-transformative approaches are advantageous in terms of quick development and testing, no regulation in

development of dsRNA as like in a GM product and silencing of genes without introduction of heritable changes into the host plant genome. However, there are several concerns for use of this approach as crop protection method like up-take restrictions, requirement of periodical applications and temporary protection [47].

4. Plant-mediated RNAi for the control of lepidopteran pests

The use of RNAi to silence the insect genes through topical application of dsRNAs has several concerns for use of this approach as crop protection method like up-take restrictions, degradation under field condition thus requirement of periodical applications and temporary protection [47]. Also, the production of large amount of dsRNA is not only expensive, but requires expertise for handling and storage. These limitations can be dealt with a transformative approach of crop protection, generation of transgenic plants expressing double-stranded RNAs (dsRNAs) that target essential genes of insect pests. Feeding upon the plant induces an RNAi response, which either harms, or ideally kills, the pest. Most transgenesis events perform nuclear transformation with agrobacterium vectors carrying inverted repeats of target insect gene sequences. Target gene dsRNA is transcribed by plants RNAi machinery and is processed into siRNAs. However, these plant-processed siRNAs are less efficient in insect cells as compared to longer dsRNA [48]. Another approach to create transgenics is transformation of chloroplast (plastid) DNA. Lack of RNAi machinery in the organelle prevents chopping of dsRNA by Dicer and thereby permits accumulation of much higher amounts of long dsRNA [49].

Popularly known as plant-mediated RNAi or Host-Induced Gene Silencing (HIGS), this strategy has been demonstrated for protection of a range of crops against their specific pest insects, mites, ticks, plant pathogens, viruses, nematodes, and weeds [50–56]. Recently, two regulatory authorities, the Canadian Food Inspection Agency and US Environmental Protection Agency, have declared the approval of the RNAi-based corn event Monsanto MON87411, the "SmartStax PRO" for release and commercialization. The transgenic plants harbor a dsRNA construct that specifically targets the SUCROSE-NON-FERMENTING7 gene of WCR (DvSnf7), together with two insecticidal proteins Cry3Bb1 and Cry34Ab1/Cry35Ab1 [57]. This is in concurrence with the approval granted for apple and potato expressing dsRNAs for quality enhancement [58, 59]. In the past decades, lepidopteran pests have been successfully managed by the first-generation insecticidal plants expressing the Bt proteins. However, with reports of resistance evolution to Bt proteins, scientific community searched for alternatives to manage these pests. The caterpillar pests were one of the first and main targets for RNAi transgenics.

4.1 Model plants

To prove a hypothesis, with the available human and financial resources and carry forward the research as rapidly as possible, researchers use model systems. Model plants like *Arabidopsis thaliana* and *Nicotiana tabacum* can be easily manipulated, are genetically tractable, and about them much is already known. The very first report of plant-mediated RNAi for lepidopteran insect resistance was published by Mao et al. (2007) where they have silenced cytochrome P450 monooxygenase CTP6AE14 gene of *Helicoverpa armigera* involved in degradation of gossypol. The dsRNA was expressed in model plants *A. thaliana* and *N. tabacum* which when fed to insect *H. armigera* resulted into significant reduction in the transcript level, augmented gossypol toxicity in larvae and affected the larval weight and size [60].

The same group also tested the efficiency of plant-mediated RNAi in silencing other midgut gene, GST1, which encodes a glutathione-S-transferase and which is not affected by gossypol content. Feeding of transgenic *A. thaliana* plants resulted into decreased transcript level in the insect midgut and also resulted into larval weight reduction [60]. Insects are compelled to undergo molting through shedding the old cuticles during their growth and enter into pupal stage after which they metamorphose into adult moth. Hence, the whole process of molting is a vital way to regulate development. The major genes associated with molting are the target of insect specific chemical pesticides which have shown promising result [61]. 20, hydroxyecdysone is one of the main genes involved in molting process and metamorphosis and dsRNA expressing tobacco against this gene resulted into impaired molting, pupation and adult emergence rate in *H. armigera* and *Spodoptera exigua* [62]. Silencing of molt-regulating transcription factor, hormone receptor 3 (HR3), of *H. armigera* also resulted into significant downregulation of the target gene which affected the molting and larval growth cycle [63]. Another gene named arginine kinase, required for cellular energy metabolism when silenced through *Arabidopsis*, resulted into defective larval growth and survival in *H. armigera* [64]. Transgenic tobacco plants expressing dsRNA against chitin synthase, cytochrome P450 monooxygenase and V-ATPase genes of *H. armigera* significantly reduced the transcript level and affected the larval weight and pupation [65]. Down-regulation CYP6B46 gene of *Manduca sexta* required for nicotine degradation through genetically modified tobacco resulted into decreased transcripts of the target gene and affected the larval weight [66]. The transgenic plants expressing dsRNA can also be used for the management of closely related insects. *Nicotiana attenuate* plants expressing dsRNA of *M. sexta's* midgut-expressed genes, the nicotine-ingestion induced cytochrome P450 monooxygenase and the lyciumoside-IV-ingestion induced β-glucosidase1, was also able to silence the homologous genes in native *Manduca quinquemaculata*. Hence, careful selection of target genes will help in effective control of congeneric insect pests that share sufficient sequence similarity [67].

4.2 Food and cash crops

4.2.1 Rice

Rice, a staple food for more than half of the global population, is heavily infested by lepidopteran pest, striped stem borer (SSB), *Chilo suppressalis* Walker. The crop yield is significantly reduced by the insect pest as it causes 'deadheart' at the tillering stage and 'whitehead' at the heading stage. In an attempt to impart insect resistance, Jiang & co-workers generated transgenic rice overexpressing five important SSB housekeeping genes, but none of the acquired dsRNA-transgenic rice plants presented significant effects on SSB growth and development. In their subsequent attempt they selected 13 SSB novel microRNAs (miRNAs), and overexpressed them in rice using artificial miRNA (amiRNA) expression technology. Feeding tests on transgenics demonstrated that two out of 13 selected SSB novel miRNAs caused significant growth inhibition in SSB [68]. Recently, Zheng et al., (2020) have developed highly SSB-resistant rice (named csu260) expressing amiRNA of SSB endogenous miRNA - miR260 which negatively regulates ecdysteroid biosynthesis, through amiRNA expression technology [69].

4.2.2 Maize

Even though the only commercialized example of HIGS technology is in maize against western corn rootworm (*Diabrotica virgifera*), the technology has not been

yet used against the lepidopteran pests. One of the breakthrough research showing the use of RNAi for insect control was performed in maize. The maize plant was transformed using putative V-ATPaseA coding region from WCR. The F1 hybrid plants displayed resistance to wcr evidenced by less nodal injury and healthy root masses [70]. This provides a sufficient possibility of using this technology for controlling lepidopteran pests as well in the near future.

4.2.3 Soyabean

Soybean (*Glycine max* (L.) Merrill) is an important protein and oil-seed agricultural crop worldwide. *Leguminivora glycinivorella* is a major pest of soybean causing direct loss in yield as well as additional losses in the quality and sale price caused by damaged seeds (Edmonds et al. 2000). Silencing of ribosomal protein P0, involved in protein translation and DNA repair through transgenic plants conferred resistance against the pest. Larval mortality, lesser foliage damage, reduced SpbPo expression and developmental deformities were observed in pest after feeding upon the transgenic plants [71]. In another study transgenic soybean plants expressing Spb18S dsRNA also exhibited resistance to the soybean pod borer. Feeding upon the transgenic plants downregulated Spb18S expression levels as well as reduced second-instar larval survival rates. Also, the developed transgenic plants were less damaged by SPB larvae than control plants under field conditions [72].

4.2.4 Cotton

Cotton is cultivated for its soft fiber immensely used in the clothing industries. However, the production is hugely affected by cotton bollworm (*H. armigera*) which not only affect cotton, but also other crop plants as discussed above. Transgenic cotton plants expressing a P450 monooxygenase gene, CYP6AE14, from *H. armigera* showed enhanced resistance towards the pests suggesting the efficacy of RNAi as a tool for pest management [73].

4.3 Common vegetables

Vegetables provide a reasonable source of vitamins and minerals for maintaining good health and also offer economic revenue to combat rural unemployment. Globally, one billion metric tons of vegetables are harvested per year, with Asia being the leading producer. Tomato, *Solanum lycopersicum*, is an important vegetable crop grown and consumed worldwide. An annual production of about 160 million tonnes is harvested globally. However, the production is hugely affected by the various insect pests like fruit-worms, aphids, cutworms, tomato hornworms, tobacco hornworms, cabbage loopers, whiteflies, flea beetles, red spider mite, slugs, and Colorado potato beetles [19]. Tomato yield loss reported only due to insect infestation accounts to 5–55% [74]. RNAi-mediated crop protection in tomato crop has shown promising results. Most insects cause damage to the plants during their larval stage and hence genes regulating the metamorphosis and development are considered as potent targets for successful RNAi-mediated gene silencing. Silencing of juvenile hormone (JH), a sesquiterpene, has been reported to affect the larval growth and development in tomato fruitworm (*H. armigera*). Juvenile hormone acid O-methyl transferase gene (JHAMT), a key enzyme regulating JH titer, downregulation via tomato expressing dsRNA disrupted the metamorphosis and adult emergence in *H. armigera* [75]. Similarly, silencing of chitinase mainly found in insect midgut, integument cell walls, cuticles, shells, and intestinal peritrophic matrices (PMs) play important role during insect molting and metamorphosis [76]. Continuous feeding

of tomato transgenic leaves expressing hairpin RNA complimentary to chitinase gene of *H. armigera* led to reduced gene transcript which induced detrimental effect on the overall development and survival of insect [77]. Aphids (*Myzus persicae*) are sap-sucking pests that cause significant crop loss by direct feeding and transmitting the virus causing severe diseases in plants [78]. Tomato-mediated RNAi to silence acetylcholinesterases (AchE) which work as neurotransmitters in insects, resulted into reduced aphid fecundity [79]. The endogenous gene regulation pathway of miRNA is exploited by amiRNA technology to control the gene of interest and has shown significant silencing of the target gene with less or no off-target effects [80–83]. Silencing of ecdysone receptor gene (EcR), involved in all the stages of insect's life cycle, through tomato expressing amiRNA significantly increased the tolerance of plants towards insect attack [84].

Another popularly consumed vegetable, Potato (*Solanum tuberosum*) belongs to the family Solanaceae, and is the 4th most grown crop after wheat, rice and maize [85]. The crop is highly nutritious since it is rich in carbohydrates, proteins, minerals and vitamins [86]. Various biotic and abiotic stress factors limit the production and crop yield. Various biotic and abiotic stress factors limit the production and crop yield. Common potato infesting insects are Colorado potato beetle, potato tuber moth, green peach aphid (*M. persicae*), potato aphid, beet leaf hoppers, thirps and mites. Colorado potato beetle (CPB), *Leptinotarsa decemlineata*, is the most important pest due to the detoxification mechanism to survive various natural and synthetic chemicals [87]. RNAi- mediated silencing of *EcR* gene of CPB expressed in transgenic potato, showed 80% mortality and inability of the insect to complete the life cycle [88]. Similarly, feeding of transgenic potato expressing the hairpin RNA against JHAMT gene of CPB, led to reduced transcript level of the targeted gene and also significantly affected the growth and development of the pest specially the oviposition. Field trials of the transgenic potato showed high tolerance to the pest infestation and the surviving insects displayed low reproduction potential [89]. Potato transgenics encoding the RNAi construct targeting the host's gene Glycoalkaloid metabolism 4 (GAME4) coding for cytochrome P450, resulted into early instar mortality and accelerated insect development [90]. Similar to CPB, insect pest *Phthorimaea operculella* also causes huge losses to the production [91]. RNAi mediated control of insect pest has been demonstrated through topical application of dsRNA targeting Chitin Synthase A gene [92].

Other common vegetables like Cauliflower and Cabbage etc. are also heavily infested by insect pests. Cauliflower, belonging to species *Brassica oleracea* is profoundly infested by diamondback moth *Plutella xylostella*. It is one of the most destructive insect pests of Brassica all over the world for its short life span, high reproductive potential, lack of natural predators, and its ability to become resistant to a wide range of toxins and growth regulators [93, 94]. Cabbage, another member of the "cole" group crops is infested by many lepidopteran pests such as *P. xylostella, Pieris rapae, Mamestra brassicae* and *Trichoplusia ni* causing a major constraint of its yield [95, 96]. Various studies demonstrate the potential of RNAi mediated management of lepidopteran pest complex of cauliflower and cabbage, but dsRNA expressing transgenic plants targeting the pest complex have yet not been reported [97–100].

5. Conclusions

Post discovery, RNAi technology has been harnessed as a functional genomics tool as well as a crop improvement tool for various applications including control of insect pests. With its unique insecticidal mode of action, suppression of gene

expression, it can act individually as well as can complement the current methods deployed for pest control. The technology has been applied in a range of crops against insect pests from orders such as Coleoptera, Lepidoptera, and Hemiptera. However, RNAi efficacy varies in insects for reasons like dsRNA molecule itself, instability of dsRNA due to presence of nucleases and gut pH, incomplete or impaired dsRNA internalization, lacking core RNAi machinery, weakened systemic spreading, developmental stage used for silencing and refractory target genes [100]. Therefore, for deployment of this technology on a commercial scale these challenges need to be addressed. In the course of evolution, insects are known for their remarkable adaption, allowing them to evolve resistance to any control method, including transgenic plants with protective traits like insecticidal proteins and RNAi. Thus, to provide sustainable crop protection managing the pest resistance issue, integrated pest management (IPM) approaches using combination of various control strategies like preventive measures like crop rotation, intercropping or cultivation of pest-resistant varieties, use of natural biocontrol factors such as pathogens or predators, and genetic control via transgenic plants expressing transgenes (Insecticidal proteins/dsRNA targeting insect genes) or release of sterile insects should be deployed.

Author details

Shipra Saxena[1], Sneha Yogindran[2], Manmohan Arya[3], Yogita Sharma[3] and Chandra Pal Singh[3]*

1 Department of Genetics, University of Delhi, New Delhi, India

2 Department of Biotechnology, Cochin University of Science and Technology, Kerala, India

3 Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India

*Address all correspondence to: chandrapal203@gmail.com

References

[1] FAO. (2009). FAO's director-general on how to feed the world in 2050. Population Dev. Rev. 35, 837-839.

[2] Gautham H.R., Bhardwaj M.L. International Year of Plant Health 2020 to focus on threat of pests on food security. Curr. Sci. 2020;118:857.

[3] Deutsch CA, Tewksbury JJ, Tigchelaar M, Battisti DS, Merrill SC, Huey RB, et al.. Increase in crop losses to insect pests in a warming climate. Science (2018):919:916-919. doi: 10.1126/science.aat3466

[4] Mallet, J. Lepidoptera Taxome Project Draft Proposals and Information. Available online: http://www.ucl.ac.uk/taxome/lepnos.html (accessed on 20 August 2016).

[5] Xu J, Wang X-F, Chen P, Liu FT, Zheng SC, Ye H, Mo MH (2016) RNA interference in moths: mechanisms, applications and progress. Genes 7:88. doi: 10.3390/genes7100088

[6] Zalucki, M.P.; Shabbir, A.; Silva, R.; Adamson, D.; Liu, S.S.; Furlong, M.J. Estimating the economic cost of one of the world's major insect pests, Plutella xylostella (Lepidoptera: Plutellidae): Just how long is a piece of string? J. Econ. Entomol. 2012, 105, 1115-1129. [Google Scholar] [CrossRef] [PubMed]

[7] Asaro, C. Chamberlin, L.A. Outbreak history (1953-2014) of spring defoliators impacting oak-dominated forests in Virginia, with emphasis on Gypsy Moth (*Lymantria dispar* L.) and Fall Cankerworm (*Alsophila pometaria* Harris). Am. Entomol. 2015, 61, 174-185. [Google Scholar] [CrossRef]

[8] Cox, P.D.; Bell, C.H. Biology and ecology of moth pests of stored foods. In Ecology and Management of Food-Industry Pests (FDA Technical Bulletin Number 4); Gorham, J.R., Ed.; The Association of Official Analytical Chemists: Gaithersburg, MD, USA, 1991; pp. 181-193. [Google Schol

[9] Choudhary B and Gaur K (2009) The Development and Regulation of Bt Brinjal in India (Eggplant/ Aubergine). ISAAA Brief No.38. ISAAA: Ithaca, NY

[10] Bravo, A., Gill, S. and Soberón, M. Mode of action of *Bacillus thuringiensis* Cry and Cyt toxins and their potential for insect control. Toxicon. 2007: 49: 423-435.

[11] Crickmore N, Zeigler DR, Schnepf E, Van Rie J, Lereclus D, et al. (2010) *Bacillus thuringiensis* toxin nomenclature. http://www.lifesci.sussex.ac.uk/ home/ Neil_ Crickmore/Bt/.

[12] Roh JY, Choi JY, Li MS, Jin BR, Je YH. Bacillus thuringiensis as a specific, safe, and effective tool for insect pest control. Journal Microbiology and Biotechnology. 2007; 17: 547-559.

[13] Crickmore N. Using worms to better understand how *Bacillus thuringiensis* kills insects. Trends in Microbiology 2005; 13: 347-350.

[14] van Frankenhuyzen K. Cross-order and cross-phylum activity of *Bacillus thuringiensis* pesticidal proteins. Journal of Invertebrate Pathology. 2013; 114: 76-85.

[15] Ranjekar PK, Patankar A, Gupta V, Bhatnagar R, Bentur J, Kumar PA Genetic engineering of crop plants for insect resistance. Current Science. 2003; 84: 321-329.

[16] Sheikh AA, Wani MA, Bano P, Sajad Un Nabi SU, et al. An overview on resistance of insect pests against Bt Crops. Journal of Entomology and Zoology Studies. 2017; 5: 941-948.

[17] Allah B, Emine A, Delpasand KS, Cem KO, Cengiz S, Sebahattin O. Development of insect-resistant cotton lines with targeted expression of insecticidal gene. Archives of Biological Sciences. 2016; 68: 773-780.

[18] Bates SL, Zhao J-Z, Roush RT, Shelton AM. Insect resistance management in GM crops: past, present and future. Nature Biotechnology. 2005; 23: 57-62.

[19] Srivastava DK, Kumar P, Sharma S, Gaur A, and Gambhir G (2016) Genetic Engineering for Insect Resistance in Economically Important Vegetable Crops M. Anis, N. Ahmad (eds.), Plant Tissue Culture: Propagation, Conservation and Crop Improvement, DOI 10.1007/978-981-10-1917-3_15

[20] Stevens J, Dunse K, Fox J, Evans S and Anderson M (2012) Biotechnological approaches for the control of insect pests in crop plants. In: Pesticides-advances in chemical and botanical pesticides, (Soundararajan RP, ed.), ISBN. 978-953-51-0680-7, In: Tech, doi: 10.5772/46233.

[21] Tabashnik BE, Carrière Y. Surge in insect resistance to transgenic crops and prospects for sustainability. Nature Biotechnology. 2017 Oct;35(10):926.

[22] Saurabh S, Vidyarthi AS, Prasad D. RNA interference: concept to reality in crop improvement. Planta. 2014 Mar;239(3):543-64. Katoch R, Sethi A, Thakur N, et al. RNAi for insect control: Current perspective and future challenges. Applied Biochemistry and Biotechnology. 2013; 171: 847-873.

[23] Yogindran S, Rajam MV (2015) RNA interference strategy for crop protection against insect pests. In: Bt resistance, characterization and strategies for GM crops producing *Bacillus thuringiensis*. Toxins. Soberon M, Gao Y, Bravo A (eds.) CABI Biotechnology Series 4. CAB International, Oxfordshire, pp. 162-172.

[24] Mello CC, Conte D Jr. Revealing the world of RNA interference. Nature. 2004 Sep 16;431(7006):338-42. doi: 10.1038/nature02872. doi: 10.1038/nature02872.

[25] Fire, A., Xu, S., Montgomery, M. K., Kostas, S. A., Driver, S. E., and Mello, C. C. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. Nature. 1998; 391, 806-811. doi: 10.1038/35888.

[26] Baulcombe, D.C. RNA silencing in plants. Nature. 2004; 431, 356-363. doi: 10.1038/nature02874.

[27] Wilson RC, Doudna JA. Molecular mechanisms of RNA interference. Annu Rev Biophys. 2013;42:217-39. doi: 10.1146/annurev-biophys-083012-130404. doi: 10.1146/annurev-biophys-083012-130404.

[28] Meister G, Tuschl T. Mechanisms of gene silencing by double-stranded RNA. Nature. 2004 Sep 16;431(7006):343-9. doi: 10.1038/nature02873.

[29] Bernstein E, Caudy AA, Hammond SM, Hannon GJ (2001). "Role for a bidentate ribonuclease in the initiation step of RNA interference". Nature. 409 (6818): 363-6. doi: 10.1038/35053110.

[30] Margis R, Fusaro AF, Smith NA, Curtin SJ, Watson JM, Finnegan EJ, Waterhouse PM. The evolution and diversification of Dicers in plants. FEBS Lett. 2006 May 1;580(10):2442-50. doi: 10.1016/j.febslet.2006.03.072. doi: 10.1016/j.febslet.2006.03.072.

[31] Agrawal N, Dasaradhi PV, Mohmmed A, Malhotra P, Bhatnagar RK, Mukherjee SK. RNA interference: biology, mechanism, and applications. Microbiol Mol Biol Rev.

2003 Dec;67(4):657-85. doi: 10.1128/mmbr.67.4.657-685.2003.

[32] Baum JA, Roberts JK. Progress towards RNAi-mediated insect pest management. Adv Insect Physiol. 2014;47:249-295. doi: 10.1016/B978-0-12-800197-4.00005-1.

[33] Burand JP, Hunter WB. RNAi: future in insect management. J Invertebr Pathol. 2013;112:S68–S74. doi: 10.1016/j.jip.2012.07.012.

[34] Mamta B, Rajam MV. RNAi technology: a new platform for crop pest control. Physiol Mol Biol Plants. 2017;23(3):487-501. doi:10.1007/s12298-017-0443-x.

[35] Liu S, Jaouannet M, Dempsey DA, Imani J, Coustau C, Kogel KH. RNA-based technologies for insect control in plant production. Biotechnol Adv. 2020 Mar-Apr;39:107463. doi: 10.1016/j.biotechadv.2019.107463.

[36] Scott JG, Michel K, Bartholomay LC, Siegfried BD, Hunter WB, Smagghe G, Zhu KY, Douglas AE (2013) Towards the elements of successful insect RNAi. J Insect Physiol 59:1212-1221. doi: 10.1016/j.jinsphys.2013.08.014

[37] Baum JA, Bogaert T, Clinton W, Heck GR, Feldmann P, Ilagan O, Johnson S, Plaetinck G, Munyikwa T, Pleau M, Vaughn T. Control of coleopteran insect pests through RNA interference. Nature biotechnology. 2007 Nov;25(11):1322-6.

[38] Hunter WB, Glick E, Paldi N and Bextine BR. Advances in RNA interference: dsRNA treatment in trees and grapevines for insect pest suppression. South West Entomology 2012; 37(1):85-87.

[39] Dalakouras A, Jarausch W, Buchholz G, Bassler A, Braun M, Manthey T, Krczal G, Wassenegger M. Delivery of hairpin RNAs and small RNAs into woody and herbaceous plants by trunk injection and petiole absorption. Frontiers in Plant Science. 2018; 24;9:1253.

[40] Ghosh SKB, Hunter WB, Park AL. Gundersen-Rindal Double strand RNA delivery system for plant-sap-feeding insects. PLoS One. 2017; 12: e0171861

[41] Joga MR, Zotti MJ, Smagghe G and Christiaens O. RNAi efficiency, systemic properties, and novel delivery methods for pest insect control: what we know so far. Front Physiol 2016; 7:553.

[42] Chen X, Li L, Hu Q, Zhang B, Wu W, Jin F et al. Expression of dsRNA in recombinant Isaria fumosorosea strain targets the TLR7 gene in *Bemisia tabaci*. BMC Biotechnol. 2015; 15: 64.

[43] Kumar P, Pandit SS, Baldwin IT. Tobacco rattle virus vector: a rapid and transient means of silencing manduca sexta genes by plant mediated RNA interference. PLoS One, 2012; 7: e e31347

[44] Tian, H., Peng, H., Yao, Q., Chen, H., Xie, Q., Tang, B. and Zhang, W. Developmental control of a Lepidopteran pest *Spodoptera exigua* by ingestion of bacterial expressing dsRNA of a non-midgut gene. PLoS ONE, 2009; 4: e6225

[45] Kolliopoulou A, Taning CNT, Smagghe G, Swevers L. Viral Delivery of dsRNA for Control of Insect Agricultural Pests and Vectors of Human Disease: prospects and Challenges. Front. Physiol. 2017; 8: 1-24

[46] Cagliari D, Dias NP, GALDEANO D, dos Santos EÁ, Smagghe G and Zotti MJ. Management of pest insects and plant diseases by non-transformative RNAi. Frontiers in plant science. 2019; 10:1319.

[47] Li H, Khajuria C, Rangasamy M, Gandra P, Fitter M, Geng C, Woosely A, Hasler J, Schulenberg G, Worden S, McEwan R. Long ds RNA but not si RNA initiates RNA i in western corn

rootworm larvae and adults. Journal of Applied Entomology. 2015 Jul;139(6):432-45.

[48] Zhang J, Khan SA, Hasse C, Ruf S, Heckel DG & Bock R. Full crop protection from an insect pest by expression of long double-stranded RNAs in plastids. Science 2015; 347: 991-994.

[49] de la Fuente J, Kocan KM, Almazán C and Blouin EF. RNA interference for the study and genetic manipulation of ticks. Trends in Parasitology. 2007; 23(9):427-433.

[50] Duan CG, Wang CH, Guo HS. Application of RNA silencing to plant disease resistance. Silence. 2012; 3(1): 5.

[51] Shaner DL and Beckie HJ. The future for weed control and technology. Pest Management Science 2014; 70(9): 1329-1339.

[52] Wang M, Weiberg A, Lin F-M, Thomma BPHJ, Huang H-D, Jin H. Bidirectional crosskingdom RNAi and fungal uptake of external RNAs confer plant protection. Nature Plants. 2016; 2(10):16151

[53] San Miguel K and Scott JG. The next generation of insecticides: DsRNA is stable as a foliar-applied insecticide. Pest Management Science. 2016; 72(4): 801-809.

[54] Zotti M, dos Santos EA, Cagliari D, Christiaens O, Taning CNT and Smagghe G. RNA interference technology in crop protection against arthropod pests, pathogens and nematodes. Pest Management Science. 2018; 74(6): 1239-1250.

[55] Niu J, Shen G, Christiaens O, Smagghe G, He L, Wang J. Beyond insects: current status and achievements of RNA interference in mite pests and future perspectives. Pest management science. 2018 Dec;74(12):2680-7.

[56] Head GP, Carroll MW, Evans SP, Rule DM, Willse AR, Clark TL, Storer NP, Flannagan RD, Samuel LW, Meinke LJ. Evaluation of SmartStax and SmartStax PRO maize against western corn rootworm and northern corn rootworm: efficacy and resistance management. Pest management science. 2017; 73(9):1883-99.

[57] Waltz, E. (2015). USDA approves next-generation GM potato. Nat. Biotechnol. 33, 12-13.

[58] Baranski, R., Klimek-Chodacka, M., Lukasiewicz, A. (2019). Approved genetically modified (GM) horticultural plants: a 25-year perspective. Folia Hortic. 31, 3-49.

[59] Mao YB, Cai WJ, Wang JW, Hong GJ, Tao XY, Wang LJ, Huang YP and Chen XY. Silencing a cotton bollworm P450 monooxygenase gene by plant-mediated RNAi impairs larval tolerance of gossypol. Nature Biotechnology. 2007; 25 (11):1307-1313.

[60] Dhadialla TS, Carlson GR, Le DP. New insecticides with ecdysteroidal and juvenile hormone activity. Annu Rev Entomol. 1998;43:545-569

[61] Zhu JQ, Liu S, Ma Y, Zhang JQ, Qi HS, Wei ZJ, Yao Q, Zhang WQ, Li S (2012) Improvement of pest resistance in transgenic tobacco plants expressing dsRNA of an insect associated gene EcR. PLoS ONE 7:e38572

[62] Xiong Y, Zeng H, Zhang Y, Xu D, Qiu D. Silencing the HaHR3 gene by transgenic plant-mediated RNAi to disrupt Helicoverpa armigera development. Int J Biol Sci 2013:9:370-381

[63] Liu F, Wang XD, Zhao YY, Li YJ, Liu YC, Sun J (2015) Silencing the HaAK gene by transgenic plant-mediated RNAi impairs larval growth of Helicoverpa armigera. Int J Biol Sci 11:67-74

[64] Jin S, Singh ND, Li L, Zhang X, Daniell H (2015) Engineered chloroplast dsRNA silences cytochrome p450 monooxygenase, V-ATPase and chitin synthase genes in the insect gut and disrupts Helicoverpa armigera larval development and pupation. Plant Biotech J 13:435-446

[65] Zha WJ, Peng XX, Chen RZ, Du B, Zhu LL, He GC (2011) Knockdown of midgut genes by dsRNA-transgenic plant-medi- ated RNA interference in the Hemipteran insect *Nilaparvata lugens*. Plos One 6:e20504

[66] Poreddy, S., Li, J. & Baldwin, I.T. Plant-mediated RNAi silences midgut-expressed genes in congeneric lepidopteran insects in nature. BMC Plant Biol 2017:17:199. doi.org/10.1186/s12870-017-1149-5

[67] Jiang S, Wu H, Liu H, Zheng J, Lin Y, Chen H. The overexpression of insect endogenous small RNAs in transgenic rice inhibits growth and delays pupation of striped stem borer (Chilo suppressalis). Pest management science 2017:73(7):1453-1461.

[68] Zheng, X., Weng, Z., Li, H., Kong, Z., Zhou, Z., Li, F., ... & Chen, H. (2020). Transgenic rice overexpressing insect endogenous microRNA csu-novel-260 is resistant to striped stem borer under field conditions. Plant Biotechnology Journal.

[69] Baum JA, Bogaert T, Clinton W, Heck GR, Feldmann P, Ilagan O, Johnson S, Plaetinck G, Munyikwa T, Pleau M, Vaughn T. Control of coleopteran insect pests through RNA interference. Nature biotechnology. 2007 Nov;25(11):1322-6.

[70] Meng, F., Li, Y., Zang, Z., Li, N., Ran, R., Cao, Y., ... & 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 management science, 73(12), 2447-2455.

[71] Wang, Z., Li, T., Ni, H., Wang, G., Liu, X., Cao, Y., ... & Meng, F. (2018). Transgenic soybean plants expressing Spb18S dsRNA exhibit enhanced resistance to the soybean pod borer Leguminivora glycinivorella (Lepidoptera: Olethreutidae). Archives of insect biochemistry and physiology, 98(2), e21461.

[72] Mao, YB., Tao, XY., Xue, XY. et al. Cotton plants expressing CYP6AE14 double-stranded RNA show enhanced resistance to bollworms. Transgenic Res 20, 665-673 (2011).

[73] Selvanarayanan V, Narayanasamy P Factors of resistance in tomato accessions against the fruit worm, Helicoverpa armigera (Hubner). Crop Prot 2006; 25:1075-1079

[74] Maligeppagol M, Asokan R, Krishna V, Latha J, NK KK, Ellango R. Transgenic tomato expressing dsRNA of juvenile hormone acid O-methyl transferase gene of *Helicoverpa armigera* (Lepidoptera: Noctuidae) affects larval growth and its development. Journal of Asia-Pacific Entomology. 2017 Jun 1;20(2):559-67.

[75] Agrawal N, Sachdev B, Rodrigues J, Sree KS, Bhatnagar RK. Development associated profiling of chitinase and microRNA of Helicoverpa armigera identified chitinase repressive microRNA. Scientific reports. 2013 Jul 26;3:2292.

[76] Mamta, K. R. K. Reddy, M. V. Rajam. Targeting chitinase gene of Helicoverpa armigera by host-induced RNA interference confers insect resistance in tobacco and tomato. Plant Mol Biol 2016; 90:281-292

[77] Hogenhout, S.A.; Ammar, E.-D.; Whitfield, A.E.; Redinbaugh, M.G. Insect Vector Interactions with

Persistently Transmitted Viruses. Annu. Rev. Phytopathol. 2008; 46: 327-359

[78] Faisal M, Abdel-Salam EM, Alatar AA, Saquib Q, Alwathnani HA, Canto T. Genetic Transformation and siRNA-Mediated Gene Silencing for Aphid Resistance in Tomato. Agronomy. 2019 Dec;9(12):893.

[79] Pareek M, Yogindran S, Mukherjee SK, Rajam MV. Plant microRNAs: Biogenesis, functions, and applications. InPlant biology and biotechnology 2015 (pp. 639-661). Springer, New Delhi.

[80] Niu QW, Lin SS, Reyes JL, Chen KC, et al. Expression of artificial microRNAs in transgenic *Arabidopsis thaliana* confers virus resistance. Nature Biotechnology 2006; 24: 1420-1428.

[81] Schwab R, Ossowski S, Riester M, Warthmann N, Weigel D. Highly specific gene silencing by artificial microRNAs in Arabidopsis. Plant Cell 2006; 18: 1121-1133

[82] Alvarez JP, Pekker I, Goldshmidt A, Blum E, Amsellem Z, Eshed Y. Endogenous and synthetic microRNAs stimulate simultaneous, efficient, and localized regulation of multiple targets in diverse species. Plant Cell. 2006: 18: 1134-1151.

[83] Yogindran S and Rajam MV. Host-derived artificial miRNA-mediated silencing of ecdysone receptor gene provides enhanced resistance to *Helicoverpa armigera* in tomato. Genomics. 2020 Oct 12.

[84] FAOSTAT data (2017) http://www.fao.org/home/en/. Retrieved 30 July 2018

[85] Calıskan ME, Onaran H, Arıoğ lu H. Overview of the Turkish potato sector: challenges, achievements and expectations. Potato Res 2010; 53:255-266

[86] Zhu F, Xu JJ, Palli R, Ferguson J, Palli SR. Ingested RNA: interference for managing the populations of the Colorado potato beetle (*Leptinotarsa decemlineata*). Pest Manag Sci. 2011; 67:175-182

[87] Hussain T, Aksoy E, Calıskan ME, Bakhsh A. Transgenic potato lines expressing hairpin RNAi construct of molting-associated EcR gene exhibit enhanced resistance against Colorado potato beetle (*Leptinotarsa decemlineata*, Say). Transgenic Res. 2019; 28:151-164

[88] Guo W, Bai C, Wang Z, Wang P, Fan Q, Mi X, Wang L, He J, Pang J, Luo X, Fu W. Double-stranded RNAs high-efficiently protect transgenic potato from *Leptinotarsa decemlineata* by disrupting juvenile hormone biosynthesis. Journal of agricultural and food chemistry. 2018 Nov 6;66(45):11990-9.

[89] Paudel JR, Davidson C, Song J, Maxim I, Aharoni A, Tai HH. Pathogen and Pest Responses Are Altered Due to RNAi-Mediated Knockdown of GLYCOALKALOID METABOLISM 4 in *Solanum tuberosum*, MPMI. 2017; 30(11): 876-885.

[90] Rondon, SI. The potato tuberworm: a literature review of its biology, ecology, and control. American Journal of Potato Research 2010; 87: 149-166.

[91] Mohammed AM, Diab MR, Abdelsattar M, Sayed MS. Characterization and RNAi-mediated knockdown of Chitin Synthase A in the potato tuber moth, *Phthorimaea operculella*. Scientific Reports. 2017 Aug 25;7(1):1-2.

[92] Furlong MJ, Wright DJ, Dosdall LM. Diamondback moth ecology and management: problems, progress, and prospects. Annual review of entomology. 2013 Jan 7;58:517-41.

[93] Talekar NS, Shelton AM. Biology, ecology, and management of the diamondback moth. Annual review of entomology. 1993 Jan;38(1):275-301.

[94] Iqbal, M., Khan, A.R., Rafique, M., Suthar, V. and Solangi, B.K. Comparative Efficacy of Botanical and Synthetic Pesticides against Major Insect Pests of Cabbage. European Academic Research, 2015; 3(9):10038-10057.

[95] Cartea, M. E.; Francisco, M.; Lema, M.; Soengas, P.; Velasco, P. Resistance of Cabbage (*Brassica oleracea* capitata Group) Crops to Mamestra brassicae. Journal of Economic Entomology, 2010; 103(5), 1866-1874.

[96] Israni B and Rajam MV. Silencing of ecdysone receptor, insect intestinal mucin and sericotropin genes by bacterially produced double-stranded RNA affects larval growth and development in Plutella xylostella and Helicoverpa armigera. Insect Mol Biol, 2017; 26: 164-180.

[97] Wang Y, Liu Z, Xu J, Li X, Bi H, Andongma AA, Niu C, Huang Y. Mutation of doublesex induces sex-specific sterility of the diamondback moth Plutella xylostella. Insect biochemistry and molecular biology. 2019 Sep 1;112:103180.

[98] Ellango R, Asokan R, Chandra GS, Kumar NK, Mahmood R, Ramamurthy VV. Tyrosine hydroxylase, a potential target for the RNAi-mediated management of diamondback moth (Lepidoptera: Plutellidae). Florida Entomologist. 2018 Mar;101(1):1-5.

[99] Su Liu, Yu-Xing Zhang, Wen-Long Wang, Bang-Xian Zhang, Shi-Guang Li. Identification and characterisation of seventeen glutathione S-transferase genes from the cabbage white butterfly *Pieris rapae*, Pesticide Biochemistry and Physiology, 2017; 43:102-110.

[100] Cooper AM, Silver K, Zhang J, Park Y, Zhu KY. Molecular mechanisms influencing efficiency of RNA interference in insects. Pest management science. 2019 Jan;75(1):18-28.