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RNA INTERFERENCE AND ARTIFICIAL MICRO-RNA TECHNOLOGY AS NEW TOOLS FOR ENGINEERING INSECT RESISTANCE IN CROP PLANTS

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Worldwide, insect pests cause extensive damage to the economically important crops. With the advancement of genetic engineering technology, attempts have been made to achieve insect resistance in crop plants. The discovery of RNA interference (RNAi) phenomenon opens a realm of gene expression regulation, which is highly sequence-specific. Since then, this technology has been proved to be promising for crop improvement. RNAi technology is being used to develop crop plants for abiotic and biotic stress tolerance, and many other agronomically important traits. Insect resistant crops have been developed by expressing exogenous small interfering RNAs (siRNAs) or artificial miRNAs (amiRNAs) for silencing vital genes of the target insect. This review summarizes the progress made in the field of crop improvement to achieve insect resistance by RNAi technology.

Keywords: RNA interference; artificial miRNA; gene silencing; transgenic plants; crop protection; insect control

The upsurge of global population, which is expected to hit 9.6 billion by 2050 calls for the food security in the future (Singh and Prasad 2016). The potential yield of the agricultural crops is obstructed by many factors such as insufficiency of arable land, climate change and different biotic and abiotic stresses. Among biotic stresses, insect pests cause a considerable amount of loss of annual turnover from crops. Some examples of notorious crop insect pests are given in Table 1. The damage can occur in the pre-harvest stages or post-harvest phase (Oerke 2006). The morphological and physiological traits, higher reproductive ability, polyphagous nature and fast rate of evolution to resistant biotypes confer success to the establishment of the insects as pests (Mamta and Rajam 2017). The control strategies mainly rely upon the manual methods by picking and trapping of the insects or the use of chemical insecticides. The first one has severe limitations and can be applied over a small area only and latter has significant negative impact on the environment and human health. Plant breeding approaches have also marked their places in the crop improvement but has constraints of being labour intensive and time consuming and lack of resistance source in some cases.

Therefore, an imperative call to look for the alternative strategies to develop insect resistance and concomitant yield improvement was needful. Recombinant DNA technology, comes to a rescue by the advent of genetic engineering and transgenic approaches. In recent years, insect resistant crops expressing δ-endotoxins genes from *Bacillus thuringiensis* have been developed and remarkably changed the global agricultural scenario. However, insects have been shown to develop resistance against such insecticidal proteins over the course of time, which limits the success of Bt technology. The ideal pest control strategy should be precise, effective, economical and environment friendly. RNA interference (RNAi), phenomenon was discovered by Fire *et al.* (1998), where the dsRNA-mediated gene silencing in *Caenorhabditis elegans* was demonstrated. RNAi involves the silencing of an essential gene of the target organism in a highly sequence-specific manner at the post-transcriptional level. This strategy is being used as a novel alternative approach for insect pest management in different crops (Kola *et al*. 2015, Yu *et al.* 2016, Mamta and Rajam 2017).

Genetic engineering strategies and their limitations for insect resistance in crop plants: Some plants are found to be naturally

Table 1: Examples of some of the major insect pests of economically important crops

Insect pest	Order	Affected crops
Helicoverpa armigera	Lepidoptera	Cotton, tomato, pigeonpea, chickpea, maize, okra
Nilaparvata lugens	Hemiptera	Rice
Bemisia tabaci	Hemiptera	Cotton, tomato, okra, brinjal, beans
Plutella xylostella	Lepidoptera	Cabagge, cauliflower, broccoli
Sitophilus oryzae	Coleoptera	Rice, wheat, maize
Chilo suppressalis	Lepidoptera	Rice
Rhopalosiphum maidis	Hemiptera	Barley, wheat, rice
Sitobion avenae	Hemiptera	Wheat
Sesamia inferens	Lepidoptera	Wheat, maize
Leucinodes orbonalis	Lepidoptera	Brinjal
Spodoptera sp.	Lepidoptera	Okra, cauliflower, cabbage, pigeonpea, beans, cotton and several other vegetables
Maruca vitrata	Lepidoptera	Cowpea, pigeonpea, soybean and several other vegetables

resistant to a broad group of insects. Investigations have suggested the presence of several substances which play an important role as a part of defence mechanism in plants. These compounds have been explored by the scientific communities to generate transgenic plants to impart resistance against the pest population using genetic engineering technologies.

Resistance to insects has been achieved by inserting the genes of *B. thuringiensis*, which encode for crystal proteins (Cry proteins). Upon intake, these protoxins become activated by intestinal proteases to form active toxins and recognize the binding site on the midgut membrane surface. Consequently, pores are formed on the epithelial membrane leading to cell lysis and death of the insects (Soberón *et al*. 2007; Deist *et al.* 2014). The genetically modified GM) or transgenic plants expressing *Bt* toxins were grown commercially in 1996 (James 2004) and since then significant advancement has taken place in order to obtain insect resistance. The continuous expression and effectiveness against a vast array of insects made this technology a popular choice for the farmers. Cry1A family of *Bt* toxins has been the mostly used, several crops including cotton, maize, tomato, soybean, potato, brinjal, etc. expressing Cry proteins have been generated so far (Sheikh *et al.* 2017). Report on resistance against Bt protein was first reported in 2005 and since then population of 5 out of 13 major insect species have been reported to exhibit resistance (Tabashnik *et al.* 2013). Rapid evolution of insects to impart resistance to insecticide is the anticipated possibility behind the resistance towards Cry toxins.

In addition to Cry proteins, a number of other insecticidal toxins are secreted by *B. thuringiensis*. For example, vegetative insecticidal protein (VIP) is also being used to engineer cotton plants for insect resistance (Estruch *et al.* 1996). The mode of action of VIPs is different as compared to Cry proteins, and Cry1Ac-resistant strains of *Heliothis virescens* have been shown to be susceptible to VIP3A (Jackson *et al.* 2007).

Protease inhibitors (PIs) are a group of naturally occurring plant substances which play a role as a defence mechanism against insect herbivory. The exploration of the insecticidal properties of PIs led to the production of first transgenic soybean plants expressing PIs (Hilder *et al.* 1987), following that a number of transgenic crops have been generated expressing this group of insecticidal proteins (Reviewed by Malone *et al.* 2008).

Alpha- amylase inhibitors are another group of plant defence molecules, reported to interfere with the ability to digest starch in insects (Franco *et al.* 2002). Legumes have been particularly exploited to express this class of proteins to confer resistance against a number of weevils (Shade *et al.* 1994; Schroeder *et al.* 1995; Ishimoto e*t al.* 1996; Sarmah *et al.* 2004). It has been shown that the expression of α- amylase inhibitor from legume to non-

legume plant confers resistance against insect pest (Barbosa *et al*. 2010).

Biotin binding proteins (BBPs) such as avidin and streptavidin from chicken egg white and *Streptomyces avidiniii* respectively, have been shown to be tox*ic against a wide range of insects (Markwick et al.* 2001). Following the discovery, a number of transgenic plants such as rice, tobacco, maize, apple, potato, etc. have been developed expressing BBPs (Yoza *et al.* 2005; Burgess *et al.* 2002b, Kramer *et al.* 2000; Markwick *et al.* 2003; Meiyalaghan *et al.* 2005).

Though, the above mentioned strategies are promising but still associated with some limitations. Plants expressing *Bt* toxins have been shown to be successful against vast majority of lepidopteran pests but is found to be ineffective against other insect orders, *viz*. coleoptera, hemiptera (Gatehouse and Price 2011). PIs play major role in achieving insect resistance but there is no report of commercialization till date (Rajam and Yogindran 2018). A better understanding of the underlying mechanisms in the above mentioned strategies holds the possibility to achieve crop yield improvement by controlling insect pests.

Small RNAs: Biogenesis and functions

Over the years, small RNAs (sRNAs) have been recognized as an important group of regulatory elements involved in the regulation of gene expression. sRNAs are broadly classified into two classes namely small interfering RNAs (siRNAs) and microRNAs (miRNAs). Though they are more or less structurally similar (20-24 nts) but differ in the process of biogenesis and mode of action. siRNAs are derived from long double-stranded RNAs (dsRNAs) and play the role in target mRNA cleavage in response to foreign nucleic acids such as invading RNA viruses, transgenes, endogenous repeat elements and transposons (Zamore 2002, Finnegan and Matzke 2003). On the other hand, miRNAs are endogenous transcription products which are highly conserved across diverse species and control the endogenous gene expression (Vaucheret *et al.* 2004). Dicer and Argonaute (AGO) proteins are the core components of the RNAi pathway, although many other proteins are involved in the process. Both siRNAs and miRNAs are generated from precursor molecules by Dicer, a RNase III enzyme. The double-stranded siRNAs and miRNAs, then get associated with RNA induced silencing complex (RISC), Argonaute and other effector molecules leading to the target mRNA cleavage or repression of translation (Figure 1).

Movement of plant sRNAs occurs in two ways: cell-to-cell and systemic movement (Melnyk *et al.* 2011). The first of movement is symplastic and takes place by plasmodesmata (Lough and Lucas 2006) and long-distance systemic movement takes place via vascular phloem tissue and eventuates in several days (Voinnet *et al.* 1998). The success of RNAi in different orders of insects has confirmed that the insect cells are capable of dsRNA uptake. In *C. elegans*, two proteins namely SID-1 (Systemic RNAi defective-1) and SID-2 have been identified to be involved in the process of dsRNA uptake and systemic RNAi. SID-1 is essential and play role in the spread of RNAi signals across cells and SID-2 acts in association of SID-1 and is gut specific (Winston *et al.* 2002, 2007). Orthologs of *sid* genes have been identified in a number of insect species belong to different orders (Reviewed by Huvenne and Smagghe 2010).

siRNAs

They are derived from dsRNAs or hp-RNAs and Dicer enzyme processes this dsRNA into siRNA molecules. siRNAs are generally short, with 5'-phosphorylated dsRNAs and 2 nt overhangs at the 3' end(Bernstein *et al.* 2001). siRNA- induced silencing complex (siRISC) is activated by the accumulation of many other proteins to form the RISC-loading complex (RLC) (Carthew and Sontheimer 2009). AGO protein gets recruited to form pre-RISC complex and capable of binding to the dsRNAs

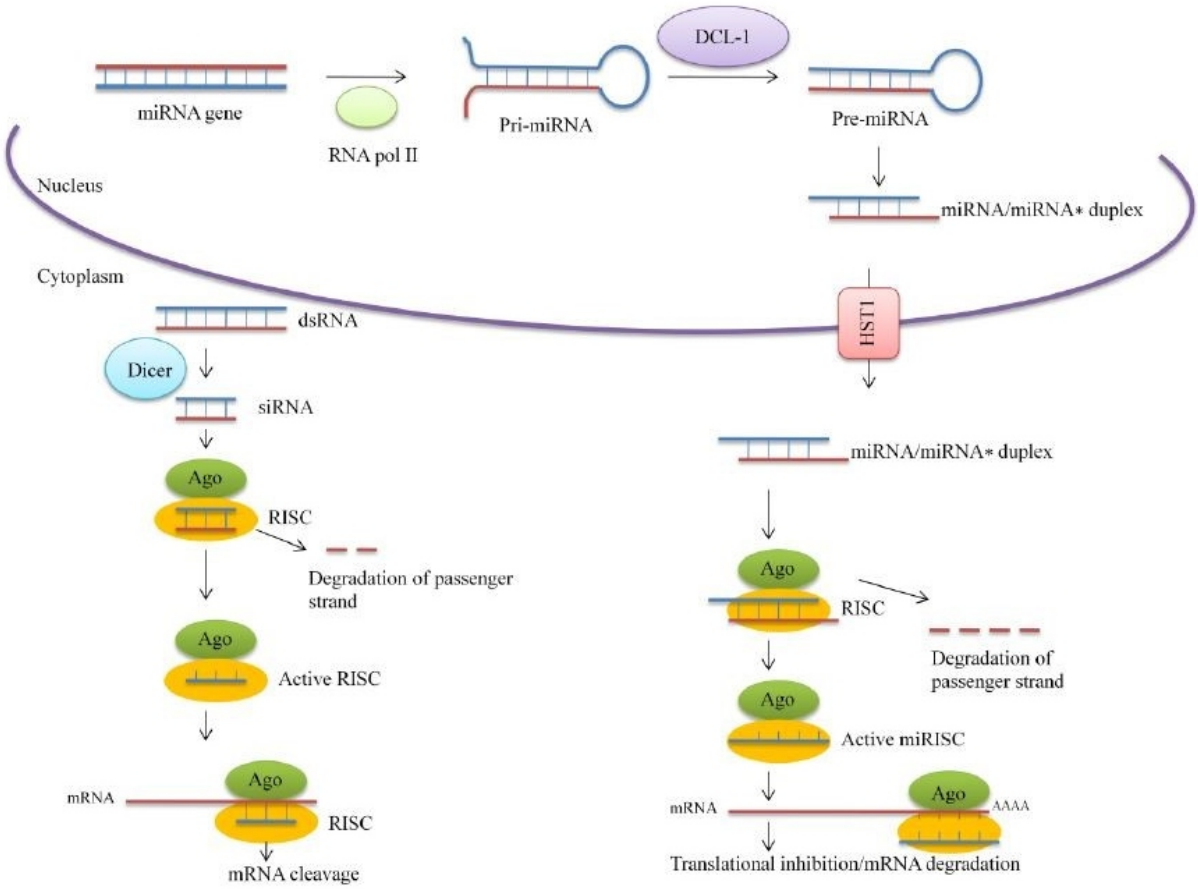

Figure 1: siRNA and miRNA biogenesis pathways in plants

(Kim *et al*. 2007). Degradation of the passenger (sense) strand takes place to form functional RISC. The guide (antisense) strand then directs the cleavage of the targeted mRNA leading to post-transcriptional gene silencing (PTGS). AGO is an important component of the active RISC complexes. PIWI domain of the AGO protein induces the process of mRNA degradation (Figure 1). Ten different *ago* genes have been reported to participate in the process of RNAi. The effector phases of siRNA-mediated silencing are reported to occur in the cytoplasm.

miRNAs

miRNAs are found in eukaryotic organisms and are encoded by a diverse array of genes. miRNAs are transcribed by RNA polymerase II into primary miRNAs (pri-miRNAs) having a hairpin loop structure. Next, the pri-miRNAs are cleaved by a member of RNase III family (DCL1 in plants and Drosha in animals) to produce pre-miRNAs and mature miRNA duplex (miRNA/miRNA*)(Kim 2005).These steps occur in the nucleus and the mature miRNA duplex exported to cytoplasm for further processing. The export is mediated by HASTY (an ortholog of exportin-5 protein). The mature miRNA duplex then loaded onto RISC and degradation of target mRNA takes place. The cleavage of the target mRNA takes place at the 10th or 11th nucleotide from the 5' end of the miRNA (Mallory *et al.* 2004). The downregulation of mRNA can occur either by translational inhibition or by cleavage (Figure 1). In animals, mostly translational inhibition takes place by binding to the motifs in the 3' UTRs of the targets, containing several mismatches to the miRNA. However, in plants target motifs contain fewer mismatches and lead to target cleavage. In plants, miRNAs are

less conserved usually consist of 20–24 nts. In animals, it is mostly 20–22 nucleotides long and are highly conserved (Bartel 2004).

RNAi- mediated silencing for developing insect resistant crop plants

Agronomic insect pests cause an enormous amount of yield loss annually. A number of insect genes have been tested so far for RNAi (reviewed by Kola *et al.* 2015, Mamta and Rajam 2017). According to the reports, engineered plants can produce dsRNA, which further direct gene silencing in the insects upon feeding. The advantages of dsRNA expressing transgenic plants are manifold, including heritability and stability of the structures. Production is continuous under the use of constitutive promoters for plant expression. Thus host-induced or plant-mediated RNAi technology opens up a new arena of insect pest control. A number of efforts have been put forth to develop dsRNA producing transgenic plants. In 2007, two independent groups reported the generation of transgenic plants producing dsRNAs targerted against insect pests. In the first report, transgenic *Arabidopsis thaliana* has been raised to express dsRNA targeted against an insect cytochrome P450 gene (*CYP6AE14*), which resulted in retardation of larval growth of *Helicoverpa armigera* upon feeding (Mao *et al.* 2007). Cytochrome P450 monoxygenases are present to provide tolerance to plant toxins like gossypol (present in cotton leaves) in insects. Baum *et al.* (2007) reported the RNAi-mediated knock-down of insect vacuolar ATPase (*V-ATPase*) gene of western corn rootworm (WCR), *Diabrotica virgifera virgifera*. Transgenic maize (*Zea mays*) lines have been generated expressing dsRNA against *V-ATPase*. Feeding assay with the transgenic plants on WCR larva resulted in stunted growth and mortality. Later on, *CYP6AE14* of *H. armigera* has been targeted to generate transgenic cotton plants expressing dsRNA against *CYP6AE14* (Mao *et al.* 2011).Cotton plants contain gossypol, which acts a part of plants defence mechanism against herbivory but insects express a group of cytochrome P450 monoxygenase genes to catabolize such defence molecules to breach the anti-herbivory barrier. Down-regulation of *CYP6AE14* by feeding on transgenic cotton plants was associated with retarded growth of cotton bollworm (*H. armigera*). *Nilaparvata lugens* is one the most destructive pests of rice. Towards the goal to combat this insect, RNAi technology has been applied to check the efficacy. Three genes, *viz*., hexose transporter (*NlHT1*), carboxypeptidase (*Nlcar*) and trypsin like serine protease (*Nltry*), have been targeted to develop transgenic rice to confer resistance against brown planthopper (*N. lugens*) (Zha *et al.* 2011). Feeding on transgenic plants demonstrated significant amount of reduction in the transcripts of these midgut genes but lethal phenotypic deformity has not been observed. Sufficient up-take of dsRNA, optimal RNAi penetrance, choice of genes are some of the major factors to be taken into account to achieve the maximum RNAi effects (Terenius *et al.* 2011, Zha *et al.* 2011). An ecdysone receptor gene of *N. lugens* was targeted to generate transgenic rice, where feeding on transgenics showed decreased fecundity due to reduction in target gene expression (Yu *et al.* 2014). Receptor of Activated Kinase C (*Rack-1*) and *MpC002* genes have also been targeted in another aphid, *Myzus persicae* by raising transgenics, feeding on dsRNA expressing *A. thaliana* exhibited knock-down of the targeted genes and concomitant reduction in number of insect progeny (Pitino *et al.* 2011). Transgenic tobacco plants expressing dsRNA against a number of genes including 20 Hydroxyecdysone, transcription factor *HaHR-3)*, chitin synthase, cytochrome P450 monooxygenase and v-ATPase genes were generated to examine the potential of RNAi on *H. armigera* (Zhu *et al.* 2012, Xiong *et al.* 2013; Jin *et al.* 2015). More recently, a non-midgut gene, chitinase of *H. armigera* has been targeted through host-induced gene silencing (HIGS) for the control of tomato fruit worm (Mamta *et al.* 2016). Transgenic tomato and tobacco plants were raised to express dsRNA

Table 2: Plant-mediated RNAi for insect pest control

RNAi plant	Target insect	Target gene	Effects	References
Arabidopsis thaliana	*Helicoverpa armigera*	Cytochrome 450 monooxygenase *CYP6AE14*	Reduced tolerance to gossypol and retarded larval growth	Mao *et al.* (2007)
Zea mays	*Diabrotica virgifera virgifera*	Vacuolar ATPase (V-ATPase)	Stunted larval growth and mortality	Baum *et al.* (2007)
Gossypium hirsutum	*H. armigera*	Cytochrome 450 monooxygenase *CYP6AE14*	Retarded larval growth and enhanced resistance against the insect	Mao *et al.* (2011)
Oryza sativa	*Nilaparvata lugens*	Hexose transporter gene *NlHT1* Carboxypeptidase gene *Nlcar* Trypsin like serine protease gene *Nltry*	Reduction in target gene transcript in the midgut but no lethal phenotype observed	Zha *et al*. (2011)
A. thaliana *Nicotiana benthamiana*	*Myzus persicae*	Receptor of Activated Kinase C (*Rack-1*) and *MpC002*	Reduction in number of progenies	Pitino *et al*. (2011)
Nicotiana tabacum	*H. armigera*	20-hydroxyecdysone (20E)	Defective molting and lethality in larva	Zhu *et al*. (2012)
A. thaliana	*Myzus persicae*	Serine protease	Reduced fecundity	Bhatia *et al.* (2012)
N. tabacum	*H. armigera*	Molt-regulating transcription factor *HaHR3*	Larval deformity and lethality	Xiong et al. (2013)
O. sativa	*N. lugens*	Ecdysone receptor (*EcR*)	Reduction in number of offsprings	Yu *et al.* (2014)
N. tabacum	*Bemisia tabaci*	*V*-ATPase subunit A (V-ATPaseA)	Mortality in insects	Thakur *et al*. (2014)
Triticum aestivum	*Sitobion avenae*	Carboxylesterase (*CbE E4*)	Impairment of larval tolerance to Phoxim insecticides	Xu *et al*. (2014)
N. tabacum	*H. armigera*	Cytochrome 450 monooxygenase *CYP6AE14* V-ATPase A Chitin synthase B	Reduction in larval growth and pupation	Jin *et al.* (2015)
Hordeum vulgare	*Sitobion avenae*	Structural sheath protein (*SHP*)	Reduced growth and rate of survival	Abdellatef *et al*. (2015)
N. tabacum *Solanum lycopersicum*	*H. armigera*	Chitinase	Developmental deformities and reduced survival	Mamta *et al.* (2016)
N. tabacum	*Bemisia tabaci*	Acetylcholinesterase (*AChE*) Ecdysone receptor (*EcR*)	Mortality in adult flies	Malik *et al*. (2016)
Solanum tuberosum	*Leptinotarsa decemlineata*	Ecdysone receptor (*EcR*)	High rate of mortality	Hussain *et al*. (2019)

against chitinase gene of cotton bollworm (*H. armigera*) and 1.5–6.2 fold down-regulation in the transcripts of the targeted gene was observed, which led to insect developmental deformity and mortality (Mamta *et al.* 2016). The potential use of serine protease gene was explored by Bhatia *et al.* (2012), where *A. thaliana* transgenics were raised for the expression of dsRNA against serine protease gene for the control of *M. persicae* . Whitefly (*Bemisia tabaci*) is another serious insect pest of a diverse group of crop plants. It not only causes damage by ingestion but also play a role in transmission of several viral diseases. Recently, v-ATPase, acetylcholinesterase and ecdysone receptor have been targeted through transgenic tobacco plants and insect feeding assays exhibited reduction of *B. tabaci* growth and fecundity (Thakur *et al.* 2014, Malik *et al.* 2016). Carboxylesterases are a group of enzymes, widely distributed in microbes, plants and animals and play an important role in detoxification of insecticides. *CbE E4* is one such gene, associated with hydrolysis of organophosphate insecticides. Xu et al. (2014) cloned *CbE E4* from *Sitobion avenae* and generated RNAi transgenic wheat, which showed reduction in target gene expression along with stunted growth of the target insect after feeding. Interestingly, siRNAs have found to be transported from phloem to intestinal tissues via stylet of aphids (*S. avenae*). Sheath protein of *S. avenae* has also been targeted by developing transgenic barley plants, and down-regulation of the sheath protein gene has been shown to affect feeding and survival rate of the aphids (Abdellatef *et al.* 2015). Moreover, RNAi has found to be transmitted through several generations though weakening of silencing was observed across the generations (Abdellatef *et al.* 2015). More recently, Hussain *et al.* (2019) developed transgenic potato expressing dsRNA against ecdysone receptor gene of colorado potato beetle (*Leptinotarsa decemlineata*), and up to 80% mortality was achieved with significant growth retardation. The work on HIGS for the control of crop insect pests is summarized in Table 2.

Artificial microRNAs (amiRNAs)

The amiRNAs are designed by replacement of miRNA/miRNA* duplex in an endogenous miRNA precursor, with a sequence which is complementary to a target gene of interest intended for silencing, and the endogenous miRNA processing machinery is used for the generation of amiRNAs (Tiwari *et al.* 2014; Yogindran and Rajam 2016). The biogenesis of new precursor is not hampered as long as the hairpin loop structure remains intact (Sablok *et al.* 2011). This concept was validated by the development of transgenic *A. thaliana* through the manipulation of precursor miR159 to express amiRNAs for targeting viruses (Niu *et al.* 2006). The amiRNA technology is said to be the second generation RNAi and holds on to several advantages over hpRNAi, such as minimal off-target effects and high precision. Moreover, the easy optimization of amiRNA sequences to target one or several desired mRNAs without affecting the expression of other genes is another added advantage (Schwab *et al.* 2006; Ossowski *et al.* 2008). amiRNAs are being exploited for targeting insect pest genes for their control in crop plants (Yogindran 2017; Yogindran and Rajam 2016).

amiRNAs-mediated insect resistance in plants

After the tremendous success of miRNA-mediated silencing for crop improvement, the use of amiRNAs to generate transgenic crops for resistance against different biotic stresses has also been explored. A handful of reports about targeting insect physiological and endocrinological processes through amiRNAs are available. After the identification of miR-24 which targets the chitinase gene of *H. armigera* effectively and subsequent disruption of molting process (Agrawal et al. 2013), miR-24 was used to raise tobacco transgenics to check the effect on *H. armigera* upon feeding, which led to cessation of molting and larval death. (Agrawal *et al.* 2015). A natural pre-miRNA of *Solanum lycopersicum* (Sly-miR-159) was used as a backbone and miRNA duplex was replaced by custom

synthesized miR-24 to generate amiR-24 and cloned in binary expression vector under the control of constitutive CaMV35S promoter with an enhancer. The amiRNA-24 construct was used to develop transgenic tomato and used for insect feeding, which resulted in stunted growth, disrupted molting and mortality of *H. armigera* larva. Moreover, this was shown to be highly specific for the particular insect and did not affect other insects. Acetylcholinesterase (*AChE*) has been proven to be a good target for insect pest control (Kumar *et al.* 2009). This enzyme hydrolyses a neurotransmitter, acetylcholine which acts in the process of transmission of nerve impulse and targeting *AChE* shown to disrupt the process of neurotransmission and paralysis and death of the organisms. *AChE* of *M. persicae* has been targeted via transgenic tobacco plants, which were generated by using two amiRNA constructs (Guo *et al.* 2014). The CaMV 35S promoter has been used to drive the expression of amiRNAs in plants and feeding on which demonstrated a significant down-regulation of *AChE* and proved the significance of amiRNA-based approach in aphid control. *Chilo suppressalis* is one of the major pests of rice, causes extensive damage. amiRNA technology has been applied to confer resistance to rice against this insect pest where 13 novel insect endogenous miRNAs have been over-expressed to check the efficacy (Jiang *et al.* 2017). Among the miRNAs selected, transgenic rice over-expressing csu-novel-miR15 and csu-novel-miR53 demonstrated maximum RNAi effects. More recently, He et al. (2019) reported the development of transgenic rice expressing amiR-14 under the ubiquitin promoter. The feeding of larvae of *Chilo suppressalis* on transgenics exhibited significant developmental deformities and mortality. An insect-specific miRNA, *Csu-miR-14*, which targets two genes, i.e., *CsSpo* and *CsEcR*, involved in the ecdysone signalling pathway of the insect was used to examine its effects (He *et al.* 2019). Interestingly, plant-expressed insect pre-amiRs (plin-amiRs), a new strategy of insect pest control has been reported by Bally et al. (2020). A segment of insect pre-miR sequences has been replaced with sequences that target an insect gene, Acetylcholinesterase 2, in *H. armigera* and this was cloned in a plant expression vector to transform *Nicotiana benthamiana*. Insect larva showed notable developmental delay and mortality upon feeding of tobacco leaves.

Conclusions and future prospects

Over the past two decades, RNAi has emerged as a promising tool in the area of insect pest management. Extensive studies have demonstrated the effectiveness of this technology in insect gene silencing. The breakthrough studies by Mao *et al.* 2007 and Baum *et al.* 2007 showed a new way of insect pest control by generation of transgenic plants to express small RNAs to be fed by insects. With the advancement of second generation sequencing technology, better understanding of insect genome study could pave the way to the innovations of more efficient control strategies. As there is no expression of transgene proteins, so there is no extra metabolic load on protein synthesis in transgenic plants which is an advantage of RNAi along with its high specificity. Off-target effect is a serious limitation of this technology, which can lead to the unintended silencing of the related genes in beneficial organisms. Besides that tissue-specific expression of siRNAs is not possible because of the systemic spread nature of this group of small RNA molecules. Artificial miRNA technology exhibits to overcome this problem. This technology shows up to be as effective as siRNA-mediated silencing but with minimal off-target effects. amiRNAs are suggested to deliver enhanced silencing when expressed under strong promoters. Till date, only conventional promoters have been used to raise transgenics. Exploration of other constitutive, tissue and developmental stage specific promoters holds the possibility towards more effective silencing. Though a handful of studies demonstrated the promising nature of these technologies but these are mainly

restricted to the model plants. Application on plants of commercial interest is needed in order to assess the efficacy. Substantial studies on risk assessment are required to overcome the regulatory barriers and GM related concerns among public. Commercialization of the insect-resistant GM crops is needed to meet the food demand in the coming years. It is also noteworthy for the scientific communities to look for credible diagonostic tools for the detection and quantification of small RNA molecules in the vicinity. These tools are preferred to be cost-effective, functional at field level and should be capable of detection in low amount with high specificity (Auer and Frederick 2009). A better understanding of the mechanism in depth along with addressing the bio safety concerns can lead to the successful application of this technology for crop improvement and human welfare in the near future.

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