RNA interference: A potential method of crop pest management: A review

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Abstract
Suppression of genes that code for specific proteins through RNA interference is an alternative strategy for insect pest management. RNA interference is a powerful tool that exemplifies the gene function by making changes in post transcriptional gene silencing phenomenon. This ability of suppressing a particular gene expression helps in obtaining a new trait by elimination or accumulating particular plant trait that are desired which are phenotypic or biological changes which are not present in non-transgenic plants. Clear evidence is obtained from the recent studies that RNAi has potential application in controlling insect pests. However environment and non target organism and risk resistance is yet to be determined.

Keywords: Gene silencing, insect pest management, RNAi, RNAi based insecticides

1. Introduction
RNA interference is a method of regulating or manipulating the process of gene silencing to disturb a particular gene expression and in turn their function. This method was first discovered in Cenorhabditis elegans [1] and Drosophila melanogaster Megnin [2] which was published back in 1998. The RNAi machinery utilises a particular sequence of information in dsRNA and produces a RNA-protein complex that ultimately degrade the corresponding mRNA [3-5]. This is a highly specific process where genes sharing a complimentary sequence with dsRNA are affected. Both in plants and animals the very most important function of RNAi is to maintain integrity of genome, which is possible only by subduing the moment of transposons and the accumulation of repetitive DNA in germline [6, 7]. Since then this method was constantly being explored in a much deeper manner to find the functioning of host genes and to generate and enhance the plant traits that correspond to stress tolerance, qualitative and quantitative yield characteristics [8, 9]. Apart from regulating host genes RNA interference has also been successfully employed for making changes in the genes of insect pests and pathogen carrying vectors which pose a critical threat for crop production [10, 11, 12]. Thus it is much important to understand RNA interference technique exemplifying core RNA interference mechanism, players involved, new developments regarding dsRNA uptake, current RNAi based insecticides and lastly we also discuss recent studies of using RNAi transgenic plants for controlling agriculturally important insect pests.

2. Players involved in RNAi
Many compounds serving as initiators, effectors, an amplifier, transmitters has been identified. It is hopefully expected that in future many other components and their interrelations may be unveiled. Here we discuss some of the components known so far.

2.1 Dicer
Dicer belongs to RNase III family are among the few nuclease that show specificity for dsRNAs [13]. Dicer cleaves dsRNA into short ssRNA approximately of 20-25 base pairs long with 3’ overhangs of 2-3 nucleotides of 5’ phosphate and 3’ hydroxyl termini. Usually some species specific RNase III like enzymes called dicer act upon these long dsRNAs resulting in small dsRNA now these short RNAs gets binded to RISC complex and thus helps in finding mRNAs that are to be blocked for translation in post transcriptional silencing [14, 15].

2.2 RNA and DNA helicases
Small interfering RNAs of 21-25 nt have a very prominent role in post transcriptional
This method is in vitro production and storage of dsRNA is quite complicated and involves in high production costs. Additionally the wounds generated due to injection pressure also limit its application.

4.2 Feeding of artificial diet
Feeding dsRNA is more convenient and easy to manipulate method as it is one of the natural method of inducing dsRNA in to the body of insects, without causing any physical injury to the target organism. For small insects which are difficult to handle through micro injection method can be operated easily by feeding artificial diet. A pheromone binding protein EposPBP1 in antenna of adult Epiphyas postvittana successfully inhibited when their larvae are fed with dsRNA, along with inhibition of carboxyl esterase gene EposCXEL in larval midgut [19]. Successful attempts were also reported in orders like hemiptera, coleopteran, and lepidoptera [20, 21]. The main constrain remains that silencing has been shown to be incomplete and also need a large amount of material for delivery [22].

4.3 Soaking
In an experiment, when embryos of D. Melanogaster were soaked in dsRNA solution the results for inhibition of gene expression were much efficient. Moreover the amount of solution with dsRNAs in this method is less when compared with injection method which requires a higher concentration of dsRNA [23]. Soaking method is limited to few insects and life stages which can readily absorb dsRNA from solution.

4.4 Transgenic crop technique
Transgenic crop technique is one method that was found much better than direct feeding with dsRNA [20, 21]. This method has an advantage of producing dsRNA continuously. Studies showed that gene silencing was not successful in D. melanogaster when fed with dsRNAs produced from genetically engineered yeast strand [24]. However dsRNA produced by bacteria were effective when fed to C. elegans [25]. Since then bacteria is widely used for production of dsRNA in RNAi.

4.5 Virus-mediated uptake
In virus mediated RNAi host organisms get infected with dsRNA which are produced during the replication of virus. The target gene of interest into host organism is degraded by small interfering RNA produced from dicing dsRNA. For example when Bombyx mori cells were infested with recombinant Sindbis virus the dsRNA then produced inhibits BR-C gene expression which has lead to the poor pupation and defects in adult insects [20]. Though pathogenicity and rapid spreading ability of virus in host population in high virus mediated RNA studies are still rare.

5. Current RNAi-based insecticides
An extensive research on the response of the RNA interference in western corn root worm Diabrotica vergifera vergifera [20, 27-30] showed very sensitive results. The oral administration of dsRNA targeting some specific genes of interest showed a significant positive interference with gene function [20]. Based on these positive results United States environment protection agency waved green signal for developing a first RNAi based insecticides to control insect pest. Monsanto and Dowagro sciences has made a proposal for making an RNAi based insecticide which will later be
called as SmartStax pro® a plant incorporated protectant (PIP) pyramiding several Bt proteins, dsRNA targeting western corn rootworm Snf7 gene [31]. Bt proteins in this insecticide may act on gut epithelium leading to gut paralysis and death of insect [32]. Besides down regulation of Snf7 gene leads to protein trafficking which leads to insect’s mortality [33]. So this combined strategy would not only result in immediate mortality of insect but also prevent from resistance development in insects against this PIP. It would only be a question of time before SmartStax Pro® or other yet to be discovered insecticidal strategies will appear in the market with such a great pace in RNAi technology.

6. RNAi studies for controlling agriculturally important insect pests

<table>
<thead>
<tr>
<th>Target organism</th>
<th>Target gene</th>
<th>Function of target gene</th>
<th>Intron</th>
<th>Binary Vector</th>
<th>RNAi plant M</th>
<th>Effect of HI- RNAs on target insect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bemisia tabaci</td>
<td>Acetylcholinesterase (AChE) and edysone receptor (EzR)</td>
<td>1. Required in signaling 2. Role in molting and metamorphosis</td>
<td>--</td>
<td>pJIT163+pCAM bia2300</td>
<td>Tobacco</td>
<td>Silencing of targets gene in chimeric construct produced detrimental effect on survival (approx. 80% mortality) with decrease in target genes transcripts</td>
<td>Malik et al. (2016)</td>
</tr>
<tr>
<td>Helicoverpa armigera</td>
<td>Chitinase</td>
<td>Role in molting and metamorphosis</td>
<td>Chalcone synthase (ChaS) gene intron</td>
<td>pMVR-hp</td>
<td>Tobacco and tomato</td>
<td>Detrimental effects on larval growth and survival, decrease in larval and pupal weight, pupation and adult emergence with significant reduction in target gene transcript level</td>
<td>Mantra et al. (2016)</td>
</tr>
<tr>
<td>Helicoverpa armigera</td>
<td>Arginine kinase</td>
<td>Play role in role in cellular energy metabolism</td>
<td>GUS linker fragment</td>
<td>pANDA35HK</td>
<td>Arabidopsis thaliana</td>
<td>Detrimental effects on larval growth and survival, decrease in target gene transcripts</td>
<td>Liu et al. (2015)</td>
</tr>
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<td>pANDA35HK</td>
<td>Arabidopsis thaliana</td>
<td>Detrimental effects on larval growth and survival, decrease in target gene transcripts</td>
<td>Liu et al. (2015)</td>
</tr>
<tr>
<td>Myzus persicae</td>
<td>hunchback(hb)</td>
<td>Play role in insect axilial patterning</td>
<td></td>
<td>pUCCRNAi</td>
<td>Tobacco</td>
<td>Reduced Mphb mRNA level in the fed aphids and inhibited insect</td>
<td>Mao and Zeng (2014)</td>
</tr>
<tr>
<td>Bemisia tabaci</td>
<td>v-ATPase</td>
<td>Encode for v-ATPase subunit A, role in cellular energy production</td>
<td>Arabidopsis RTM gene</td>
<td>pBH101</td>
<td>Tobacco</td>
<td>Depletion in the transcript level and affected insect survival</td>
<td>Thakur et al. (2014)</td>
</tr>
<tr>
<td>Nilaparvata lugens</td>
<td>0-Hydroxycedysone</td>
<td>Role in molting and metamorphosis</td>
<td>pSK-int vector</td>
<td>pCantG-HA</td>
<td>Rice</td>
<td>Depletion in the transcript level, reduction in fecundity</td>
<td>Yu et al. (2014)</td>
</tr>
</tbody>
</table>

Source: adopted from Mantra and Raimam (2014)

7. Challenges for successful RNAi in insects

Though there has been an enormous utility of RNAi as a promising technique for control of crop pests there is still a long way awaiting to be explored to decide do’s and don’t’s in this technique before establishing it as a long term effective pest control method in the field. There are still many challenges in adopting this potential technique; here we discuss a few of those challenges.

7.1 Digestion of dsRNA

The very first challenge is to protect these ingested dsRNA which triggers the whole process of RNAi. These dsRNA has threat from nucleic acid degrading enzymes in the insect gut which awaits for their degradation, but clearly very less is known about fate of these dsRNA inside insect gut after the ingestion of plant material by insect. For example when polygalacturonase dsRNAs were injected to tarnish plant bug (Lygus lineolus) an immediate down regulation of gene was observed where as when these dsRNA was ingested by feeding approach did not show any response this maybe due to the presence of nucleases in insect saliva and midgut [34-36].
7.2 Chemical hydrolysis of dsRNA

Preventing the breakdown of dsRNA antagonistic environment of insect gut nucleases and pH variation can be done by coating dsRNAs was observed that degradation of dsRNA is quit faster in case of lepidopteran than coleopteran insects this maybe due to differences in haemolymph composition, uptake and processing methods of dsRNA [37-39]. In certain cases a very robust and positive RNAi response was observed even in hostile environmental conditions of insect gut which may be due to additional factors that gives stability to dsRNA in insect gut [40-42].

7.3 Quantity of dsRNA molecules

The optimal dose of dsRNA is influenced by many external and internal factors like insect species, their life stage, method of delivery of dsRNA and also the amount of target gene transcripts in addition, ability of spreading dsRNA molecules also play a major role. For example in D. melanogaster dsRNA can be induced only by haemocytes as the y have haemocytes as the

7.4 Length of dsRNA molecules

Uptake of dsRNA and gene silencing efficacy in organisms usually depends on length of dsRNA which later get diced to produce siRNA molecules [21, 47]. It was reported that longer DNA usually greater than 200 bp produces more siRNA through dicer activity. But reports have shown that even a single chemically synthesized siRNA was also successful in producing silencing effect in H. armigera and tsetsefly [48, 49]. This shows that both long and short dsRNA is effective in producing silencing effect conditioned target gene or pest.

7.5 Life stage of insects

Younger stages of organisms show much greater efficacy in gene silencing due to their small size or less developed body. In an experiment when a gene nitripin-2 was silenced in both second and fourth instar stage with same dose of dsRNA promising gene silencing effects were observed in second instar larva in comparison to fourth instar the difference in physiological and genetic characteristics of insect might be reason for difference in RNAi efficacy [50].

8. Future prospects and Conclusion

RNAi has been a promising technology in controlling crop pest by enhancing resistance traits in plants. Moreover this mechanism do not depend on the plants ability to produce foreign protein that would be toxic to invading pests, which makes this technique much widely acceptable than transgenic approaches, but one cannot solely rely on this particular technique for complete eradication of crop pest but can substantially reduce the application of toxic synthetic pesticides. Effect of RNAi on human and other mammals health is still unfound further additional studies should be determined to test the safety of transgenic dsRNA PIPs which are ought be used in food and feed products, further a potential research is required for understanding the effect of dsRNA on environment and their potential for uptake by target organisms.

However, development of effective delivery mechanisms and creation of transgenic plants producing dsRNA still remains lacunae in adopting such large scale projects. Overtime, the use of transgenic insects will also lead to more efficient pest control. Therefore all the potential risks associated with RNAi technology needs to be evaluated in order to have a successful pest control strategy.

9. References

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