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RNA Interference: An Environment Friendly Approach for Targeted Pest Management

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Editorial

Modern civilization has achieved dramatic expansion in terms of agricultural yield based on mechanical labour intensive approaches and through introduction of chemical based fertilizers and pesticides. According to thermodynamic point of view, these approaches are not energetically favourable as they involve far more energy inputs to power the machinery and provide the synthetic fertilizers and pesticides per unit of every output.

Even after this huge energy investment global need of food security is still below expectation. The major reason behind this under-attained food security comes from altered climatic changes and growing resistance of insects over conventional pesticides. Particularly, in tropics and sub-tropics the climate provides a highly favourable environment for a wide range of insects. Here, massive efforts are required to suppress population densities of the different field and store grain pests to achieve an adequate supply of food. Moreover, there is always an environmental concern from the residual toxicity of these chemical pesticides. Use of bacterial toxins as potential larvicide although found to be effective, is too much arduous, operation intensive and sometimes with unpredictable control efficiency due to stability issues. Moreover, there are numerous reports from around the globe on widespread resistance in several insects to common insecticides including synthetic pyrethroids, organophosphates, carbamates, chlorinated hydrocarbons, *Bacillus thuringiensis*, botanicals and fumigants. Prolonged exposure to chemical insecticides is reported to cause neuronal and hormonal disorders and may also lead to environmental contamination. Therefore, there is an urgent need to develop alternative strategies for more scientific and targeted management of the agriculture sector, especially in the use of pesticides.

Gene silencing using RNA interference (RNAi) is an emerging technology which can be a biologically safe alternative to control insect pests. The technology relies on controlling gene expression and regulation of protein function through small interference RNA (siRNA) tools. Briefly the mechanism of RNAi starts with the presence of exogenous dsRNA into a cell which in presence of a cellular dicer enzyme are processed into siRNA's of 18-21bp in length. These siRNA's are ultimately channelled to the RNA-induced silencing complex (RISC). RISC machinery consists of a huge number of proteins which facilitates complementary binding of siRNA's to polynucleotide strands of mRNA. This particular event leads to blocking of one or more steps of central dogma, eventual outcome of which is either reduction (knockdown) or complete inhibition (knockout) of protein production of the target gene.

Starting from the emergence of RNAi technology, it has been applied broadly for regulating heterologous and endogenous gene expression related to disease control. Due to massive importance of the technology in the field of biological research and drug development, RNAi was awarded the Nobel Prize for medicine in 2006. Since then the technology is being applied in many other directions. siRNA mediated regulation of gene expression is a very safe regulatory tool that operates at post-transcriptional level minimizing the chance of mutation and terratogenic risk. It also has the efficiency to be very target specific due to self restricted choice of target ensured by complementary base pairing. With the advent of knowledge coming from genomic databases of almost all higher organisms, the technology is flourishing all the way to its heights gradually.

According to the literature survey, most of the applications of RNAi technology in agriculture sector are concentrating upon studying high throughput structural and functiontional genomics studies to determine the function of each gene in the insect and plant genome. Based on these studies transgenic plants containing dsRNA of targeted insect pest is being developed which exhibits reduced and affected survival of them on transgenic plants. RNAi studies with fruit flies, *Drosophila melanogaster,* revealed functional aspects of genes that encode proteins related to growth, reproduction, development, behaviour; encompassing almost all the aspects of insect's life. Vigorous optimization of RNAi target for each and every gene in case of vertebrates is not necessary for studying insect genome which further reduces the complexity in understanding and developing basic research related to insect health. This information leads towards the potential role of RNAi technology in pest management. Although the technology is on its immerging phase, but showing a very good potential as an environmentally safe alternative to bypass harmful effect of chemical pesticides. Based on this, siRNA mediated genetic knockdown strategy is recently being applied in a number of insects like *Tribolium castaneum* [1], *Phyllotreta striolata* [2], *Nilaparvata lugens* [3], *Schistocerca gregaria* [4], *Helicoverpa armigera* [5], *Apis mellifera* [6] and *Diabrotica virgifera virgifera* [7] to identify potential target genes related to growth, development, reproduction for target specific pest management.

Three main strategies have been applied so far in entomological research for gene silencing. Microinjection is the most popular method used for dsRNA delivery in insects. But the limitation of this approach comes from its delicate, laborious, time consuming technique and also the dependence on certain optimization like needle choice, place of injection and volume of material - all these factors are critical in this aspect. As for example, volume of injection becomes critical for

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Acyrthosiphon pisum aphids, as it is directly related to their survival [8] As insect vector control or pest control in agricultural field and large water bodies' deal with a huge number of insect pests, the dsRNA delivery through microinjection is not a feasible method for pest control at field level. Delivery of dsRNA through soaking and transfection is another method of choice to control insects mainly because of its convenient operation. Soaking of nematodes (*Caenorhabditis elegans*) in the dsRNA solution was used for large-scale analysis of gene function to accomplish high-throughput RNAi technology [9]. But transfection-mediated gene silencing found to be more efficient RNAi response compared to simply soaking, most likely because of more efficient introduction of dsRNA into the cell. The major disadvantage of this mechanism is that this method is effective only to cell line systems and application of this technique in field level is limited. Development of dsRNA expressing plants which will target insect pest genes is also another approach for pest control [10]. But till now people are reluctant to widely accept genetically modified food crops. Oral delivery of dsRNA is most widely accepted method and so broadly being used in various insects such as *Spodoptera exigua*, *Diabrotica virgifera virgifera*, and *Epiphyas postvittana* [10,11]. The process is labour-saving, cost-effective and easy to perform [10]. Furthermore, it is highly suitable for high-throughput gene screening, especially genes for pest control. As dsRNA is delivered by mixing with food or as solution droplets in oral delivery, it is less invasive and also is a more practical delivery method for small insects such as aphids and first- and second-instar larvae or nymphs and mosquito larvae. But this method also suffers from limitations of its poor delivery and requirement of higher dose for successful inhibition.

Though from the environment point of view RNAi mediated insect management is very much eco friendly due to target specificity of the technology, RNAi in this field has not become much successful because of some major challenges. One of the most important amongst those is instability of siRNA under physiological condition. Net negative charge, hydrophilicity, size and sensitivity to nuclease mediated degradation are the major factors related to siRNA delivery and successful RNAi to control insect pest. Most of the laboratory studies related to RNAi in insects relies on dsRNA delivery through systemic injection, which restricts their practical application. Even after administration, nuclease enzymes in hemolymph are major threat to these foreign entries. These highly anionic double stranded nucleic acid entities cannot cross biological membranes which induces very low cellular uptake to generate sufficient RNAi. It also activates innate immune response which reduces the efficiency further by phagocytosis mediated degradation processes. Highly acidic physiological environment within lysosome imposes further challenge of possible degradation by nucleases. Endosomal escape of these tiny nucleic acid machineries for successful cellular uptake and target specific mRNA hybridization is also a rate limiting factor for consideration.

More strategic approach is further needed for application of RNAi technology in insect pest management which can ensure better stability in physiological condition, evade immune response, provide better cellular intake, escape endosomal degradation, resist renal clearance and ultimately establishes delivery of sufficient amount of dsRNA to generate RNAi that can retard the growth and survival of deadly insect pests in a cost-effective and environment safe approach. Chemical modification of siRNA to ensure better stability is most commonly adopted approach for addressing the issue. Modification at 2' position of the ribose ring of siRNA makes them stabilized enough to resist nuclease degradation. Similar to these some other strategies, such as replacement of the phosphodiester (PO_4) group with phosphothioate at the 3' end of RNA backbone, or the introduction of novel conjugation of 40-thiolation with 20-O-alkyl modification are also being employed nowadays. But still siRNA stability and delivery needs to be protected further by encapsulating them in a non-toxic carrier material such as, lipid-based nanovectors, cataionic polymer-mediated delivery system, cationic nanocarriers etc [12].

Although RNAi in agriculture sector is still in very nascent stage, it has undoubtedly bright future potential as this technology can easily be advantageous for long run, limiting the chances of insect resistance. There is always an option to switchover to different target genes. This is definitely an advantageous approach over chemical pesticides that are generally prone to insect resistance over time.

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