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Review

Role of RNA-interference in Crop Pests and Disease Vector Control

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Abstract

Insect pests are a threat to meeting food demands of the ever-increasing human population. They are also the cause of many vector borne diseases in humans leading to countless deaths. Present insect pest control strategies including chemical pesticides, developing transgenic plants and organic certified chemical pesticides have numerous limitations in terms of their effectiveness and target specificity. However, genetic method that makes use of the sequence specificity of RNA interference (RNAi) has great potential in controlling pest insect populations. RNAi is a naturally occurring conserved process responsible for protection against viral pathogens. Efficiency of RNAi is variable among different pest insects. It is dependent on method of double stranded RNA (dsRNA) delivery, gene selection techniques, dsRNA expression and presence of off-target effects. Moreover, environmental risks involved in use of RNAi based insecticides in natural crop field scenario is debatable. Despite the challenges faced, RNAi mediated gene knockout of different pest insect genes has potential usefulness in controlling pest insect growth and survival.

1 Introduction & Background

Climate change is a universal phenomenon that is impacting all the organisms on this planet. Insect population growth is linked to increases in temperature due to global warming[1], leading to increases in the frequency and intensity of periodic insect outbreaks[2]. The most worrisome increase in insect population outbreaks is of the disease vectors and pest insect populations as they cause human suffering and destruction of crops. Every year more than 1.5 million human lives are lost to vector borne diseases[3], when the easiest method of prevention of such diseases is elimination or population control of vectors and pest insects.

Insect population control is key to preventing the spread of vector borne diseases like malaria, dengue, yellow fever, chikungunya and lymphatic filariasis that are targeted to be eradicated globally in the near future[4, 5]. To control insect populations, more efficient, target specific and cost-effective insecticides and delivery methods must be explored[6]. Presently, narrow and broad-spectrum insecticides both are heavily reliant on the use of chemical insecticides.

Two basic types of insecticides, narrow and broad-spectrum include wide varieties of chemical insecticides that act through inhibition of enzyme activities in pests[7]. Chemical insecticides are delivered mostly by aerial spraying, however very small percentage (0.003-0.0000001%) of insecticide actually reaches the target crop pests. Spraying, although instantly effective, has many side effects including high dosage administration and off-target effects like death of pollinators[8]. To deal with these side effects, genetic methods are being incorporated into insecticide development and delivery[9]. Genetic methods incorporate wide variety of techniques including chromosomal replacement, translocation formation, sterile insect technique and gene knockout using RNA-interference (RNAi)[10, 9, 11]. RNAi has been used for gene silencing to produce sterile insect males[10]. Gene silencing via RNAi is achieved by administering dsRNA (Table 1) using various introduction techniques to specific cells or the whole organism[10]. It would be pertinent to discuss various issues related to the conventional methods of pest insects and disease vector eradication vis-à-vis genetic method of RNAi. The objective of this literature review is to compare and contrast the effectiveness of last generation chemical insecticides with future generation insecticides using modern genetic technologies such as RNAi, to control crops and disease vector insect populations.

2 Review & Discussion

2.1 Techniques in crop pest control

Some of the earliest attempts at population control of crop pests included biological control by prey species such as use of bats as predator of moths in pecan orchards[12]. Biological control method was introduced as an alternative to chemical pesticides since chemical pesticides had negative impact in terms of biomagnification and off-target effects as was observed in honey bees with use of imidacloprid, a broad spectrum insecticide[13, 6]. Even though the biological method was potentially non-harmful with no off-target effects, researchers faced the difficulty of controlling prey-predator interactions as sometimes the presence of large herds of bats over the pecan orchard was enough to deter the prey moths[6]. The strong interaction between the populations of predator and prey limited this method, as when prey population declined so did the predator population because of resource separation[14, 15]. Additionally, the interspecific competition between introduced predator species and the native predator species for the same prey lead to

population imbalance between the two[16]. It appears that the biological method is limiting in delivering the desired results by not being appropriately controllable and is also not very cost effective.

In addition to biological methods, efforts were also made in development of organic certified pesticides to minimize harm to other off-target species and humans as consumers of crops. Although these organically certified pesticides were safer and much preferred over traditional synthetic pesticides because of absence of effects on non-targeted species, these new pesticides also had issues. The residual persistence of organic certified pesticides was higher than synthetic pesticides despite having low toxicity under laboratory conditions[17, 18, 19]. Thus, organic certified pesticides need further complementary bioassay treatment before being used in integrated pest management (IPM) programs dealing with wide variety of crops[18]. Therefore, despite seeming promising, organic certified pesticides were not found to be of significant advantage over the synthetic pesticides[18, 19].

Emergence of transgenic plant technology led to development of bio-insecticides using Bt-toxin (*Bacillus thurigiensis*) (Table 1), which showed promise in controlling plant as well as disease vector insect populations[20]. The development of transgenic crops that produce insecticidal Bt-toxin against specific insect pests lead to death of target pests upon ingestion of the plant[21]. This technique has been proposed to be useful for mosquito control as it can be administered via feeding through water to the targeted mosquito larvae species[22]. However, Bt-toxin administration in water bodies may impact other aquatic organisms that share the same water body with the target mosquito species[22].

Hence further investigation of off-target effects is required in administration of Bt-toxin via feeding through water. Another common method of administration of Bt-toxin is through the development of transgenic crops that selectively express Bt-toxin in their non-edible parts. The selective expression prevents pest ingestion as observed in crops like soybean, corn and cotton that are affected by defoliating pests including cotton bollworm[23, 24, 21]. Transgenic soybean plants specifically express Bt-toxin in their leaves and the pests are killed by gut perforation[23]. Transgenic plants produced by this method show Bt-toxicity trait retention and generational transfer from the parent plant to offspring plant making bio-insecticides commercially viable[13]. However, evolution of Bt-resistance in pest insects is an issue that has been observed by several different studies[13, 20, 24, 21].

2.2 RNAi in Agriculture Pest Control

Existing pest control strategies have limitations as discussed above. The techniques are not very successful in limiting pest insect populations. Sequence specific gene silencing via RNAi holds great promise for effective agricultural pest management[25]. RNAi mediated gene silencing of genes involved in various key physiological processes of pest insect was found to be detrimental to the growth, development into fertile adult and overall survival of the plant[26]. RNAi has an edge over bio-insecticide technology because of its highly conservative nature. For its activation and function, sequence specific nucleotide base pairing complementarity is required[27]. This implies that there will be minimum off-target effects. Hence, plant mediated RNAi is a powerful weapon in the fight against agriculture insect pests.

One example of efficiency of dsRNA in controlling plant pests is the use of RNAi in development of bollworm resistant cotton plants. When RNAi specific to critical bollworm genes was introduced, cotton yields increased significantly[28, 29]. Cotton plants expressing dsRNA of a reductase gene HMGR (Table 1) showed regression in the growth and development of cotton bollworms, however, it was unclear if expression of dsHMGR affected the number and time of bollworm pupation since most of the tested larvae died before pupation[27]. Therefore, further analysis of role of dsHMGR expression in cotton bollworm is required to explore the pest insect developmental stage that is targeted by RNAi of HMGR gene in transgenic cotton plants.

Efficiency of RNAi is variable among different pest insects. It is dependent on many factors, including the method of double stranded RNA (dsRNA) delivery, the dose that the insect acquires, the choice of the gene target (as not all gene knockdowns will have lethal effects), and the barriers within the insects, such as gut nucleases or reduced uptake mechanisms[30, 31]. One method that shows some promise in terms of increasing efficiency is the use of microorganisms to not only produce the dsRNA, but to deliver it to the feeding insects. Transgenic bacteria[32] and yeast[33] have been produced that express dsRNAs targeting mosquito gene transcripts, and as larval mosquitoes will readily consume these microorganisms in their diet, effective RNAi has been achieved in these insects. It is not yet clear if the microorganism-mediated delivery system is providing protection of the dsRNA from gut nucleases, or if it is providing higher doses of dsRNA to the gut cells, through different uptake mechanisms[34]. Nevertheless, this delivery system is worth further exploration for mosquito control applications.

Off-target effects observed in pest insecticides are undesirable have major concern. To address this issue, dsRNAs was designed to be delivered orally using sequence specificity of RNAi to selectively kill target species. The study also looked at a wide variety of pests including fruit flies *Drosophila melanogaster*, beetles *Tribolium castaneum*, aphids *Acyrthosiphon pisum* and hornworms *Manduca sexta*. These pest insects were selectively killed when fed with species specific dsRNA targeting vATPase transcripts[35].

Moreover, the study also demonstrated the selection ability of RNAi by use of two closely related fruit flies which were both given dsRNA specific for □-tubulin gene. Only those fruit flies that possessed the gene were affected in terms of their development whereas the others showed normal growth and became normal adults[35]. These results show that RNAi is not only specific in attack but can also be used in wide variety of insects.

2.3 Risk Management of RNAi

Environmental risk assessment (ERA, Table 1) involves producing an analysis plan describing relevant exposure scenarios and their potential consequences[36]. It is important to consider risk assessment before field release of any transgenic organisms. For RNAi, many aspects of ERA are similar to those of other genetically modified crops and pesticides[37]. However, difference in

mode of action of dsRNAs make RNAi and other insecticidal technologies very different.

For wide range of species subjected to RNAi, dsRNAs have off-target binding elsewhere in a nontarget species' genome making prediction of toxic effects and designing maximum-hazard dose assays challenging[37]. There is also an issue of safety concerning open field use of RNAi. ERA of RNAi is a necessary step towards widespread its practical utilization in plant pest control. Crop plants are host and food source of herbivorous insects, have tons of biomass, can accumulate a large amount of dsRNAs to provoke the RNAi response and dsRNAs can be continuously produced under varying environmental conditions[38]. Therefore, looking into the importance of crop plants, the challenge of unpredictable ERA of RNAi should not be discouraging in investing further into different ways of RNAi expression in plants.

2.4 RNAi in medicinal disease vector control

Controlling medicinally important disease vector pests using RNAi has not attracted many proponents due to the unique challenges of delivering dsRNA to these pests. As a result, chemical pesticides are still used extensively to control disease vector pests causing large scale harm to pollinator species through the common use of aerial spraying as the delivery method[18, 39].

The basic components of the RNAi machinery are found in all eukaryotes to protect them from the potentially harmful long viral dsRNA[37]. Therefore, the use of RNAi is widely exploited as a reverse genetics tool to assess gene functions in a broad range of species. Application of RNAi in most species under study is only limited by how easily the dsRNA can be delivered to the target cells[39]. The importance of any delivery method of RNAi is in reducing the loss of model insects during the delivery procedure to create loss of function mutants. Ideal pest control methods have some, if not all, of the following characteristics: species specificity, absence of side effects on crops and no or negligible environmental pollution[38]. There are different types of RNAi related delivery methods that show such potential.

Chitosan is an inexpensive, non-toxic and biodegradable polymer. It was found that chitosan-interfering RNA nanoparticles derived from chitosan, when introduced in Aedes aegypti via feeding, were successful in gene knockout[40]. These findings are of great importance as chitosan has potential for use in natural crop fields.

Microinjections are common mode of delivery of dsRNA to whole organisms like adult mosquito larvae[22]. There are two types of micro injections widely used for delivery of dsRNA, including the more common haemocoel injections and the direct injections. Direct injections are preferred over haemocoel injections as they can be administered during any stage of insect development, whereas the use of haemocoel injections is a laborious delivery technique and many insects die during the procedure[39]. Overall, currently both methods are in use depending upon the chosen model species under study.

Ever since the discovery of resistance of dsRNA to gut exonucleases, feeding has become the most favoured form of delivery as it is non-invasive and causes no physical harm to the model insect. dsRNA containing bacteria fed Caenorhabditis elegans had RNAi interference in broad region of their body. It was due to the spreading effect involving inhibition of several genes in bacterial-mediated delivery of dsRNA via feeding. Spreading effect could be due to differences in the susceptibility of some cells or developmental stages to the consequences of ingested dsRNA. Thus, bacterial-mediated delivery of dsRNA via feeding was less effective as RNAi method than direct microinjection[26, 41]. Although bacterial-mediated delivery of dsRNA via feeding method is an example of RNA-mediated transfer of information between organisms and between species, however, it is not yet known whether such RNA-mediated interference-transfer mechanisms participate in natural ecological interactions, such as antiviral defence or communication during biological symbiosis[26]. Soaking is another delivery method used to introduce dsRNA to create sterile males in various mosquito species. This sterile insect technique uses aerial release of sterile males to reduce wild local populations[42, 5]. In one study, spermatogenesis-specific dsRNAs were administered to mosquito larvae by soaking larvae in dsRNA solutions. At higher concentration, dsRNA sufficiently induced sterility in most (72-92%) of the males[22]. However, lower concentrations were found to be inefficient in inducing sterility in males (only 20-35%). Advantageously, upon mixing of low concentrations of different dsRNAs together, sterility frequencies were near 100%[22]. This experiment is an example of the efficiency of the soaking method. Furthermore, in these dsRNA soaking treatments, the dsRNA entered the insects by ingestion, although entry through other routes (e.g. cuticular penetration) could not be excluded. Nevertheless, RNAi clearly spread beyond the initial entry points to reach the testes. Soaking technique for making sterile insects is relatively simple compared to other approaches of transferring dsRNA[10, 22].

3 Conclusion

Looking at the studies performed to improve insecticide delivery and their pros and cons, it is evident that RNAi has great potential for control of insect pest populations. However, all studies involving RNAi were restricted to laboratory conditions only. No references were found pertaining to RNAi expression under open field conditions because of lack of knowledge about the gene sequence of other off-target and economically viable species. In addition, the coupling of other technologies, like Bt-toxin, with RNAi could expand the possibilities of further improvement in pest control and research in the field of molecular biology. It might even provide a solution to the existing problem of Bt resistant pests. To further improve and establish RNAi technology, large scale field tests need to be conducted along with evaluations of the potential risks of this technology. To confirm no off-target effects and better risk assessment, further studies need to be done in closely related members of the same species by administering RNAi specific for certain desired genes present only in one of the members of the species and comparing it with the control. Further, as information on off-target effects of RNAi is limited, future study will focus on prevalence of off-target effects in two closely related disease vector insect.

Despite all the apprehensions, RNAi is still a promising technology of the future as dsRNAs can be chosen from a vast number of potential target genes. In addition, owing to the advancement in technology, identification of more crucial genes involved in the growth and development of various insects can be used as target genes in RNAi based pest resistance in the future.

Table 1: *Full description of scientific terms and terminology used in the literature.*

Terms	Full Form
RNA	Ribonucleic acid
RNAi	RNA-interference
dsRNA	Double stranded RNA
IPM	Integrated Pest Management
SIT	Sterile Insect Technique
Bt-toxin	Bacillus thuriegenis toxin
WHO	World Health Organization
HMGR	3-Hydroxy-3-methylglutaryl reductase
vATPase	Vacuolar Adenosine Triphophatase
ERA	Environment Risk assessment
PF	Problem Formulation
MCL	Mantle Cell Lymphoma
TLRs	Toll Like Receptors

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