SEMINAR REPORT

Gene silencing- An effective tool in insect pest management

By

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COLLEGE OF HORTICULTURE

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CERTIFICATE

This is to certify that the seminar report entitled **"Gene silencing- An effective tool in insect pest management"** has been solely prepared By Sachin G Pai(2018-11-058) under my guidance and has not been copied from seminar reports of any seniors, juniors or fellow students.

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1.Introduction

Agriculture faces many challenges and among them management insect pest is a major challenge. Insect pest management strategies have evolved over a period of time from the insecticidal era in the early 1930's, biorational pest management during 1970's and genetic manipulation at the end of 20th century and more recently by introduction of gene silencing through RNAi (Fire *et al* 1998).

RNAi is a natural process present in eukaryotic cells for gene regulation and antiviral defense. Although, from a crop protection perspective, RNAi refers to double-stranded RNA (dsRNA) mediated gene silencing that involves the blocking of the expression of specific target genes by destroying the corresponding mRNA molecules affecting only the translation process. Due to its sequence-dependent mode of action, the RNAi technology, as referred nowadays by industry, has a vast range of potential crop protection application, including genetic studies and pest control research in insects (Gordon and Waterhouse 2007, Price and Gatehouse 2008). These RNAi practical applications have been pursuing over the last decade for the development of novel crop protection methods.

Through the discovery of RNAi and its regulatory actions, it has become evident that RNAi has immense potential in opening a new vista for crop protection. Delivery of dsRNA to a target organism is the easiest through transformative RNAi approach *i.e.*, by transgenic plants (Baum and Roberts 2014, Mao and Zeng 2014), but it is not practical to every target and crop since there is lot of procedures for commercial cultivation of GM crops. Therefore, the development of non transformative approach (*i.e.*, sprayable dsRNA) (Hunter *et al.*, 2012, Joga *et al.*,2016) for RNAi delivery will boost up its use in the field.

2. Gene silencing

2.1 Definition:

"Gene silencing is the regulation of gene expression in a cell to prevent the expression of a certain gene" (Redberry, 2006)

2.2 History:

History of gene silencing dates back to the early 1990s, when the Jorgson *et al.*, attempted to manipulate gene expression in petunias for developing purple petunias by overexpression of *chalcone synthase* gene resulted in unexpected gene silencing. Guo and Kemphues, 1995 later found out that injection of antisense and sense RNA into the germ layer of *C. elegans* could cause gene silencing of complementary mRNA. Mello and Fire, 1998 injected both antisense and sense RNA into the *Caenorhabditis elegans* and it was found to effectively silence the target gene expression.

2.3 Types of gene silencing

There are mainly two types of gene silencing based upon the stage of occurrence *ie* transcriptional and post transcriptional gene silencing

2.3.1 Transcriptional gene silencing

This is a type of gene silencing which takes place inside the nucleus wherein, the gene targeting for a specific protein gets heterochromatinized, hence making the gene inaccesible for transcription.

2.3.2 Post transcriptional gene silencing

The ability of exogenous or endogenous RNA to suppress the gene expression by degrading the complimentary m-RNA sequence. There are two types of post transcriptional gene silencing which are Anti sense RNA technology, RNA interference.

3. Post transcriptional gene silencing

3.1 Anti sense RNA technology :

Antisense RNA has the opposite sense or complimentary sequence to m-RNA. The presence of complimentary sense and antisense RNA in the same cell can lead to the formation of a stable duplex which interferes with gene expression at the level of RNA processing or possible translation. This can suppress gene expression.

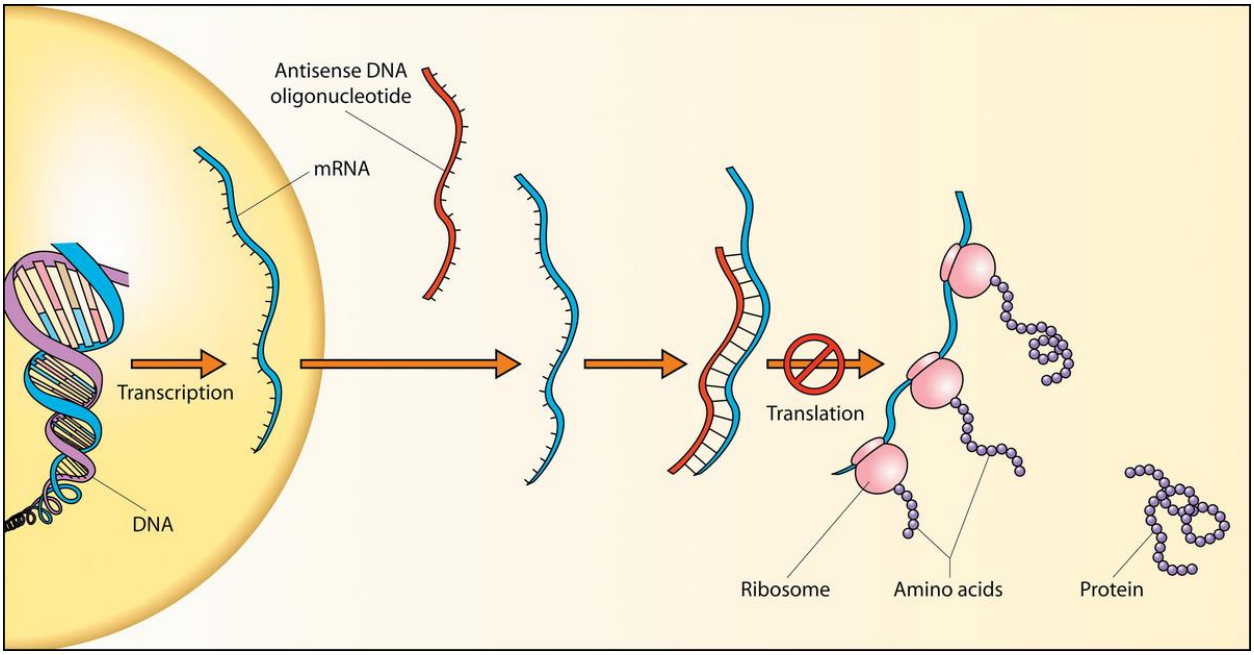

Plate 1. Mechanism of antisense RNA technology

3.2 RNA interference : RNA interference or Post-Transcriptional Gene Silencing is a conserved biological response to double-stranded RNA that mediates resistance to both endogenous parasitic and exogenous pathogenic nucleic acids, and regulates the expression of protein-coding genes. This natural mechanism for sequence-specific gene silencing promises to revolutionize experimental biology and may have important practical applications in functional genomics, therapeutic intervention, agriculture and other areas.

3.2.1 Components of RNAi :

Dicer: It is an enzyme and is named as RNAse III-like dsRNA-specific ribonuclease. It is able to digest dsRNA into uniformly sized small interfering RNAs (si RNA). They belong to the family of proteins known as ATP - dependent nucleases.

RNA-Induced Silencing Complex (RISC) : Nuclease complex composed of proteins and siRNA. Targets and destroys endogenous mRNAs complementary to the siRNA. It consists of both protein and RNA. Preferentially incorporates one strand of unwound RNA. Argonaute protein present will help to cleave the target mRNA.

siRNA- Short Interference RNA : It is 21-23 nucleotide dsRNA that mediate gene silencing. It is produced *in vivo* by cleavage of dsRNA and gets incorporated into the RISC guiding it to mRNA. Each strand of Si RNA has a 5'-phosphate terminal and a 3'-hydroxyl terminal and two or three nucleotide overhangs at 3' end.

3.2.2 Mechanism of RNAi .

RNA interference (RNAi) is a gene silencing mechanism at the cellular level triggered by double-stranded RNA (dsRNA).

- dsRNA are chopped into short interfering RNAs (siRNA) by the action of Dicer.
- The siRNA-Dicer complex recruits additional components to form an RNA Induced Silencing Complex (RISC). The siRNA unwinds by the action of Helicase.
- The unwound siRNA base pairs with complementary mRNA, thus guiding the RNAi machinery to the target mRNA.
- The target mRNA is effectively cleaved and subsequently degraded–resulting in hampering of gene expression

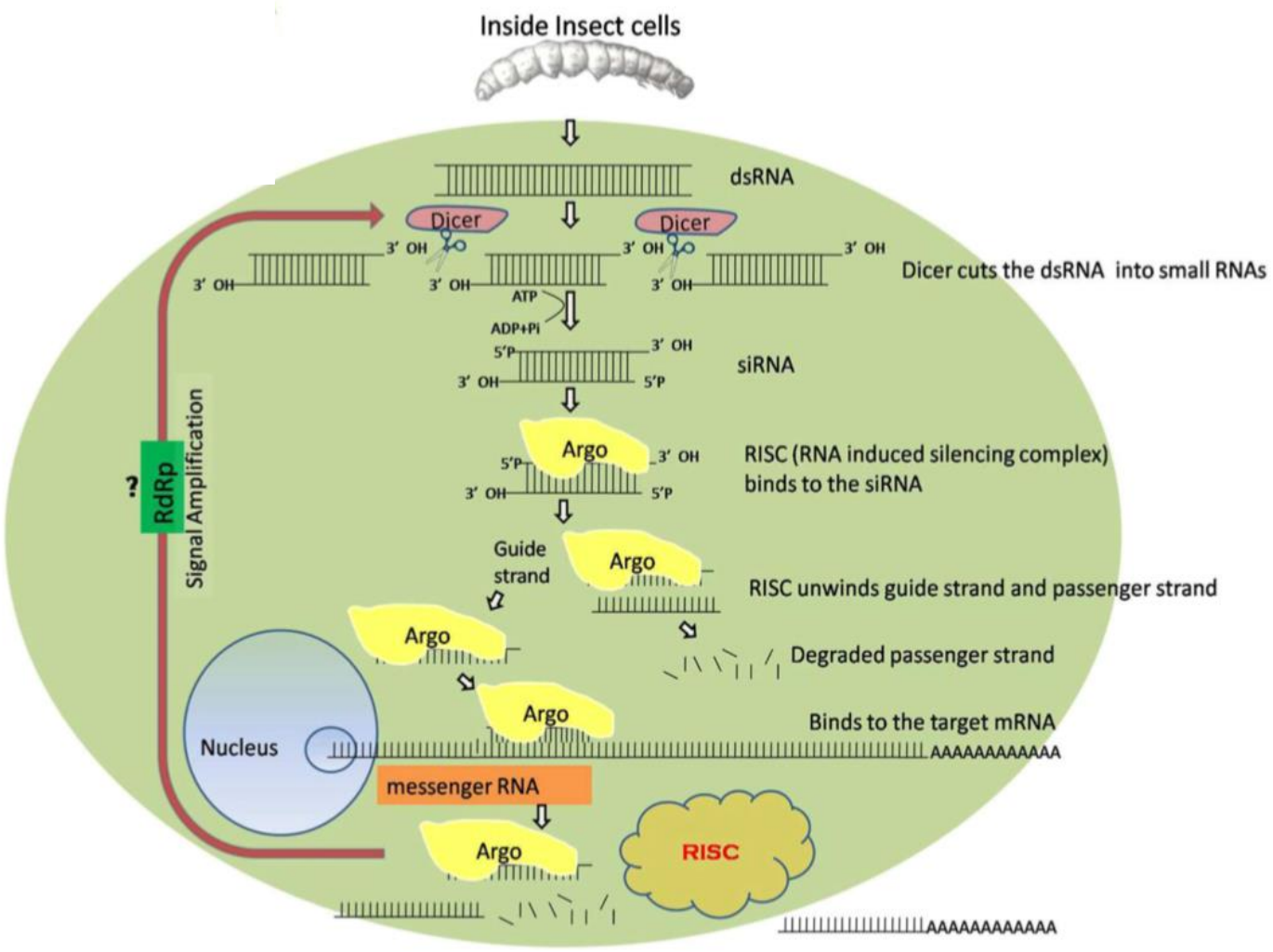

Plate 2 : Molecular mechanism of gene silencing

4. RNAi in pest management :

Case 1 : Baum *et al*., 2007 gave the first experimental proof that RNAi can be successfully used in management of the pest, western corn rootworm (WCR) *Diabrotica virgifera virgifera* LeConte. First successful - oral delivery of dsRNA for pest management. To assess coleopteran sensitivity to ingested dsRNAs, candidate target genes from several WCR cDNA libraries were selected and dsRNAs prepared for testing in a WCR feeding assay. Genes encoding proteins with essential functions would be the best RNAi targets for causing lethality. Bioassays were conducted by applying dsRNA on to the agar based diet resulted in both larval stunting and mortality. Subsequent feeding assays demonstrated that certain dsRNA samples, including

dsRNAs targeting putative genes encoding vacuolar *ATPase (V-ATPase)* subunit A, D and E, as well as a-tubulin, were active at applied concentrations. To confirm that, ingestion of dsRNA triggered a specific RNAi response in WCR larvae, and they have analyzed total RNA from dsRNA-fed and untreated larvae on northern blots to demonstrate downregulation of specific target genes. WCR larvae that ingested dsRNA targetted against the *V-ATPase A* exhibited a dramatic suppression of the mRNA within 24 h of ingestion. Maize plants were transformed to express dsRNA *V - ATPase* and significant mortality was observed during the pot culture experiments suggesting that the RNAi pathway can be exploited to control insect pests *via* in planta expression of a dsRNA.

Case 2 : Cotton bollworm (*Helicoverpa armigera*) is a major pest of cotton and various control measures including *Bt* cotton was developed to manage the pest. Mao *et al.*, 2007 conducted a study which exploits the basic understanding of ill effects of plant secondary metabolites against the pest. Cotton plant produces gossypol, a secondary metabolite which provides resistance against the pests. It was identified that *cytochrome P450* gene is responsible for detoxification of gossypol. So in this study the gene coding for *cytochrome P450 (CYP6AE14)* was identified and dsRNA constructs were fed to larvae of the bollworm using artificial diet and was observed to cause significant mortality. Later in 2010 Mao *et al.,* did transformation studies in cotton plants to express *CYP6AE14* gene and the transgenics were resistant to the bollworm attack.

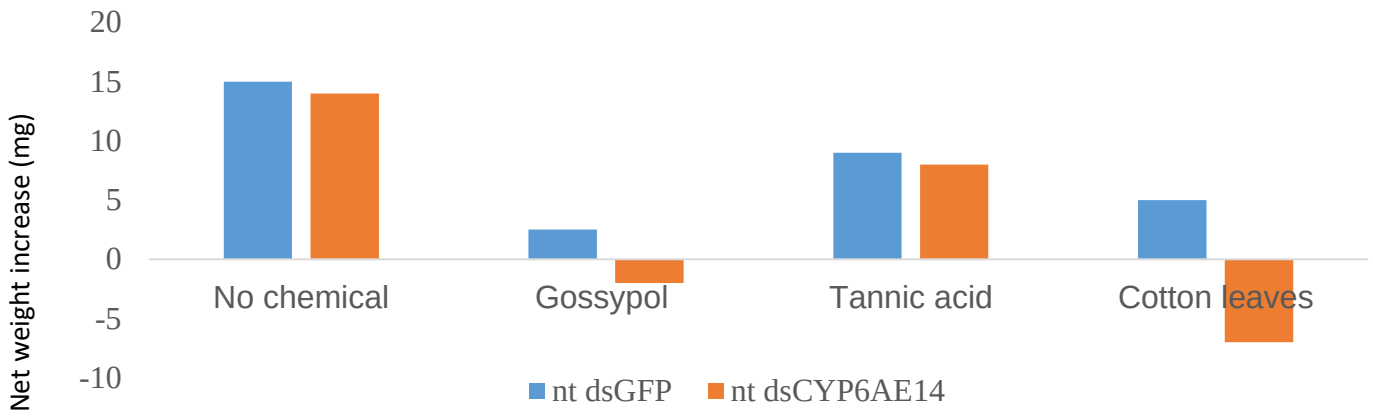

Fig .1. Enhanced effect of gossypol on larval growth fed on dsRNA leaves

5. Target gene identification:

Genes encoding for key enzymes or proteins - great potential.

Insects	Target genes
Coleoptera ***Diabrotica virgifera*** ***Leptinotarsa decemlineata***	***Vacuolar ATPase subunit A & E, Tubulin, Arginine kinase***
Hemiptera ***Acyrthosiphon pisum*** ***Rhodnius prolixus***	***Water specific aquaporin*** ***Nitroporin 2***
Lepidoptera ***Helicoverpa armigera*** ***Plutella xylostella***	***Pheromone binding protein*** ***Cytochrome p450 monoxygenase***

Table 1. Candidate genes for RNAi

6. dsRNA delivery :

The delivery of dsRNA is one of the most important step in the application of the RNAi in pest management strategy. The following methods were used for the delivery of dsRNA - Host induced gene silencing (HIGS), Spray induced gene silencing (SIGS), Virus induced gene silencing (VIGS), Vegetable mediated gene silencing.

6.1 Host induced gene silencing (HIGS) :

Western corn rootworm (WCR) has evolved field resistance to different control methods including resistance to *Bt, Cry3Bb1*gene. Gene pyramiding to express multiple CRW traits can delay resistance development compared to single trait products. **Monsantos' SmartStax PRO** (expressing *Cry3Bb1, Cry34Ab1/Cry35Ab1* and *DvSnf7*) has been proved effective and has been approved for commercial cultivation in Canada, USA, Brazil (Head *et al*., 2017),

6.2 Spray induced gene silencing :

This method of gene silencing does not involve transformation of the host, here we spray dsRNA on the plants after formulating it. The different methods for delivery of dsRNA are as follows : Bacteria mediated gene silencing, Symbiont mediated gene silencing and Guanylated polymers.

6.2.1 Bacteria mediated gene silencing :

Zhang *et al*., 2019 developed a cost-effective production and stable delivery of double-stranded RNA (dsRNA) to the target insects *via* transformed bacteria expressing the target genes. Six potential genes were targeted, including actin (*ACT*), signal recognition particle protein 54k (*SRP54*), heat shock protein 70 (*HSC70*), shibire (*SHI*), cactus (*CACT*), and soluble N-ethylmaleimide-sensitive fusion attachment proteins (*SNAP*), Cloning and transforming of *Escherichia coli* HT115 was done *in vitro* and the resultant clones were cultured in Luria Bertani media overnight, the culture was centrifuged to obtain the bacterial pellet and was was suspended in distilled sterile water to get a bacterial suspension. For conducting bioassays these bacterial suspensions were painted on to the leaf of the Salix plant and it was found that feeding bacteria-expressed dsRNA successfully triggered the silencing of the five target genes tested and the suppression of *ACT* and *SRP54* genes caused significant mortality. Results suggest that the oral delivery of bacteria-expressed dsRNA is a potential alternative for the control of *P. versicolora*, and that *ACT* and *SRP54* genes are the potent targets.

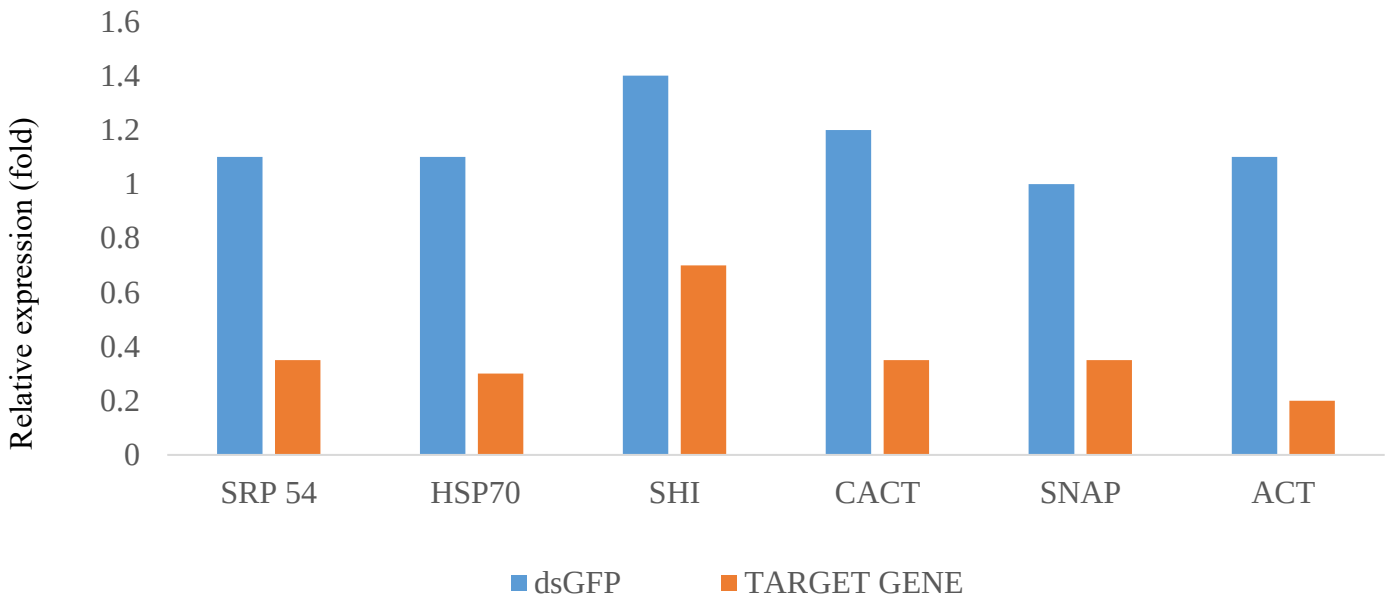

Fig. 2. Relative gene expression in *Plagiodera versicolora* larvae

6.2.2 Symbiont mediated gene silencing

Whitten *et al*., 2016 studied the possibility of use of insect symbiotic bacteria for: (i) constitutive dsRNA synthesis and (ii) trauma-free delivery. RNaseIII-deficient, dsRNA-expressing bacterial strains were created from the symbionts of Western flower thrips (*Frankliniella occidentalis*). *BFo2* a gram-negative facultative symbiotic bacterial species from *F. occidentalis.* An RNaseIII-deficient mutant, BFo2α, was obtained into which plasmids, directing expression of dsRNA, were introduced. To demonstrate symbiont-mediated gene silencing, the targeted genes was *alpha-tubulin* (*Tub*), an essential gene for survival of the insect. Stable bacterial expression of ds*Tub* and the off-target control ds*dagA*, was confirmed by qRT-PCR prior to introducing the bacteria into insects of different ages *via* an artificial sucrose-based feeding solution. After 4 days, a highly significant mortality phenotype was observed among larvae exposed to ds*Tub*, particularly in the first (L1) larval stage. This suggests that when ingested, the manipulated bacteria colonized the insects, successfully competed with the wild-type microflora, and sustainably mediated systemic knockdown phenotypes that were horizontally transmissible

6.2.3 Guanylated polymers:

Lepidopteran insects, which are characterized by a very alkaline gut environment (pH > 9.0) and a strong intestinal nucleolytic activity causes failure of orally delivered RNAi. So Christiaens *et al.*, 2018 conducted a study in which guanidine-containing polymers were developed to protect dsRNA against nucleolytic degradation, specifically in high pH environments. Polymers with high guanidine content provided a strong protection against nucleolytic degradation at pH 11, protecting the dsRNA for up to 30 h. Next, cellular uptake of the dsRNA and the polyplexes in lepidopteran CF203 midgut cells was investigated by confocal microscopy, showing that the polymer also enhanced cellular uptake of the dsRNA. in vivo feeding RNAi bioassays demonstrated that using these guanidine-containing polymer nanoparticles led to an increased RNAi efficiency in *S. exigua*. Targeting the essential gene chitin synthase B, they have observed that the mortality increased to 53% in the polymer-protected dsRNA treatment compared to only 16% with the naked dsRNA and found that polymer-protected dsRNA completely blocked the development of the caterpillars. These results have shown that using guanylated polymers as a formulation strategy can prevent degradation of dsRNA in the alkaline and strongly nucleolytic gut of lepidopteran insects

6.3 Virus induced gene silencing (VIGS) :

Citrus is a very important commercial crop which id affected by a wide range of pests and diseases. Citrus greening is one among such disease which is caused by *Candidatus liberibacter asiaticus* and is transmitted by Citrus psylla. There are no current methods for the control of citrus greening so we are left with controlling the vector. Hajeri *et al.,* 2014 utilized the coincident cohabitation of phloem. A transient expression vector based on Citrus tristeza virus (CTV) is unusually stable . CTV-RNAi vector, engineered with truncated *abnormal wing disc* (*Awd*) gene of *D. citri*, induced altered Awd expression when silencing triggers ingested by feeding *D. citri* nymphs. Decreased *Awd* in nymphs resulted in malformed-wing phenotype in adults and increased adult mortality. This impaired ability of *D. citri* to fly would potentially limit the successful vectoring of CLas bacteria between citrus trees in the grove

6.4 Vegetable mediated gene silencing:

Brown marmorated stink bug (BMSB) *Halyomorpha halys* (Heteroptera: Pentatomidae) is the invasive insect pest in North America and the experiments conducted by Ghosh *et al*., 2017 developed an oral delivery approach for biocontrol of *H. halys* using RNAi. This study utilized thin organic green beans; *Phaseolus vulgaris* L., as the medium for delivery of dsRNA or other treatments to the animal. BMSB feeds on this cultivar crop by piercing into the vascular tissue using their needlelike stylets. The BMSB feeds by alternate salivation and ingestion with slow movement of stylets in a lacerate-and-flush feeding method causing considerable damage to the cultivar. The advantage of the feeding style by testing the delivery via green bean of green food coloring as compared to water. After confirmation of the movement of colour in the green pods, the target gene dsRNA *JHAMT* and *Vg* were synthesized. These were fed into the green beans by dipping the pods in dsRNA solution. dsRNA - green beans- resulted in targeted gene depletion by 2.2 fold in *Vg* and 4.5 fold in *JHAMT.* So by using this method there wont be any requirement for spraying dsRNA in the field so it will be environmentally safer.

7. Commercialization of RNAi

One of the major obstacle is the mass production of dsRNA making mass amounts of RNA expensive and storage without degradation is yet another task. Cost-effective methods will allow real-world applications of exogenous dsRNA for RNAi-mediated crop protection. Since the discovery of dsRNA and its potential for crop protection, some companies and academic scientists are seeking to develop more cost-efficient methods for large production of dsRNA

In the last few years, we have experienced an ever-growing interest in the market for dsRNA that has pushed long-established companies and startups toward better production, cost efficient, and stable delivery systems. In instance, the cost to produce 1 g of dsRNA (100 up to 800 pb) has dropped from $12,500 USD in 2008 to $100 USD in 2016, and to less than $60 USD today (July 2018) (http://www.agrorna.com/sub_05.html). The agroRNA [produces bulk amounts of dsRNA that could be used in agriculture; however, it is worth noting that naked dsRNA as sold by agroRNA needs to be formulated if the objective is a long-lasting crop protection; otherwise, the dsRNAs will last only a few days. For crop protection, dsRNA does not need to be as pure as for medical application; however, at least for gene silencing in insects, the efficacy of dsRNA increased using purified RNA.

Considering the rapid half-life of dsRNA mainly regulated due to action of RNases and sunlight in the hostile environment, a biotechnology company RNAagri (former APSE) developed a technology “Apse RNA Containers” (ARCs) that allows the mass production of encapsulated ready-to-spray dsRNA with costs near $1 USD per 1 g. In brief, this technology is based on plasmids engineered to produce naturally occurring proteins such as capsids that are co transformed with another plasmid coding for the target dsRNA with a sequence called the “packing site.” The proteins produced by bacteria self-assemble around RNAs, resulting in RNA protected and resistant to environmental hostile conditions. For long-lasting crop protection with exogenous applications, the dsRNAs should be protected with coating of nanoparticles, liposomes, or polymers, which will increase the efficacy by reducing dsRNA.

8. Factors affecting the silencing effect and RNAi efficiency :

The RNAi approach to control insect pests had been considered for many years, but application of this technology was just realized after it was shown that ingestion of dsRNA would trigger RNAi. The concept of RNAi-plant mediated pest control was demonstrated in 2007 by the development of transgenic plants producing dsRNAs against specific insect genes, with the consequent effect on the target species. The main prerequisites to generate successful RNAi insect-resistant transgenic plants are: (i) identification of a specific gene with an essential function in the insect that can cause developmental deformities and/or larval lethality when

knocked down or knocked out; and (ii) dsRNA delivery by oral ingestion and uptake by the insect cells, and spread systemically in the insect body.

Some factors can affect the efficiency of the dsRNA uptake and systemic silencing spread in different insects

- **Target gene :** The choice of the target gene should be carefully considered. Among the target genes, those involved in immunity were more effectively silenced, and, in contrast, genes expressed in epidermal tissues seem to be most difficult. There is differences for RNAi sensitivity among genes in the same tissue (Terenius *et al* 2010)
- **dsRNA design :** The design of the dsRNA determines the specific gene to be silenced, but off target effects can occur if siRNAs have some sequence similarity with unintended genes, this can also affect nontarget insects, becoming a biosafety issue.
- **dsRNA length :** The length of the dsRNA fragments plays an essential role in the effectiveness of molecular uptake in insects, which is directly involved in the success of the target gene silencing. In most of the RNAi experiments, the insects are fed with long dsRNAs. This may be due to the fact that a long dsRNA, with 100% match of the target mRNA, after processing into siRNA will provide a greater diversity of siRNAs available to cause specific suppression of target gene.
- **dsRNA concentration :** Optimal concentration of dsRNA delivered to the insect is required to induce sufficient gene target silencing. It is noteworthy to mention that exceeding the optimal dsRNA concentration may not result in more silencing.
- **Molecular silencing confirmation :** An efficient molecular confirmation of the RNAi silencing should be conducted, which includes target RNA expression, and analyses of protein amount and/or enzyme activity. In RNA analysis, additional care should be taken for expression analysis. The method of choice for RNA expression analysis is the quantitative amplification of reversed transcripts or RTqPCR, considered a very sensitive and accurate method.
- **Insect tissues, life stage, nucleases, and gut pH :** Some insect characteristics should also be considered before starting an RNAi experiment including the developmental stage of insects. Another consideration that can affect the RNAi silencing efficiency is the presence of insect nucleases and gut pH. The stability of the dsRNA in the midgut could be affected not only by enzymatic but also by chemical hydrolysis, gut pH is an important

factor; particularly, it is quite variable among insect orders, with variation even among gut regions.

9. Environmental safety :

RNAi is considered to be environmentally safe compared to other methods of pest management as they are sequence dependent silencing mechanism and it is highly specific. RNAs are known to be highly unstable and their persistence in the environment is very short lived and it doesn't have inherent capacity for protein production.

10. Future line of work

- Detailed mechanism of dsRNA uptake, signal amplification and systemic spread and gene regulationneeds to be studied.
- The affect of RNA on insect biology needs to be studied insect biology
- Development of efficient commercial formulation for field application
- Cost effective mass production of dsRNA
- Formulation of protocol for environmental risk assessment
- Detailed mechanism of the otential resistance development

11. Conclusion:

Crop protection against pathogens and insect pests relies mostly on the widespread use of chemical pesticides that are applied to the environment in large quantities every year. Some of these chemicals are in use for almost half a century. Therefore, there is a need for novel tools which are more sustainable and less detrimental to the environment. Therefore, scientists have harnessed RNAi technology that has been an effective tool in functional genomics studies and its application towards pest management is already close to a reality. The effectiveness of the RNAi mechanism is mainly depending on the delivery, stability, and uptake of dsRNA by target species.

The systemic RNAi is still a matter of investigation in insects, and so far there is no consensus on what mechanism is involved behind spreading the silencing signal. The holistic understanding of systemic properties of dsRNA along with improvements toward delivery methods is underway, and in the coming years will provide innovative breakthrough applications for management of pest insects with a unique mode of action. Furthermore, because of the high specificity as a consequence of its sequence-dependent mode of action—typically targeting a single gene—RNAi will be safer than any pesticide currently available in the marked. The high specificity reduces the negative impact produced by broad spectrum insecticides, and preserves the natural enemies and beneficial fauna in the crop area. The beneficial fauna helps for a more efficient pollination process as well as the natural enemies help to keep the pest insect populations below economic thresholds.

Transgenic plants, which express dsRNA can be a potent method to suppress insect pests selectively. However, due to the extensive regulatory process, non-transformative strategies can be used with similar efficiency. Hence, delivery of dsRNA using chemically modified molecules, polymer nanoparticles, liposomes, viruses or bacteria, could increase efficacy in attaining a potent RNAi response. The choice of the delivery method and the choice of formulation would of course depend on the circumstances, on the target insect and on the reason for impaired RNAi-efficiency.

Studies on environmental safety, off target effects and resistance development needs to be studied. Risk assessment protocol, cost effective method of mass production and delivery needs to be formulated.

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14. Discussion:

14.1 How does the dsRNA enter into the cell of the insect?

Detailed mechanism of entry of dsRNA into the cell is unknown. But according to Jaba and Preethi 2017 there are transmembrane proteins like sid1 and sid2 which will help in the transfer of dsRNA into the cytoplasm of the cells.

14.2 Is this technology cost effective?

Yes, there are startups like agroRNA and RNAagri which massmultiply and sell dsRNA in large quantities and they also offer it at low price.

14.3 Is this environmentally safe ?

Since this method involves sequence specific gene silencing, only the if the complimentarity with target sequence is found the cleavage of mRNA takes place hence it is and environmentally safer compared to braod spectrum chemical pesticides.

14.4 RNA is highly unstable, how can it exist in the field for longer time for the action to take place?

The field trials of RNAi has not been undertaken, so the persistence studies needs to be undertaken. If the persistence is less the chance of resistance development is also low.

14.5. Which is the best method among non transformative methods?

Virus induced gene silencing would be the best method as there wont be need of repetative application wont be necessary once we apply or inoculate in the plant.

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ENT 591 : Masters Seminar

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Venue : Seminar Hall
Date : 13-12-2019
Time : 11.30 am

Gene silencing- An effective tool in insect pest management

Abstract

Insect pest management in the modern era has evolved from synthetic pesticides, bio-rational strategies and then to genetic manipulation. This progress over the last years or so has seen a gradual but steady shift towards increased safety and specificity. Genetic manipulation represents the latest addition to this continuum, amongst which gene silencing is one of the novel advancements.

Gene silencing is the suppression of the expression of a gene at transcriptional or post transcriptional stage. Post transcriptional gene silencing, especially through RNA interference (RNAi), has considerable potential for application in pest management. RNAi is a natural process present in eukaryotic cells for gene regulation. It refers to double stranded RNA mediated gene silencing that involves the blocking of the expression of specific target genes by destroying the corresponding mRNA.

The feasibility of an insecticidal RNAi approach in plants has been demonstrated in corn (Baum *et al*., 2007) as well as in tobacco (Mao *et al*.,2007). Transgenic corn plants expressing dsRNA demonstrated RNAi activity against the *vacuolar ATPase A* (*V-ATPase A*) of the western corn rootworm (WCR), *Diabrotica virgifera virgifera*. Similarly tobacco plants engineered to have RNAi activity targeting *cytochrome p450 monoxygenase gene* (*CYPAE14*) of cotton bollworm, *Helicoverpa armigera* inhibited the expression of the gene which detoxifies the natural defence compound gossypol.

For an insecticidal RNAi based strategy to be effective, selection of the target gene coding for essential proteins is important. It must also employ an appropriate mechanism for the delivery of the dsRNA to the target within the host. The different delivery mechanism for dsRNA application includes host induced gene silencing (HIGS), spray induced gene silencing (SIGS), virus induced gene silencing (VIGS) and vegetable mediated gene silencing.

Host induced gene silencing is the development of transgenic crops expressing dsRNA against the target pest. Spray induced gene silencing (SIGS) involves spraying of transformed bacterial suspension expressing target dsRNA (Zhang *et al*., 2019) and also by means of guanylated polymers (Christiaens *et al*., 2018).

A key advantage of RNAi-mediated resistance is that dsRNA has no inherent translational ability to produce a functional protein. This means that non-target effects should be minimal and lower than those of even highly specific transgenic proteins. The success of RNAi is highly variable across insect orders, Coleoptera being the most susceptible, followed by Hemiptera, Diptera and Lepidoptera. It also depends upon various factors *viz*, target gene, dsRNA design, dsRNA length, dsRNA concentration, molecular silencing confirmation, insect tissues, life stages and gut pH (Rodrigues, B. T. and Figueira, A., 2016).

The US based AgroRNA. Inc produces bulk amounts of dsRNA that could be used in agriculture and considering the half-life of RNA, another company named RNAagri. Inc developed a technology 'Apse RNA Container (ARCs)' that allows mass production of encapsulated ready to spray dsRNA.

Gene silencing is a promising tool in insect pest management. However, this technology needs to be refined by way of effective and simple delivery mechanism, understanding long term response of insects and arising environmental impact before it can be used as a mainstream tool in integrated pest management.

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