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Role of RNAi (RNA Interference) in Insect Pest Management

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Introduction:

- Insect pests - major threats to global food grain production.
- Threats posed by insect pests to agricultural production is the major adversity that led to food security.
- Overall estimate of annual yield loss due to insect pests in major crops account approximately 18 percent in the absence of control measures.
- RNA interference (RNAi) is one of the latest tool used to reduce the problems of insect pest and for their management.
- RNAi is sequence specific gene silencing and holds a great promise for effective management of agricultural crop pests.

Gene Silencing:

- Gene is a segment of DNA that codes, or holds instructions, for producing a protein (Kowalski, 2015). Silencing is down regulation or "switching off "of specific genes.The interruption or suppression of the expression of a gene at transcriptional or Post transcriptional level is called as gene silencing(Brigneti *et al*., 1998).

Types of Gene Silencing:

- **Transcriptional gene silencing:** Result of modifications of either the histone or DNA.
- **Post-transcriptional gene silencing (PTGS)**: Result of the mRNA of a particular gene being destroyed or blocked. A common mechanism of PTGS is RNAi.

Why to go for RNA interference??

- Reduce the dependence on chemical pesticides.
- Reduce cost of production.
- Reduce environmental and health hazards.

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- Precise and reliable.
- Effective management of target pests.
- Excellent strategy for reducing specific gene expression in several insect orders, including Diptera, Coleoptera, Hymenoptera, Orthoptera, Blattodea, Lepidoptera and Isoptera.

RNAi (RNA Interference):

- ❖ A biological process in which dsRNA molecules inhibit gene expression by causing the destruction of specific mRNA molecules
- ❖ In plants dsRNAs may be generated from three sources:
  1. Viral replication intermediates
  2. Products of the endogenous RNA-directed RNA polymerase
  3. Transcribed inverted repeats

Mechanism of Gene Silencing

Step- 1

- ➢ dsRNA is processed into sense and antisense RNAs
  - 21-25 nucleotides in length
  - have 2-3 nt 3' overhanging ends
  - Done by Dicer (an RNase III-type enzyme)

Step- 2

- ➢ siRNAs associate with RISC (RNA- induced silencing complex) and unwind

Step- 3

- ➢ Antisense siRNAs act as guide for RISC to associate with complementary single stranded mRNAs.

Step- 4

- ➢ RISC cuts the mRNA approximately in the middle of the region paired with the siRNA.
- ➢ mRNA is degraded further.

Causes of gene silencing:

- Methylation of transgenes
- Degradation of transgenic mRNA in cytoplasm
- Inactivation of homologous gene by transcriptional and post-transcriptional regulation

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Methods of dsRNA uptake in insects:

1. Microinjection:

- Direct injection of dsRNA into the body of insects.
- Most effective delivery.

Advantage:

- High efficiency of inhibiting gene expression.

Limitations:

- Costly
- Complicated steps

2. Soaking:

- Suitable only for certain insect that readily absorb dsRNA from the solution.
- It is rarely used.

Disadvantage:

- Less effective compare to the injection method.

3. Feeding of Artificial diet :

- Convenient and easy.
- More natural method of introducing dsRNA into insect body
- Popular in very small insects that are more difficult to manipulate using microinjection.

Limitations:

- Greater amount of material needed, otherwise incomplete silencing (Chen *et al.*, 2010).

4. Transgenic plants:

- Pest control using transgenic plants expressing dsRNA.
  - ❑ Transgenic corn dsRNA against the vacuolar ATPase gene (v-ATP) increase the mortality decreases the damage caused by *Diabrotica virgifera virgifera*.
  - ❑ Introduction of RNAi elements in Tobacco and Arabidopsis plants supress the CYP6AE14 gene, which is directly related to gossypol detoxification in *H. armigera*, increases the toxicity of gossypol feeding on such transgenic plants.

Some cases of RNAi based insect pest control:

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1. Silencing of Chitin Synthase A gene in *Spodoptera litura* (Fabricius) Using RNAi Approach :

- dsRNA mediated strategy for the management of *S. litura* targeting chitin synthase (SlCHSA) gene.
- In this study, the SlCHSA gene specific dsRNA was synthesized and assessed their effect on growth and development of *S. litura* under in vitro conditions.
- Comparatively lower larval body weight and moulting rate was observed on the larvae fed with dsSlCHSA treated leaves than in untreated leaves.
- The results suggested that SlCHSA can be used as a good candidate gene for RNAi based insect pest control.

2. **Transgenic cotton plants expressing the HaHR3 gene conferred enhanced resistance to *H. armigera* and improved cotton yield.**

- RNAi insect-resistant transgenic plants expressing double-stranded RNA (dsRNA) that is ingested into insects to silence target genes can affect the viability of pest or even lead to death.
- HaHR3, a molt-regulating transcription factor gene was selected as a target expressed in plants to control *Helicoverpa armigera* by RNAi technology.
- In this work, they selected dsRNA-HaHR3 fragment to silence HaHR3 in cotton bollworm for plant mediated-RNAi research.
- A total of 19 transgenic cotton lines expressing HaHR3 were successfully cultivated, and seven generated lines were used to perform feeding bioassays. Transgenic cotton plants expressing dsHaHR3 were shown to induce high larval mortality and deformities of pupation and adult eclosion when used to feed the newly hatched larvae, and 3rd and 5th instar larvae of *H. armigera*.

3. Oral Delivery of Double-Stranded RNAs Induces Mortality in Nymphs and Adults of the Asian Citrus Psyllid, *Diaphorina citri.*

- Asian citrus psyllid (ACP) is the vector of the phloem-limited bacteria *Candidatus Liberibacter americanus* and *Candidatus Liberibacter asiaticus*, the causal agents of the devastating citrus disease huanglongbing (HLB).
- RNA interference (RNAi) has proven to be a promising tool to control pests and explore gene functions.

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- Targeted the cathepsin D,chitin synthase and inhibitor of apoptosis genes of adult and nymph ACP by feeding artificial diets mixed with dsRNAs and *Murrayapaniculata* leaves placed in dsRNAs solutions, respectively.
- Both nymphs and adult ACP fed dsRNAs exhibited significantly increased mortality over time compared with that of the controls.
- Controls ACP and thus helpful in reducing HLB disease.

Pros of RNAi Strategy

- ✓ High degree of specific gene silencing (Sequence-specific).
- ✓ Highly molecular potent and effective.
- ✓ Silencing can be introduced in different developmental stages.
- ✓ Systemic silencing.
- ✓ High level of safety to the non-target organisms
- ✓ Silencing effects passed through generations.
- ✓ Compatible with other insect pest management approaches (e.g. Bt)

Future Remark

- Undoubtedly, there is broad potential for the application of RNAi technology in pest control, mainly if combined into IPM strategies.
- Dependance on chemical pesticides could be lower and thus reduce production cost.
- Success rate of RNAi could be enhanced by selecting a biologically crucial target gene and refining dsRNA molecules and its delivery method.
- Efforts are needed to reinforce the RNAi effectiveness on agricultural pests by silencing multiple genes using combine dsRNA molecules.

Conclusion:

- RNAi is a potential tool for effective management of insect pests.
- It has become a popular topic in last few years.
- It reveals a entirely new level of post transcriptional gene regulation.
- An extremely useful technique for molecular biology.
- However, a better and comprehensive understanding of RNAi would allow the researchers to work effectively and efficiently in order to improve crop plants nutritionally and manage various insect pests and diseases of crop plants.