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RNA INTERFERENCE (RNAi) FOR INSECT CONTROL

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Introduction:

In agricultural sector around the world, billions of dollars are lost every year due to over 20,000 species of different types of insect pests and upto 40 % of crops are lost due to insect pest attack. From many years, the widespread use of chemically synthesized, broad-spectrum insecticides is being considered a significantly satisfactory and permanent solution for pest control. But, the excessive use of these agrochemicals has led to development of pesticide resistant insects, reduction in beneficial insect population and ill effects to human health, natural ecosystem and environment. To meet the growing demand for food, it is essential to increase the production of food. This can be achieved by employing biotechnological tools to study various aspects like insect identification, insect control and insect genetic relationships. In the 21st century, transgenic technology has emerged as a vital tool for the control of insect pests mainly due to the toxin producing *Bacillus thuringiensis* (Bt-toxin encoded by cry genes) which protects a wide range of crops. However, many crops are not covered under Bt protection and some species of insects developed Bt resistance. So, there is a need to look for a different approach and in recent years, one such approach found is the use of RNA interference or RNAi for the control of insect pests. RNAi is a type of post-transcriptional gene silencing mechanism (PTGS) which is initiated by the introduction of dsRNA into a cell to silence gene expression through binding, cleaving, and degrading complementary endogenous mRNA. For this purpose autonomously take up of dsRNA by insect able to feed and digest in its midgut.

RNAi in insects:

RNAi has considerable potential applications in agriculture for insect pest control due to its high specificity in controlling pests.

Cell-autonomous RNAi: The silencing process is limited to the cell having the dsRNA which is exogenously introduced and encompasses the RNAi process within individual cells. It uses broadly conserves mechanism and similar strategies in many organisms.

Non-cell autonomous RNAi: The interfering effect takes place in cells or tissues different from the production of the dsRNA and therefore, it happens exclusively in multicellular organisms and it has high applicability in insect pest control. There are two different kinds of non-cell autonomous RNAi.

Choudhary1. Environmental RNAi: It inovlves taking up of dsRNA by a cell from the environment and this process can also be observed in unicellular organisms.

2. Systemic RNAi: In this process of RNAi, silencing signal is transported from one cell/tissue to another or from one cell/tissue type and it can only take place in multicellular organisms.

RNAi Mechanism:

The introduction of double-stranded RNA (dsRNA) in cells by specific down regulation or knockdown of gene expression leads to the degradation of mRNAs containing homologous sequences by sequence-specific cleavage of mRNAs. Two RNA silencing pathways are known to exist which are mediated by miRNAs; uses endogenous products transcribed from the cell's genome with dsRNA structure to regulate developmental processes and siRNAs; the pathway is involved in defense response against exogenous dsRNAs.

1. The transmembrane channel-mediated uptake mechanism: The dsRNA uptake via the gut is still not very clear and the mechanisms in *Caenorhabditis elegans* and *Drosophila melanogaster* revealed two proteins being involved in non-cell autonomous RNAi *viz.*,

SID-1: Is a type of multispan transmembrane protein, essentially required for systemic RNAi in multicellular organism and it functions probably as a multimer, transporting dsRNA passively into the *C. elegans* cells.

SID-2: Facilitates environmental RNAi and is mainly found in the intestine tissue of the worm.

Three hypotheses are proposed on the relation between the two proteins:

- SID-2 modifies the SID-1 transmembrane protein for activation of transport.
- SID-2 binds to the dsRNA from the environment and delivers it to SID-1.
- SID-2 induces the endocytosis pathway of the dsRNA.

2. Microinjection: The direct injection of dsRNA into the body of insects, has been one of the most effective delivery methods for systemic RNAi types. Short dsRNA have had the most success rates with this mechanism. Injecting dsRNA into the insect body is the highly efficient method of inhibiting gene expression.

3. Soaking: It involves dsRNA solution for soaking the organism in extracellular RNAi for triggering RNAi response and this technique was subsequently used for analysis of gene function in the organism. This method of dsDNA delivery is more applicable for certain insect cells and tissues as well as for the developmental stages of insects rather than the adults due to their hard cuticles and therefore it is rarely used. *D. melanogaster* embryos, require a higher concentration of dsDNA that can inhibit gene expression.

4. Feeding of artificial diet: Compared to other methods, dsRNA feeding through artificial diet is unique and most attractive method as it is easy to manipulate and is a more natural strategy for introduction of dsRNA into insect's body. It causes less damage to the insects, when compared to other methods like microinjection. *Epiphyas postvittana* larvae have shown to inhibit the expression of the carboxylesterase gene EposCXE1 and pheromone-binding protein EposPBP1 in the larval midgut and adult antennae, respectively by feeding of dsRNA. It is especially popular in very small insect species that are more difficult to manipulate using microinjection.

5. Virus-mediated uptake: This method involves, uptake of viruses that carry dsRNA which infect host during viral replications and target the gene. Some studies (Uhlirova*et al.*, 2003) reported that through electroporation method of gene transfer, recombinant sindbis virus introduced into *Bombyx mori* cells could inhibit the expression of BR-C gene which ultimately prevented the larvae to pupate. It has great advantage over other methods as it does not require screening for transgenic insects or tissues.

Conclusion:

RNAi or PTGS can also provide broad spectrum resistance against pathogens like virus, bacteria *etc.*, with high degree of variability. Recent studies have hinted possible role of RNAi related processes in plant stress adaptation. In the recent years, much progress has been made in the field of RNAi, but the full potential of RNAi in crop protection as well as crop production remains to be realized. The complexities of RNAi mechanism, the molecular machineries or pathways and how it relates to plant development are still to be elucidated.

Future thrust:

Tissue specific or organ specific RNAi vectors are required to be identified to achieve targeted gene silencing in specific plant tissues and organs with minimal interference to the normal plant lifecycle. Future dissection of miRNA gene structures will greatly facilitate the development of RNAi vectors with high silencing efficiency and fewer side effects to plants. Future research should focus on the development and fine tuning of RNAi based gene silencing vectors that can operate in a temporarily and spatially controlled manner.

References:

Katoch, R., Sethi, A., Thakur, N. and Murdock, L.L. (2013). RNAi for Insect Control: Current Perspective and Future Challenges. *Appl. BiochemBiotechnol,* **171**: 847–873.

Dong, Y. and Friedrich, M. (2005). Nymphal RNAi: systemic RNAi mediated gene knockdown inn juvenile grasshopper. *BMC Biotechnol,* **5**: 25–32.

Chen, J., Zhang, D., Yao, Q., Zhang, J., Dong, X. and Tian, H. (2010). Feeding-based RNA interference of a trehalose phosphate synthase gene in the brown planthopper, *Nilaparvata lugens. Insect Mol. Biol,* **19**: 777–786.