

NEWPORT INTERNATIONAL JOURNAL OF RESEARCH IN EDUCATION (NIJRE) Volume 2 Issue 1 2022

Evaluation of RNA Interferences in Pest Control

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ABSTRACT

Chemical pesticides are still the major approach for control of insect pests, but they are associated with significant hazards to the environment and human health. The alternative commercial biotechnological system relies mostly on the expression of *Bacillus thuringiensis*, insecticidal proteins. Its effectiveness however is threatened by the development of resistance in some species such as *Ostrinia nubilalis* (Hubner) (Lepidoptera, Pyralidae) and *Heliothis virescens* (Fabricius) (Lepidoptera: Noctuidae). As a result, there is an urgent need to develop and adopt economically and ecologically sound alternative strategy for insect pest management. RNA interference (RNAi) mediated gene silencing has been suggested as one of the new alternative to reduce damage from insect pests; Consequently, this paper assessed the capacity of RNA interference in pest control. The paper concluded that feeding is very popular in insect RNAi research and may have the most promising future in pest control, especially with the creation of transgenic plants producing dsRNA. Eventually, the use of transgenic insects will also lead to more efficient pest control.

Keywords: Pest control, RNA, Pesticides, Biotechnology

INTRODUCTION

For The Control of Insect Pests, Farmers Mostly Rely On Chemical pesticides. Chemical pesticides are still the major approach for control of insect pests, but they are associated with significant hazards to the environment and human health. The alternative commercial biotechnological system relies mostly on the expression of *Bacillus thuringiensis*, insecticidal proteins (Cry toxins). Its effectiveness however is threatened by the development of resistance in some species such as *Ostrinia nubilalis* (Hubner) (Lepidoptera, Pyralidae) and *Heliothis virescens* (Fabricius) (Lepidoptera: Noctuidae) [1-3]. As a result, there is an urgent need to develop and adopt economically and ecologically sound alternative strategy for insect pest management. RNA interference (RNAi) mediated gene silencing has been suggested as one of the new alternative to reduce damage from insect pests. RNA interference (RNAi) refers to a group of post-transcriptional or transcriptional gene silencing mechanisms conserved from fungi to mammals. It is a phenomenon primarily for the regulation of gene expression, self or oneself depending upon the surrounding factors or conditions. This paper evaluates the capacity of RNA interference in pest control.

The Concept of RNA Interference

RNA interference' refers collectively to diverse RNA-based processes that all result in sequence-specific inhibition of gene expression, either at the transcription, mRNA stability or translational levels [4]. It has most likely been evolved as a mechanism for cells to eliminate foreign genes. The unifying features of this phenomena includes the production of small RNAs 21-26 nucleotides (nts) that act as specific determinants for down-regulating gene expression and the requirement of one or more members of the Argonaute family of proteins. RNAi operates by triggering the action of dsRNA intermediates, which are processed into RNA duplexes of 21-24 nts by a ribonucleic III-like enzyme called Dicer. Once produced, these small RNA molecules or short interfering RNAs (siRNAs) are incorporated in a multi-subunit complex called RNA induced silencing complex or RISC. RISC is formed by a siRNA and an endonuclease among other components. The siRNAs within RISC act

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as a guide to target the degradation of complementary messenger RNAs (mRNAs). The host genome codifies for small RNAs called miRNAs that are responsible for endogenous gene silencing.

Components of RNA interference

Both genetic and biochemical approaches have been undertaken to understand the basis of silencing. Genetic screens were carried out in the fungus, *Neurospora crassa* Shear and B. O. Dodge, the alga, *Chlamydomonas reinhardtii* P. A. Dang, the nematode *Caenorhabditis elegans*, and the plant *Arabidopsis thaliana*(L.) to search for mutants defective in quelling, RNA interference, or PTGS. Analyses of these mutants led to the identification of host-encoded proteins involved in gene silencing and also revealed that a number of essential enzymes or factors are common to these processes. Some of the components identified serve as initiators, while others serve as effectors, amplifiers, and transmitters of the gene silencing process. In the years to come, many other components as well as their interrelations will be revealed. Here, some common components of RNA interference mediated gene silencing. In brief, upon entry into the cell, the exogenous dsRNA is processed by a ribonuclease III enzyme, called Dicer-2, into small interfering RNAs (siRNAs). These 21-24 nucleotide duplexes are subsequently incorporated in the so called RNA-induced silencing complex (RISC) where the duplex is unwound. Subsequently, an Argonaute2 (AGO2) protein cleaves the passenger (sense) strand and the guide (antisense) strand remains connected with the RISC. Afterwards, the guide strand of the siRNA guides the RISC and allows Watson-Crick base pairing of the complex to complementary target mRNA for cleavage of target mRNA by AGO2 protein. By this degradation of the target mRNA, specific post-transcriptional gene silencing occurs.

MECHANISM OF RNA INTERFERENCE

Knowledge on RNAi has expanded dramatically in a short time since its discovery. In the last few years, important insights have been gained in elucidating the mechanism of RNAi, although it seems to be very complicated. RNAi appears to be a highly potent and specific process, which starts when a dsRNA is introduced either naturally or artificially in a cell. An endoribonuclease enzyme cleaves the long dsRNA into small pieces of miRNA or siRNA depending upon the origin of long dsRNA, i.e., endogenous or exogenous, respectively. A dsRNA may be generated by either RNA-dependent RNA polymerase or bidirectional transcription of transposable elements. Based on the in vitro and in vivo experimental results, a two-step mechanistic model for RNAi has been established. The first step referred to as the RNAi initiating step, involves the binding of RNA nucleases to a large dsRNA and its cleavage into discrete 18 to 23 nucleotide RNA fragments (siRNA). In the second step, these siRNAs join a multinuclease complex, RISC, which degrades the homologous single-stranded mRNAs.

DELIVERY METHODS OF dsRNA INTO INSECTS

Delivery of dsRNA is a major challenge in RNAi-based plant protection method. After identifying the target gene, choosing a convenient strategy to deliver the dsRNA into the insect body is very important. The main delivery methods include injection, soaking, feeding, transgenic technique and viral infection. The artificial RNAs could be exogenously applied onto the plant surface using additional techniques to increase the RNA absorption/uptake viz., cationic nanoparticles, clay nanosheets, surfactants, peptide-based

RNA DELIVERY SYSTEMS

Microinjection

Microinjection involves direct injection of dsRNA into the body of insects. It is one of the most effective delivery methods for systemic RNAi. Short dsRNA have had the most success with this mechanism [5-6]. The major advantage of injecting dsRNA into the insect body is the high efficiency of inhibiting gene expression. However, there are some limitations with micro-injection. First, the cost for vitro synthesis and storage of dsRNA is relatively high, and the steps are complicated. In addition, injection pressure and the wound generated inevitably affect the insects. In practice, this delivery method would have very limited application as pest control in the field level.

Soaking

This involves direct application of the solution of dsRNA on the insect body. Soaking dsRNA solution can inhibit gene expression, and its effectiveness is comparable to the injection method in that it requires a higher concentration of dsRNA [7] The soaking method is suitable only for certain insect cells and tissues as well as for specific insects of developmental stages that readily absorb dsRNA from the solution, and therefore, it is rarely used.

Feeding of Artificial Diet

Compared to other methods, dsRNA feeding is the most attractive primarily because it is convenient and easy to manipulate. Since it is a more natural method of introducing dsRNA into insect body, it causes less damage to the insect than microinjection. It is especially popular in very small insects that are more difficult to manipulate using microinjection.

DEVELOPING TRANSGENIC INSECTS

The advantage of using transgenic insects that carry the dsRNA is that as it is inheritable, the expression can be stable and continuous. The technique has been proposed to help either reduce population through introduction of sterile insects or for population replacement. In this case, dsRNA must be first injected in the host insect. Tests are being conducted on several species with promising results. There is a need to understand environmental and genetic influences when assessing the potential use of such transgenics. The transgenic method has been first used in *D. melanogaster* with the GAL4/UAS transgenic system that leads to the expression of hairpin RNA

Virus-Mediated Uptake

Virus-mediated RNAi methods involve the infection of the host with viruses carrying dsRNA formed during viral replication and targeting the gene of interest in the host. Virus-mediated RNAi studies are still rare. However, this method takes advantage of the infection and ability of the virus to spread rapidly in a host population. Viruses that are commonly employed for this purpose include lentiviruses, adenoviruses, and adeno associated viruses (AAVs).

Applying of dsRNA through Root Absorption or Injection into Plant

Delivery of dsRNA to phytophagous insects could be achieved by supplying dsRNA through root absorption or injection into plant vessels, where these insects can naturally acquire dsRNA through sucking or chewing. However, it is important to bear in mind that this strategy will demand mass production of dsRNA, which may be costly using molecular biology kits.

FACTORS AFFECTING THE SILENCING EFFECT AND RNA INTERFERENCE EFFICIENCY FOR INSECT MANAGEMENT

In recent years, the research on the potential of using RNA interference (RNAi) to suppress crop pests has made an outstanding growth. But some factors can affect the efficiency of the dsRNA uptake and systemic silencing spread in different insects. Some important points that must be considered in developing an RNAi approach against insect pests include:

1. Concentration of dsRNA
2. Length of the dsRNA fragment
3. Species-specific nucleotide sequence
4. Persistence of the silencing effect
5. Life stage of the target organism

CONCLUSION

Extensive increase in the application of RNAi technology in insect research has facilitated the identification of insect gene function. Research has shown that the dsRNA is particularly conservative; there are various functions and development factors among insect species. Such variations are yet to be fully understood but certainly can serve as a basis for determining their capacity to control insect genes. The main challenge for moving towards larger scale projects remains the development of effective delivery mechanisms. Feeding is very popular in insect RNAi research and may have the most promising future in pest control, especially with the creation of transgenic plants producing dsRNA. Overtime, the use of transgenic insects will also lead to more efficient pest control.

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