UDC 632.9

APPLICATION AND DEVELOPMENT PROSPECT OF RNA INTERFERENCE TECHNOLOGY IN PEST CONTROL

Cao Zhishan
PhD student
Sumy National Agrarian University, Sumy, Ukraine
School of Resources and Environment,
Henan Institute of Science and Technology, Xinxiang, China
ORCID: 0000-0003-3127-4592
caozhishan123@163.com

Vlasenko Volodymyr
Doctor (Agricultural Sciences), Professor
Sumy National Agrarian University, Sumy, Ukraine
ORCID: 0000-0002-5535-6747
vlasenkova@ukr.net

Chemical pesticides have been gradually replaced by other methods for controlling pests and pathogens in the agriculture, due to the serious "3R" problems (resistance, resurgence, and residue) caused by them. Meanwhile transgenic crops expressing Bacillus thuringiensis (Bt) toxins have been extensively planted for insect pest control, but the evolution of Bt resistance in target pests threatens the sustainability of the approach. The researches to find new ways for controlling pests effectively never stop. Biological control for pest has gained acceptance in recent years with the advantages of no pollution and continuous effects. However, the biological methods are also facing many problems: their effectiveness are strongly affected by environmental factors or the insect host; it is difficult to get large numbers of natural enemies' insects by artificial reproduction and it is very expensive for producing insect natural enemies in laboratories. With the development of molecular biology technology, it has become a new trend for biological pest control by using modern molecular biological targets. RNA interference (RNAi) is a highly conserved post-transcriptional gene silencing mechanism that existed in insects by which the mRNA is targeted for degradation by the double-stranded RNA (dsRNA) or the inducing homologous mRNA, resulting in the sequence specific inhibition of gene expression. RNAi technology not only plays an important role in the study of insect functional genome, but also has great potential in pest control. RNAi was listed as one of the top ten scientific breakthroughs by Science magazine in 2001, and it was also awarded of the Nobel Prize for its discoverers in 2006. RNAi has high efficiency and strong specificity, and it is widely used for studying the function of the target gene or explore experimental treatment for diseases. When the target genes in insects was knocked down via RNAi, it always led to insect death or behavioral defects. This method is an environmentally friendly biotechnological one for pest control, and it rarely causes resistance with other insecticides. Therefore, RNAi technology is considered as a potential pest control strategy, which has great potential, beneficial in insect protection, development of new pesticide, etc., and this has been successfully used in Hemiptera, Orthoptera, Diptera insects and others. In this paper the silencing mechanism of RNAi, several ways of dsRNA transplanting into insects, and factors determining RNAi efficiency on application of insect are mainly described. Finally, we also reviewed the existing problems and some current solutions of RNAi technology, in order to discuss further the mechanism and existing problems of RNAi that applied in pest control. This will provide a new insight in pest management by RNAi technology.

Key words: *RNAi technology, target gene, biological method of pest control, protection against pests in agricultural production.*

DOI https://doi.org/10.32845/agrobio.2022.2.1

Introduction. RNA Interference (RNAi) is a post-transcriptional gene silencing mechanism by which the expression of homologous endogenous mRNA will be inhibited by the introduction of exogenous dsRNA or small interfering RNA (siRNA). (Moritz et al., 2010; Liu et al., 2012; Xu et al., 2016; Itsathitphaisarn et al., 2017). This phenomenon is widely existing in fungi, plants, animals and other eukaryotes, and it can not only participate the defense and differentiation regulation of cells, but can also resist the invasion of viruses and other exogenous nucleic acids, so that to keep the organism itself in a genetically stable state. (Tijsterman et al., 2002; Voinnet, 2008; Walker & Allen, 2011; Armas-Tizapantzi & Montiel-González, 2016). Fire et al (1991) firstly discovered the silencing ability of antisense RNA in *Caenorhabditis elegans*. Then in 1998, he described the interfering mediator as being a dsRNA, rather than a single-stranded antisense RNA which led to a Nobel Prize in medicine, and also with possible applications in other fields (Fire et al., 1998; Zotti et al. 2018). During the last thirty decades, significant progresses have been made to understand and unravel several aspects of dsRNA mediated gene silencing. RNAi is a highly protected gene regulation mechanism, which inhibits gene expression and silences specific genes by degrading the mRNA of target genes or binding to the non-transcriptional region at the 3' end of mRNA (Jarvinen et al., 1991; Wianny & Zernicka-Goetz, 2000;

Carthew & Sontheimer, 2009; Berezikov, 2011; Liu et al., 2017). Additionally, RNAi has been proved to be very promising in many research fields, in particular, study the gene function determination in genomics and control of cancers and viral disease by the mechanism of gene knockdown in eukaryotes. Meanwhile, as a new specific method, RNAi has shown great potential for pest control in agriculture (Borovsky, 2005; Gordon & Waterhouse, 2007; Price & Gatehouse, 2008).

As a gene silencing method, RNAi played an important role in advancing insect science and helped in identifying functions of many genes involved in physiological, metamorphic, behavioral and reproductive processes of insects (Palli, 2014; Mallikarjuna et al., 2016). Silencing important gene expression will lead to the death or behavioral defect of some insects, so the technology is considered as a potential pest control strategy (Bettencourt et al., 2002; Zotti & Smagghe, 2015; Whitten et al., 2016). RNAi has great potential in insect control and protection, and it has been widely applied in the *Hemiptera, Orthoptera, Diptera* insects, etc. (Wang et al., 2006; Turner et al., 2006; Zha et al., 2011; Huvenne & Smagghe, 2010; Swevers & Smagghe, 2012; Li et al., 2013). In recent years, the researches about pest control and target agents have been developing rapidly and they have attracted more attention. In this paper the mechanism, introducing methods of RNAi, biological control, factors effecting the efficiency of RNAi, insect resistance are synthetically analyzed and evaluated and provided the reference for researchers in related fields. We also reviewed the feasibility, application methods, existing problems and some current solutions of RNAi technology.

The principle of RNAi and its application in agricultural pest control

It is clear that in many eukaryotes the dsRNA-mediated gene silencing is a relatively conserved mechanism (Hannon, 2002; Geley & Muller, 2004). The dsRNA can be delivered to insects by microinjection (Ren et al., 2018) and artificial diet feeding or introduced into insect systems by spraying onto infested crops (Yu et al., 2014; Guo et al., 2016). The whole of dsRNA silencing process contains the three stages: initiation, maintenance, signal amplification (Cao et al., 2016). Once the dsRNA entered into the insect cell, Dicer RNase III can recognize and cleave it into siRNA which was duplexes composed of approximately 21 to 23 nt. The siRNAs are then loaded in the RNA-induced silencing complex (RISC) that guides degradation or translational inhibition of homologous mRNA, resulting in RNAi-mediated silencing of the target gene in insects (Huvenne & Smagghe, 2010; Terenius et al., 2011).

The advent of RNAi represents a new way for pest control, and it also open the door for studying gene functions. Bettencourt et al. (2002) firstly applied this technology on *Lepidoptera* insect by injecting the dsRNA into *hemolin* inhibiting hemagglutinin gene in the pupal stage of *Hyalophora cecropia*, which lead to the deformity and death of the next generation of embryos. Since then, this technology has been effectively carried out in a wide range of insect species with some promising results (Zhang et al., 2017; Niu et al., 2018; Zotti et al., 2018). Recently reports shows that when the *tim* gene related to rhythmic behavior of *Laodelphax striatellus* (*Hemiptera: Delphacidae*) are knockdown, it will lead to the rhythm disorders of its adult (Jiang et al., 2018). Deng et al. (2018) synthesized dsRNA of insulin/insulin-like growth receptor of *Leptinotarsa decemlineata*, then he fed to the larvae by the *Solanum tuberosum* leaves that soaked in dsRNA suspension, as a result, the larval development was postponed and larval growth was inhibited. Majidiani et al. (2019) immersed the root system of tomato plant in a solution containing dsRNA, and mortality rate of the tomato pest reach to astounding 80%. RNAi prevents protein synthesis by interfering with the transcription and translation process of genes related to pest growth and development, resistance, immunity and oviposition, which resulting in reduced adaptability or death of pests. This technique has been seen as an alternative to usage of pesticide and also reduced the damage to the environment.

Factors determining RNAi efficiency on application of insect

The feasibility of RNAi technology has been widely accepted, due to its sequence specificity, it can get good result for controlling of species-specific pests. RNAi showed great potential on agriculture applications and it has made great progress in pest control (Joga et al., 2016; Guan et al., 2017, 2018; Mamta & Rajam, 2017; Qi et al., 2019; Rani Das & Sherif, 2020). However, the efficiency of RNAi is easily affected by a variety of factors, which is the most important issues at present. The RNAi efficacy is depended on various insect species, target genes, mode of dsRNA delivery, stable expression of dsRNA (Prentice et al., 2015). The length of expressed dsRNA is also an important factor affecting RNAi efficiency in some insect species (Khan et al., 2013; Wynant et al., 2014a, b; Wang et al., 2016; Song et al., 2017).

Mode of dsRNA delivery ways into insects

Microinjection. Microinjection is a classical method of dsRNA delivery to target insect tissues to initiate an RNAi response. It is considered the preferred approach and offers unique benefits by delivering precise amounts of RNAi molecules into egg, nymph and adults easily and effectively (Yu et al., 2012). The *Drosophila melanogaster*, *Tribolium castaneum, Anopheles gambiae* were the first insects that using RNAi method by microinjection of dsRNA (Blandin et al., 2002; Barinova et al., 2007; Kennerdell & Carthew, 1998; Mahaffey et al., 2003). Since then, this technique was widely applied to many other insects, including *Bombyx mori*, *Bees*, *Blattodea*, and other species of *Diptera* and *Orthoptera* (Bellés, 2010; Gu & Knipple, 2013; Scott et al. 2013). In vitro synthesis of dsRNA has been commercialized. The key step of this delivery method is the microinjection process. However, it is difficult to achieve RNAi via microinjection for small insects, meanwhile the mechanical damage and using of anesthetic during the injection also affected RNAi efficiency (Svoboda et al., 2000; Wuriyanghan et al., 2011; Liu et al., 2010; Yan et al., 2020).

Feeding. Feeding is a more popular strategy than microinjection with little damage to insect body, and it has higher application value for pest control based on RNAi. Feeding may also lead to novel methods for pest control by dsRNA transgenic plants (Price & Gatehouse, 2008). At present, a fixed method of feeding operation has been developed in insects such as *Bees, Aphids*, *Bemisia tabaci* and Species of *Delphacidae* (Jarosch & Moritz, 2011; Li et al., 2011; Mutti et al., 2006; Thakur et al., 2011; Whyard et al., 2009). The dsRNA was fed to bees mixed with the appropriate amount of honey. In *Aphids*, *Bemisia tabaci* and *Delphacidae*, dsRNA was mixed with liquid artificial diet, and the solution is sealed with parafilm, so insects can absorb and deliver by piercing-sucking mouthparts. However, in *Diptera* insects, the RNAi efficiency and the consistent by feeding of synthetic dsRNA directly are worse than injection (Cooper et al., 2018; Scott et al., 2013). In *Lepidoptera* and *Orthoptera* species, compared to injection, feeding of dsRNA also could not achieve of good RNAi efficiency (Garbutt et al., 2013; Luo et al., 2013; Wang et al., 2016). While in *Coleoptera*, both feeding and injection have equal RNAi efficiency (Liu et al., 2015; Chitvan et al., 2018; Price & Gatehouse, 2008). Researchers speculated that the degradation of dsRNA in the intestinal tract of insects and the different absorption capacity of cells might affect the feeding effect of dsRNA (Christiaens et al., 2014; Garbutt et al., 2012; Luo et al., 2013).

2.3. Other methods. Another approach is to express dsRNA in host plants, which the insects then feed on. At present, this technology has been applied in corn, tobacco and other crops, which can control tobacco moth and corn root and leaf pests effectively (Baum et al., 2007; Joga, et al., 2016; Zhang et al., 2017). But the biggest problem of this technology is safety, and long-time basic researches are needed to determine its potential threat to humans, the environment, and other non-conventional organisms (Heinemann et al. 2013). Besides, delivery of dsRNA using nanoparticle carriers can enhance RNAi efficiency effectively, and it is the most innovative approaches at present which attracted more and more people's attention (Das et al., 2015; Christiaens et al., 2018; Zheng et al., 2019).

3. Life stage and insect species

The life-stage of the insect is often a key factor for RNAi efficiency. Although adult stage insects are often more easily handled than larvae stage, gene silencing effects are generally more prominent in the early and immature development stages. For example, in *Rhodnius prolixus*, the dsRNA of nitropin 2 gene had no silencing effect on the 4th instar larvae, but had 42% silencing effect on the 2nd instar larvae (Araujo, et al., 2006); in *Spodoptera frugiperda*, the 5th instar larvae induced a stronger silencing effect than the adults stage (Griebler et al., 2008). Insect species can also affect the RNAi efficiency. Some studies have shown that the RNAi efficiency of *Coleoptera* is much higher than that on *Lepidoptera* species (Zhu et al., 2011; Scott et al., 2013; Palli, 2014). The gene knockdown rate in *Coleoptera* is often 90% or higher, in contrast, in other order insects, that is around 60% or lower (Baum et al., 2007; Bolognesi et al., 2012; Rangasamy & Siegfried, 2012). In *Diptera*, the efficiency differentiation of RNAi is more obvious. For example, the model organism of *Drosophila melanogaster* is much less sensitive to RNAi than *Aedes aegypti* (Scott et al., 2013). Both feeding and injection of dsRNA can produce significant RNAi effects in mosquitoes, while injection of dsRNA cannot stimulate effective RNAi responses in *Drosophila melanogaster* (Miller et al., 2008).

4. Target gene selection of insects

Target gene is the most important factor for the success of RNAi. Firstly, the RNAi sensitivity of different target genes in the same insect was significantly different. Terenius et al. (2011) reported that, the immune-related genes had better RNAi efficiency than epidermis gene among *Lepidoptera* insects. Secondly, different dsRNA segments of the same gene also produce differences in RNAi efficiency. Li et al. (2011) demonstrated that in *Nilaparvata lugens*, the RNAi efficiency was lowest when the dsRNA fragment located in the 5' coding region, while the RNAi efficiency was the highest when the fragment located on the 3' coding region or non-coding region. Finally, the length and optimal concentration of dsRNA are also very important for RNAi efficiency (Bolognesi et al., 2012). Studies have shown that RNAi efficiency is positively correlated with length of dsRNA. For example, in *Drosophila* S2 cell, the longer fragment of dsRNA has higher RNAi efficiency (Saleh et al., 2006). In *Tribolium castaneum*, length of dsRNA between 69 bp to 520 bp had high RNAi efficiency, but long fragment stimulated RNAi more obviously (Miller et al., 2012). However, in practical applications, especially in pest control, longer dsRNA fragments may bring more off-target effects, or cause harm to non-target insects.

5. The advantages and challenges of RNAi in pest control applications

RNAi is a potential bio-insecticide, compared with traditional chemical method, its advantages are as follows: (1) it is highly specific technology, and act as a pesticide resistance repressor by targeting essential genes; it can kill the target specific pests while leaving other species unharmed; (2) it is environmental-friendly method and has unknown any toxic effects on the ecosystem; (3) it can also solve the problem of pesticide resistance, if the insecticide resistance associated gene be silenced by RNAi; it will increase insect sensitivity to insecticides, then improve insecticide efficiency and reduce the amount of chemical pesticide use. (Kurreck et al., 2009; Bautista et al., 2009; Scott et al., 2013; Borel, 2017; Sparks & Nauen, 2015 ; Liu et al., 2020). Recent research suggests another desirable prospect of RNAi. We can silence the insects' pheromone receptors by RNAi, which will block the communication systems of mating behavior or host-seeking pests, then the pest population will effectively be controlled (Turner et al., 2006; Trivedi, 2010; Zhao et al., 2011).

This technology demonstrates its potential to control pests, however, there are a few challenges in the process of pest management use, such as dsRNA uptake, stability and recalcitrance of insect species (Zhang et al., 2013). The dsRNA is easily degraded before ingested, and variability in specificity within species is differ (Kourti et al., 2017, Kunte et al., 2020). In addition, we also need to focus on the practicability of RNAi production applications. At present, the dsRNA production has been commercial one, but it is quite expensive, limited by small-scale production, and prone to false amplification (Dubrovina et al., 2019; Voloudakis et al., 2015; Alvarez-Sanchez et al., 2017).

Conclusions. In summary, RNAi is a natural mechanism found in most eukaryotic organisms. Researches on applications of RNAi for pest control have made an outstanding growth. It is becoming apparent that RNAi-based approaches can make a major contribution towards integrated pest management and sustainable agriculture. The application of RNAi has helped scientists to find a possible solution to the global problems of agricultural losses attributed to insects and pathogens in a sustainable way, and this technology has raised more attentions. At present, there are also some problems in the application of RNAi technology in pest control. The RNAi efficiency varies greatly among different target genes and different insects. Especially, *Lepidoptea* species are serious agricultural pests, but most species are not sensitive to RNAi. At present, more and more researches are applied to improving the RNAi efficiency, such as liposome modification and nano particle embedding method, which can reduce the degradation efficiency of dsRNA and promoting the study of insect RNAi technology. We need to do more research in order to applicate better this technology in pest control.

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Цао Чжишань, *аспірантка, Сумський національний аграрний університет, м. Суми, Україна, Школа ресурсів і навколишнього середовища, Інститут науки і технологій Хенань, Сіньсян, КНР*

Власенко В.А., *доктор сільськогосподарських наук, професор, Сумський національний аграрний університет, м. Суми, Україна*

Перспективи застосування та удосконалення технології інтерференції РНК у боротьбі з шкідниками

Через серйозні проблеми, які спричиняють хімічні пестициди – «3R» (формування стійкості до них, переродження та шкідливі залишки), вони поступово замінюються в сільському господарстві іншими методами боротьби з шкідниками та патогенами. Водночас, трансгенні культури, що експресують токсини Bacillus thuringiensis (Bt), були широко залучені для боротьби з комахами-шкідниками, але еволюція стійкості до Bt у задіяних шкідників *загрожує надійності такого підходу. Дослідження щодо пошуку нових способів ефективної боротьби з шкідниками ніколи не припиняються. В останні роки отримав визнання біологічний метод боротьба з шкідниками, оскільки дає перевагами щодо відсутності забруднення та безперервного впливу на довкілля. Проте, біологічні методи також стикаються з багатьма проблемами: на їхню ефективність сильно впливають фактори зовнішнього середовища або комаха-господар; важко отримати велику кількість комах природних ворогів шляхом штучного розмноження, це дуже дорого для виробництва у лабораторіях комах – природних ворогів. З розвитком технологій молекулярної біології за допомогою сучасних молекулярно-біологічних мішеней отримано нову тенденцію для біологічної боротьби з шкідниками. Інтерференція РНК (RNAi) є висококонсервативним механізмом приглушення генів після транскрипції, який існував у комах. За допомогою цього мРНК націлена на деградацію дволанцюгової РНК (dsRNA) або гомологічної мРНК, що призводить до інгібування специфічної послідовності генів. Технологія RNAi не тільки відіграє важливу роль у вивченні функціонального геному комах, але також має великий потенціал у боротьбі з шкідниками. У 2001 році технологію RNAi було занесено журналом Science до складу десяти найкращих наукових проривів, а також його першовідкривачів удостоєно Нобелівської премії у 2006 році. RNAi має високу ефективність і сильну специфічність, широко використовується для вивчення функції цільового гена або дослідження експериментального лікування хвороб. Коли цільові гени комах знищувалися за допомогою РНК-і, це завжди призводило до загибелі комах або поведінкових дефектів. Це є екологічно чистий біотехнологічний метод для боротьби з шкідниками, він рідко викликає стійкість до інших інсектицидів. Тому технологія RNAi розглядається як потенційна стратегія боротьби з шкідниками, яка має великий потенціал, корисна для захисту від комах та розробки нових пестицидів тощо. Ця технологія успішно використовується у комах Hemiptera (напівкрилих), Orthoptera (прямокрилих), Diptera (двокрилих) та інших. У цій роботі в основному описано механізм приглушення RNAi, кілька способів трансплантації dsРНК комахам та фактори, що визначають ефективність RNAi при застосуванні на комахах. Нарешті, ми також розглянули існуючі проблеми та деякі поточні рішення технології RNAi, щоб додатково обговорити механізм та існуючі проблеми RNAi, які застосовуються у боротьбі з шкідниками. Це додає нове уявлення про боротьбу з шкідниками за допомогою технології RNAi.*

Ключові слова: *технологія RNAi, ген-мішень, біологічний метод боротьби з шкідливими комахами, захист від шкідників у сільськогосподарському виробництві.*