RESEARCH

A Comprehensive Review on Potential of RNA Interference for Pest Control

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ABSTRACT

The invasive human population needs the event of latest agricultural technologies to satisfy consumers´ demand and minimize the environmental impacts. Currently the management technologies are relying more on the use of pesticides instead of transgenic crops which could be one of the reasons these crops are facing efficacy challenges. Chemical pesticides causes a great deal of damage due to which there is an exigent need to develop economically and ecologically more opportune alternatives for pest control and RNAi is one of such incipient alternative which reduces the damage from insect pests. RNA interference. (RNAi)is a new generation technique which incorporates gene silencing mechanism triggered by providing double-stranded RNA (dsRNA), which when ingested into insects can lead to death or affect the viability of the target pest. This process basically relies on plants stably expressing double-stranded RNAs (dsRNAs) that target essential genes in pest insects. Recent studies have shown that plant-mediated RNA interference (RNAi) shows great potential in crop protection. A better understanding of the mechanisms that verify the variability within the sensitivity of insects would accelerate the worldwide unleash of business RNAi-based approaches. The purpose of this review article is to discuss in detail the mechanism of RNAi as a sustainable tool for pest control Management and the potential of this technology as an alternative to the traditional chemical approaches.

KEYWORDS. RNAi, Gene Silencing, Double Stranded RNA, Transgenic crop, Pest Control Management.

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INTRODUCTION

Despite the frequent use of insecticides and other ongoing efforts to increase productivity, approximately 18-20% of the worldwide crop harvest remains lost mainly due the persistent complications caused by pest insects (Sharma et al., 2017). It has greatly reduced the productivity in major crops to a extent that the yield is far from usual average global estimates. To state the total global potential loss in numbers, amid the food crops it has varied from 50% in wheat to an estimate of 31% in maize and 37% in rice (Oerke, 2006). One of the major underlying cause is the resistance of insect population against the foremost commonly used insecticides which poses a persistent threat to agriculture (Tabashnik et al., 2013; Zhu et al., 2016). Moreover, the catastrophic impact of chemical insecticides used on the environment and other organisms, like beneficial insects, cannot be overseen. Taking the previous statements into consideration, it becomes evident that the present array of insect pest combating methods is insufficient to secure global food production for subsequent decades. Finding alternative options to enhance plant protection strategies is therefore critical. In this context, a stimulating perspective is represented by the RNAi technique (Vogel, Santos, Mingels, Verdonckt, and Broeck, 2019).

The prospects of RNAi mechanism is inherent in its mode-of-action, i.e. the degradation of complementary target mRNA upon entry of specific dsRNA into the cell (Agrawal et al., 2003). Therefore, by delivering dsRNA targeting any endogenous gene transcript to the intended pest organism, expression of this gene is often knocked down at the post-transcriptional level. Thus, through careful selection of an important target gene, this mechanism can cause insect mortality. The sequence-specific nature and therefore the possibility to theoretically target any non-conserved, lethal gene, makes RNAi a perfect candidate for further application as a selected insecticide.

In a more relevant and comprehensible terms, RNA interference (RNAi) is a biological mechanism which results in post transcriptional gene silencing (PTGS) trigger by double stranded RNA (dsRNA) molecules to stop the expression of specific genes. RNAi mechanism has the potential in identification and functional assessment of thousands genes within any genome that's liable for crop improvement. This propitious approach imparts an effective and efficient role to knock down the expression of any particular gene through short interfering RNA molecules in any target cell and moreover to assess the variation that occur in signalling pathways. Recently, RNAi has become a strong and more reliable technique to inhibit the expression of targeted genes and also determine gene loss-of-function phenotype which results in gene functional analysis when no mutant alleles are unavailable. RNAi technique was first time applied on hybrid petunia L. plants to reinforce anthocyanin pigment through introducing chalcone synthase gene. New pattern of flower color transgenic Petunia was observed, credits to overexpression of chsA gene that encodes major enzymes in anthocyanin biosynthesis pathway (Younis, Siddique, Kim, and Lim, (2014).

MECHANISM OF RNA INTERFERENCE

As stated above RNAi is a simple gene silencing method expressed in variety of organisms. The gene silencing is the result of degradation of a double stranded RNA, exogenously introduced into various eukaryotic organisms, into short interfering RNAs with the aid of activated ribo-nuclease enzyme known as Dicer. The outcome of this cleavage is ~25 nucleotides of sense and anti sense polarity short Interfering RNA strands. Based on the most well studied results, one of the strand pairs with the

complementary sequence of mRNA resulting in post-transcriptional gene silencing (FIgure 1.)

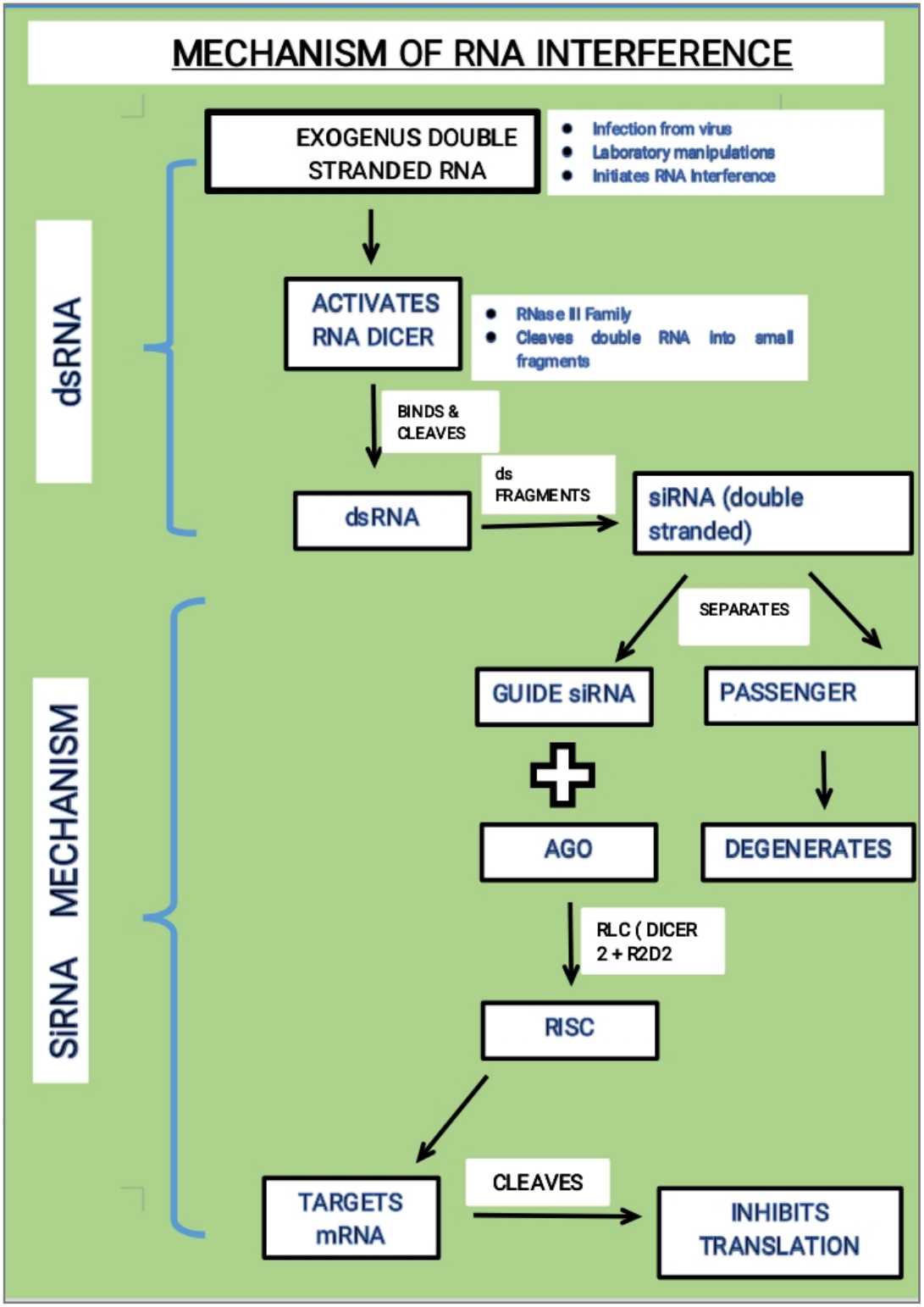

Figure 1. Flow chart on Mechanism of Gene Silencing

To elaborate, the mechanism of RNA interference involves:

1. Transgenic crops
2. Insect feeding and dsRNA uptake
3. siRNA Mechanism

1. TRANSGENIC CROPS

- The introduction of exogenous double-stranded RNA (dsRNA) into the cells of numerous eukaryotic organisms has been shown to induce speedy and sustained degradation of mRNAs containing sequences complementary to the dsRNA (Mello and Conte,2004).

- Developing Transgenic Plants: In dsRNA expressing plants, an inverted recurrent sequence are cloned into a vector followed by the introduction of cloned vector into a selected bacteria. Then the plant to be transformed is injected with the bacteria which enables the integration of plasmid vector into the plants genome (Kunte, McGraw, Bell, Held and Avila,2020)

2. INSECT FEEDING AND DOUBLE STRANDED RNA UPTAKE

Once the transgenic plants are ready with the dsRNA, they have to be delivered into the cells of insects to initiate the gene silencing method. The delivery of these RNA molecules can occur through two ways:

I. Direct Uptake: Direct feeding the transgenic plants is one of the most common strategies to achieve RNAi effects. It occurs when the RNA molecules are directly fed to the insects or through topical contact(Cagliari et al., 2019)

II. Indirect Uptake: It demands the insect to initially enter the plant vascular system followed by the uptake of RNA molecules from the system (Cagliari et al., 2018)

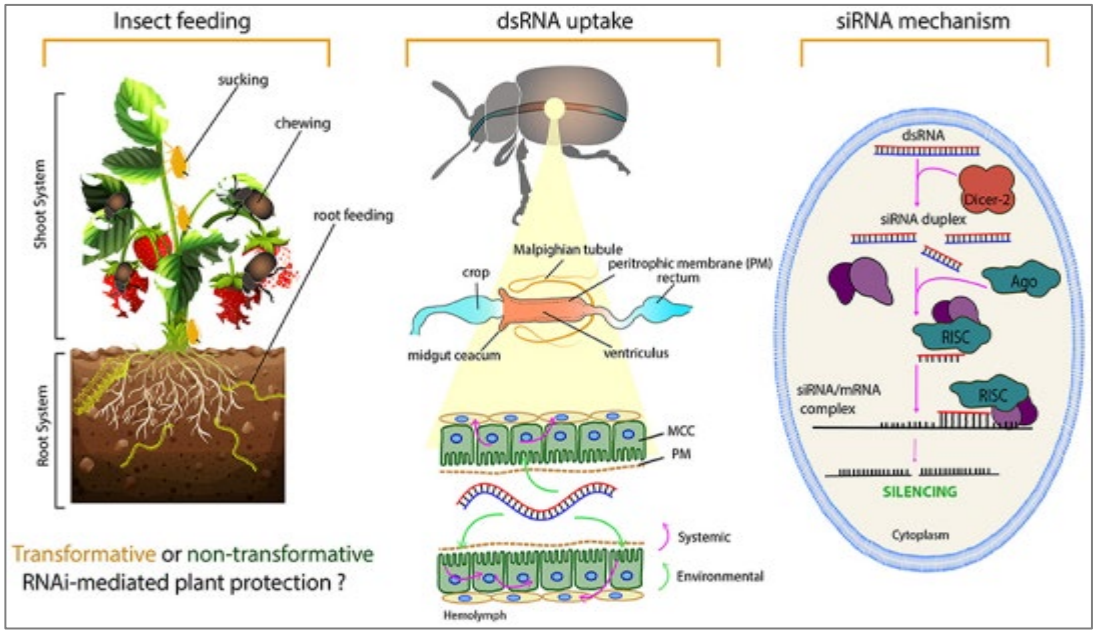

Figure 2. Image reference: Joga MR, Zotti MJ, Smagghe G and Christiaens O (2016) RNAi Efficiency, Systemic Properties, and Novel Delivery Methods for Pest Insect Control: What We Know So Far. Front. Physiol. 7:553. doi: 10.3389/fphys.2016.00553

3. SiRNA MECHANISM

Once the RNA molecules are taken up by the insect cells, it activates the Dicer enzyme (RNase III family) that initiates the cleavage of long double stranded RNA into short ~21-25 nucleotides double stranded fragments(siRNA). The cleavage is followed by the unwinding of these double stranded RNA molecules into two single stranded RNAs, the guide strand and the passenger strand. The passenger strand is discarded eventually leading to its degradation. Rest of the process of Gene silencing is carried out by the Guide strand.

Eventually the guide strand becomes incorporated into a supermolecule referred to as the RNA Induced silencing complex (RISC). After the RISC is formed it is guided to a specific mRNA which is complementary to one of the strands of the small interfering RNA that eventually causes its degradation. Here Argonaute protein is the major component in the RISC eventually mediating recognition and cleavage of the target (Hammond et al., 2001).

According to Whangbo and Hunter (2008) three forms of RNAi response can be defined: cell autonomous, environmental and systemic, with the latter ones referred additionally referred along as the non-cell autonomous. With relevance to the effort, the focus has been mainly on non-cell autonomous RNAi (Gu and Douglas, 2013).

The discovery of the mechanism of RNAi has revolutionized the experimental gene regulation. Particularly siRNAs targeting complementary mRNAs can be effortlessly designed and swiftly synthesized.

CONCEPT STUDY ON RNAi BASED PEST CONTROL

A feasible study results that a proof-of-concept study was carried out in the year 2007 by Baum et al. In this research, a transgenic corn crop was genetically engineered to modify the precision of dsRNA against the V-ATPase. The transcript of the Western corn rootworm that is Diabrotica virgifera virgifera. Feeding D. virgifera with this modified plant caused in larval stunting resulting in the premature death of insect Additionally, dsRNA behaved as a crop protectant as it vastly reduced the feeding damage of the transgenic crop (Baum et al., 2007).

Table 1. Examples of recent studies of RNAi technology.

Transgenic Crop	Target Pest/Insect	Insect/Pest Order	Target Gene	Aim	References
Citrus aurantifolia	*Citrus tristezavirus*	Virus - Martellivirales	CTV - CP	Transgenic plant with coat protein	(Domínguez, de Mendoza, A.H., Guerri, J. et al., 2002)
Medicago sativa	*Acyrthosiphon pisum*	Homoptera	C002	Knockdown of salivary gland gene and toxic to insects.	(Mutti, N. S., Park, Y., Reese, J. C., & Reeck, G. R., 2006)

Oryza sativa	*Magnaporthe grisea*	Fungus - Magnaportha los	OsSSI2	Knockdown for gene responsible for fatty acid desaturase activity.	(Jiang, C. J., Shimono, M., Maeda, S., Inoue, H., Mori, M., Hasegawa, M., Sugano, S., & Takatsuji,2009).
Nicotiana Benthamiana	*Myzus persicae*	Hemiptera	M. persicae Rack1	Knockdown of Salivary gland and gut tissues with reduced aphid proliferation	(Pitino, M., Coleman, A. D., Maffei, M. E., Ridout, C. J., & Hogenhout, S. A.,2011)
Arabidopsis thaliana	*Myzus persicae*	Hemiptera	Serine protease gene (MySP)	Serine protease gene (MySP)"Reduced ability to produce the offspring but no harmful effects"	(Bhatia, V., Bhattacharya, R., Uniyal, P. L., Singh, R., & Niranjan, R. S.,2012)
Nicotiana tabacum	*Helicovrpa armigera*	*Helicovrpa armigera*	Transcription factor gene HR3	Deformation in developmental stage and larval mortality.	(Xiong, Y., Zeng, H., Zhang, Y., Xu, D., & Qiu, D., 2013)
Nicotiana rustica	*Benisia tabaci*	Hemiptera	vATPase	Mortality in dsRNA expressing tobacco plants.	(Thakur, N., Upadhyay, S. K., Verma, P. C., Chandrashekar, K., Tuli, R., & Singh, P. K.,2014)
Zea mays	*Diabrotica v. virjifera*	Coleoptera	vATPase	High efficiency in root protection but not lethal to insects.	(Li, H., Khajuria, C., Rangasamy, M., Gandra, P., Fitter, M., Geng, C., et al.,2015)
Solanum tuberosum	*Leptinotarsa decemlineata*	Coleoptera	Hairpin version of beta and Shrub	Mortality after 5 days	(Zhang, J., Khan, S. A., Hasse, C., Ruf, S., Heckel, D. G., & Bock, R.,2015)
Arabidopsis thaliana	*Helicovrpa armigera*	Lepidoptera	Arginine kinase (HaAK)	Mortality rate of 55% in develpmental stage and growth retardation in adult stage.	(Liu, F., Wang, X. D., Zhao, Y. Y., Li, Y. J., Liu, Y. C., & Sun, J.,2015)

- A cognate study was implemented for the corn earworm Helicoverpa armigera. In this research, Mao et al. (2007) showed that plant mediated expression of dsRNA which targets the cytochrome P450 mono oxygenase gene (CYP6AE14) could intensify the toxic effect of gossypol which is a cotton metabolite which was otherwise tolerated by the cotton bollworm Silencing of CYP6AE14 led to delayed growth of larvae when gossypol was added within the diet (Mao et al., 2007)

On the basis of studies mentioned above, it is apparent that RNAi technology has a prodigious potential for control of different types of insect pests. In addition, RNAi technology integrated with Bt or other technologies extends an eminent choice in controlling the insects pests, that have high

probability to develop resistance against the insecticidal proteins. However, to initiate the true potential of RNAi to combat the insect pests, more intense development and refinement of technology in large-scale is required. Likewise, for any other pest control strategy to succeed, the potential risks associated with the same should be evaluated.

CHALLENGES

In order to use RNAi as a tool to control and manage pest there are various challenges including the cost of dsRNA triggers, efficient system to delivery dsRNA to site of action, other non target effects of dsRNA, and possible resistance development. (Whyard,Singh, & Wong,2009) fed in vitro synthesized dsRNA to insects and successfully silenced target genes; this suggests that it is possible to achieve silencing of target genes by feedings dsRNA. On the other hand it becomes challenging to use dsRNA feeding method for insect control as it highly expensive to produce dsRNA. Another major complication is to deliver the dsRNA effectively to the site of action. Also, the half-life of naked siRNA in serum ranges from several minutes to about an hour. (Bartlett, & Davis, 2007) A high dose of dsRNA or siRNA is required as the trigger degrades rapidly in an organism.

The most important concern is that insects developing resistance to the dsRNA based products. There are various mechanisms through which insect can develop resistance including mutations of target genes, mutations of RNAi core machinery genes, enhanced dsRNA degradation, increased dsRNA excretion, decreased dsRNA uptake and transport.Insects can cause resistance by the method of mutation and polymorphism which might cause mismatch between the dsRNA and mRNA sequences within the target genes leading to the evolution of resistance. Polymorphism is common in nature and associated with diversity and evolution. Mismatching because of polymorphism are often decreased by screening a lot of potential targets and their frequency of polymorphism to spot preserved domains across species by bioinformatics tools. RNA interference through feeding holds a great value as a pest control technique. However, there are many information gaps that are required to be considered before it is commercialized. Considerations associated with the potential effects on non target organisms, fate of dsRNA within the environment, adequate quantity, and an appropriate methodology of administration should be taken into account. However, the question rises here that a replacement era of insect management supported RNAi guarantees new opportunities for effective and a lots of target selective pest management. (Zhu, & Palli,2020)

CONCLUSIONS AND FUTURE PROSPECTS

In the process of insects pest management RNAi can play an important role once the research related issues are solved. For the employement of RNAi as a poster product we need to spot the target genes which are accountable for the killing of the pest or inhibiting the toxicity of insects.

Since the thought of a transgenic plant expressing dsRNA targeted a specific essential sequence within the insect that affects its viability was first developed in 2007, the technology has been extended to several variety of insect species from numerous orders. Elucidating the various mechanisms and elements of the RNA interference pathway has progressed, however several aspects stay to be processed. Several variations in elements and mechanisms among insect orders and between insects and alternative organisms still have to be compelled to be found out.

A number of those variation (example: gene concerned, gene number, and level of expression)

could justify variation in refractoriness among insect species any generates a necessity of investigation. Further insect- or order-specific characteristics, like gut pH, presence of dsRNA-degrading activity in gastrointestinal system, among others that would be related to variations in refractoriness to RNAi should be cleft and processed.

Due to the variability of RNAi response to RNAi in insects, no single protocol is appropriate for all species. Issues associated with the selection of effective target genes, together with deciding the scale of best dsRNA length and ideal sequence region.

Assuming that the strategy of option to deliver dsRNA is transgenic plants, a serious question still to be addressed is that the impact of plant dsRNA process within the effective RNAi-induced silencing. There's still a necessity for investigation during this space. The selection of an acceptable inducible promoter for expressing the dsRNA construct is another purpose barely explored.

Based on the recent publications studied it is evident that, the progress in developing RNAi-plants to control the damage caused by insect pests widely shows the potential of this technology to enhance Bt crops and replace the current chemical insecticides and thus providing resistance against a broad type of insect pests. However, to be applied on a commercial level, many problems associated with the RNAi mechanism and safety still got to be self-addressed. As a brand new technology, risk assessments and government rules still ought to be developed. However, in terms of acceptance and safety and efficacy challenges RNAi posses a wider acceptance in the society due to the specific RNAi traits than a protein incorporated into a plant, like Bt transgenic crops(Scott, Michel, Bartholomay, Siegfried, Hunter, Smagghe, Zhu, & Douglas, (2013) Thus, RNAi-mediated pest management can open a brand new paradigm in insect pest management (Rodrigues and Figueira, 2016).

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Conflict of Interest

Authors declare no conflict of interest.

Author's Contribution

P.K. and R.S. have collected the data from different sources, reviewed, analysed it and prepared the final draft of the manuscript. A.S. helped in reviewing the final draft and approved it.

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