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RNA interference: A Sustainable Approach for Pest Management in field crops

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Abstract

In modern Agriculture, Pesticide resistance in pest leads to development of new ways to control the pests. Raising the formulation of pesticides causes significant damage to the environment and on other side percentage of risk for humans & other beneficial organisms which depends on this crop also crossed new levels. To maintain Sustainability of environment scientists came up with new eco-friendly approach popularly known as RNAinteference (RNAi). The RNAi works at mRNA level by utilizing sequence dependent mode of action with high target specificity due to development of complementary dsRNA sequences. By exploiting this complementarity, eradication of pest will become more precise compare to chemical pesticide. RNAi works at Molecular level by silencing the genes of pests by complementary dsRNA leads to lethal effects, this can be achieved by both transgenic and non-transformative approaches, For, instance non-transformants like biopesticides can be applied topically on the leaf surfaces, once after the application the dsRNA can take up by plant cell then transferred to pest once it chews the plant parts or dsRNA directly transferred to pest directly. Since the development of transgenic plants are costly these non-transforming methods are making waves in market. Though it is effective for pest management, developing of transgenic plants faces many restrictions such as issues like RNA efficiency, dsRNA degradation, environmental risks. Overall, this review aims to discuss about various mechanisms for effective control of pests in sustainable manner.

Introduction

In Agriculture system pest become major problem and causing significant losses in yield production. Global population number spikes rapidly over past few decades which ultimately leads to increase in food production. But for instance, insects alone responsible for yield loss about 20-40% (Oerke, 2006). Researchers expecting a spike in insect damage about 10-25% as the global temperature increase in next few years.

To overcome this challenge resistant genotype has been developed over the decade which shows a minimal impact on pest control. Alternatively, Transgenic crops are also coming into market which created a revolution but it cost of production is enormous and transgenic may also develop allergic

reaction for consumers. At last chemical pesticides has been using over from since 1930s. These pesticides allow growers to minimize pest damage, increase yield production and earns better profits. The chemical usage is rapidly increasing from year to year which leads significant raise in pesticide resistance among the insects and it also effect environmental health. RNAinteference is a natural defensive mechanism works by silencing the gene at Transcription or Post Transcriptional level. RNAi, also called as Post Transcriptional gene silencing (PTGS). This gene silencing system can defend many abiotic stresses like Pest, virus and disease attack. The process of RNAi was effective at mRNA level where it degrades the mRNA by cleaving it leads to elimination of target gene expression. Silencing of essential gene ultimately shows effects in growth and development of insect finally death of the Insect. The transfer of dsRNA into the insect body can be achieved by both Transformative method (plant Incorporated protectant) and non-transformative method (non PIP). PIP is conquered by developing Transgenic plants. The resistant transgenic plant possesses the genes which codes for the double stranded RNA which is transferred into pest by sucking plant parts. On Contradictory non-transformative methods like foliar spray, root irrigation, seed treatment is done by bio pesticides. There are still many issues to be addressed such as cost of production of these products, environmental factor, danger to beneficial insects, RNAi efficiency. Overall, this study focuses on developing the RNAi based mechanism for future research and approach.

Mechanism of RNAinteference in insect pests

RNAi mechanism works at post transcriptional level by inducing the expression of gene using dsRNA. This RNAi was 1st discovered in *C elegans* (a nematode). Since then RNAi well explained in almost every organism inclusive of protozoans, fungi, plants. Small RNAs (miRNA and siRNA) formed from dsRNA degrade the mRNA by complementary base pairing and halts mRNA from protein formation. Small interfering RNAs (SiRNAs) produced from long dsRNA precursors processed by the dicer a RNase III endonuclease into 21-24 nucleotides sequence. The SiRNA, functions to find specific mRNA sequence by pairing complementary to it. Almost all eukaryotes contain dicers as they perform various functions in producing several types of small RNA. R2D2 a dsRNA binding protein carries the dicer generated dsRNA to RISC complex (RNA induced silencing Complex). The dsRNA in RISC complex than unwind forming passenger and guide strand. RISC complex later destroys passenger strand and the guide strand navigates RISC complex on mRNA until it finds complementary sequence. Then Argonaute (Ago) proteins in RISC complex degrades the target mRNA, preventing it from translation.

Another group of small silencing RNA mole are miRNAs (MicroRNAs) are small endogenous non-coding RNAs present in the genome of plants which regulate gene expression post

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transcriptionally by influencing the growth of insects. In this type of pest control, artificially synthesized RNA introduced into the insect cells and then leads to gene knockdown.

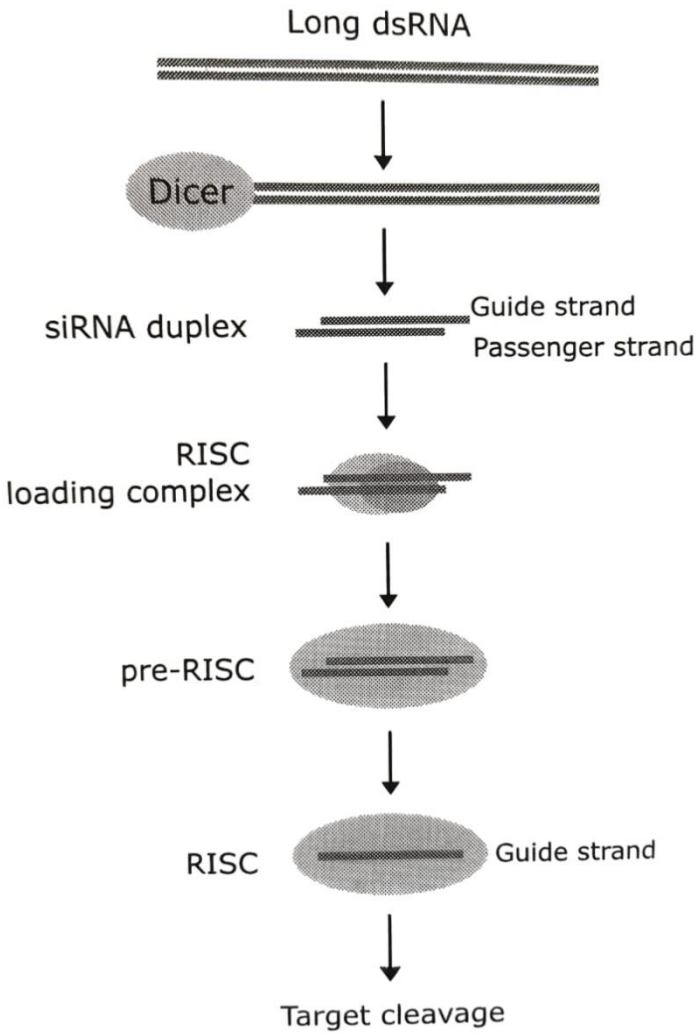

Fig 1: dsRNA precursors are processed by Dicer to generate siRNA duplexes containing guide and passenger strands. RISC-loading complex loads the duplex into RISC. The passenger strand is later destroyed and the guide strand directs RISC to the target RNA.

RNAi Delivery Methods in Crops

` RNAi is a strong tool to control pest by suppressing the gene expression. In many studies it is used a tool for reverse genetics to know the function of newly identified gene. RNAi as the pest control method has been effective in many pest orders such as coleoptera, Lepidoptera, Hemiptera.

Application of RNAi in agriculture for pest control can be done by different ways namely Host induced gene silencing (HIGS), Spray Induced gene silencing and virus Induced gene silencing (VIGS). The mechanism of HIGS is carried out by developing the transgenic plants which can codes the dsRNA specific for the pest. We can control numerous insects such as *H armigera*, *Bemisia tabaci*,

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H.zea. However, the development of transgenic crops for dsRNA against target pest takes many years and consumes dollars to make Plant commercialize against strict rules from regulatory committee. Alternatively, VIGS is novel method for delivering the dsRNA into the pest itself by engineered viral vectors. Virus attacking the insects can be modified to carry an insect target sequence. Then, after viral infection and the dsRNA are produced inside the insect during virus replication. A Successful transfer of dsRNA through VIGS has registered in which Flock House virus induced gene suppression in Fruit flies (*Drosophila melanogaster)*. However, dsRNA delivering through recombinant virus method have many limitations as the viruses possess RNAi suppressors. SIGS, is a non-transgenic method solely works by spraying (foliar application) and also used for root absorption. After dsRNA spraying it is transferred into insect after insect feed upon the plant. Due to temporary lasting nature of dsRNA in SIGS method it could be less effective as compared to transgenic method. Ledprona, a dsRNA based biopesticide is currently under review in USA.

Tab 1: HIGS mediated dsRNA coding transgenic crops in pest control

Transgenic Plants	Insects	Target genes	References
Nicotiana tobacum	*Helicoverpa armigera*	CYP6AE14 Chitinase	Mao et al. (2007) Mamta et al. (2016)
Oryza sativa	*Nilaparvata lugens*	Hexose transporter Carboxypeptidase	Zha et al. (2011)
Triticum aestivum	*Sitobion avenae*	Carboxylesterese Chitinase	Xu et al. (2014) Zhao et al. (2018)
Glycine max	*Tetranychus urticae*	Coatomer subunit α Aquaporin 9	Dubey et al. (2017)
Zea mays	*Diabrotica virgifera virgifera*	v-ATPase A vitellogenin receptor	Baum et al. (2007) Niu et al. (2017)
Saccharum spp	*Sphenophorus levis*	V-ATPase E	Mohan et al. (2021)
Brassica juncea	*Lipaphis erysimi*	sucrase1	Dhatwalia et al. (2022)

Table 2: Transfer of dsRNA into insects by non-transformative method

Crop	Target pest	Target gene	Reference
Zea mays	*Ostrinia furnacalis*	KTI	Gogoi et al., (2017)
Solanum lycopersicum	*Myzus persicae*	ZYMV HC-Pro	Gogoi et al., (2017)
Solanum tuberosum	*Leptinotarsa decemlineata*	Actin	San Miguel and Scott., (2016)

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Nicotiana tobacum	*Planococcus citri*	CHS1 V-ATPase	Khan et al., (2013)

Advantages of RNAi Based Pest Control

RNAi possess numerous advantages over conventional method of pest controlling. As the RNAi based biopesticides lacks chemical it shows no effect on Environment and it promotes sustainable Agriculture. Normal chemical pesticides play major role in environment damage and polluting water bodies and it also shows significant effect of human heath as well. In alternative RNAi based biopesticides are effective in pest control as well as they a biodegradable doesn't have any side-effects

RNAi based pest control doesn't harm any beneficial organisms as they are highly specific for pest to show effect unlike conventional broad-spectrum pesticides. This specificity approach is significant for preserving biodiversity. Furthermore, many insect developing resistances against chemical pesticides and this obstacle can be achieved by implementing RNAi based bio pesticides or transgenic plants. As this RNAi specific it doesn't show any effect to human health. Development of pest resistance can be achieved by RNAi. It reduces cost of production to farmers by eradicating pest in effective way and avoid the use of chemical pesticide. In modern Agriculture, multiple pests are attacking same crop and farmers are depending upon different pesticides to control. RNAi customization for these pests can be done to control at once avoiding use of multiple pesticides.

Challenges and limitations.

RNAi made a revolution in modern agriculture pest control methods, in contrast it has many limitations and challenges which needs to addressed before it gets adopted in agriculture. one of the main concerns regarding RNAi technology in the effect on non-target organisms. Various Pest species reacts differently to dsRNA due to dissimilarity in RNA intake, processing and its response to dsRNA molecules. Variations in uptake of dsRNA show significant effect on pest control. Insects of coleopteron order exhibit strong RNAi responses and whereas insects of Lepidoptera uptake less dsRNA and shows reduced RNAi efficiency. Instability of dsRNA molecules in field conditions is biggest drawback. Environmental effects like Temperature and UV radiation makes dsRNA degrade quickly turning down their effectiveness in open field conditions. Though RNAi is effective, when it comes to production of better-quality dsRNA requires more investment which ultimately raises the purchase cost for farmers. At present chemical pesticides are available at half cost of RNAi based bio pesticides and farmers are not interested to purchase at higher costs.

Efficient transfer of ds RNA into pest remains as a major challenge, as ingestion is mandatory to express it effects by silencing gene expression. Many of the pest's possess strong nucleases in their

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gut, which immediately breaks down the ingested dsRNA. Even, it may have many off-target damages. Some dsRNA sequences may farm complementarity in some beneficial insects like pollinators, Pest predators showing effect on their growth often leads to death. Transgenic crops may cause allergic effects in humans. To commercialize the RNAi based products could faces many challenges like safety regulations and effectiveness of RNAi based biopesticides causing delay in registration and production approvals can be halted until it ensures no effect on environment as well as on human health.

Furthermore, the major risk is development of pesticide resistance by insects. Mutations in genome of insects changes the sequences in genome as a result dsRNA can't find complementary Sequence making the pest resistance to RNAi. Efficiency will be limited if dsRNA uptake by Insect is reduced. In order to unlock full potential of RNAi researches should put more efforts in improving RNAi stability, cost efficiency, delivery methods and need to focus on regulatory guidelines for approval. By unlocking its immense potential, RNAi brings revolution in pest management in agriculture by reducing dependency on chemical pesticides.

Future Prospects and Research Directions

Increase in the global population should meets Food demands which can be increased by mitigation the yield loss in Agriculture. The significant problem of reducing in yield is due to attack of pest which in turn can be reduced by spraying conventional pesticides. Continuous usage of the pesticides damages the environment and causes significant loses in ecosystem. In order to reduce these problems RNAi mechanism will be the solution. RNAi based pesticides have many advantages over chemical pesticides such as pest specificity and more sustainability. Biopesticides are made by improving quality, environment safety and Safe for human consumption.

As the new class of biopesticides made a revolution in Pesticide usage in commercial agriculture. dsRNA action mechanism, finding of target genes by screening has been addressed and solved. However, most of the developed biopesticides are effective in controlled micro environment. Many issues will have to face during field conditions as dsRNA uptake and its effectives are highly dependent on environmental conditions. In future directions, the cultivation of dsRNA expressing transgenic plant varieties should be improved. Furthermore, the major obstacle in developing transgenic crop varieties is that not all plants can be transformed into transgenic. Even, if a crop transgenic for specific pest it can't altered further for developing resistance to another pest. To overcome all these hurdles researchers should apply multidisciplinary knowledge including Molecular biology, Nano biotechnology and Bioinformatics. Additionally, integrating RNAi with genome editing technology like CRISPR cas-9 should enhance Pest Control Strategies.

Conclusion

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RNAi emerged as revolutionary mechanism for precise pest control, show less effect on environment and Sustainable method of for pest protection. Based on effectiveness of RNAi, researchers, are interested in identification of genome of specific crop pests. Further, the main challenge to apply RNAi in large scale commercial level is the lack of valid mechanism for dsRNA delivery into pest. Developing of transgenic plant makes way for successful transferring method, on other hand non-transformative application like foliar spray, root absorption is also proved as effective transfer method. Undoubtedly, the RNAi based pest control method will dominate over conventional pest control methods and also stand out as efficient pest protection mechanism.

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