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RNAi-Based Biopesticides: Commercialization, Regulatory Hurdles, and Public Perceptions

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Abstract
The biggest group of disease-causing organisms on agricultural plants, plant pathogenic fungi provide a chronic and serious danger to agriculture all over the world. Alternative control strategies are desperately needed since traditional methods that rely on the use of pesticides increase societal concern about the impact on the environment and human health. This article primarily focused on the use of RNAi technology to enhance plant disease resistance and its applicability in the modern world in terms of exogenous dsRNA application.It also concentrated on the development of dsRNA-based biocontrol products, their dissemination to the public, and eventual commercialization.

Introduction
The longstanding practice of utilizing synthetic pesticides for pathogen control has faced significant challenges in recent years. The emergence of pathogen resistance to these pesticides, combined with growing apprehensions about environmental and human health impacts, has fueled a growing need for alternative methods that are more targeted, eco-friendly, and cost-efficient in managing pathogens and pests Nicolopoulou-Stamati et al.,2016.

In recent times, the concept of gene silencing via RNA interference (RNAi) has emerged as a promising avenue for precise breeding and the creation of innovative solutions aimed at safeguarding plants against pathogens and pests. RNAi is a well-preserved mechanism within eukaryotic organisms, activated by double-stranded RNA (dsRNA) molecules. This mechanism is involved in various regulatory functions across eukaryotes, such as defending against viral infections, managing the mobility of transposons, maintaining genome stability, controlling gene expression, and establishing heterochromatin structures Ketting et al.,2011; Castel.

Long double-stranded RNAs (dsRNA) are converted into small interfering RNAs (siRNA) by the RNAi pathway. These siRNAs precisely attach to and break the targeted viral messenger RNAs (mRNA) in the cytosol, providing excellent plant defence Mezzetti et al.,2021. This versatile tool's vast potential is actively harnessed for the manipulation of genes associated with pests and pathogens. Methods like host-induced gene silencing (HIGS) (Nowara et al. 2010) and spray-induced gene silencing (SIGS) (Koch et al. 2016) are being employed at a significant scale. Their application holds great promise in revolutionizing the field of integrated pest management, as outlined (Koch et al. in 2019).

Mechanism of RNAi

A) In the process of RNAi-induced gene silencing, lengthy dsRNA or hpRNA is cleaved into short interfering RNA duplexes (siRNAs) that typically consist of 21 to 23 nucleotides. These siRNA duplexes uncoil, and one of the strands, known as the Guide strand, becomes part of the RISC complex, ultimately leading to the degradation of the targeted mRNA with a complementary sequence. The RISC complex can then be reused.
B) Within Arabidopsis thaliana, the processing of long dsRNA is facilitated by Dicer enzymes. DCL2 produces 22-nucleotide siRNAs, which interact with argonaute proteins (AGO1) and bring RDR6 into play, resulting in transitive silencing. DCL3 generates 24-nucleotide siRNAs that engage with AGO4 and trigger DNA methylation by enlisting DNA PolV. Meanwhile, DCL4 generates 21-nucleotide siRNAs, which interact with AGO1 and guide the cleavage of mRNA sequences with matching complementary segments.

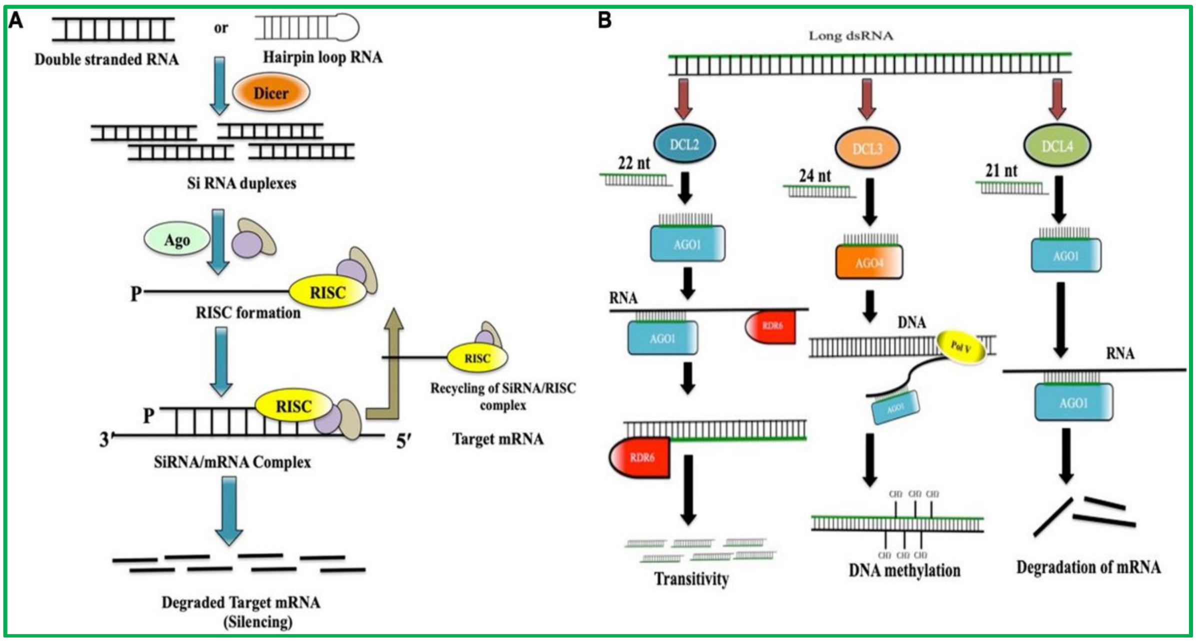

Figure 1. RNAi mechanism (Source: Halder *et al*.. 2022**)**

Significance of RNAi in the Contemporary World: Exploring the Exogenous Use of RNAi-Inducing dsRNAs

For a considerable duration, the creation of GMOs/transgenics has sparked widespread controversy globally. Furthermore, following the age-old adage that "Necessity is the mother of invention," the extensive public outcry and opposition from the anti-GMO scientific community prompted the exploration of alternative methods. These novel approaches circumvent transgenics and instead involve the direct application of RNA molecules (specifically dsRNA-containing end-use products, referred to as dsRNA-EPs) to initiate the necessary RNAi response.

Exogenously applied dsRNA on plants has the capacity to initiate RNAi-mediated gene silencing of the invading pest or the pathogen genes. The effectiveness of RNAi-based methods in pest control relies on various factors, including the dynamics of host-pathogen interactions, structural attributes, RNAi machinery. The technique used to deliver the dsRNA biomolecules—the spray approach or mechanical inoculation—determines the extent to which exogenously delivered RNAi is effective. The delivery method differs depending on the plant type chosen and the targeted pest (Dalakouras et al., 2018).

The external use of naked dsRNAs, liposome-encased dsRNA, and synthetic extracellular vesicles (EV) that may be combined with protein carriers or nanoparticles has demonstrated substantial reduction in the expression of transgenes and numerous endogenous plant genes, as indicated by numerous studies Huang et al.,2019. The commercial usage of nanocarriers for crop protection against diseases has become very common in the current environment. They are mostly employed as RNA-based sprayable pesticides. In order to maximize advantages in the pest and disease control sectors, RNAi technology and nanotechnology have been successfully combined. The creation of Bioclay, or "layered double hydroxide clay nanoparticles," employed as a possible delivery or application method for dsRNA, is a development from the lab to the field (Fletcher et al.,2020).Recent findings suggest that DNA nanostructures, have been identified as effective carriers for RNA, particularly siRNA, in plant cells (Zhang et al. in 2019).

Commercialization, regulatory guidelines, and public acceptance of RNAi-based biopesticides

Leading the way in this endeavor is the worldwide corporation Monsanto, with their "Biodirect" brand currently in the stages of creating RNA-based biopesticides for pest management. This is closely followed by other major multinational companies like Bayer, Syngenta, and others (Taning et al.,2020).

Emerging companies like Greenlight Biosciences, AgroRNA, and RNAgri have initiated large-scale production of dsRNA at a cost-effective price point. Greenlight Biosciences, in particular, has introduced a pioneering cell-free bioprocessing method that can efficiently produce RNA sequences on a scalable basis. This innovation ensures the accessibility of affordable dsRNA for use in the field, all without the associated GMO label. A promising startup called AgroSpheres is focusing on safeguarding dsRNA from degradation while enhancing its targeted delivery to achieve maximum crop protection. They've achieved this by creating bioparticles consisting of tiny, non-chromosomal spherical cells capable of encapsulating dsRNA. Recent reports reveal that GreenLight and AgroSpheres have joined forces to further develop these bioparticles, creating a protective shield around dsRNA to shield it from degradation caused by RNases and ultraviolet radiation.

Drawing inspiration from these recent advancements, another biotech startup, Nanosur, has specialized in manufacturing modified RNA (MdsRNA) products designed for smooth and rapid translocation through cell membranes, thus improving effectiveness and minimizing degradation. Following in Nanosur's footsteps, another startup named TrilliumAg has launched its own agricultural platform, Agrisome, specializing in the production of self-assembled protein-based nanoparticles known as modified RNA molecules (MV-RNA). These molecules exhibit enhanced stability and efficient delivery to their intended targets.

Risk assessment within this domain primarily revolves around potential adverse effects on non-target organisms (NTOs) exposed to biopesticides. Potential concern arises when RNAi-inducing dsRNAs accumulate to optimal concentrations in NTOs. This can activate the NTOs' endogenous RNAi mechanisms, inadvertently causing unintended gene knockdowns, which can result in health hazards. To ensure safety, a series of experimental field trials must be conducted to rule out the possibility of such events and establish the RNA-based biocontrol compound as safe for use. Another major concern regarding biopesticides (dsRNA) is the formulations used to improve its retention power and delivery inside the target organism.

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Science Advisory Panel has proactively undertaken measures to tackle the possible ecological and human health hazards, seeking strategies to mitigate these risks. The European Food and Safety Authority (EFSA) plays a noteworthy role in evaluating the risk elements associated with RNAi-based genetically modified (GM) plants.

The agriculture industry is a highly intricate system with numerous intermediaries at various levels. From the research and development scientists who uncover these innovative biopesticides to the point where they become available to local farmers, the product must navigate through a series of steps. These include field trials, endorsement by appropriate biosafety committees for commercial release, production facilities of agritech startups, distribution channels, and engagement with other stakeholders along the way.

Therefore, the agricultural industry and the research and development sector serve as the essential link between farmers and the general public. It is their responsibility to step forward and provide transparent information about the advantages and disadvantages of recently introduced biopesticides in the market. The overarching goal is to underscore the importance of rigorous research and evidence-based product approval by authorized biosafety committees, facilitating the adoption of these innovative tools to promote a sustainable agricultural system. Exogenously used dsRNA or "Biopesticides" have the potential to significantly reduce social opposition to GMOs and worries about chemical pesticides. Its successful use in pest control ensures that RNAi is still relevant today. The public's widespread awareness of these biopesticides and good communication to hasten their commercialization are ultimately key to their success.

Conclusion

RNA interference, a straightforward natural mechanism within eukaryotic organisms, has been extensively harnessed and extended in the agricultural sector. The resulting products, whether they originate from internal or external applications, provide strong evidence of their substantial potential in the realm of crop protection. This method has already established a distinct role in integrated pest management and the promotion of sustainable agriculture.

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