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Extracellular Vesicle-Mediated RNA Warfare: A New Frontier in Sustainable Crop Protection

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Abstract. The increasing pressure of plant diseases, pesticide resistance and environmental degradation has created an urgent need for sustainable crop protection strategies. Plant extracellular vesicles (EVs) have emerged as nanoscale, membrane-bound carriers that mediate intercellular and cross-kingdom communication. In plant-pathogen interactions, EVs may function as vehicles for "RNA warfare", enabling plants to deliver regulatory RNAs into pathogens and suppress virulence-associated genes [1, 3]. Conversely, fungal pathogens can exploit EV-mediated RNA transport to interfere with host immune responses [2]. This mini-review summarizes the emerging role of EV-mediated RNA communication in plant-pathogen interactions, critically evaluates current mechanistic and translational limitations, and discusses its potential as a conceptual basis for RNA-based biopesticides, immune-priming tools and biologically derived nanocarriers for sustainable crop protection [5, 6].

1. Introduction

Crop production is increasingly threatened by fungal, bacterial and viral pathogens, while the overuse of conventional pesticides raises concerns regarding environmental contamination, non-target toxicity, resistance development and food safety. Sustainable agriculture therefore requires new approaches that can enhance plant defense while reducing chemical dependency. In this context, plant molecular biology is revealing that plant immunity is not limited to receptor-mediated pathogen recognition or defense hormone signaling. Plants also use mobile molecular signals to communicate across cells, tissues and even kingdoms [1-3].

Extracellular vesicles are lipid bilayer-enclosed nanoparticles released by cells into the extracellular space. In plants, EVs have been detected in apoplastic fluids and plant-derived materials, and they are increasingly recognized as mediators of plant-microbe communication [5, 7]. Their cargo may include proteins, lipids, metabolites, small RNAs and messenger RNAs, suggesting that EVs act as mobile information carriers rather than simple cellular debris [1, 4, 7]. Although plant EV biology is still developing, accumulating evidence indicates that EVs participate in plant immunity, pathogen interaction and stress responses [3, 7].

One of the most exciting developments in this area is the discovery that plant EVs can transport RNA molecules into pathogens. This process has led to the concept of extracellular vesicle-mediated RNA warfare, in which host plants and invading pathogens exchange regulatory RNAs to influence each other's gene expression [1-3]. This concept is important for agriculture because it connects fundamental plant immunity with the possibility of RNA-based crop protection.

2. Plant EVs as molecular carriers in plant immunity

Plant immunity relies on rapid and coordinated communication between infected and non-infected cells. EVs are well suited for this role because they can protect their cargo from degradation and deliver molecular signals to extracellular or inter-organismal targets [1, 7]. In immune responses, plant EVs have been associated with defense-related proteins, membrane trafficking components and RNA molecules [3, 5].

A landmark study demonstrated that *Arabidopsis* secretes exosome-like EVs containing small RNAs that can be delivered into the fungal pathogen *Botrytis cinerea* [1]. These plant-derived small RNAs were shown to target fungal genes associated with pathogenicity, thereby reducing fungal virulence [1]. This finding provided direct evidence that plants can use EVs as natural delivery systems for cross-kingdom RNA interference.

This discovery changed the way plant-pathogen interactions are interpreted. Traditionally, plant defense was mainly described through antimicrobial compounds, pathogenesis-related proteins, reactive oxygen species, cell wall reinforcement and hormone-mediated signaling. EV-mediated RNA transfer adds another layer to this model: plants may actively export regulatory RNAs into pathogens to silence genes required for infection [1, 3]. Therefore, EVs should not be viewed merely as transport vesicles, but as functional participants in immune communication. Recent evidence also suggests that EV-mediated communication is not restricted to plant–fungal systems, but may contribute more broadly to plant–microbe interactions, including immune modulation by fungal and bacterial EVs [8].

3. RNA warfare as a bidirectional molecular arms race

The term RNA warfare reflects the dynamic molecular conflict between host plants and pathogens. In this process, RNA molecules are used as regulatory weapons to suppress the opponent's gene expression. Plant-derived small RNAs may target pathogen virulence genes, while pathogen-derived small RNAs may target host immunity genes [1-3]. This bidirectional RNA exchange can be conceptualized as an extracellular vesicle-mediated molecular arms race between host plants and fungal pathogens, with direct implications for RNA-based crop protection strategies (Fig. 1).

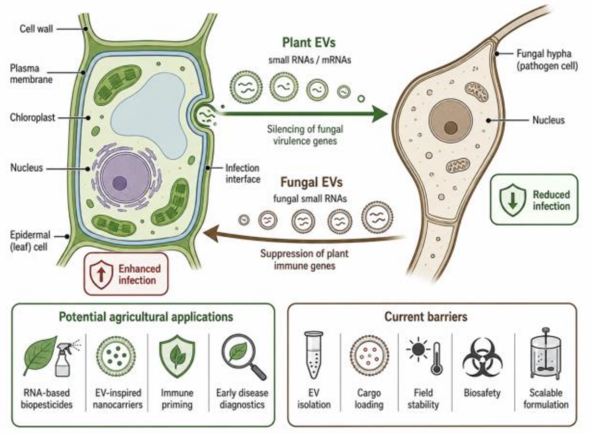

Fig. 1. Extracellular vesicle-mediated RNA warfare in plant–pathogen interactions. Plant EVs may deliver small RNAs and mRNAs to fungal pathogens, leading to silencing of fungal virulence genes and reduced infection, whereas fungal EVs may transfer small RNAs into plant cells and suppress host immune genes, thereby enhancing infection. This bidirectional RNA exchange provides a conceptual basis for RNA-based biopesticides, EV-inspired nanocarriers, immune priming and early disease diagnostics, although EV isolation, cargo loading, field stability, biosafety and scalable formulation remain key barriers.

Recent evidence indicates that this exchange is bidirectional. *Botrytis cinerea* can release EVs containing fungal small RNAs that enter plant cells and suppress host defense-related transcripts [2]. He et al. further showed that these fungal EV-associated small RNAs are internalized by plant cells through clathrin-mediated endocytosis, supporting the idea that pathogens have evolved mechanisms to exploit vesicle-mediated RNA trafficking for immune suppression [2]. The interaction between plant and pathogen therefore resembles a molecular arms race in which both organisms use EV-associated RNAs to manipulate the outcome of infection.

Moreover, EV-mediated RNA trafficking is not limited to small RNAs. Wang et al. reported that *Arabidopsis* mRNAs can move into *B. cinerea* via EVs and contribute to reduced infection [4]. This expands the functional significance of EV cargo and suggests that plant EVs may transfer multiple classes of regulatory nucleic acids. The presence of both small RNAs and mRNAs in EV-mediated communication indicates a complex and multilayered regulatory system [3, 4].

4. Relevance to sustainable crop protection

EV-mediated RNA communication has several potential applications in sustainable agriculture. The most direct application is RNA-based crop protection. Spray-induced gene silencing is an emerging approach in which double-stranded RNA or small RNA molecules are applied to plants or pathogens to silence target genes [5, 6]. However, naked RNA molecules are often unstable under field conditions because they can be degraded by nucleases, ultraviolet radiation and environmental factors. EVs, or EV-inspired nanocarriers, may help overcome this limitation by protecting RNA cargo and improving delivery efficiency [3, 5]. In parallel, spray-induced gene silencing has gained increasing attention as an environmentally compatible RNA-based strategy for crop protection, strengthening the applied relevance of EV-inspired RNA delivery systems [9].

Barley EV studies have provided an important experimental connection between plant EVs and RNA spray-based crop protection. Schlemmer et al. (2021) isolated and characterized barley extracellular vesicles to assess their potential role in RNA spray-based crop protection [5]. A later study reported that EVs isolated from dsRNA-sprayed barley plants did not cause growth inhibition or gene silencing in *Fusarium graminearum*, highlighting that EV-based RNA delivery remains promising but technically unresolved [6]. This limited-effect result is important because it prevents overstatement and shows that practical EV-mediated crop protection still requires mechanistic optimization.

Another potential application is the development of biological nanocarriers. Plant EVs are biodegradable, biocompatible and naturally derived, making them attractive alternatives to synthetic nanoparticles [7]. If scalable production and loading methods can be optimized, EV-like systems may be used to deliver pathogen-targeting RNAs, defense elicitors or stress-protective molecules [3, 5]. EV-associated RNAs and proteins may also serve as biomarkers of plant stress or disease status, supporting early diagnosis in precision agriculture and high-value horticultural production [5, 7].

Compared with conventional spray-induced gene silencing approaches based on naked dsRNA or small RNAs, EV-inspired delivery systems offer three theoretical advantages: protection of RNA cargo from extracellular degradation, potential improvement of cellular uptake through membrane-associated trafficking, and biological compatibility as naturally derived carriers. Compared with fully synthetic nanocarriers, plant EVs may also reduce concerns related to environmental persistence and non-target accumulation. However, these advantages should not be overstated. Current evidence indicates that EV association alone does not guarantee functional gene silencing, as shown by dsRNA-sprayed barley EVs that failed to inhibit Fusarium graminearum growth or induce target gene silencing [6]. Therefore, EV-mediated RNA delivery should presently be considered a complementary and hypothesis-generating platform rather than a direct replacement for established SIGS or nanoparticle-assisted RNA delivery systems.

5. Current challenges and future perspectives

Despite its promise, EV-mediated RNA-based crop protection is not yet ready for routine field application. First, plant EV isolation and purification methods require further standardization. Differential centrifugation, density gradient purification, size-exclusion chromatography and precipitation-based approaches may yield different vesicle populations and may also co-isolate proteins, cell wall fragments or other nanoparticles [5, 7]. Without standardized methods, it is difficult to compare results across studies.

Second, EV heterogeneity remains a major issue. Plant EVs likely consist of multiple subpopulations with different origins, markers, cargo profiles and biological functions. Identifying which EV subtypes are most important for RNA delivery, pathogen interaction or immune signaling is essential for future applications [3]. Third, cargo selection mechanisms are still poorly understood. Although clathrin-mediated endocytosis has been demonstrated for fungal EV-associated small RNAs entering plant cells, many aspects of plant-to-pathogen EV uptake remain unresolved [2-4]. The existence of distinct *Arabidopsis* EV subpopulations under biotic stress further indicates that EV heterogeneity is both a biological feature and a technical barrier for future agricultural translation [10].

Future research should integrate plant molecular biology, nanotechnology, plant pathology and sustainable agriculture. EV cargo profiling under different plant-pathogen systems should be expanded to identify conserved and species-specific RNA signatures. Functional validation is also necessary to distinguish biologically active molecules from passive cargo. Crop species beyond *Arabidopsis* should be studied, especially economically important horticultural and field crops [3, 5, 6]. Together, these studies show that EV-associated RNA trafficking is not only a mechanistic feature of plant–pathogen interactions but also a conceptual basis for future RNA-based crop protection strategies (Table 1).

Table 1. Key evidence for EV-mediated RNA communication in plant–pathogen interactions.

System / focus	Key evidence and crop-protection relevance	Ref.
Arabidopsis–Botrytis cinerea	Plant EV-associated small RNAs enter *B. cinerea* and silence fungal virulence genes; this provides a natural model for RNA-based pathogen suppression.	[1]
B. cinerea EVs–host plants	Fungal EV-associated small RNAs enter plant cells and suppress host immune genes, showing that RNA warfare is bidirectional.	[2]
Cross-kingdom EV-RNA communication	EV-mediated RNA exchange is emerging as a molecular conflict between plants and pathogens, supporting the RNA warfare concept.	[3]
Arabidopsis–B. cinerea mRNA transfer	Plant mRNAs can move into fungal cells via EVs and reduce infection, expanding EV cargo relevance beyond small RNAs.	[4]
Barley EVs and RNA spray	Barley EVs have been characterized in the context of RNA spray-based crop protection, linking EV biology with crop-level RNA delivery.	[5]
dsRNA-sprayed barley–*Fusarium*	EVs from dsRNA-sprayed barley did not induce fungal gene silencing, highlighting current technical limits.	[6]
Arabidopsis EV subpopulations	Distinct EV subpopulations respond differently to biotic stress, indicating that EV heterogeneity must be resolved before application.	[10]

Table 2. Future research priorities for translating EV-mediated RNA communication into sustainable crop protection.

Priority area	Future perspective
Mechanistic validation	Identify EV subtypes and RNA cargos with causal roles in plant-pathogen interaction.
Cargo loading and targeting	Optimize RNA loading, pathogen specificity and delivery to the correct biological target.
Formulation and field stability	Improve storage, spray stability and environmental persistence under field conditions.
Crop-level testing	Validate EV-mediated RNA effects beyond Arabidopsis in economically important crops.
Biosafety and regulation	Assess off-target effects, ecological persistence and regulatory requirements.

Another important direction is the connection between EVs and the rhizosphere. Roots interact with beneficial microbes, pathogens and soil-derived signals. EV-mediated molecular exchange in the rhizosphere may influence microbial community structure, root immunity and plant resilience [7]. Understanding this communication could open new possibilities for soil-compatible biological crop protection. The major future research priorities for translating EV-mediated RNA communication into sustainable crop protection are summarized in Table 2.

6. Conclusion

Extracellular vesicle-mediated RNA warfare represents a modern and conceptually powerful frontier in plant-pathogen interaction research. The ability of plants and pathogens to exchange regulatory RNAs through EVs reveals a sophisticated layer of molecular communication that directly influences immunity and virulence [1-4]. For sustainable agriculture, this knowledge provides a foundation for future RNA-based biopesticides, biological nanocarriers, immune-priming tools and molecular diagnostics [3, 5-7]. Although important technical and regulatory challenges remain, plant EV research has the potential to reshape how crop protection is designed in the coming decades. Rather than relying solely on chemical suppression of pathogens, future agriculture may increasingly exploit the plant's own molecular communication systems to develop precise, biodegradable and environmentally compatible disease management strategies. Moreover, extracellular RNA-based protection may extend beyond fungal pathogens, as extracellular plant small RNAs have been shown to contribute to immunity against bacterial pathogens such as *Pseudomonas syringae* [11, 12].

Recent literature further supports this translational perspective. Plant EVs are increasingly recognized as active participants in defense against pathogens rather than passive by-products of cellular activity [13]. In particular, RNA-containing EVs provide a mechanistic link between vesicle biology, cross-kingdom RNA trafficking and the regulation of host–pathogen interactions [14]. Together with advances in nanotechnology-assisted RNA delivery, these insights suggest that future crop protection may benefit from EV-inspired platforms that improve RNA stability, target specificity and environmental compatibility [15]. Thus, the next challenge is to move from describing EV-mediated RNA exchange toward developing reproducible, field-adaptable and biosafe RNA-based disease management strategies.

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