Extracellular Vesicles - A Shuttle Facilitating Dialogue Between Plants and Microbes in Micro Environment

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Abstract

Plants engage in intricate communication with their associated microorganisms through the exchange of functional molecules, particularly via extracellular vesicles (EVs). These EVs are single-membrane-bound structures that encapsulate a diverse array of cargos, including lipids, proteins, and nucleic acids. Both plants and their microbial counterparts release these vesicles into the extracellular space, facilitating a highly regulated system of intercellular communication. Recent research has highlighted the pivotal role of plant EVs in mediating plant-microbe interactions. These vesicles are not only involved in waste and nutrient transport but also serve as vectors for immune responses and RNA silencing mechanisms. Despite their importance, the study of plant EVs has been historically limited due to challenges in isolation and detection techniques. However, advancements have been made in understanding the composition and function of these vesicles. Plant EVs have been found to carry various bioactive molecules that play crucial roles in modulating interactions with microorganisms, thus influencing plant health and pathogen resistance. In this short review, we focus on the recent advances in our understanding of plant EVs and their role in plant–microbe interactions.

Introduction

Plants are constantly exposed to a wide array of microorganisms in their environment. To defend against pathogenic invaders, plants have evolved sophisticated immune systems. Extracellular vesicles (EVs) are emerging as key players in plant-microbe interactions, facilitating communication between plants and neighbouring cells, as well as between plants and microbes. EVs are lipid bilayer-enclosed vesicles secreted by nearly all cell types. Based on their size, eukaryotic EVs are generally categorized into three groups: apoptotic bodies (1000-5000 nm), micro vesicles (100-1000 nm), and exosomes (30-150 nm). Apoptotic bodies are the largest and most heterogeneous, formed from membrane blebs of dying cells. Micro vesicles bud directly from the plasma membrane, while exosomes originate from endosomal compartments called multivesicular bodies (MVBs) and are secreted when MVBs fuse with the plasma membrane. Although EVs were first observed in carrot suspension cells in 1967, research on their functions in plants has lagged behind that in mammals due to technical limitations in isolation and detection. However, exosome-like EVs were successfully isolated from sunflower apoplastic fluids in 2009, demonstrating that plants can secrete EVs despite their rigid cell walls. Recent studies have revealed that both plants and phytopathogens can release EVs that play crucial roles in regulating plant-microbe interactions. EVs facilitate cross-kingdom communication by transferring small RNAs (sRNAs), mRNAs, proteins, and lipids between species. Interestingly, EVs are also present in fruits like grapes and coconuts. This review highlights the potential of EVs in plant-microbe interactions, their mechanisms of action, and their roles in facilitating immunity or susceptibility signals in plants, integrated with other signalling pathways.

Functions of extracellular vesicles

The capacity to transport nucleic acids is a fundamental characteristic of extracellular vesicles (EVs) across all three domains of life. In plants, the phloem serves as a conduit for the systemic transport of viral RNAs, mRNAs, microRNAs (miRNAs), and small interfering RNAs (siRNAs). RNA loading into the phloem is believed to occur via plasmodesmata (PD), and EVs may provide an alternative pathway for RNA transport through both the phloem and the apoplast. During fungal infections, EVs and multivesicular bodies (MVBs) have been observed to accumulate around plasmodesmata, potentially facilitating the deposition of callose to block connections between healthy cells and those undergoing hypersensitive cell death. This suggests that EVs could modulate plasmodesmata function in both positive and negative contexts. Furthermore, there is an intriguing possibility that plant EVs mediate interspecies RNA transfer. They may also play a role in transporting important defence compounds such as glucosinolates (GSLs), which are nitrogen- and sulphur-containing secondary metabolites predominantly found in *Brassicaceae* plants. Additionally, the lipid components of plant EVs might act as signalling molecules. Emerging evidence highlights the critical role of plant EVs in communication with interacting microbes, particularly in antimicrobial defence. For instance, infections caused by the fungal pathogen *Botrytis cinerea* or the bacterial pathogen *Pseudomonas syringae* pv *tomato* DC3000 trigger the secretion of plant EVs, underscoring their significance in plant-pathogen interactions. The functions of EVs in plant pathogen interactions has been depicted in Figure 1.

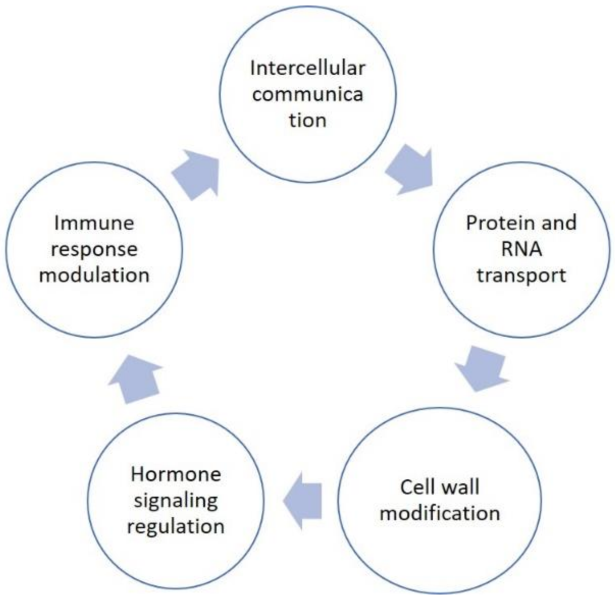

Fig 1. Infographics highlighting EV-mediated processes in plant pathogen interaction

Plant Extracellular Vesicles in Interactions with Fungi: Key Roles and Functions

The abundance of defence bioactive compounds in plant EVs indicates their crucial role in plant immunity. In interacting with fungal pathogens, plants deploy EVs loaded with sRNAs to inhibit the activation of virulence genes in the fungi. Sunflower EVs have been found to inhibit the growth of *Sclerotinia sclerotiorum* by triggering cell death. The role of EVs in plant defence against fungi is confirmed by this discovery. Arabidopsis EVs harbour sRNAs targeting genes related to fungal virulence and vesicle trafficking. *B. cinerea* can detect and internalize sRNAs carried by EVs from infected plants. Plant-derived EVs promote fungal silencing of virulence genes and boost plant defence through sRNA transfer. Fungal cells have been found to take in plant EVs, resulting in the transference of sRNAs into these cells. Arbuscular mycorrhizal fungi suppress fungal virulence genes through EVs. EVs are also involved in arbuscular mycorrhizal symbioses and have been observed in the interface of plants and symbiotic arbuscular mycorrhizal fungus. Plant MVBs have been observed fusing with the PAM during the development of arbuscular mycorrhiza. It is undetermined whether EVs carry RNAs, specifically small RNAs.

Plant Extracellular Vesicles and Their Role in Bacterial Interactions

In Arabidopsis, the secretion of extracellular vesicles (EVs) is significantly increased upon infection with *Pseudomonas syringae* and in response to salicylic acid treatment. This observation indicates that plant EVs may play a crucial role in the immune response against bacterial pathogens. The constitutive expression of small RNAs (sRNAs) that target bacterial virulence factors can induce silencing of these genes, resulting in reduced water-soaking symptoms and a diminished stomatal reopening response caused by the bacteria. Notably, EVs derived from Arabidopsis expressing these sRNAs have demonstrated the ability to suppress stomatal reopening induced by Pto DC3000 in wild-type plants. This suggests that plant EVs can inhibit bacterial growth through the delivery of sRNAs directly to the pathogens. Such findings highlight the potential of EVs as important mediators in plant defence mechanisms against bacterial infections.

Potential Dual Role of EVs in Plant-Virus Interactions

The recent study found that TuMV-infected plants' EVs possess both the viral RNA and the 6 K2 membrane-associated viral protein, crucial for the creation of viral replication vesicles. EVs from TuMV-infected plants contain proteins linked to the virus's replication and infection, as revealed by their proteome profile, implying a potential role in intercellular virus transmission. TuMV RNA was also detected in the EVs. The EVs carry proteins involved in signal transduction and immune response triggered by TuMV infection. This finding suggests that plant extracellular vesicles could facilitate both viral and immune signal transfer between nearby cells. EVs in plant-virus interactions may facilitate virus propagation by transferring viral replication complexes from infected to healthy cells through the phloem. Studies need to determine if EVs derived from virus-infected phloem sap harbour viral replication complexes, despite the difficulty of obtaining substantial quantities of phloem sap; additionally, these EVs may function as vehicles for PAMPs or signalling molecules in transmitting antiviral immunity between infected and neighbouring cells. The ability of plant EV viral components to function as PAMPs and trigger plant immune responses is intriguing.

Roles of fungal EVs in plant-fungus interactions

Fungal extracellular vesicles (EVs) were first identified in 2007 from the opportunistic human pathogen *Cryptococcus neoformans*. Since then, EVs have been characterized in various plant pathogenic fungi, including *Colletotrichum higginsianum, Fusarium graminearum, Botrytis cinerea, Phytophthora sojae, Alternaria infectoria* and *Zymoseptoria tritici*. However, the mechanisms by which fungal EVs can penetrate the plant cell wall remain unclear. These fungal EVs contain a variety of components such as protein effectors, mRNAs encoding virulence factors, small RNA molecules that can manipulate host immunity genes, and metabolites linked to immune and toxic responses. This suggests that fungal EVs may play a significant role in plant-fungal interactions. Notably, recent findings indicate that EVs from B. cinerea can transport small RNAs into plant cells via clathrin-mediated endocytosis (CME), leading to the suppression of host immunity-related genes and increased susceptibility to the fungus. A proteomic analysis of EVs isolated from sunflower seedlings revealed a rich array of defense-related proteins, including pathogenesis-related (PR) proteins (PR4, PR5, PR9, and PR14), disease resistance dirigent proteins, Gnk2 antifungal proteins, as well as GDSL lipase acyl hydrolases and lectins. Additionally, research has shown that small RNAs targeting fungal transcripts in barley and wheat can influence the development of *Blumeria graminis* (the powdery mildew fungus) through host-induced gene silencing. This indicates that RNA molecules can be transferred from plants to invading fungi to silence specific target genes. These findings underscore the complex interactions between plants and fungal pathogens mediated by EVs, highlighting their potential roles in both promoting fungal virulence and facilitating plant defense responses.

Roles of bacterial EVs in plant-bacteria interactions

All gram-negative bacteria are capable of releasing extracellular vesicles (EVs) through a process known as outer membrane vesicle (OMV) formation, which involves pinching off their outer membrane. These OMVs, which range in size from 20 to 400 nm, are enveloped by a lipid bilayer and contain various macromolecules, including signalling compounds, nucleic acids, virulence factors, toxins, and lipopolysaccharides. OMVs play a crucial role in numerous bacterial functions such as cell-to-cell communication, biofilm formation, tolerance to physiological stressors, adherence to host tissues, and modulation of host immune responses. For instance, the release of OMVs by the plant pathogenic bacterium *Xylella fastidiosa* inhibits bacterial attachment to plant cell walls. This mechanism not only aids in the spread of the bacteria throughout the plant but also contributes to disease progression (Ionescu et al., 2014). Additionally, OMVs derived from *P. syringae* and *P. fluorescens* have been shown to activate plant immune responses against both bacterial and oomycete pathogens. These findings underscore the significant role of OMVs in facilitating interactions between bacteria and their hosts, impacting both pathogenicity and plant defence mechanisms.

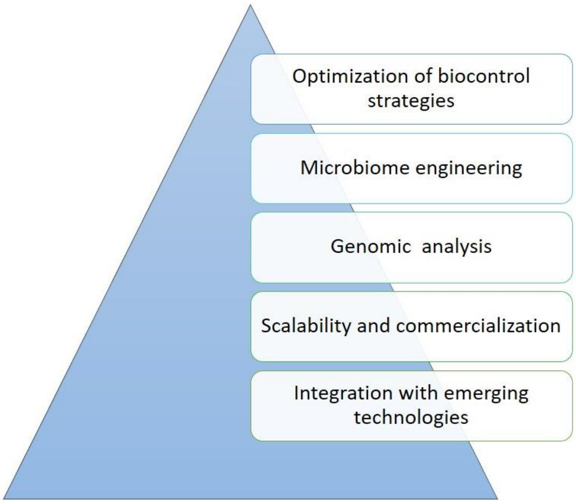

Fig 2 Illustration of EVs in the future for next generation disease management

Conclusion

Plant extracellular vesicles (EVs) were first identified in the 1960s, approximately fifteen years before mammalian exosomes were discovered. Despite this early start, research into plant EVs has lagged for over fifty years. While extensive studies have been conducted on EVs in animal systems, plant researchers are only beginning to explore the complex roles that EVs play in plant biology. A significant knowledge gap remains regarding the role of EVs in facilitating the transfer of bioactive substances between plant cells and between plants and microbial cells. Recent advancements in high-resolution microscopy and detection methods offer promising opportunities to bridge this gap. Further advancements has been mentioned in Figure 2. Although this review primarily focuses on the involvement of plant EVs in defense responses, it is likely that these vesicles also contribute to various aspects of plant physiology, including reproduction and symbiotic relationships. Further investigation into the contents of plant EVs and their specific roles in plant-pathogen communication mechanisms, such as cross-kingdom RNA interference (RNAi), will be crucial for developing innovative strategies for plant protection.

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