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Research Progress of Exosomal Proteomics in Plant-Microbe Interactions

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Abstract:

Exosomes, containing various biological information molecules such as proteins, nucleic acids, lipids, and small molecule compounds, play a crucial role in plant-microbe interactions and are currently a cutting-edge focus in this research field. As carriers of multiple biological information molecules, the composition of exosomes determines their functions. Therefore, analyzing the protein composition of exosomes using proteomic techniques can provide insights into their role in plant-microbe interactions. This article systematically summarizes the research progress of exosomal proteomics in plant interactions with fungi and bacteria, emphasizing the correlation between exosomal protein composition and plant disease resistance signaling as well as pathogen virulence. Based on this, we propose future directions for exosomal proteomics research, aiming to provide a reference for a deeper understanding of the mechanisms underlying plant-microbe interactions.

Keywords:

Exosome
Extracellular vesicle
Plant immunity
Proteomics
Pathogen

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1. Introduction

Extracellular vesicles are lipid bilayer membrane vesicles secreted by cells into the external environment, playing crucial roles in regulating growth and development, immune responses, cancer initiation and progression, as well as virus transfer [1]. Cells secrete three distinct types of membrane vesicles, including apoptotic bodies, microvesicles, and exosomes. Exosomes are lipid bilayer membrane vesicles with a diameter ranging from 30 to 150 nm, containing various biological information molecules such as nucleic acids, proteins, lipids, and small compounds, which have important functions in intercellular communication [2,3]. Exosomes secreted by animal cells can be taken up by adjacent or distant target cells via the humoral system, delivering biological information molecules to regulate the function of target

cells [4]. Recent studies have shown that plants and plant-derived microorganisms can also secrete exosomes. These exosomes mediate the cross-kingdom transfer of biological information molecules between plants and microorganisms, participating in plant-microorganism interactions [5-9]. Microbial exosomes contain various microRNAs, virulence proteins, and pathogen-associated molecular patterns (PAMPs), which can inhibit or induce plant resistance [5,10-12]. On the other hand, plant-derived exosomes can transport disease-resistant proteins and microRNAs targeting microbial virulence genes into microorganisms, thereby inhibiting microbial growth [6,8,9]. Proteins are the direct executors of cellular functions and play a crucial role in cellular life activities. Therefore, proteomic analysis of exosome protein composition can help to deeply understand the molecular mechanisms of plant-microorganism interactions. This study systematically summarizes the research progress of exosome proteomics in plant interactions with fungi and bacteria, including the antibacterial mechanisms of plant exosomes, the signaling mechanisms mediating disease resistance, the functions of fungal exosomes during fungal infection of plants, and the activation of plant innate immune mechanisms by bacterial exosomes. The aim is to provide a reference for further revealing the functions of exosomes in plant-microorganism interactions.

2. Exosome isolation techniques

2.1. Isolation methods for plant exosomes

Currently, the most commonly used method for extracting plant exosomes is ultracentrifugation, and the first step is to obtain high-quality plant apoplastic fluid [6-9,13]. The specific isolation method involves the following steps: (1) Immersing plant leaves in an osmotic solution, applying vacuum until the leaves are saturated, and separating the plant apoplastic fluid by centrifugation at 700 g; (2) Filtering with a 0.45 μm membrane to remove cellular debris and large vesicles; (3) Performing differential centrifugation at 5,000 g and 10,000 g to further remove cell fragments and impurities; (4) Sedimenting exosomes by ultracentrifugation at 100,000 g (**Figure 1**). The obtained exosomes can be resuspended and further purified using density gradient centrifugation. Additionally, He *et al.* [14] used immunoaffinity capture to purify exosomes enriched in the tetraspanin protein TET8 from *Arabidopsis thaliana*. This method allows for the isolation of specific subpopulations of exosomes. In animal systems, other methods for extracting exosomes include microfluidic separation and size exclusion chromatography [15]. However, there are currently no reports on the use of such methods for the isolation of plant exosomes.

2.2. Isolation methods for microbial exosomes

Similar to plants, ultracentrifugation is the most commonly used method for isolating bacterial and fungal exosomes (**Figure 1**). The specific isolation steps are as follows: (1) Obtaining bacterial or fungal culture supernatant; (2) Performing differential centrifugation to remove impurities and cell fragments from the culture supernatant; (3) Filtering with a 0.45 μm membrane to further remove impurities and large extracellular vesicles; (4) Sedimenting exosomes by ultracentrifugation at 100,000–150,000 g. Currently, exosomes have been successfully isolated from various bacteria, fungi, and oomycetes using ultracentrifugation (**Figure 1**). Additionally, researchers have used size exclusion chromatography to extract exosomes from *Fusarium* culture supernatants [16]. However, Rutter *et al.* [17] found through transmission electron microscopy that exosomes secreted by *Colletotrichum* fungi are confined between the cell wall and plasma membrane, and can only be isolated from the culture supernatant of *Colletotrichum* protoplasts. This suggests that the cell walls of a few fungi can hinder the secretion of exosomes.

3. Proteomics research methods based on mass spectrometry

Traditional proteomics research typically involves the separation of proteins based on their isoelectric points and molecular weights using two-dimensional electrophoresis. After image comparison and analysis, mass spectrometry is employed to identify the proteins in the differential spots. However, due to the limitations of two-dimensional electrophoresis, such as low throughput, low resolution, and poor reproducibility [18], it has gradually been replaced by proteomics techniques based on high-performance liquid chromatography-tandem

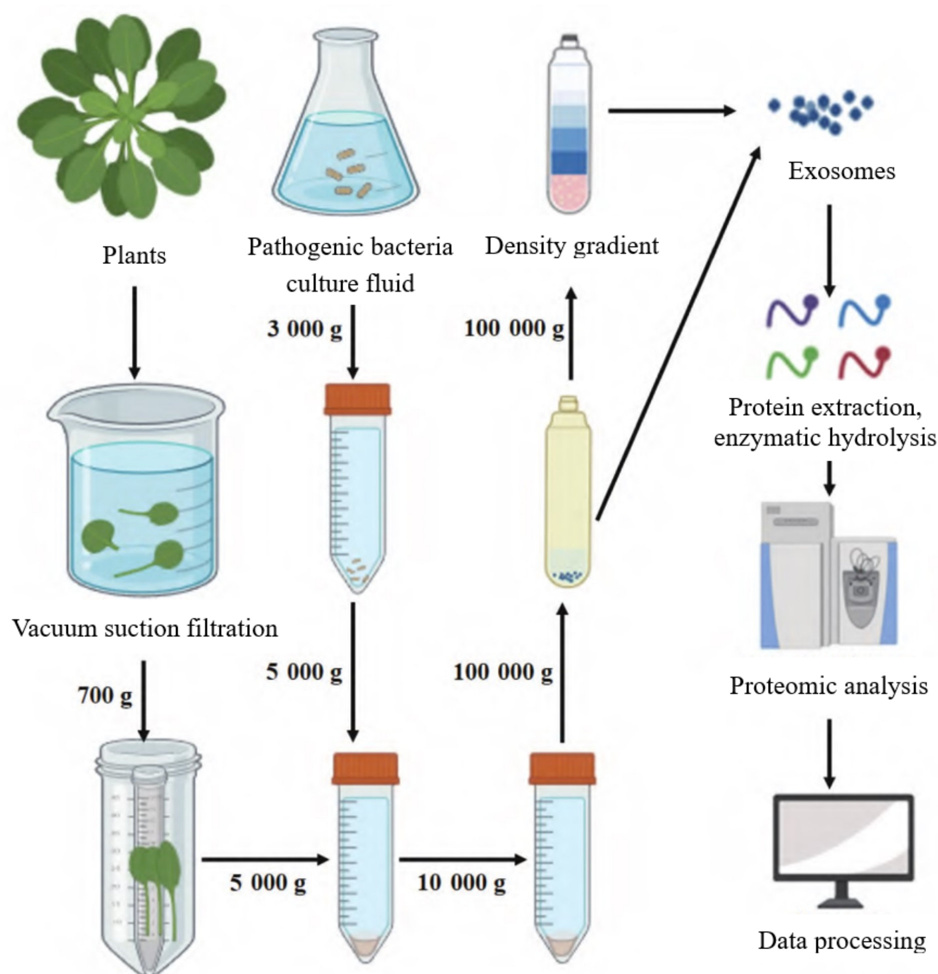

Figure 1. Isolation methods for plant and microbial exosomes

mass spectrometry (LC-MS/MS). With improvements in mass spectrometry accuracy and scanning speed, it is now possible to perform qualitative and quantitative analysis on tens of thousands of proteins in a sample [19]. The core processes of LC-MS/MS-based proteomics technology are as follows: (1) Extraction of sample proteins; (2) Proteolytic digestion of proteins into peptide segments using proteases; (3) Separation of peptide segments using high-performance liquid chromatography, followed by ionization and introduction into the mass spectrometer; (4) Qualitative and quantitative analysis of peptide segments through primary and secondary mass spectrometry; (5) Database searching and bioinformatics analysis. According to different detection purposes, proteomics can be divided into qualitative proteomics and relative quantitative proteomics. Qualitative proteomics is mainly used to detect protein components in samples or the types of proteins with certain post-translational modifications, while quantitative proteomics focuses on detecting changes in protein content or modified protein content across different samples. Unlike conventional proteomics, modified proteomics requires the enrichment of target modified peptide segments from the total peptide pool, as the proportion of proteins with a specific modification among the total proteins is relatively low. The basic principle involves the use of materials that can specifically adsorb modified peptide segments to enrich them. For example, phosphorylated peptide segments can be enriched using IMAC (Fe^{3+}) technology containing cation-affinity adsorption phosphate groups, while ubiquitinated, acetylated, and methylated peptide segments can be enriched using specific antibodies.

Glycosylated peptide segments can be enriched using lectins [20]. Currently, commonly used quantitative proteomics methods based on LC-MS/MS include label-free quantitative proteomics and labeled quantitative proteomics.

Label-free quantitative proteomics refers to the separate analysis of peptide segments from each sample. The peptide segments are separated by high-performance liquid chromatography, ionized, and then introduced into the mass spectrometer. Quantitative analysis of the peptide segments is performed based on the peak area of the primary mass spectrum (MS1), while qualitative analysis is achieved through the secondary fragmentation (MS2) pattern of the peptide segments. Data-dependent acquisition (DDA) mode is typically used to collect MS2 information for the peptide segments. However, in DDA mode, the mass spectrometer can only collect MS2 information for peptide segments with higher abundance, missing a large amount of MS2 information for low-abundance peptide segments. Therefore, label-free quantitative proteomics based on DDA mode has poor stability and a relatively small data volume [21]. Currently, this method is widely used for the identification of exosome protein components in almost all plants and plant-derived pathogens (**Table 1**).

In recent years, with improvements in mass spectrometry performance, it has become feasible to collect MS2 information for peptide segments in data-independent acquisition (DIA) mode. In DIA mode, the mass spectrometer can collect all MS2 information, greatly improving data reproducibility and volume [31]. Labeled quantitative proteomics refers to the use of isotopes containing different reporter groups to label peptide segments from different samples, enabling qualitative and quantitative analysis of the peptide segments in the samples through mass spectrometry. Commonly used commercial isotope labeling reagents include ITRAQ, TMT, and SILAC [32]. Among them,

Table 1. Application of proteomic technology based on LC-MS/MS in the identification of exosomal protein components in plants and microorganisms

Organism	Isolation method	Detection method of proteins	Detection method of protein number	Reference
Sunflower	Ultracentrifugation	Label free	2	[7]
Arabidopsis	Density gradient centrifugation	Label free	598	[9]
Sunflower	Ultracentrifugation	Label free	237	[8]
Nicotiana benthamiana	Ultracentrifugation	Label free	105	[22]
Arabidopsis	Ultracentrifugation	Label free	399	[22]
Arabidopsis	Ultracentrifugation	Label free	981	[14]
Alternaria infectoria	Ultracentrifugation	Label free	20	[23]
F. oxysporum	Ultracentrifugation	Label free	482	[24]
Zymoseptoria tritici	Ultracentrifugation	Label free	240	[25]
Fusarium graminearum	Size-exclusion chromatography	Label free	647	[26]
Colletotrichum higginsianum	Density gradient centrifugation	Label free	1033	[17]
Botrytis cinerea	Density gradient centrifugation	Label free	2 461	[27]
Phytophthora sojae	Density gradient centrifugation	Label free	468	[28]
Xylella fastidiosa	Ultracentrifugation	Label free	199	[10]
Liberibacter crescens	Kit	Label free	55	[12]
Sinorhizobium fredii	Density gradient centrifugation	Label free	889	[29]
Pseudomonas syringae	Density gradient centrifugation	Label free	369	[30]

ITRAQ and TMT reagents are used for *in vitro* labeling, which means they directly label the peptide segments after enzymatic hydrolysis. On the other hand, SILAC reagents are mainly used for *in vivo* labeling, where isotope-labeled amino acids are added to the culture medium during cell cultivation. Through cellular metabolism, these isotope-labeled amino acids are introduced into newly synthesized proteins. ITRAQ and TMT reagents can simultaneously label up to eight and 16 samples, respectively, while SILAC reagents can label up to three samples simultaneously. Compared to label-free quantitative proteomics, isotope-labeled proteomics offers the following advantages: (1) Good reproducibility: After isotope labeling of peptide segments from different samples, the mass spectrometer can distinguish which group of samples the same peptide segment belongs to based on the different isotopes. This allows for the mixing of isotope-labeled peptide segments from different samples and performs qualitative and quantitative analysis of all samples in a single experiment, avoiding errors introduced due to the stability of the mass spectrometer. (2) Large data volume: Mixing isotope-labeled peptide segments from different samples enables two-dimensional high-performance liquid chromatography separation. This involves first separating the peptide segments into different fractions based on their characteristics (such as ionic properties, pH, etc.), followed by LC-MS/MS analysis of each fraction. This method effectively reduces the interference of high-abundance peptide segments on the detection of low-abundance peptide segments [33]. However, due to the large sample size required for isotope labeling technology, there are currently no reports on the use of this method for proteomic analysis of plant and plant-derived pathogen exosomes.

4. Plant extracellular vesicle proteomics and plant immunity

4.1. Plant exosome proteomics and trans-kingdom delivery of plant disease resistance components

During the interaction between plants and microorganisms, the trans-kingdom delivery of biological information molecules such as nucleic acids and proteins is crucial for microbial infection and plant immunity. For example, pathogenic bacteria secrete effector proteins and virulence factors into host cells to promote infection, while plant cells secrete nucleic acids, antimicrobial peptides, and disease-resistant proteins to inhibit the growth of pathogenic bacteria. However, the mechanisms by which these biological information molecules pass through plant or microbial cell membrane barriers remain unknown [34]. Recent research has shown that exosomes play an important role in mediating the trans-kingdom delivery of biological information molecules [6,11]. Regente *et al.* [8] identified 237 proteins in sunflower exosomes through proteomics. Besides proteins related to biological processes such as glycolysis/tricarboxylic acid cycle and vesicular transport, sunflower exosomes also contain various disease-resistant proteins such as pathogenesis-related proteins PR4, PR5, PR6, PR9, and PR14 (**Figure 2**). Further incubation of sunflower exosomes with *Sclerotinia sclerotiorum* spores revealed that the exosomes could be taken up by the spores and inhibit their growth, indicating that plant exosomes mediate the trans-kingdom delivery of disease-resistant proteins from plants to fungal cells. Similar to the delivery of proteins, researchers found that *Arabidopsis* exosomes can transport microRNAs targeting fungal virulence genes to *Botrytis cinerea*, thereby inhibiting the expression of these virulence genes [6]. To explore the mechanism of microRNA loading into exosomes, He *et al.* [14] analyzed

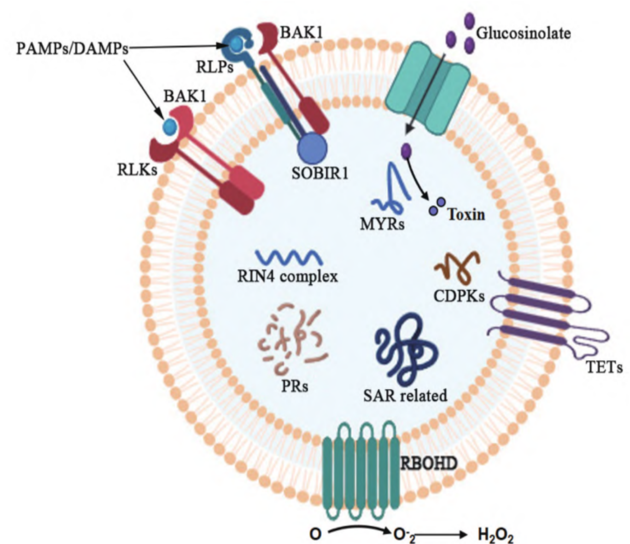

Figure 2. Disease-resistant proteins in plant exosomes. Abbreviations: MYRs, myrosinase; TETs, tetraspanin.

the protein composition of *Arabidopsis* exosomes through proteomics and identified various RNA-binding proteins such as argonautes (AGOs), RNA helicases (RHs), and annexins (ANNs). Further combining reverse genetics experiments revealed that AGO1, RH11, and RH37 load microRNAs into exosomes, indicating that exosome proteins also mediate the trans-kingdom delivery of microRNAs.

Additionally, proteomic analysis of exosomes by researchers suggests that they may be associated with the chemical defense of plants [9]. Glucosinolates are unique secondary metabolites found in cruciferous plants, and their hydrolysis products serve as natural toxins against various bacteria and fungi. Early research indicates that glucosinolates and their hydrolyzing enzymes are stored separately in vacuoles and endoplasmic reticulum bodies (ER bodies). Pathogen infection leads to the release of glucosinolates and hydrolyzing enzymes, triggering a reaction that inhibits pathogen infection. Through proteomic analysis of *Arabidopsis thaliana* exosomes, researchers have discovered the presence of glucosinolate hydrolyzing enzymes such as epithiospecifier 1, myrosinase 1, myrosinase 2, as well as glucosinolate transporters pentacyclic triterpene synthase 3 and PTI Family 2.10 (**Figure 2**) [9,22]. These findings suggest that plant exosomes may be involved in the metabolism and transport of glucosinolates. When pathogens infect plants, glucosinolates are transported to exosomes and hydrolyzed into toxins, which are then transported via exosomes to exert antifungal functions against the pathogens.

4.2. Plant exosome proteomics and PAMP-triggered immunity

Although progress has been made in understanding the function of exosomes in plant-microbe interactions, it remains unclear whether exosomes are involved in plant disease resistance signaling processes. Proteomic analysis of exosomes from *Arabidopsis thaliana*, sunflower, and tobacco has revealed that plant exosomes carry multiple proteins related to innate immune signaling, suggesting that plant exosomes may mediate the transmission of disease resistance signals [8,9,14,22]. When pathogens infect plants, pattern recognition receptors (PRRs) located on the cell membrane recognize pathogen-associated molecular patterns (PAMPs), triggering PTI (PAMP-triggered immunity) [35]. PRRs mainly include receptor-like kinases (RLKs) and receptor-like proteins (RLPs). Among them, RLKs contain an extracellular ligand recognition domain capable of binding PAMPs and an intracellular kinase domain, while RLPs only contain an extracellular ligand recognition domain [36,37]. Proteomic analysis has identified various types of PTI signal transduction-related proteins in plant exosomes (Figure 2) [8,9,14,22]: (1) RLKs, including FLS2 (flagellin-sensitive 2), EFR (EF-Tu receptor), CERK1 (chitin elicitor receptor kinase 1), LYK4 (LysM-containing receptor-like kinase 4), LYK5, KIN7 (kinase 7), and NILR2 (nematode-induced LRR-RLK 2). RLKs primarily mediate the recognition of PAMPs and transmit PTI signals through phosphorylation to the cell interior. Among them, FLS2, EFR, and KIN7/NILR recognize bacterial flagellin, elongation factor EF-Tu, and lipopolysaccharide, respectively, while LYK4/5 and CERK1 mainly recognize fungal chitin [36,38]. (2) RLPs, such as RLP23 and RLP54. Similar to RLKs, RLPs recognize pathogen PAMPs through their extracellular ligand recognition domains and mediate PTI signal transduction. (3) PTI co-receptors BAK1 (BRI-associated receptor kinase), BKK1, and the adaptor protein SOBIR1 (suppressor of BIR1 1). After recognizing PAMPs, the extracellular ligand recognition domain of RLKs forms a complex with BAK1 and BKK1, which then transmits the PTI signal through phosphorylation cascades to the nucleus. Since RLPs do not have an intracellular kinase domain, they first bind to the adaptor protein SOBIR1 before forming a complex with BAK1 and BKK1 to transmit the PTI signal [36,39,40]. (4) Pseudokinases BRI1 (brassinosteroid insensitive 1), BRI2, and BRI3. Under normal conditions, BRI proteins interact with BAK1, impeding the formation of the RLKs-BAK1 complex and activation of PTI. However, recognition of PAMPs by RLKs/RLPs induces the dissociation of BAK1 from the BRI-BAK1 complex, allowing it to form a complex with RLKs/RLPs and activate PTI [41,42]. These proteomic results suggest that exosomes may mediate plant PTI signal transduction. Specifically, PTI-related kinase complexes in exosomes recognize pathogen PAMPs, become activated, and are then taken up by plant cells to transmit the PTI signal to the nucleus. In mammalian systems, exosomes secreted by cells can be transported

long distances via bodily fluids and taken up by distant target cells, regulating their function [4]. Therefore, plant exosomes may serve as carriers of PTI signal complexes, mediating the transmission of PTI signals from pathogen infection sites to adjacent or distant uninfected cells.

4.3. Plant exosome proteomics and DAMP-triggered immunity

In addition to PAMPs, the infection of pathogenic bacteria can lead to the degradation of plant cells, resulting in the production of damage-associated molecular patterns (DAMPs). DAMPs can be recognized by receptors located on the cell membrane, triggering plant DTI (DAMP-triggered immunity) [43]. Both PTI and DTI constitute the first line of defense for plants against pathogenic bacterial infection. The production of DAMPs relies on the degradation and modification of the cell wall. Through proteomics research, it has been found that plant exosomes contain a large number of enzymes related to cell wall degradation and modification [8,9], suggesting that exosomes may be involved in the production of DAMPs. Furthermore, exosomes also contain various DAMP receptors such as WAK1 (cell wall-associated kinase 1), WAK2, THE1 (theseus 1), MIK2 (mdis1-interacting receptor-like kinase 2), and PEPR1 (proline-rich extensin-like receptor kinase 1) (**Figure 2**) [8,9,14,22], indicating that exosomes may mediate signal transduction in DTI. Oligogalacturonides (OGs) are DAMPs produced by the depolymerization of cell wall pectin during pathogenic bacterial infection, and they play important roles in inducing plant defense responses and regulating plant development [44,45]. THE1 and MIK2 have crucial functions in mediating signal transduction of unknown cell wall-derived DAMPs [43,46,47]. Additionally, during interaction with pathogenic bacteria, plants produce a class of peptide DAMPs (Pep) consisting of 23–29 amino acids. Pep can induce resistance responses similar to PTI in plants. Studies have shown that PEPR1 is the receptor for Pep and plays a significant role in mediating Pep-induced resistance signal transduction.

4.4. Plant exosome proteomics and effector-triggered immunity

To overcome PTI and DTI, pathogenic bacteria secrete various effector proteins into host cells. These effector proteins can inhibit the activity of key signaling proteins in the PTI system, thereby suppressing plant PTI [48]. NLRs located inside plant cells can recognize or sense the activity of effector proteins, triggering a more intense immune response in plants. This process is known as effector-triggered immunity (ETI) [49,50]. Rutter *et al.* [9] identified 598 proteins in *Arabidopsis* exosomes through proteomic analysis. Bioinformatics analysis revealed that 11% of these proteins are related to plant stress responses. This includes RIN4 (RPM1-interaction protein 4) and its interacting proteins such as ATPases, early-responsive to dehydration 4, remorin, and delta(24)-sterol reductase (**Figure 2**). RIN4 can be degraded by various pathogenic bacterial effector proteins such as AvrB, AvrRpm1, and AvrPt2. Changes in its state are sensed by the receptor kinase RPS2 inside host cells, which further activates calcium-dependent protein kinases (CDPKs). These kinases then transmit ETI signals to the cell nucleus through a series of protein phosphorylations [36,51]. Since CDPK3 and CDPK6 are also located in exosomes [9,14,22], it suggests that plant exosomes may mediate the transmission of plant ETI signals.

4.5. Plant exosome proteomics and systemic acquired resistance

Pathogenic bacterial infection can stimulate plant PTI, DTI, and ETI. Additionally, various signaling molecules can be synthesized at the infection site. These signaling molecules can be transported to uninfected parts of the plant via the plant's phloem, inducing broad-spectrum resistance to bacteria, fungi, and viruses in these areas. This process is known as systemic acquired resistance (SAR) [52]. In mammalian systems, exosomes can enter the bodily fluid system for long-distance transport, regulating the function of distant target cells. Similarly, proteomic research has found that *Arabidopsis* exosomes contain various SAR-related proteins, indicating that exosomes may be involved in the long-distance transport or amplification of plant SAR signals [1,9]. Although most identified SAR long-distance signals are small molecule metabolites, studies have reported that some proteins can be transported long distances via the phloem from pathogenic infection sites, mediating the transmission of SAR long-distance signals [53-55]. Chanda *et al.* [56] discovered that the *Arabidopsis* lipid transfer

protein DIR1 can be transported long distances via the plant's phloem, and exogenous DIR1 can induce SAR in plants, indicating that DIR1 mediates the transport of SAR long-distance signals. Carella *et al.* [57] identified multiple mobile proteins related to long-distance SAR signaling in *Arabidopsis* through phloem proteomics. Among them, 16 proteins, including CML27, MLP43, and TRXh3, are also present in the *Arabidopsis* exosome proteome [1,9], suggesting that plant exosomes can move long distances via the plant's phloem, mediating the transport of protein-based SAR long-distance signals to systemic locations.

4.6. Plant exosome proteomics and reactive oxygen species

Research has shown that reactive oxygen species (ROS) play a crucial role in plant signaling pathways, including PTI, DTI, ETI, and SAR [43,52,58]. ROS mediate the transmission of PTI, DTI, and ETI signals, and participate in the amplification of SAR signals. Recently, proteomic studies have identified various proteins in plant exosomes that are related to the production and transmission of ROS signals, including phospholipase Da, annexin1, ascorbate peroxidase 1, glutathione S-transferase PHI2, and respiratory burst oxidase homologue D (RBOHD) [9]. Among these, the NADPH oxidase RBOHD is particularly critical in the generation of ROS [59]. Located in the cell membrane, RBOHD is mainly involved in the formation of superoxide anions, which can be further catalyzed into hydrogen peroxide by the action of superoxide dismutase [60]. Therefore, plant exosomes may be involved in the production and signal transmission of ROS.

5. Fungal exosome proteomics and fungal virulence

During the infection process, fungi secrete cell wall hydrolases and effector proteins into host cells to penetrate plant cell walls and inhibit the plant's immune system. Most proteins are released through traditional secretory pathways, where proteins containing N-terminal signal peptides are secreted extracellularly via the endoplasmic reticulum, Golgi complex, secretory vesicles, and cell membrane. However, proteomic analysis of fungal exosomes has revealed that they contain various cell wall hydrolases and effector proteins [23-25], indicating that fungal exosomes are associated with fungal virulence. Silva *et al.* [23] were the first to isolate exosomes from the plant-derived fungus *Alternaria* and identify their protein components. The results showed that *Alternaria* exosomes contain multiple proteins related to host cell adhesion and cell wall degradation. Similarly, exosomes from wheat leaf blight fungus and cotton wilt fungus also contain virulence proteins such as cell wall hydrolases and proteases [24,25]. To explore whether fungal exosomes mediate the secretion of effector proteins, researchers analyzed the protein composition of *Fusarium* exosomes through proteomics and identified multiple effector proteins such as NIS1-like, SnodProt1-like, and LysM domain-containing proteins [26]. Further studies have found that fungal exosomes can be taken up by plant cells through clathrin-mediated endocytosis [27], suggesting that exosomes mediate the cross-boundary delivery of virulence proteins from fungi to host cells, thereby assisting fungal infection of plants. To elucidate the function of exosome virulence proteins in the process of fungal infection of plants, He *et al.* [11] constructed a mutant of the exosome marker protein PLS1 in *Botrytis cinerea*. Through molecular biology and physiology experiments, they found that the PLS1 mutant had reduced virulence towards *Arabidopsis thaliana*; the release of exosomes from the PLS1 mutant was reduced; and wild-type *Botrytis cinerea* exosomes could restore the virulence of the PLS1 mutant towards *Arabidopsis thaliana*. This indicates that fungal exosomes mediate the cross-boundary delivery of virulence proteins and play an important role in the process of fungal infection of plants.

6. Proteomics of bacterial exosomes and plant immunity

Unlike the secretion pathways of plant and fungal exosomes, Gram-negative bacteria have an outer membrane that can secrete vesicles directly into the external environment through budding, hence they are also known as outer membrane vesicles (OMVs) [61]. In recent years, proteomic analysis of OMVs from bacteria such as *Pseudomonas syringae* and *Candidatus* Liberibacter asiaticus has revealed that bacterial OMVs

contain various effector proteins, components of the Type II/III/IV secretion systems, and protein-like PAMPs [10,12,30,62,63], suggesting that OMVs may stimulate plant PTI and ETI. Researchers have confirmed through molecular biology experiments that OMVs from *Xanthomonas* and *Pseudomonas syringae* can stimulate the expression of multiple PTI and ETI-related genes in plants, enhancing plant resistance to bacteria and oomycetes [5,64-66]. However, current research mainly focuses on the mechanism of exogenous OMVs inducing plant disease resistance, and the function of OMVs in the interaction between plants and bacteria is still unclear. Proteomic results show that OMVs also contain various proteins that determine bacterial virulence, such as lipases, adhesins, proteases, and cell wall lytic enzymes [10], indicating that OMVs may be related to bacterial virulence during the interaction between plants and bacteria.

7. Prospects

Exosomes have been a focus of attention in the field of plant-microbe interactions in recent years. Although proteomic analysis has made significant contributions to understanding the protein composition of exosomes and their functions in plant-microbe interactions, there are still many unresolved issues. Firstly, current methods for detecting the protein components of plant and plant pathogen exosomes are based on DDA-mode proteomics technology. With improvements in mass spectrometry accuracy and scanning speed, it has become possible to analyze the protein composition of exosomes in DIA mode. Compared to the DDA detection mode, the DIA-based detection method can greatly improve the detection rate of low-abundance proteins in exosomes. Secondly, in mammalian systems, the protein composition and function of exosomes secreted by different cells or the same cells under different environments vary. Using quantitative proteomics to analyze differences in the protein composition of exosomes secreted by cells under different conditions can help clarify the biological functions of exosomes. However, current proteomics studies on plant and plant pathogen exosomes are qualitative analyses, and there have been no reports on detecting changes in the protein content of exosomes secreted by plants or plant pathogens under interactive conditions. Therefore, using quantitative proteomics to analyze changes in exosome protein composition during plant-pathogen interactions will help elucidate the mechanism of plant-microbe interactions. Lastly, proteomic analysis has shown that plant exosomes contain various key kinases that mediate PTI, DTI, and ETI signaling. The activation or inhibition of these kinases is regulated by post-translational modifications such as phosphorylation and acetylation. Therefore, analyzing changes in post-translational modifications of plant exosome proteins before and after pathogen infection through modomics will help to further understand the function of exosomes in plant immune signaling.

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