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Review

Plant fluid proteomics: Delving into the xylem sap, phloem sap and apoplastic fluid proteomes☆

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ABSTRACT

The phloem sap, xylem sap and apoplastic fluid play key roles in long and short distance transport of signals and nutrients, and act as a barrier against local and systemic pathogen infection. Among other components, these plant fluids contain proteins which are likely to be important players in their functionalities. However, detailed information about their proteomes is only starting to arise due to the difficulties inherent to the collection methods. This review compiles the proteomic information available to date in these three plant fluids, and compares the proteomes obtained in different plant species in order to shed light into conserved functions in each plant fluid. Inter-species comparisons indicate that all these fluids contain the protein machinery for self-maintenance and defense, including proteins related to cell wall metabolism, pathogen defense, proteolysis, and redox response. These analyses also revealed that proteins may play more relevant roles in signaling in the phloem sap and apoplastic fluid than in the xylem sap. A comparison of the proteomes of the three fluids indicates that although functional categories are somewhat similar, proteins involved are likely to be fluid-specific, except for a small group of proteins present in the three fluids, which may have a universal role, especially in cell wall maintenance and defense. This article is part of a Special Issue entitled: Plant Proteomics— a bridge between fundamental processes and crop production, edited by Dr. Hans-Peter Mock.

1. Introduction

The vascular system of land plants is essential for long- and short-distance transport and distribution of nutrients and signals. The vascular transport system consists of the xylem and phloem conduits, with the apoplast compartment acting as an interface between them and cells and also between cells. The xylem is composed of long treachery elements and shorter cells that are dead at maturity and have primary and secondary cell walls; these elements are connected together forming long tubes known as xylem vessels. The xylem mainly transports water and mineral nutrients taken up by the roots from the soil to the aerial part of the plant, although it also contains a number of other organic compounds, including carboxylates, hormones, amino acids, peptides and proteins [1]. Transport in the xylem is passive and driven by shoot transpiration, which causes a negative pressure that pulls the xylem fluid from the roots, and/or by root pressure, which can occur after soil solution enters the root by osmosis, and causes the sap to move towards the shoot [2]. A major role of the xylem is to provide water and nutrients to the shoots, but it also plays an important role in the root to shoot signaling system.

In contrast, the phloem conduit is a living tissue, whose major role is to transport the photosynthates from a photosynthetically active source to sink tissues. The conducting portion of the phloem is composed of two cell types, the sieve elements and the companion cells. At maturity, sieve elements are arranged to form the sieve tube; sieve elements lack a nucleus and contain pores at their ends, forming sieve plates that allow sap flow. The companion cells are metabolically active and usually contain a large number of ribosomes and mitochondria [1]. These cells are in close association with the sieve elements and support their function [3,4]. The exchange of molecules between these two cells occurs *via* plasmodesmata [3]. While transport in xylem sap is unidirectional (upwards), transport of the phloem sap is multidirectional and driven by positive hydrostatic pressures created by the gradients in sugar concentration between source and sink tissues. The phloem sap is rich in sugars, but also contains inorganic ions, other organic compounds including proteins and small signaling molecules such as hormones, systemic wound signals and mRNAs [3–5]. Furthermore, the phloem sap is also a conduit for the trafficking of pathogens and foreign compounds such as herbicides and other xenobiotics [4,6].

The apoplast is the free diffusional space outside the plasma membrane and comprises the cell wall matrix and the fluid in the

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intercellular spaces. The apoplast occupies less than 5% of the plant tissue volume in aerial organs [7,8] and the root cortex [9] and its composition reflects the exchange between xylem and phloem compartments and the export and import of molecules by individual cells [7]. Therefore, small changes in these fluxes could produce large differences in the solute composition of the apoplast. The apoplast plays important roles in a wide array of physiological processes, including water and nutrient trafficking [10], plant defense, cell interactions, cell wall maintenance, and in the transduction of environmental and developmental signals [11–15].

The study of plant fluid dynamics can be approached by means of non-destructive methodologies such as those used to measure sap flow velocity and pressure or to visualize vessel structures (reviewed and discussed in [16]). Other approaches include the use of isotope labeling to study fluxes of specific compounds [17]. However, studies of the chemical composition, including their proteomes, often require the use of harsh techniques to collect these plant fluids. While information about the chemical composition of these plant fluids is ample with regard to minerals, sugars and small organic compounds such as carboxylates and amino acids [18], information about their proteomes has arisen more recently. Proteomic studies can provide useful information about processes occurring in plant fluids and also to target proteins putatively involved in them for future studies. Most of the proteomic studies in plants were initially carried out with whole tissue samples [19,20], and later on with subcellular organelles such as the mitochondria [21], chloroplast [22], and different membrane systems [23–25], as well as with cell walls [26,27] and plant fluids (see references in Table 1).

A major constraint for the proteomic analysis of plant fluids is the limited amount of sample that can be obtained. In the case of the xylem sap, another constraint is the low protein concentration, which is only in the ng μl^{-1} range. An additional constraint is an inherent consequence of the sampling methodologies: the presence of proteins not intrinsic to these fluids, which can be considered either as contaminants or as the result of protein–protein interactions occurring *in vivo* or during isolation (see below). Methodological progress in the proteomic field has shed light not only into the protein composition of these plant fluids, but also into their functionality. This includes the development of high-throughput technologies such as shotgun proteomics and LC–MS/MS that allow for the analysis of small sample volumes and for the detection of low abundance proteins, as well as the constant improvement in plant proteome databases, which have led to the construction of several mapping datasets (see Table 1 for references).

The aim of this review is to provide a general overview of the proteomic studies carried out in the three plant fluids mentioned. We have considered as outside the scope of this review, which is mainly focused into describing these plant fluid proteomes in healthy plants, a number of differential proteomic studies focused on the specific effects of biotic stresses, including pathogen systemic dissemination and plant defense mechanisms.

2. Plant fluid collection

The main limitations for obtaining reliable plant fluid proteomes have been the technical difficulties in the collection of plant fluids, which usually imply a disruption of the vascular tissues, and the small volumes and sometimes dubious purity of the samples obtained.

Xylem sap is frequently obtained by de-topping plant shoots, cleaning the cut area, and subsequently collecting the fluid that bleeds

Table 1
Summary of proteomic bibliography reviewed. Functional classification and number of identified proteins are depicted as reported in the original publications.

Reference	Plant species	Technique	Material	Separation	Identified	Functional classification
[51]	*Brassica napus* *B. oleracea* *Cucurbita maxima* *Cucumis sativus*	Stem de-top	Xylem	1-DE	14	Redox response, plant defense, proteolysis
[52]	*Brassica napus*	Stem de-top	Xylem	2-DE	69	Plant defense, cell wall
[50]	*Zea mays*	Stem de-top	Xylem	2-DE	154	Cell wall, plant defense, proteolysis
[53]	*Glycine max*	Stem de-top	Xylem	1&2DE, 2D-LC	24	Proteolysis, cell wall, redox stress, metabolism
[30]	*Oryza sativa*	Stem de-top	Xylem	2D-LC	118	Metabolism, cell wall, pathogenesis related, redox stress
		Stylectomy	Phloem	1DE followed by 2D-LC	107	Metabolism, pathogenesis related, redox stress, signal transduction
[32]	*B. oleracea*	Stem de-top	Xylem	1DE followed by 2D-LC	189	Carbohydrate metabolism, proteolysis, cell wall, oxido-reductases
[55]	*G. max*	Stem de-top	Xylem	2-DE	38	
[54]	*Gossypium hirsutum*	Stem de-top	Xylem	LC	455	Carbohydrate metabolism, cell wall, stress response, signal transduction
[40]	*Arabidopsis thaliana*	VIC	Leaf apoplast	2-DE, 1-DE	93	Cell wall, defense related, protein interaction, proteinases
[45]	*Medicago truncatula*	VIC and IB	Leaf apoplast	2-DE	81	Defense, redox, transport, cell wall, pathogenesis related
[66]	*Populus deltoides*	Pressure chamber	Leaf apoplast	2-DE, 2D-LC	144	Cell wall, stress defense, proteolysis
[44]	*Z. mays*	VIC	Leaf apoplast	2-DE	67	Cell wall, defense, transport
[48]	*Vitis vinifera*	VIC	Leaf apoplast	2-DE	89	Defense, cell wall, proteolysis
[46]	*Beta vulgaris*	Leaf centrifugation	Leaf apoplast	2-DE	164	Stress and defense, cell wall, metabolism
[68]	*Ricinus communis*	Stem puncture	Phloem	2-DE	18	Sugar metabolism, redox regulation, chaperones
[39]	*C. sativus* *C. maxima*	Stem puncture	Phloem	1-DE, 2-DE	45	Redox response, proteinase inhibitors, signaling, defense proteins
[34]	*B. napus*	Stem puncture	Phloem	2-DE and 1-DE	140	Redox stress, signaling, structural, RNA binding, metabolism
[69]	*Populus trichocarpa x P. deltoides*	Phloem flow from a cut stem into solution	Phloem	2-DE	48	Metabolism, signaling, stress, structural
[37]	*C. maxima*	Stem puncture	Phloem	2D-LC	1121	Embryo development, ubiquitination, proteolysis, RNA binding, metabolism
[38]	*Lupinus albus*	Stem puncture	Phloem	2-DE	86	Metabolism, protein modification, redox regulation, stress and defense, structural components
[36]	*Lupinus texensis*	Stem puncture	Phloem	2-DE and 1-DE	54	Protein modification, metabolism, redox stress, cell wall