Vascular bundles

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Vascular bundles play important roles in transporting nutrients, growth signals, amino acids, and proteins between aerial and underground tissues.

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1. Introduction

Higher plants contain vascular bundles that connect all of their organs and act as a long-distance communication system to transport carbohydrates, nutrients, and growth signals throughout the plant, in order to regulate growth and development [1]. Plant vascular bundles contain two major types of transport units, the xylem and the phloem, with the apoplast compartment acting as an interface between them. Xylem tissues are composed of tracheary elements, parenchyma cells, and fiber cells that provide physical support for plant growth in woody plants[2][3]. During differentiation, tracheary elements lose their nuclei and cellular contents, which leaves a hollow tube that becomes part of the xylem vessel, which is used to transport the water and minerals taken up by the roots from the soil. In contrast, the phloem tissue facilitates the movement of photosynthates and other macromolecules among different organs within the plant. Phloem tissues include phloem parenchyma cells (PPCs), companion cells (CCs), and sieve elements (SEs). During differentiation, the SE undergoes selective autophagy, which results in the breakdown of the nucleus, tonoplast, and some other organelles such as ribosomes, Golgi, and microtubules. Consequently, the enucleate SE loses its protein biosynthesis ability and has limited metabolic activity, and it depends on companion cells for many of its functions via the establishment of a functional SE–CC complex between the two cell types[2][4].

In recent years, evidence has accumulated that various components, including hormones, mRNAs, amino acids, proteins, and lipidic molecules, might participate in the coordination of the developmental and physiological events at the whole-plant level[5][6]. Many highly-informative data sets derived from microarrays and the recent sequencing-based omics analyses have been obtained from plants. Xylem and phloem tissues have been shown to harbor various RNAs and proteins[7]. Specific information related to the vascular RNA repertoire is available in recent reviews[8][9]. In addition, thousands of vascular proteins have been identified from plants, including cucurbits, rice, *Ricinus*, and *Brassica napus* and *Populus*, using 1-D PAGE [10][11][12], 2-D PAGE[5][13][14][15], iTRAQ[16], and three-dimensional gel electrophoresis [17]. In this review, we focus on the vasculature located proteins with functions in growth, development, defense, and biotic and abiotic stresses. We also discuss the technical advances and challenges in the area of vascular sampling.

2. Sample Collection Methods from Vascular Tissues

2.1. Xylem Samples

Xylem sap has frequently been obtained by decapitating plant shoots and collecting from the cut shoot due to root pressure [18]. This can be performed for most monocots and many dicots, including *Solanum lycopersicum*, *Brassica napus* and *B. oleracea*, because their stems are large enough to collect sufficient volumes of xylem sap[2][10][13]. The cutting of plant stems followed by the application of external pressure using a Scholander chamber may lead to the excessive release of intracellular proteins. A prerequisite for the use of this technique is that the studied plant needs to have turgid stems. Another weakness related to this technique is the underestimation of the concentrations of the components in the xylem sap due to the dilution effect of the pressure.

The collection of exudates facilitated by root pressure is more suitable for non-woody plants. For woody plants, e.g., *Populus* and *Vitis*, additional manipulations, such as external bark removal, is required [18][19]. When a piece of bark is removed from a tree, new periderm is formed and wound cambium is developed from the callus on the surface of the secondary xylem. New phloem is subsequently formed from the wound cambium[20]. After the separation of the phloem and cambium from the stem, the wood-forming tissue can be collected from the exposed surface by scraping it with a

razor blade. This scraped secondary xylem is expected to contain cell types that include secondary cell-wall–forming xylem vessels and fibers. Changes in protein expression patterns corresponding to the different stages of secondary vascular system regeneration provide the opportunity to monitor[5][21][22] the biological events during the different stages of wood formation[23][24].

2.2. Phloem Samples

Phloem sap is much more difficult to obtain than xylem sap in most plant species, and the major challenge of phloem sap proteomics is therefore to obtain sufficient authentic materials for analysis. Several different methods have been used, and their feasibilities largely depend on the plant species of interest.

2.2.1. Stylectomy

Some plants exude sap spontaneously when their phloem tissues are cut. Phloem sap can be collected by making shallow incisions into the bark of the hypocotyl. Each cut severs one-quarter to one-third of the bark, and attention should be paid to avoid damaging the underlying woody tissues (xylem). This stylectomy method has been used in rape [25], cucumber[26], melon[27], mulberry[28], apple trees[29], and tomato[30].

Recently, an alternative method of sap collection has been reported, which involves peeling off the outermost (phloem) layer, and placing the peels into a plastic centrifuge tube with stainless steel balls, followed by centrifugation to acquire the phloem sap[30]. This method is straightforward and rapid, but it is not feasible with most plants because the accumulation of callose and P-proteins at the wound site can block the flow of sap.

2.2.2. Insect Stylectomy

Another classical method for phloem sampling is to take advantage of insects, such as aphids and leaf-hoppers, that feed on phloem. In order to collect the phloem sap using this method, the stylet is first severed from the insect body [29]. Due to the high turgor pressure of the SEs, the stylet that is left behind exudes phloem sap for a period of time. The sap can be collected and used for transcriptomic and proteomic analysis. An improved technique has been developed recently using aphid stylectomy with many plants. In this technique, aphids are placed in sealed cages, which are flooded with water-saturated silicon oil immediately after the micro-cauterization of the stylets. The phloem sap exuded from the stylet is then collected with a capillary connected to a pump[31]. In addition, phloem sap has also been obtained by puncturing the inflorescence stems of plants, such as those of *Brassicaceae* species, with a small hypodermic needle[23], and concentrating the phloem sample using centrifugation with a molecular weight cutoff (MWCO) of 10,000 Daltons (Da) [32].

The insect stylectomy method provides relatively-pure phloem sap. However, it remains challenging due to its labor-intensive nature, the low amount of sap collected, and its limitation to certain insect–plant combinations. The collected sap might be contaminated with a trivial amount of xylem components, because many phloem sap-feeders also feed on xylem sap occasionally[29].

2.2.3. EDTA-Facilitated Exudation

EDTA-facilitated exudation is the most popular method; it enables phloem sap to be collected from many plants, including those species that do not naturally exude sap[5][21][22]. In this method, phloem sap is collected by submerging the ends of severed petioles in an EDTA-containing solution for several hours. The EDTA inhibits the accumulation of callose and P-proteins in the phloem sieve tube, and results in a continuous outflow of the SE contents into the EDTA solution. The disadvantage of this method is that samples are easily contaminated with the contents of xylem and other non-phloem tissues, which probably come from the damaging of plant tissues by the EDTA[33].

2.3. Laser Capture Microdissection (LCM)

A different strategy from the aforementioned phloem sap analysis methods is to directly collect the vascular tissues before the proteomics profiling[34]. Vascular bundles from *Arabidopsis thaliana* have been collected by laser micro-dissection/pressure catapulting (LMPC) and subjected to high-efficiency liquid chromatography (LC) in conjunction with tandem mass spectrometry (LC/MS)[35]. A similar strategy that dissected the vascular components from the stem of broccoli was also reported[36]. A more extensive adoption of LCM for species that are less feasible for the sap methods will lead to promising discoveries related to vascular-tissue–specific proteomics.

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