Photoassimilate Translocation

6

Rashmi Shakya and Manju A. Lal

During evolution, early land plants were challenged by serious environmental pressures for their survival. The main challenge was the absorption and retention of water. This pressure for survival led to the differentiation of roots for absorption of water and inorganic nutrients and leaves for light absorption, photosynthesis, and gaseous exchange. Development of leaves rendered plants capable of carrying out photosynthesis. Xylem strands are responsible for transport of water and minerals from roots to the aerial parts of the plants, while translocation of photosynthetic products (**photoassimilates**) is facilitated by phloem elements. It is estimated that as much as 80% of the photosynthetically fixed carbon can be exported out of mature leaves. Storage or photosynthesizing organs, which have surplus sugars, can either metabolize or export them. These are known as **source**. On the contrary, actively metabolizing organs or the ones which store carbohydrates need to import them. These plant parts are known as **sinks**. Life cycle of a plant is characterized by source-sink transitions due to changes in sink strength. A plant consists of series of sources and sinks, with many sinks competing for sugars exported by the source organs. Phloem plays a major role in connecting source and sink. In the early developmental stage of a plant, roots and shoots majorly compete for receiving photoassimilates, and later on many other organs become effective sinks. These include reproductive structures, buds and flowers, and developing grains or the underground storage organs, such as tubers. **Sink strength** or **sink dominance** refers to the capacity of sink organs to acquire sugars from the transporting vascular strands. Distribution of sugars in the sink is the key factor in determining the **harvest index (HI)**, which refers to the ratio of dry weight of harvestable part (economically important) of the plant to the total dry weight of the plant. The higher the ratio (high value of HI), the higher the plant productivity. Thus, transport of photoassimilates is targeted as the key factor determining plant productivity. Various abiotic and biotic factors

S. C Bhatla, M. A. Lal, *Plant Physiology, Development and Metabolism*,
https://doi.org/10.1007/978-981-13-2023-1_6

adversely affect translocation of sugars. Accumulation of sugars in the cytosol of mesophyll cells at the source is the key factor for inhibiting photosynthesis. Studying phloem structure is important since it would facilitate in learning about the molecular mechanism involved in sugar translocation. Mineral deficiency may result in increase in the ratio of roots to shoots. Various sucrose transporter proteins (SUTs), facilitating intracellular sugar transport between various subcellular compartments as well as from cell to cell, have potential roles in controlling sucrose movement to the desired sinks. The chapter deals with the source and sink concept, pathways involved in photoassimilate transport, and unique features of sugar translocation. Later part of the chapter shall explore the mechanisms of phloem translocation, including phloem loading and unloading of photoassimilates and distribution of photoassimilates that encompasses allocation and partitioning.

6.1 Source-Sink Relationship

Translocation of photoassimilates occur in phloem which can be functionally characterized into three different zones along the source-to-sink pathway (Fig. 6.1). At the sources, these are often referred as **collection phloem**, while at the sinks, these are termed as **release phloem**. The connecting pathways of the two

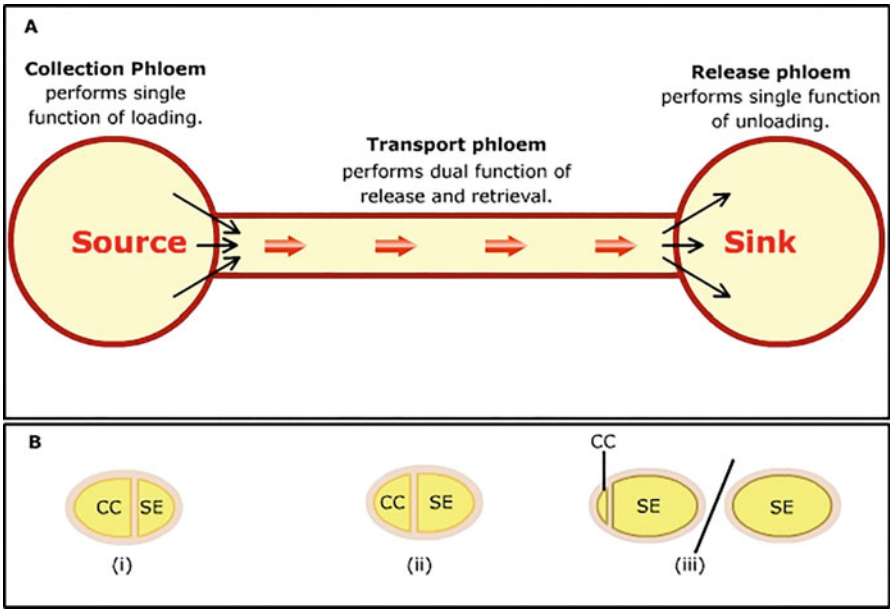


Fig. 6.1 (**a**) Three functional zones identified in phloem. (**b**) Three different phloem zones can be characterized by the size ratios between sieve element and companion cell. *CC* companion cell, *SE* sieve element

are known as **transport phloem**. Supply of photoassimilates from all sources does not reach all sinks. Instead, specific sinks are preferred over others by certain sources. Factors affecting the movement of photoassimilates from source to sink are as follows:

1. *Proximity of source to sink*—Mature leaves (sources) located on the upper region of the aerial parts of plants usually provide photoassimilates to the developing immature leaves (sinks) on the same orthostichy (a vertical row of leaves arranged one directly above another). Leaves present on the lower portion of stem predominantly supply underground parts of plants, whereas leaves present in the middle portion of the stem supply in both upward and downward directions.
2. *Developmental stage*—Root and shoot apices are usually the major sinks during vegetative growth, whereas developing fruits become the major sinks during reproductive phase. At the time of senescence, mature leaves serve as sink. Thus, there is change in the source and sink status of the growing organs during plant development.
3. *Vascular connections*—Sinks which have direct vascular connections with source are preferred.
4. *Modification in translocation pathways*—Wounding interferes with the translocation pathway and leads to the alteration of translocation patterns in relation to proximity and vascular connections. In fact, vascular interconnections, known as anastomoses (reconnection of leaf veins that were previously branched out), act as alternative pathway for translocation in the absence of direct vascular connections between source and sink.
5. *Sink strength*—Ability of a sink to store or to metabolize sugar imports determines its capacity to compete for sugars exported by various source tissues.

Removal of a sink results in increased translocation of sugars to other available and competing sinks. Young leaves act as stronger sinks in comparison to roots when supply from a source is compromised. Rapid utilization of sugars by sink cells results in lowering the concentration of photoassimilates in sieve elements of the young leaves resulting in lowering of the hydrostatic pressure. As a result, an increase in pressure gradient between source and sink is evident, and it leads to change in the translocation of photoassimilates. This ability of young leaves to mobilize sugars toward themselves is due to their relatively high **sink strength**. Sink strength is dependent on the size (total weight of sink tissue) and activity of sink (rate of uptake of transport sugars per unit weight of sink tissue). Translocation patterns may be modulated because of alteration in either sink size or activity.

$$\textit{Sink strength} = \textit{sink size} \times \textit{sink activity}$$

6.2 Transition of Leaf from Sink to Source

In eudicots, leaves act as sinks during the initial stages of their development. The transition of leaves from sink to source takes place gradually. This transition is initiated when the leaf attains 25% of its mature size and transition is usually completed in 40–50% expanded leaves. Transition during leaf development is accompanied by several anatomical and physiological changes responsible for the reversal of function from sinks to source. Sugar export from the leaf is initiated at the apex of the leaf blade and gradually moves toward the base until the entire leaf functions as a photoassimilate exporter. During this transition, leaf tip exports sugars, whereas the base imports it from other source leaves. Initiation of export and cessation of import are two independent processes. In tobacco leaves, it has been shown that loading and unloading of sugars occurs through entirely different veins (Fig. 6.2a). When leaf is young, it is in its sink phase, and it imports photosynthates from mature leaves, which gets distributed throughout lamina via major veins (Fig. 6.2b, thick lines marked with arrows). The photosynthate import unloads from the major veins into the mesophyll. The minor veins (marked with IV) which are enclosed by major veins of third-order (III) do not function in import and unloading as they are immature. The minor veins do not attain maturity until the import ceases from the source leaves. The cessation of import involves blockage of unloading from the major veins for sometime during the leaf development. The closing of plasmodesmata and a decrease in plasmodesmatal frequency are instrumental in stopping the import of sugars. Leaves begin to export sugars after the closure of sugar import route and accumulation of sufficient photoassimilates in the sieve elements necessary to initiate translocation (acts as source). In a source leaf, photoassimilates get loaded into the minor veins, and the major veins function for export only, i.e., they can no longer unload the photosynthate (Fig. 6.2c).

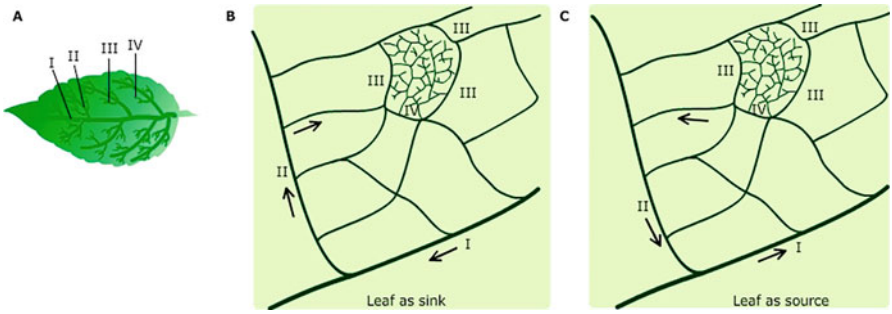


Fig. 6.2 (**a**) Veins in the leaves show division of labor. (**b**) When leaf is immature, it acts as a sink. (**c**) When the leaf is mature, it starts functioning as source. (I) Larger major vein of first order (midrib), (II) major veins of second order, (III) major veins of third order, (IV) minor veins. Arrows indicate the direction of flow of photoassimilate

6.3 Pathway of Photoassimilate Translocation

6.3.1 Experimental Evidence

6.3.1.1 Girdling

In 1686, Marcello Malpighi (an Italian anatomist) performed a classical experiment on the translocation of organic solutes. In this experiment, bark of a tree was removed in the form of a ring around the trunk. In 1727, Stephan Hales (an English clergyman) repeated the girdling experiment. In this experiment, a strip of bark around a tree trunk was removed which effectively eliminated the phloem elements. Subsequently, swelling in the region of the bark just above the girdle was observed. Contrary to this, bark region which was present immediately below the girdle shrank (Fig. 6.3). The experiment demonstrated that due to girdling, transport of sugars from photosynthesizing leaves to the roots was obstructed. However, transport of water through xylem remained unaffected. The experiment demonstrated that transport of sugars from leaves to roots occurs through phloem. The plant died after some time which demonstrated that the photoassimilates are essential for growth of those plant parts which cannot perform photosynthesis.

6.3.1.2 Autoradiography

Availability of radioactive compounds after World War II provided scientists an opportunity to use them in various experiments. **Reverse-flap** technique was used

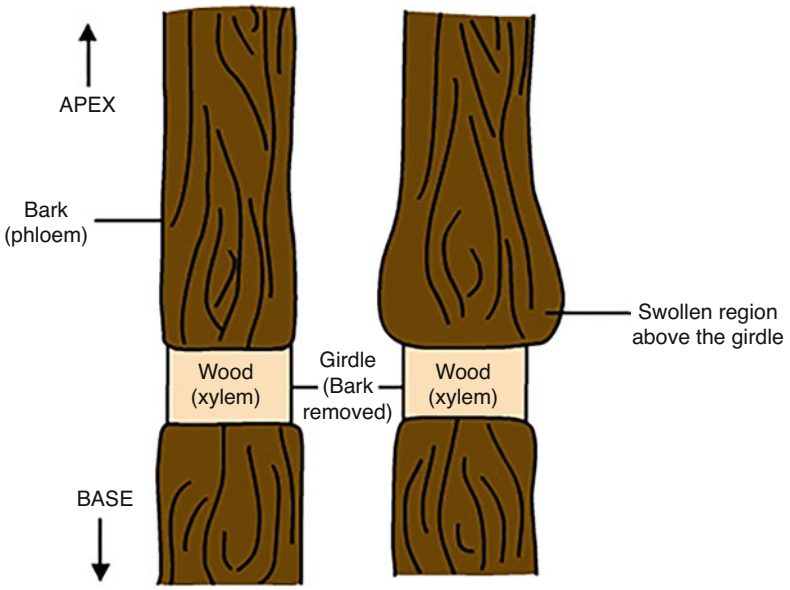


Fig. 6.3 Diagrammatic representation of girdling experiment in stem of an intact plant, showing swelling of the region above the girdle due to accumulation of sugars

for application of radioactive tracers on the leaf. In another approach, the leaf cuticle was removed by abrasion, and then radioactive compounds were applied directly to the leaf. Alternatively, leaf was exposed to labeled carbon dioxide ($^{14}CO_2$) in a closed chamber. Subsequently, $^{14}CO_2$ incorporated into the photoassimilates was analyzed which then got exported via translocation stream. The labeled ^{14}C first gets incorporated into sucrose, but later it is incorporated in many other organic compounds. Localization of these radioactive compounds can be done with the help of autoradiography technique.

6.4 Features of Phloem Cells with Reference to Photoassimilate Translocation

Phloem consists of sieve elements (SEs), companion cells, and phloem parenchyma. In addition, phloem tissue may include fibers and sclereids also which primarily provide strength and protection. Phloem parenchyma stores and releases food molecules. Mature sieve elements (SE) are phloem cells which are specialized for translocation. Sieve elements were discovered by Theodor Hartig in 1837, and their role in long-distance transport was demonstrated by him in 1860. A sieve element is a composite term used for sieve-tube elements (in angiosperms, STE) and sieve cells (in gymnosperms). Sieve areas are present on the cell walls of sieve elements. STEs in angiosperms are characterized by the presence of sieve plates which are differentiated sieve areas, unlike in gymnosperms which have no sieve plates and all sieve areas are similar. The adjacent conducting cells are interconnected by pores, forming tube-like structures. The diameter of pores in other sieve areas ranges from <1 to ~15 μm. Plasma membrane is in continuation at the sieve pores. STE are unique among living plant cells and are characterized by the lack of many structures usually found in the living cells. In angiosperms, sieve elements are associated with one or more companion cells. STEs and their associated companion cell(s) are derived from unequal division of a common mother cell. Numerous plasmodesmata are present between STE and companion cells indicating of their functional association. During development, sieve elements lose nuclei. First, chromosomes are lost and then nuclear envelop is lost. Nucleolus is retained the longest. However, cytoplasm is not destroyed. Generally, sieve elements lack microfilaments, microtubules, ribosomes, and Golgi bodies. Besides retaining plasma membrane at maturity, sieve elements contain modified mitochondria, plastids, and smooth endoplasmic reticulum, and their walls are non-lignified (Fig. 6.4). Tonoplast is not continuous in mature sieve elements and is absent at the cross walls. As a result, there is no clear distinction between the cytoplasm and the vacuole, and the inner space is called lumen. Sieve plates are characterized by relatively larger pores than other sieve areas and are present on the end walls of STEs. The end walls of SEs are longitudinally joined together to form sieve tubes. Thus, long sieve tube consists of sieve-tube elements which are connected with each other via sieve pores. Unlike

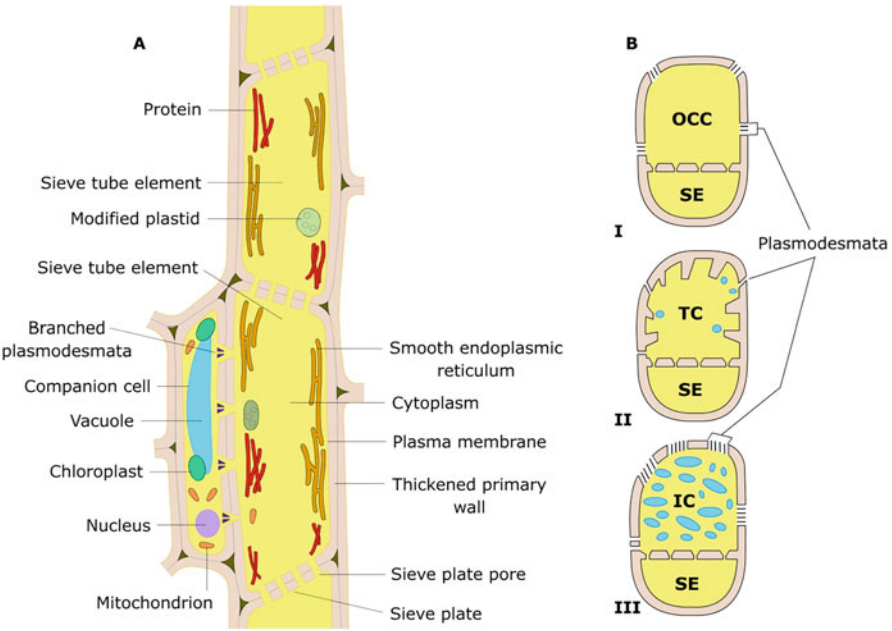


Fig. 6.4 (**a**) Diagrammatic representation of the ultrastructural features of a mature sieve element. (**b**) Diagrammatic representation of three different types of companion cells (arrows pointing plasmodesmata). *SE* sieve elements, *OCC* ordinary companion cell (I), *TC* transfer cell (II), *IC* intermediary cell (III)

xylem vessels, these are living cells. These cells can be plasmolyzed since plasma membrane of these cells is maintained because of which sieve-tube elements retain the capacity to generate turgor pressure.

6.4.1 Phloem Sealing Mechanism

During translocation process, sieve tubes are under very high internal turgor pressure. Whenever there is damage to a sieve tube (e.g., a cut), the release of pressure results in oozing of its contents from the cut end which causes loss of **phloem sap** containing photoassimilates. Plants employ sealing mechanism to avoid loss of photoassimilates. Short-term plugging involves P-proteins, while callose is involved in the long-term damage control mechanism. **P-proteins** (earlier known as slime) are found in STE of most of the angiosperms (except few monocots) but are absent in gymnosperms. P-proteins may exist in tubular, granular, fibrillar, or crystalline forms depending upon the species and maturity of the cell. In immature cells P-proteins appear as discrete bodies and are referred as **P-protein bodies**. P-protein bodies may be spindle-shaped, spheroidal, coiled, or twisted. In *Cucurbita*

maxima, P-proteins constitute two major proteins, phloem filament protein (PP1) and phloem lectin (PP2). They are synthesized in companion cells and later transported to sieve elements symplastically (via plasmodesmata). The gene encoding PP1 shows sequence similarity to the genes which encode for **cysteine proteinase inhibitors**. This homology indicates that PP1 might play a possible role in defense against phloem sap feeding insects. Damaged sieve elements are sealed off by P-proteins and other cellular inclusions which plug the sieve plate pores. This leads to prevention of further loss of phloem sap. Biosynthesis of callose, a β-1, 3-glucan, in sieve pores also prevents loss of phloem sap caused by damage to STEs. Callose synthase synthesizes callose in the plasma membrane which is then deposited between the cell membrane and cell wall. **Wound callose** is synthesized in response to damage in sieve elements. Gradually, deposition of wound callose in the sieve pores seals off the damaged sieve elements from the surrounding tissue, leading to prevention of phloem sap loss. Sieve pores of the STE are constricted due to deposition of callose. During the recovery of damaged sieve elements, wound callose disappears due to activity of callose-hydrolyzing enzyme.

6.4.2 Sieve Tube-Companion Cells Interaction

Companion cells are developmentally and functionally closely associated with the STE. Each STE is associated with one or more companion cells. These are connected to STE through numerous plasmodesmatal connections. Since companion cells are not longitudinally continuous, they do not constitute pathway for photoassimilate transport. Transport of photoassimilates to STEs in minor veins of the leaves is facilitated by companion cells. Venation pattern in leaves is designed to optimize photoassimilate transport from mesophyll cells to SE/CC complex. Minor veins in the leaves are the catchment area which consists of one or few SE/CC complexes. Structurally, minor veins have few sieve elements and much larger parenchyma cells. Minor veins constitute collection phloem. CCs are believed to provide energy to STEs in the form of ATP (by virtue of having several mitochondria) and proteins because the cytoplasm of STEs lacks mitochondria, which are crucial for maintenance of cell structure. Companion cells have nucleus with large degree of endoploidy. The presence of dense cytoplasm with large amount of RNA and many mitochondria in CCs suggest that they are metabolically active. High ATPase activity has been demonstrated in companion cells. Species have been categorized depending on apoplasmic and symplasmic continuity of the minor veins with the phloem parenchyma cells which surround them (discussed later). Type 1 species include the ones in which there are symplasmic connections in between the STE/CC complex and the surrounding parenchyma cells because of the presence of many plasmodesmatal connections. The companion cells in type 1 species are known as intermediary cells (Fig. 6.4b). Intermediary cells possess several small vacuoles but

have chloroplasts with poorly developed thylakoids. They also lack starch grains in the chloroplasts. Several plasmodesmata are present at the interface of these types of companion cells with the surrounding cells, especially with the bundle sheath cells. Type 2 species include those in which there are moderate-to-low number of plasmodesmata between STE/CC and the surrounding parenchymatous cells. In type 2A category, ordinary companion cells are characterized by relatively fewer plasmodesmatal connections with their surrounding phloem parenchyma cells. They possess chloroplasts with well-developed thylakoids, and the inner surface of the cell wall is smooth. Apparently, it appears that these might be involved in phloem loading mainly through apoplastic way or at times symplastic way since they either have none or variable number of plasmodesmatal connections with the surrounding cells. The second category of type 2, i.e., type 2B, possess companion cells which have finger-like wall ingrowths on the cell walls away from the sieve elements. These are known as **transfer cells**. The wall ingrowths increase the surface area of plasma membrane, thus increasing the transfer of solutes across the membrane. Similar to ordinary companion cells, transfer cells also possess very few plasmodesmatal connections with the surrounding cells and appear to be symplastically isolated. It is believed that these cells are specialized in solute uptake via apoplastic pathway. However, the role of few plasmodesmatal connections is presently not known. Transfer cells may also possess chloroplasts with well-developed thylakoids. Ordinary companion cells and transfer cells are features of those plants in which photosynthates are transferred apoplastically from mesophyll cells to sieve elements. On the other hand, intermediary cells function in symplastic transport of photosynthates from mesophyll cells to sieve elements. Table 6.1 depicts the relationship between the structure of various types of companion cells in minor veins and their role in phloem loading.

6.4.3 Composition of the Phloem Sap

It is difficult to obtain pure sap from STE of the phloem for analysis because of the possibility of contamination from the other injured tissues at the time of sample collection. Another difficulty in collecting phloem sap is due to the sealing

Table 6.1 Relationship between the structure of companion cells in minor veins and mode of phloem loading

Type of companion cells	Ordinary companion cell	Intermediary companion cell	Transfer cells
No. of plasmodesmata at the interface of companion cell and its neighboring cells	10–0.1 per μm^2	More than 10 per μm^2	Less than 0.1 per μm^2
Predominant transport sugar	Sucrose, sugar alcohols, and RFOs[a]	Sucrose, galactosyl oligosaccharides (RFOs)	Mainly sucrose

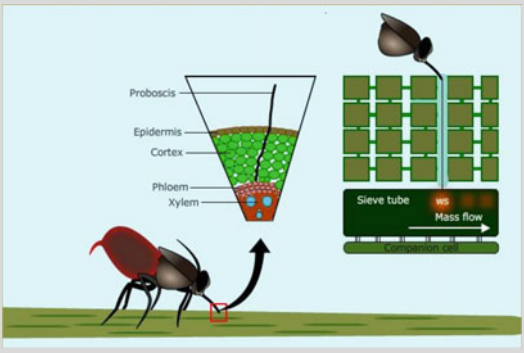
[a]*RFO* Raffinose family oligosaccharides

mechanism which occurs in the STEs at the time of mechanical injury. One of the methods used to reduce sealing due to injury is by a chelating agent such as EDTA, responsible for chelating Ca^{2+} ions required for callose synthesis. Another mechanism involved is collecting the sap from the severed stylet of an aphid after it has been anesthetized (Box 6.1). Possible presence of some compounds in the stylet does not allow the sealing in STE during suction by aphids. Since sap in the STE is under pressure, it keeps on oozing out of the stylet and can be collected. Chemical

Box 6.1: Technique for Collection of Phloem Sap

High turgor pressure in the sieve-tube elements and wound reactions make the collection of the phloem sap a challenging task. Sometimes during the process of severing, phloem damage is caused, and this leads to the contamination of the phloem sap. Sudden pressure released due to damage causes disruption of cellular organelles and proteins. Dilution of the phloem sample occurs due to influx of water from the xylem. Thus, analysis of phloem sap collected after severing the phloem is usually not a preferable approach.

A reliable approach for collection of phloem sap exploits aphid stylet as a "natural syringe." Aphids are small sap-sucking insects belonging to the superfamily Aphidoidea. Their mouth part consists of four tubular stylets which are inserted into shared cell wall between epidermal cells and steer them through cortical parenchyma cells (CPCs) and phloem parenchyma cells (PPCs) and finally to sieve tubes (STs). During this navigation, aphids secrete gel saliva which hardens into tubule and facilitate the forward movement of stylet. Aphids are anesthetized with CO_2 and their stylets are cut using laser. The phloem sap oozes out from the cut end due to high turgor pressure in the sieve elements which is collected for further analysis. The amount of phloem sap collected is small. This method of phloem sap collection is technically difficult but is believed to yield relatively pure phloem sap and, therefore, gives fairly accurate details about the composition of phloem sap.



analysis of the phloem sap, collected either from an aphid stylet or from wounds that have severed sieve elements, has revealed that it contains water, sugars, RNA, proteins, amino acids, organic acids, hormones, and mineral ions. Table 6.2 shows different types of materials translocated in the phloem. Ninety percent of the organic molecules present in phloem sap consists of carbohydrates which is mainly sucrose. Sucrose (0.3–0.9 M) has emerged as the most preferred sugar for long-distance translocation of photosynthates. This could probably be attributed to its nonreducing nature. The nonreducing sugars are less reactive in comparison to the reducing sugars. This is due to the fact that their ketone or aldehyde groups are reduced to an alcohol or they combine with a similar group on another sugar. The acetal link between the subunits is stable and nonreactive in alkaline conditions. On the contrary, reducing sugars such as glucose and fructose have a free aldehyde or ketone functional group which is capable of reducing mild oxidizing agents. Contrary to starch, sucrose is water soluble. In the members of families Rosaceae and Cucurbitaceae, oligosaccharides, such as raffinose (a trisaccharide), stachyose (a tetrasaccharide), and verbascose (a pentasaccharide), are also translocated in the phloem. In the stem internodes of *Cucurbita maxima*, stachyose (sucrose + two molecules of galactose) accounts for about 46% of the total translocated sugars, while in Oleaceae and Rosaceae families, polyols, such as mannitol and sorbitol, are also translocated. In *Sorbus aucuparia* (family Rosaceae), sorbitol is the main carbohydrate component of phloem sap next to sucrose. There are specific polyol transporters. pH of the phloem sap is high (7.4–8.7). Reduced glutathione, a translocated form of sulfur, is also present in phloem sap. Cations, such as K^+ and Mg^{2+}, are also found in the phloem sap besides the presence of trance amounts of Ca^{2+}.

In phloem, nitrogen is translocated in the form of amino acids, mainly glutamic acid and aspartic acid, and their respective amides, glutamine and asparagine. However, concentration of amino acids and organic acids is variable in the same species and is usually low as compared to sugars. Due to the presence of high amounts of carbohydrates, C/N ratio of phloem sap is much higher than that of xylem sap and ranges between 15 and 200. Levels of nitrogenous compounds being transported are quite high during leaf senescence since these get mobilized to the

Table 6.2 Composition of phloem exudates from the stem of castor bean (*Ricinus communis*)

Constituents	Concentration ($mg.mL^{-1}$)
Sugars	80–106
Amino acids	5
Potassium	2–4
Organic acids	2–3
Proteins	1–2
Chloride	0.4–0.7
Phosphate	0.4–0.6
Magnesium	~0.1

woody tissues for storage. Plants having nitrogen-fixing nodules transport nitrogen in the form of ureides, such as allantoic acid, allantoin, and citrulline. Proteins and RNA are also transported in low concentrations. P-proteins are the predominant form of proteins which occur in the phloem sap. Several enzymes, such as protein kinases, thioredoxin, ubiquitin, and chaperones, have also been reported. RNAs, which include mRNA, pathogenic RNAs, and small regulatory RNA, also occur in the phloem sap. RNA molecules usually form complexes with proteins as ribonucleoproteins (RNPs). RNAs and proteins probably act as signal molecules. Reportedly, phloem sap contains plant hormones, such as auxin, gibberellin, cytokinin, and abscisic acid. It has been suggested that, to some extent, long-distance transport of auxin occurs in the sieve elements. Plant defense hormone, jasmonic acid, has also been reported in the phloem sap. Recently, translocation of **xenobiotic** agrochemicals has also been reported in the phloem. Xenobiotics are biologically active molecules which are alien to an organism's body. The efficacy of xenobiotic agrochemicals as herbicides and insecticides is often determined by their rate of absorption and subsequent translocation. Leaf application of a broad-spectrum herbicide, N-(phosphonomethyl) glycine, also known as glyphosate, results in its rapid translocation to the meristematic regions, including underground roots, through phloem.

6.4.4 Photoassimilate Translocation: Unique Features

The rate of movement of various substances in the sieve elements can be expressed either in terms of velocity or mass transfer rate. Velocity is the linear distance traveled per unit time. Mass transfer rate refers to the quantity of material passing through a given cross section of sieve elements per unit time (**specific mass transfer**, i.e., SMT). The measurement of velocity can be achieved by employing simple conventional radioactive labeling technique. It involves exposing the source leaf to $^{14}CO_2$ for brief period. The arrival of ^{14}C-labeled sugars at the sink tissue is monitored with the help of a detector. The measurement of velocities through these techniques has shown that velocity of phloem transport ranges from 0.08 to 0.42 $mm.sec^{-1}$ (average ~ 0.28 mm/sec). However, more sophisticated techniques, such as nuclear magnetic resonance (NMR) spectroscopy and magnetic resonance imaging (MRI), are now being used for more accuracy. In castor bean, the velocity of photoassimilate transport, measured through NMR and MRI, has been found to be 0.25 $mm.sec^{-1}$. Presently, rates are measured as the mass (in kg) gained per unit time. Studies concerning the measurement of mass transfer rates have indicated that it ranges from 2.8 to 41.7 $\mu g.sec^{-1}.mm^{-2}$. The rate of sugar transport is higher than can be explained simply by diffusion.

6.5 Mechanism of Photoassimilate Translocation

Movement of photosynthates from source tissues to sieve elements is known as **phloem loading**. Energy is required for the movement of photoassimilates from sieve elements to the sink tissues. Movement of photoassimilates from sieve elements to the sink tissues is known as **phloem unloading**. Movement of sucrose along the transport route of phloem does not require any energy. Factors, such as structure of sieve elements, rates of translocation, and simultaneous translocation in different directions, should be taken into consideration, while any hypothesis for mechanism of sugar translocation is proposed. Thus, mechanism of photoassimilate translocation would be dealt under three headings, i.e., phloem loading, phloem unloading, and sugar movement in the conduits after the sugar has been uploaded at the source and sugars have been unloaded at the sink.

6.5.1 Photoassimilate Loading

Triose phosphate synthesized during daytime as a result of photosynthesis is transported from chloroplasts to the cytosol where these are utilized for synthesis of sucrose. Transitory starch stored in chloroplasts during daytime is converted to sucrose during nighttime. In some species, other forms of transport sugars, such as raffinose family of oligosaccharides (RFOs), are later synthesized from sucrose. Sugar alcohols, like mannitol, are also synthesized from hexose phosphates. During phloem loading sugars are transported into SE-CC complex. Phloem loading can occur via the apoplastic or symplastic pathways. In apoplastic transport, sugars have to cross the plasma membrane at least once, while sugars move from cell to cell through plasmodesmatal connections without crossing the plasma membrane during symplastic transport. In photosynthesizing leaves the initial transport of photosynthates (mainly sugars) from the mesophyll cells to parenchymatous cells adjacent to SE-CC complex is symplastic in nature. However, the subsequent movement of sugars to the companion cells can occur either through symplast via plasmodesmata, or sugars might enter the apoplast before phloem loading. In some species, one of the two transport pathways is predominant, while many species may exhibit more than one mechanism for loading of sugars.

6.5.1.1 Apoplastic Loading

In the apoplastic phloem-loading pathway, sugars are transported symplastically from mesophyll cells to phloem parenchyma cells located adjacent to SE-CC complex and are released in the apoplast (Fig. 6.5a), or alternatively sugars may be released in the apoplast near mesophyll cells and may diffuse in the apoplast region up to SE-CC and are uploaded in SE-CC complex. Sugars usually become more concentrated in the SE-CC complex as compared to the mesophyll cells which is confirmed by measurement of osmotic potential (Ψ_s) of these cells. In sugar beet, the osmotic potential of SE-CC complex has been observed to be about −3.0 MPa as compared to the osmotic potential of the mesophyll cells, which is −1.3 MPa. Since

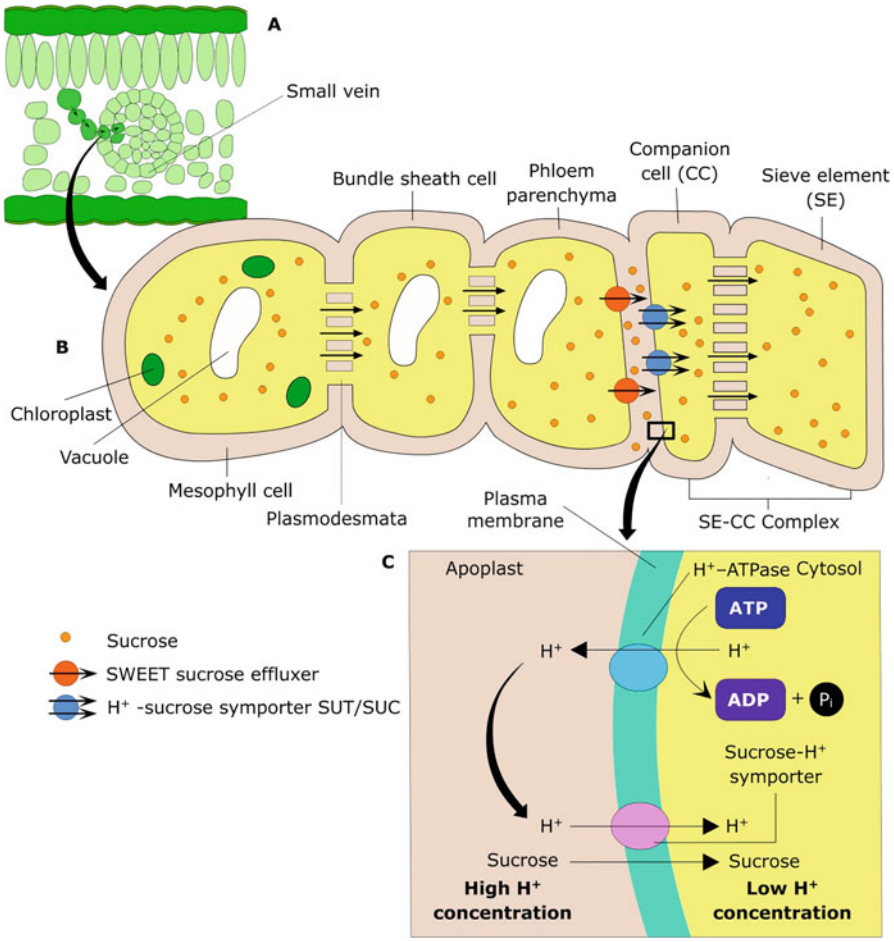


Fig. 6.5 (**a**) Vertical section of a typical dicot leaf. (**b**) A portion of leaf enlarged to show the apoplastic phloem loading. Transport of sugars from mesophyll to the phloem parenchyma cell is symplastic. Later, sugars are released in apoplast, or they may be released in apoplast near mesophyll cells and diffuse in the apoplast region near SE/CC for apoplastic loading. (**c**) ATPase located in plasma membrane of companion cell implicated in apoplastic loading of phloem

sucrose accumulation in SE-CC complex is against concentration gradient, the uptake of sucrose in the apoplastic pathway requires metabolic energy. The active loading of sucrose has been experimentally confirmed by demonstrating inhibition of loading of exogenously supplied sugars in the presence of respiratory inhibitors. There are sucrose efflux carriers located in the plasma membrane of phloem parenchyma cells or in the plasma membrane of mesophyll cells which are known as SWEET transporters (sugars will eventually be exported transporters). These are responsible for the release of sucrose in apoplastic region, and they play a significant

role in apoplastic mode of phloem loading. Pathogens induce expression of SWEET genes in order to access plant's sucrose pool. Transport of sugars from apoplast to the SE-CC complex again requires crossing of plasma membrane and is an energy-requiring process. It is facilitated by sucrose-H^+ symporters. These are specific sucrose transporters located in the plasma membrane (Fig. 6.5b). These sucrose transporters belong to SUT/SUC family. It is a secondary transport process in which energy generated by proton pump is used. ATPase located in plasma membrane pumps protons out of SE-CC complex into the apoplast (Fig. 6.5c). This leads to the establishment of higher proton concentration in the apoplast, and a membrane potential of about −120 mV is established. Energy in the proton gradient is subsequently exploited to drive cotransport of sucrose into the symplast of the SE-CC complex, which is coupled with H^+ transport. Several sucrose-H^+ symporters have been localized in phloem. To name a few, sucrose transport proteins SUT1 and SUC2 have been identified in *Solanum tuberosum* and *Arabidopsis thaliana*, respectively. On the contrary tonoplast sucrose carriers function as sucrose-H^+ antiporters. In many species polyol transporters have also been identified for transport.

6.5.1.2 Active Symplastic Loading

Several species are characterized by the presence of intermediary cells which have numerous plasmodesmata with the surrounding cells. Species such as coleus (*Coleus blumei)*, pumpkin (*Cucurbita pepo)*, and melon (*Cucurbita melo*) have intermediary cells in the minor veins. All the cells right from mesophyll cells to SE-CC complex are connected to each other through plasmodesmata (Fig. 6.6). How does SE-CC complex contain higher solute concentration as compared to mesophyll cells? How does accumulation of specific sugars take place against a concentration gradient? Following two possible mechanisms have been proposed to answer these questions.

6.5.1.3 Polymer Trapping

It is essential that sucrose concentration should be high in the mesophyll cells as compared to the intermediary cells for successful diffusion of sucrose into the intermediary cells. According to the model proposed by Turgeon (1996), larger sugar molecules belonging to RFOs (raffinose family of oligosaccharides), such as raffinose a trisaccharide (fructose-glucose-galactose), stachyose-a tetrasaccharide (Fru-Glu-Gal-Gal), and verbascose, a pentasaccharide (Glu-Fru-gal-gal-Gal) (Fig. 6.7a), are synthesized inside the intermediary cells from the transported sucrose and galactinol (a metabolite of galactose). Enzymes essential for the synthesis of RFOs are preferentially located in the intermediary cells. The utilization of sucrose for synthesis of RFOs in intermediary cells and its synthesis in mesophyll cells maintains the concentration gradient required for the diffusion of sucrose into the intermediary cells. These polymers synthesized in the intermediary cells cannot diffuse back to mesophyll cells. Possibly the size exclusion limit (SEL) of plasmodesmata at the interface of phloem parenchyma and the intermediary cells is small so that molecules larger than sucrose are excluded by them. The SEL of plasmodesmata present at the interface of the intermediary cells and the sieve

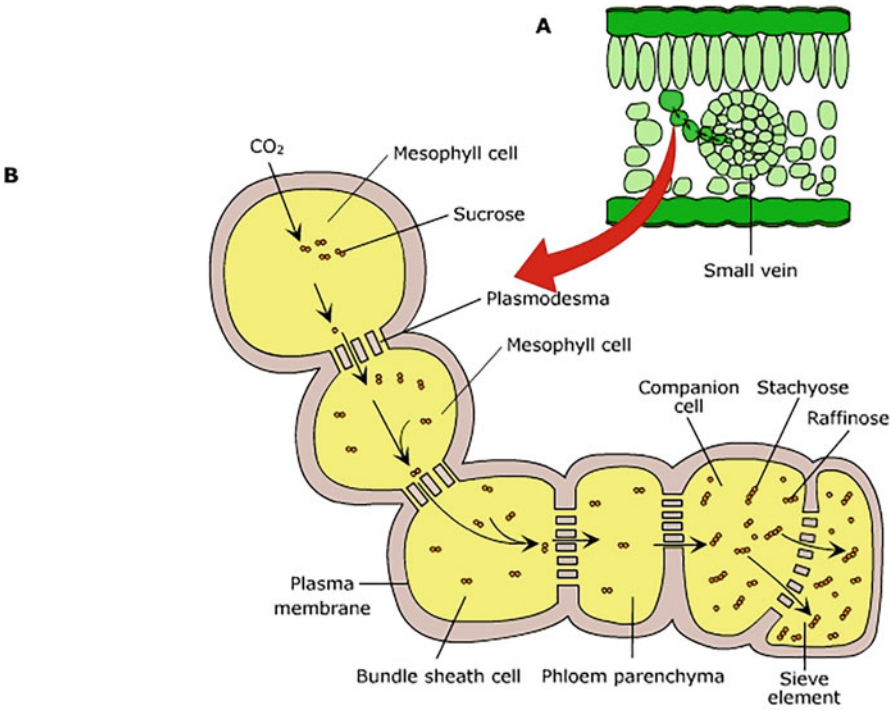


Fig. 6.6 (**a**) Vertical section of a typical dicot leaf. (**b**) A portion of leaf enlarged to show the symplastic pathway of phloem loading

elements should be large enough to allow the passage of the polymerized sugars such as raffinose, stachyose, and verbascose molecules. Since according to this model sugars in polymeric form (raffinose, stachyose, and verbascose) are trapped because of their large size and cannot flow back, this model is known as polymer-trapping model (Fig. 6.7b). Sucrose gradient maintained in between SE-CC complex and mesophyll cell facilitates diffusion of sucrose. Though sucrose moves passively from mesophyll cells to intermediary cells in response to concentration gradient, polymer-trapping model is an active mechanism. Unlike apoplastic mode of sugar translocation, energy is not consumed for the transport of sucrose across the plasma membrane; rather it is consumed for linking of sucrose to one, two, or three molecules of galactinol which is ATP-requiring process.

6.5.1.4 Passive Symplastic Loading

Symplastic loading of photoassimilates in some plants is passive and is driven by simple diffusion. Passive loading is more widespread in woody species especially in trees. In passive symplast phloem-loading transport of sucrose occurs in response to solute concentration in between that of mesophyll cells and SE-CC complex. Comparison of solute concentrations and osmotic potentials of whole leaves has shown that sucrose and sugar alcohols are more concentrated in the cytosol as

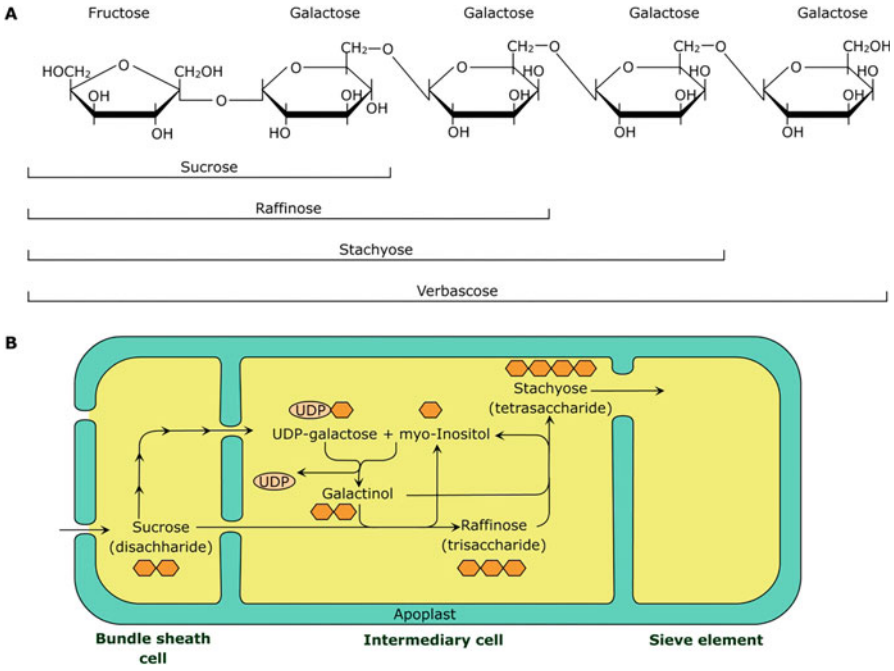


Fig. 6.7 (**a**) Different sugar polymers depicting their constituent units. (**b**) Diagrammatic representation of polymer-trapping model

compared to vacuoles of mesophyll cells. This results in increasing the driving force for passive loading in species that employ this strategy.

6.5.1.5 Patterns of Apoplastic and Symplastic Phloem Loading

In tropical and subtropical regions, trees and shrubs possess abundant plasmodesmatal connections between phloem and surrounding cells. Contrary to this, herbaceous plants found in temperate and arid regions have less plasmodesmata between phloem and the surrounding cells. This indicates that apoplasmic pathway of phloem loading is preferred in plants growing in temperate climate, while symplasmic phloem loading may be preferred in plants growing in tropical climate. This may be due to decrease in solubility of raffinose at lower temperature which can increase the viscosity of phloem sap resulting in decrease in photosynthesis. That may be the reason for apoplastic mode of photoassimilate translocation in temperate plants. Reduced capacity of photoassimilate transport in plants with symplasmic phloem loading may further be associated with limited capacity of intermediary cells to convert sucrose to RFOs. Table 6.3 summarizes the comparison in patterns observed in apoplastic and symplastic phloem-loading mechanisms. There are plants which are loaded entirely by one mechanism, such as tobacco (*Nicotiana tabacum*), an apoplastic loader, and mullein (*Verbascum phoeniceum*), a symplastic loader. Some plants, such as oyster plant (*Acanthus mollis*) using "polymer-trapping

Table 6.3 Patterns of apoplastic and symplastic phloem loading

Characteristic features	Apoplastic phloem loading	Symplastic phloem loading	
		Polymer trapping	Passive
Habit of plant	Mainly herbaceous	Herbs and trees	Mainly trees
Number of plasmodesmata connecting the SE-CC complex to the surrounding cells	Few	Several	Several
Type of companion cells	Ordinary companion cells or transfer cells	Intermediary cells	Ordinary companion cells
Transport sugar	Sucrose	Sucrose and RFOs	Sucrose and sugar alcohols
Dependence on transporter in SE-CC complex	Dependent	Independent	Independent
Overall concentration of transport sugars in source leaves	Low	Low	High

mechanism" for translocation of sugars, are also capable of apoplastic phloem loading. These plants possess both intermediary cells and transfer cells in their minor veins. In *Alonsoa meridionalis*, the expression of stachyose synthase gene (indicative of symplastic polymer trapping) and sucrose transporter (indicative of apoplastic phloem loading) has been found to be specific to the intermediary cells and ordinary companion cells, respectively.

6.5.2 Photoassimilate Unloading

Phloem unloading involves import of sugars from sieve elements to the sink tissues by an energy-demanding short-distance transport pathway. It is also called post-sieve element transport. Rapid phloem unloading occurs when an aphid feeds on phloem sap or also because of other pathogens. Aphids feed on nitrogenous compounds and carbohydrates are secreted as honeydew. Transported sugars are either stored or metabolized in sinks.

6.5.2.1 Phloem Unloading Occurs via Apoplast or Symplast

Due to variation in the structure and function of sinks, more than one possible pathway exists for phloem unloading and short-distance transport. The phloem-unloading pathway often depends on the developmental stage of the sinks. Like phloem loading, the process of phloem unloading can occur entirely through symplast via plasmodesmata. In few dicots, such as sugar beet and tobacco, phloem unloading is completely symplastic in young leaves. Root tips, the meristematic and elongating regions of roots, also exhibit symplastic pathway for phloem unloading. Mostly symplastic pathway predominates in sink tissues having involved in cell division and metabolic activities, but sinks involved in storage activities, such as in

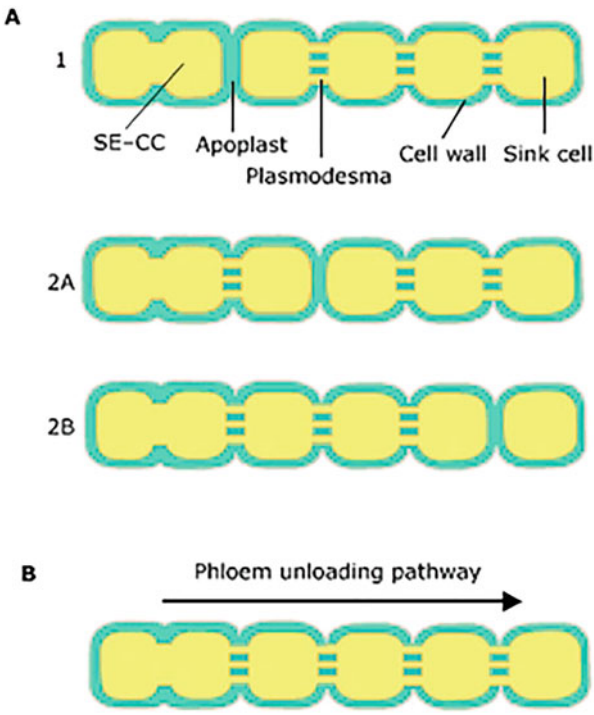


Fig. 6.8 (**a**) Apoplastic phloem unloading and short-distance transport. **1.** Apoplastic step is located at the site of unloading itself, i.e., during movement from SE-CC complex to phloem parenchyma. **2A.** Apoplastic step is located away from SE-CC complex, i.e., during movement from one parenchyma cell to another parenchyma cell or bundle sheath cell or **2B** from bundle sheath cell to sink cell. (**b**) Symplastic phloem unloading and short-distance transport

fruits and seeds, the short-distance pathway is partly apoplastic at some stage of development. In developing seeds, there is a requirement of an apoplastic pathway for uploading sugars. This is attributed to the absence of plasmodesmata between maternal and the embryonic tissues (Fig. 6.8a). There is switching of apoplastic or symplastic pathway in these sinks, with apoplastic pathway becoming functional when concentration of sugars in sinks is high. Apoplastic step could be located either at the site of unloading itself (Fig. 6.8a1) or away from SE-CC complex (Fig. 6.8a, 2A-2B). In the apoplastic pathway, the transport sugar can be partly metabolized in the apoplast or may cross the apoplast unchanged. For instance, sucrose can be hydrolyzed into glucose and fructose in the apoplast by cell wall invertase. Subsequently, glucose and/or fructose will cross plasma membrane to reach the sink cells. Cell wall invertase activity adds up to sink strength. Sucrose will be translocated in STE as long as gradient is maintained between source and sink. In the parasitic plant—*Orobanche* sp.—mannitol accumulates following haustorial connection which lowers the osmotic potential of the plant in comparison to host plant. This indicates that the enzyme responsible for mannitol synthesis in the *Orobanche sp.* can be targeted to ward off the parasitic plant. During symplastic unloading energy is not required for transport of sugar from sieve elements into sink tissues. Sugar unloading via plasmodesmata takes place passively (Fig. 6.8b). Movement of transport sugars from sieve elements to sink tissues takes place in response to the concentration gradient. However, metabolic energy is required in sink tissues for

various activities, such as respiration. In contrast, apoplastic phloem unloading involves movement of transport sugars across plasma membrane twice, i.e., plasma membrane of the SE-CC complex and sink cell. Furthermore, tonoplast is also traversed in case translocation of transport sugars takes place in vacuole for storage purposes. Experimental studies on soybean have shown that energy-dependent transporters facilitate sucrose unloading into apoplast and sucrose uptake into the embryo. The transporters implicated in efflux and uptake of sucrose have been shown to be bidirectional in some studies. The direction of the transport is determined by the sucrose gradient, the pH gradient, and the membrane potential. SUT1 symporter reported in potato tuber for phloem loading has also been reported from the sink tissues.

6.5.2.2 Long-Distance Translocation of Photoassimilates in STE-Pressure Flow Model

Mass flow is one of the widely acceptable models used to explain long-distance photoassimilate translocation in phloem. This was initially proposed in 1930 by Ernst Münch (a German biochemist). According to this model, the mechanism for the photoassimilate translocation in phloem is passive in nature (Fig. 6.9). It states that sugar loading in STE at source and unloading at sink are followed by water uptake and removal of water, respectively, which is then responsible for setting up the pressure gradient in the conduits (the sieve elements). Mass transfer of solutes from source to sink occurs along with water movement, which moves in response to a turgor pressure gradient (bulk flow). The energy-driven phloem loading in the source tissues causes accumulation of sugars in the sieve elements which generates a negative solute potential ($\Delta\Psi_s$) and leads to a drop in the water potential ($\Delta\Psi_W$). This results in development of water potential gradient, and water tends to enter sieve elements from xylem, causing an increase in turgor pressure (Ψ_P). Reverse happens in the sink tissues where unloading of sugars causes a decrease in sugar concentration in the sieve elements, leading to a decrease in solute potential and increased water potential as compared to that of xylem resulting in movement of water from sieve elements to xylem and subsequently decreased turgor pressure in the sieve elements of the sink. Movement of water in phloem takes place from source to sink in response to pressure potential gradient. Such water movement does not take place against the law of thermodynamics. The movement of water occurs by bulk flow rather than by osmosis during its movement from one sieve tube to another. Furthermore, the movement of solutes and water takes place at the same rate. Under these conditions, solute potential cannot contribute to drive water movement. Thus, in translocation pathway water movement is driven by pressure gradient rather than by water potential gradient. Sieve plates causes increase in resistance along the translocation pathway in sieve elements. Due to this substantial pressure is generated and maintained in the sieve elements between source and sink.

Certain objections have been proposed against the pressure flow hypothesis. These include the presence of sieve plates with sieve pores between STEs. Pores are likely to create obstruction to the solute transport. Studies carried out with

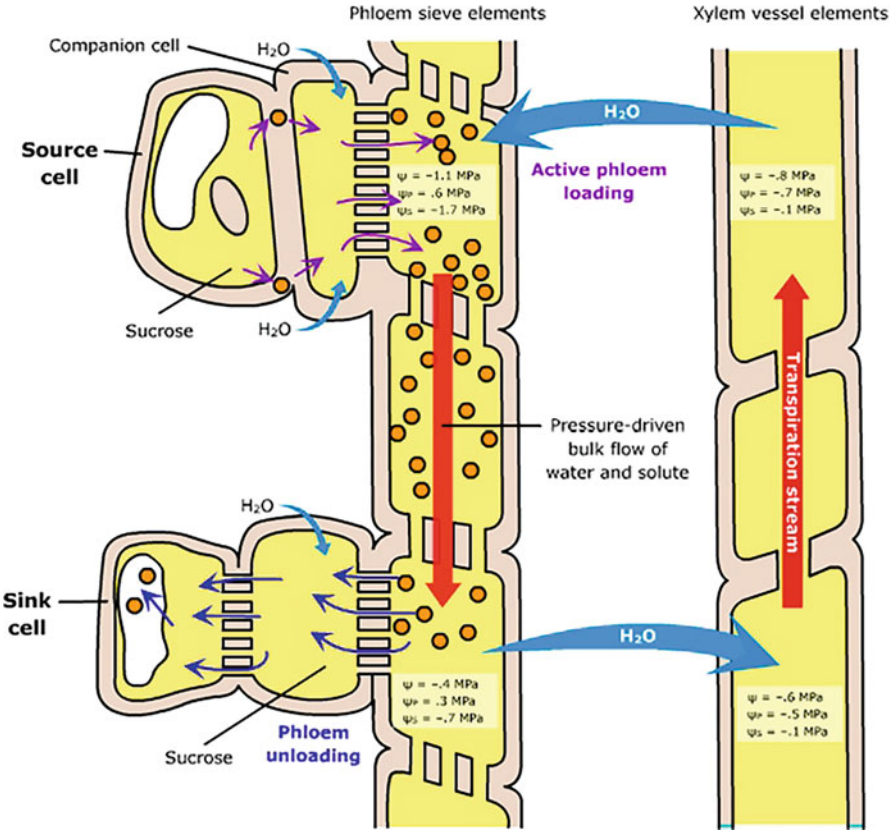


Fig. 6.9 Diagrammatic representation of pressure flow model for photoassimilate transport in phloem

confocal laser microscopy have facilitated analysis of intact. The proponents of mass flow hypothesis regard pores to be free from the obstruction in the natural state and dismiss the densely filled pores as the artifacts. Secondly, bidirectional flow of solutes has been observed for solute transport which can be explained by solute transport occurring in a single STE to be independent of another STE. Single sieve elements never exhibit bidirectional transport of photosynthates. Bidirectional transport occurs in sieve elements from different vascular bundles. Adjacent sieve elements of the same vascular bundle in petioles of leaves, which are undergoing transition from sink to source, also show bidirectional transport.

According to mass flow hypothesis, long-distance translocation of sugars is a passive process. Experiments involving treatment with low temperature or giving chilling stress or the use of inhibitors, which adversely affect the supply of ATP to the path tissues, do not influence translocation of photoassimilates. However, normal translocation is slowly resumed after a brief period of inhibition. However, ATP

turnover rate is high in phloem sap. Energy requirement may be for loading and unloading of sugars at source and sink sites, respectively. ATP may also be used for synthesis of phloem proteins which again is an energy-requiring process.

For an effective bulk flow of photoassimilates, positive pressure gradient between source and sink is necessary. Pressure gradient can be estimated with the help of pressure difference between source and sink. Solute potential and water potential provide the estimate of pressure potential. Turgor pressure should be high in sieve elements of source as compared to that of sink. The pressure difference should be sufficient enough to overcome the resistance observed along the pathway. Experiments conducted on soybean have demonstrated pressure difference of 0.41 MPa between the source and the sink. In fact, the pressure difference sufficient for translocation has been found to be in the range of 0.12–0.46 MPa. However, little or no exudation from cut phloem is observed in spite of sieve tubes having considerable hydrostatic pressure. This is because an efficient mechanism of plugging the sieve pores is operative in STE. Larger diameter of sieve tubes compensates for smaller area of phloem in vines. According to **Hagen-Poiseuille's law**, hydraulic conductance is proportional to the fourth power of the conduit diameter. Other plants do not have lesser number of sieve tubes with larger area since physical damage to lesser number of STE will cause more damage to transport capacity.

6.6 Photoassimilate Allocation and Partitioning

Diversion of photoassimilates toward various metabolic pathways is known as **allocation**. The process of differential distribution of photoassimilates among different sinks is known as **partitioning**. The coordination between allocation and partitioning is important for regulating the transport of photoassimilates to commercially important organs of plants. Research on photoassimilate allocation and partitioning is primarily focused on increasing the **harvest index** (HI) which is determined by the competition among various sinks for the photoassimilates exported by the source tissues. The signals transmitted between source and sink tissues could be physical or chemical in nature. The change in turgor pressure, which gets transmitted rapidly via sieve elements, accounts for the physical signal. Chemical signals include phytohormones found in the phloem sap, proteins, mRNAs, small RNAs, and sometimes translocated sugars. Coordination between source and sink activities in part is regulated by turgor pressure. It has been observed that if the utilization of photoassimilates is rapid in sink tissues at the time of phloem unloading, then it leads to the reduction of turgor pressure in the sieve elements of sink tissues which acts as a signal to the source tissues. Consequently, loading of photoassimilates would increase in response to this signal from the sink tissues. Conversely, if the unloading of photoassimilates is slow in sink tissues, then high turgor pressure signal present at the sink is transmitted to the source tissues along the STEs and accounts for slower loading of photoassimilates in source tissues. Phytohormones play significant roles in the regulation of source-sink relationships. Plant growth regulators, such as auxins, produced in shoot are rapidly transported to

the roots via the phloem. In some source tissues, loading is stimulated by exogenous auxin, whereas ABA inhibits it. However, a reverse response is observed in sink tissues, with ABA enhancing the uptake of sucrose in some sink tissues and auxin inhibiting it. Hormonal regulation of apoplastic loading and unloading is due to their influence on the active transporters present in plasma membrane and tonoplast (unloading only). Plant defense hormone, such as jasmonic acid, affects the photoassimilate allocation and partitioning partially due to responses against the pathogens and herbivores. Accumulation of sugars favors expression of genes involved in storage and utilization of sugars. Thus, transport sugars are not only translocated in the phloem but also act as signals in regulating the activities at sources and sinks. It has been proposed that reduced sink demand leads to elevated sucrose levels in phloem which leads to downregulation of the sucrose-H^+ symporter in the source, ultimately resulting in the increased concentration of sucrose in the source.

Summary

- Translocation in the phloem is responsible for the movement of photoassimilates from sources (mature leaves) to the areas of growth and storage (sinks). Depending on development stage, leaves can serve as sink or source for photoassimilates. The pattern of phloem translocation is independent of gravity. Factors, such as proximity of source to sink, development stage of plant, vascular connections, and modification of translocation pathway, sometimes play an important role in determining the translocation pathway.
- Transition of leaves from sink to source is a gradual process involving two separate events, first cessation of import followed by initiation of export. This transition necessitates several conditions, such as expression of the sucrose-H^+ symporter. Translocation of sugars takes place in the sieve elements of phloem. Mature sieve elements are unique structures involved in the translocation of sugars. They possess a variety of structural adaptations making them suitable for translocation process. Companion cells help in the functioning of the sieve elements. They transport photoassimilates from the mature leaves (mesophyll cells) to the sieve elements and also provide energy and proteins to the sieve elements for their maintenance as sieve elements lack mitochondria and machinery for protein synthesis.
- Sucrose is the most commonly translocated sugar in plants. However, other nonreducing sugars, such as raffinose, stachyose, and verbascose, are also transported. The rate at which various substances move in the phloem during translocation process is more than can be justified by diffusion. Pressure flow model explains the mechanism of translocation in the phloem. According to this model, the bulk flow of photoassimilates and other materials present in the phloem takes place in response to an osmotically generated pressure gradient developed as a result of phloem loading at the source and phloem unloading at the sink. Phloem loading involves short-distance transport of sugars into sieve elements via companion cells. The pathway of phloem loading can be apoplastic

or symplastic. In the apoplastic pathway, sucrose is actively transported in the SE-CC complex. The polymer-trapping model suggests that the synthesis of polymeric sugars (raffinose, stachyose, and verbascose) takes place in the intermediary cells which can easily diffuse into the sieve elements due to the presence of plasmodesmata with large SEL in between companion cell and sieve element.

- Phloem unloading involves unloading of photoassimilate into sink cells, short-distance transport, storage, and metabolism. Phloem unloading can also occur through apoplastic and symplastic pathways. The transport of sugars in sinks is an energy-dependent process.
- Allocation directs the regulation of the quantities of fixed carbon that are channeled into various metabolic pathways. It determines the quantities of photoassimilate that will be used either for storage or utilized for biosynthesis of transport compounds. Partitioning refers to differential distribution of the quantities of photoassimilate delivered to various sinks.

Multiple-Choice Questions

1. The ability of sink to mobilize photoassimilates toward it is known as:
   (a) Sink activity
   (b) Sink strength
   (c) Sink size
   (d) Sink power
2. Decrease in sink-source ratio causes rate of photosynthesis to:
   (a) Decrease
   (b) Increase
   (c) Remain unaffected
   (d) First increase followed by decline
3. Which of the following subcellular constituents is not present in mature sieve elements?
   (a) Nucleus, microfilaments, and plastids
   (b) Nucleus, Golgi bodies, and ribosomes
   (c) SER, Golgi bodies, and plastids
   (d) Microtubules, mitochondria, and plastids
4. Which type of companion cells is present in minor veins of leaf to facilitate symplastic transport of photoassimilate from mesophyll cells to sieve elements?
   (a) Ordinary companion cells
   (b) Transfer cells
   (c) Intermediary cells
   (d) Normal companion cells

5. Translocation of sugars from mesophyll cells to sieve-tube elements in leaves is known as:
   (a) Phloem unloading
   (b) Phloem loading
   (c) Sieve element unloading
   (d) Photoassimilate loading
6. Who proposed the pressure flow model to explain long-distance photoassimilate translocation in phloem?
   (a) Marcello Malpighi
   (b) Turgeon
   (c) Ernst Münch
   (d) Stephan Hales
7. During partitioning process, the distribution of photoassimilates among different sinks is:
   (a) Uniform
   (b) Differential
   (c) Normal
   (d) Similar

Answers

1. b 2. a 3. b 4. c 5. b 6. c 7. b

Suggested Further Readings

Chen LQ (2014) SWEET sugar transporters for phloem transport and pathogen nutrition. New Phytol 201(4):1150–1155

Fritz E (1973) Microautoradiographic investigations on bidirectional translocation in the phloem of *Vicia faba*. Planta 112(2):169–179

Lemoine R, La Camera S, Atanassova R et al (2013) Source-to-sink transport of sugar and regulation by environmental factors. Front Plant Sci 4:272. https://doi.org/10.3398/fpls.2013.00272

Turgeon R (1996) Phloem loading and plasmodesmata. Trends Plant Sci 1(12):418–423