

PLS 622: Plant Physiology I, Wednesday, September 27, 2006.

Vegetative development: Vascular tissue differentiation:

Just prior to the completion of seed germination, the provascular tissue in the embryo differentiates into protoxylem and protophloem, providing the rudiments of a vascular system until more permanent metaxylem and metaphloem differentiate. Thereafter, the shoot and root apical meristems produce undifferentiated cells, some of which take on the vascular cell fate as the plant body becomes larger and more complex. In some plants radial growth occurs through the production of xylem, phloem and attendant tissues by a vascular cambium (a secondary meristem). This vascular system pervades the plant body providing the means of short and long range bulk transport of liquid and dissolved solutes. In this lecture, we will learn some of the salient points known about how the vasculature develops.

Pictured below is a leaf from a dicot and a monocot showing, schematically, the typical patterning of leaf venation. In monocots, the primary venation runs longitudinally for nearly the length of the leaf. Monocot veins undergo divergence at the base of the lamina and converge and fuse toward the leaf apex. Their venation pattern is termed **striate**. The venation of grass leaves is used as an example of vascular patterning in monocots. For both dicots and monocots, veins of several different sizes (orders) comprise the vasculature and, at the level of the smallest sized veins, venation acquires an essentially reticulate pattern (forming areoles). Veins of both dicots and monocots anastomose (join together) at the margins of the leaf, with secondary veins connecting to other secondary veins at first and eventually joining with the midvein(s). Although there are typically fewer vein sizes in the monocots than there are in the dicots, as many as 6 have been identified in both. All veins diminish in size through the vein orders as they approach the leaf apex.

Vascular pattern ontogeny:

Provascular tissue and the ground meristem are both derived from the uniformly meristematic tissue of the leaf primordium and only differentiate upon the commencement of cell division and expansion associated with leaf development. It is therefore, difficult to ascertain which cells are destined to become vascular tissue prior to the commencement of their differentiation and hence, difficult to study early events in vascular tissue ontogeny. One of the first events that occurs to hint at a special developmental pathway for cells destined to become vascular tissue is that they stain darker than surrounding non-vascular cells. This is thought to be due to the greater vacuolization of the non-vascular cells at this point during development. One interesting aspect of vascular tissue ontogeny, regardless of the species in which it is studied, is that the cells do not adhere to some of the rules of cell division in a growing plant part. One is that there may be cell divisions longitudinally, perpendicular to the direction of cell growth (formative division) increasing the numbers of cells in the vascular strand.

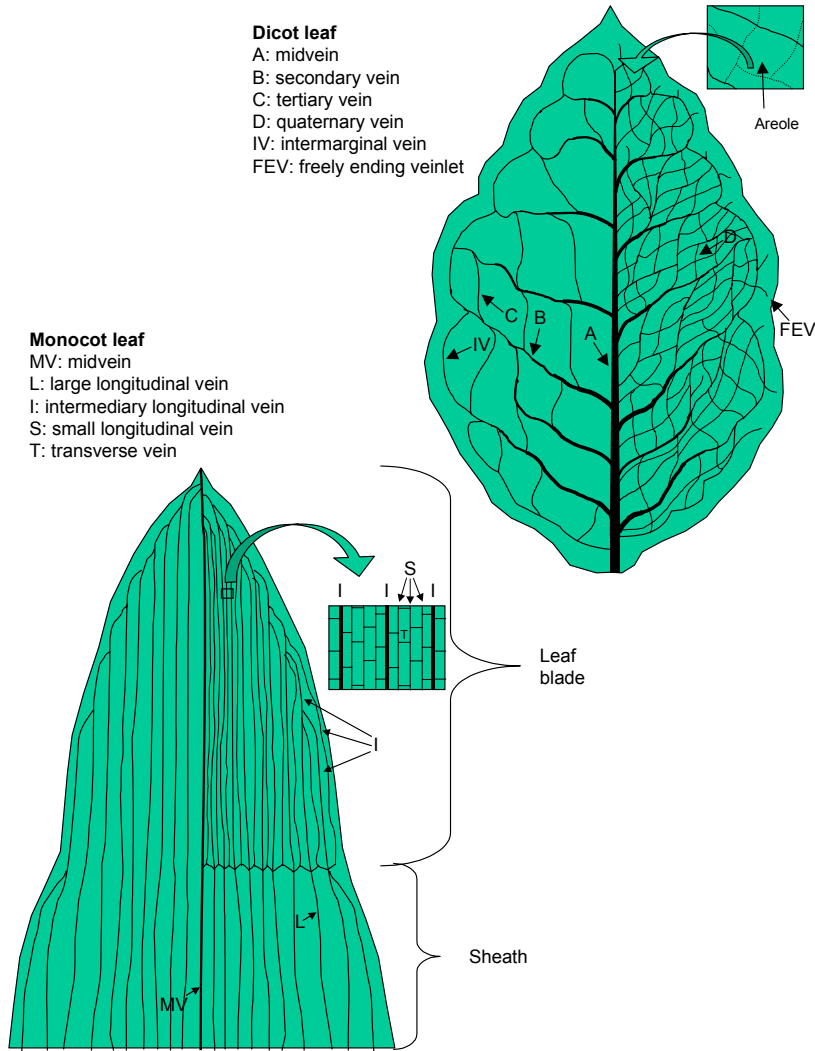
Dicot:

The vasculature of dicots develops through three major phases during leaf morphogenesis and growth. First, the midvein provascular strand invades the leaf acropetally from the stem provascular tissue into the leaf primordium. Second, secondary provascular strands proliferate from the midvein and develop in the leaf lamina towards the leaf margins. Finally, the provascular strands of tertiary and higher veins are established during intercalary expansive growth of these elements of the vasculature. In contrast with the acropetally oriented development of the midvein, secondary venation develops in a species dependant manner. In some, the secondary veins commence their outgrowth from the midvein at the leaf apex and initiation from the midvein proceeds sequentially down towards the leaf base (basipetal secondary vein development). In others the opposite is true, with secondary veins arising from the midvein in an acropetally oriented sequence. Finally, there are species that have secondary leaf vasculature arise first in the mid-part of the leaf and initiation proceeds both basi- and acro-

petally. Development usually proceeds basipetally for the higher orders of vasculature so that the minor veins are present at the leaf apex while the secondary veins are still forming near the petiole.

Monocot:

Most grasses initiate the midvein and the secondary vasculature from the disk of leaf insertion and not from the existing stem provasculature. The midvein elongates acropetally toward the leaf tip. Only after the commencement of acropetal growth does the midvein also commence development toward the stem vascular strand, forming the leaf trace. Large longitudinal veins parallel to the midvein also form in this manner, arising from the disk of leaf



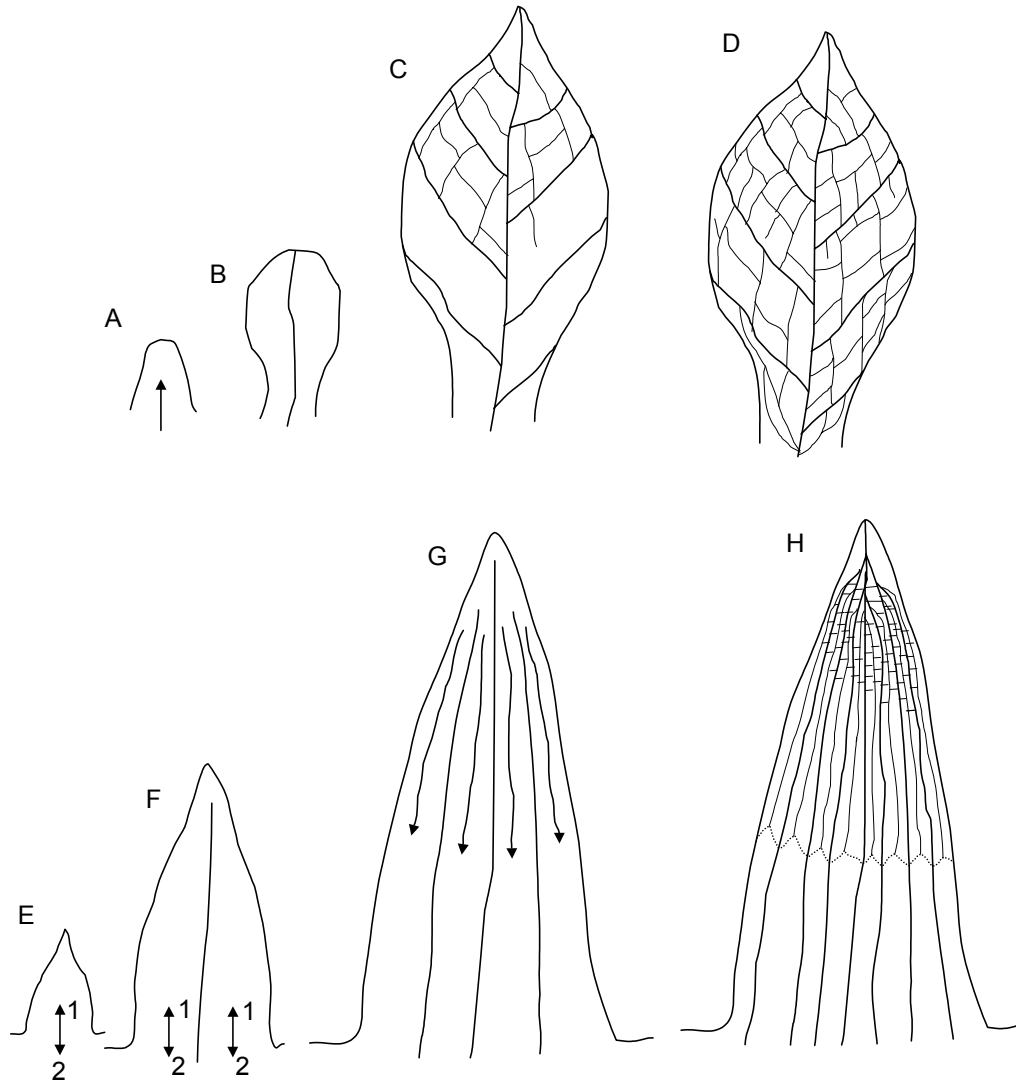
Redrawn from the Plant Cell, 1997, 9: 1122

insertion on the stem, developing first acropetally in the elongating leaf and then basipetally to the stem vasculature. Next, the intermediate provascular strands initiate in the leaf apex and development extends basipetally to connect to the existing major longitudinal veins that have developed in the leaf. Only some of the basipetally extending intermediary veins develop through the leaf sheath to connect with the stem vascular bundle through a leaf trace. Finally, small longitudinal veins develop commencing near the apex of the leaf and extending basipetally to connect to the higher order veins at about the leaf sheath-leaf junction. The transverse veins also develop starting near the apex of the leaf and extending basipetally to provide the leaf with a reticulate network of vasculature. The reader is cautioned that the rather novel developmental

sequence with the midvein and large longitudinal veins commencing development in the disk of leaf attachment without any apparent connection with the provascular trace of the stem may be just that...apparent. Our limitations identifying provascular tissue prior to its advanced development may prevent the recognition of an existing leaf trace until after the midvein in the disk of leaf attachment has commenced differentiation.

Vein spacing:

The most obvious manifestation of uniform vein spacing is seen in the leaf blade of grasses that have a constant longitudinal vein number per unit lateral blade width. In the dicots, even if the polygonal shape of the ultimate leaf areoles are remarkably diverse, the occurrence of branch points from veins and veinlets is remarkably uniform. Constant branch points from veins



Redrawn from Plant Cell 1997, 9:1121-1135

that are undergoing intercalary growth is seemingly maintained by the initiation of provascular tissue between existing branch points and the growth of the new vasculature by intercalary growth into the areole.

Models for the Regulation of Vascular Pattern Formation:

Any hypothesis that attempts to describe vascular pattern formation must account for three divergent phenomenon; 1) the acropetally oriented formation of major veins in developing dicot leaves; 2) the formation of isolated, parallel provascular tissue in expanding grass leaves and; 3) the simultaneous formation of minor veins and transverse veinlets in both dicots and monocots over large areas of the leaf. The two best models only imperfectly describe how vascular patterning might arise.

Model 1: Canalization of signal flow:

All cells start out being equivalent transporters of auxin, a hormone implicated in the induction of vascular differentiation. Stochastically, some cells transport more auxin, and this greater contact with auxin enhances their ability to transport more of it, creating a positive feedback loop. The greater auxin flux through these cells eventually induces them to become provascular cells and drains surrounding cells of auxin, inhibiting them from also becoming provascular tissue. Additionally, the auxin is passed basipetally to the next cell in the file which now accrues its own auxin plus all the auxin from the cell above it, converting it to provascular tissue. This hypothesis can account for the type of vascular development seen in dicot leaves but cannot account for how the provascular tissue in monocots appears to develop, nor the simultaneous development of minor veins throughout a large section of the leaf.

Model 2: Diffusion-reaction prepattern:

This hypothesis requires two components: 1) a localized, positive feedback loop that stimulates the further production of any transient increase in the amount of an otherwise, uniformly distributed substance, stimulatory to vascular tissue development (also known as a 'stimulatory morphogen') and; 2) the production of a rapidly diffusing, long-range inhibitory compound from the same site producing the stimulatory morphogen that radiates out from that site, inhibiting the initiation of vascular tissue in the vicinity of the developing vasculature ('inhibitory morphogen'). This hypothesis can account for the parallel, and simultaneous, formation of longitudinal veins in monocot leaves. Additionally, it can account for the intercalary growth of new veins between older veins as the leaf blade expands because the concentration of the inhibitory morphogen would be depleted the further apart the two veins moved until it was no longer sufficient to inhibit a new wave of provascular tissue formation. This would also tend to promote very uniform spacing between veins, their simultaneous formation, and produce patches where no venation would occur...areoles.

Current theory is that, in order to explain much of what we know about vascular tissue differentiation, we will have to come up with a joining of aspects of the two models above.

Comparison among leaf, stem, and root vasculature:

The most noticeable difference between the vasculature of the root, stem and leaf is in the symmetry of the organs. In roots, the vasculature forms a central pith-filled, or solid cylinder that is radially symmetrical and whose organization is not greatly influenced by the occurrence of peripheral organs. In the stem, the vasculature is organized into radially symmetrical, sympodial bundles whose organization is in direct relation to shoot phyllotaxis (i.e. it's organization is dependent on the attachment of the leaves to the stem because an amount of the vascular trace must branch off from that of the stem and enter each leaf to supply it with water and nutrients while removing photosynthate). At each dicot node, at least three vascular bundles diverge from separate sympodial bundles to serve the leaf at that node. The remainder of the sympodial bundle continues through the next internode. The divergent vascular bundles, so called leaf traces, arising as they do from independent sympodial bundles, provide redundancy in the water supply of the leaf. The architecture of the sympodial bundle seldom varies having the phloem situated to the outside of the xylem. The position of the xylem towards the adaxial (upper) portion of the typically dorsiventral leaf and the phloem towards the abaxial region reflects the architecture in the stem from whence the leaf trace originates.

Vegetative development: Phloem and Xylem:

Let us examine two components of plant vasculature, **phloem** and **xylem**.

One fundamental difference in how animals and plants transport **assimilate** is that while both use vessels made from cells, the smallest of these vessels in animals is comprised of cells but does not have majority of the transport passing through the cells themselves but rather through vessels formed by these cells capillaries. In plants of course, transport is through the phloem and xylem cells themselves.

Vascular differentiation in plants is difficult to study due to the position of the vascular elements, buried within the plant body, the relatively few cells comprising the vasculature, and the even fewer cells undergoing differentiation at any one time relative to the number of differentiated vascular cells. Much of what is known about vascular tissue differentiation at the molecular level has been acquired in the past two decades with the advent of an inducible cell culture system (*Zinnia elegans*) for xylem providing quantities of more-or-less synchronized cells following the same developmental pathway. Tissue culture systems have similarly been adopted for studies of phloem differentiation.

Development of 'axial system' (stem) Xylem and Phloem:

In the seedling there develops primary vasculature comprised of **protophloem** and **protoxylem** which are quickly crushed and torn apart as the seedling elongates. They serve to transport water and nutrients during the early stages of establishment and are quickly replaced by the **metaphloem** and **metaxylem**. This vasculature is more long lived, developing after most of the cells comprising the seedling have finished elongating. Additional files of cells are added to the existing metaphloem and metaxylem as the meristems produce them.

While the **protophloem** has no companion cells, the **metaphloem** does, enabling it to survive for considerably longer periods. Companion cells are associated with mature sieve elements and are thought to be necessary for sieve element function and survival. The role of the companion cell in phloem loading (sieve element function) will be dealt with below. Correlative evidence supporting the conjecture that companion cells are responsible for sieve element survival arises from studies of **protophloem** elements in developing leaves and stems and which lack companion cells. These protophloem elements are short-lived after they have differentiated and are replaced later in development by **metaphloem** sieve elements which have companion cells and which live much longer (years in the case of palms). The companion cells must produce the proteins for the mature sieve elements they serve because the mature elements are without ribosomes. Without a mechanism for producing proteins *de novo* the life span of any cell would be short indeed. Even the P-protein, necessary for avoiding catastrophic failure and possible infection of large portions of the phloem system and surrounding tissue upon injury of an element, is manufactured in the companion cells and transported to the mature element. This hypothesis has been demonstrated using a combination of *in situ* localization of P-protein mRNA and immunolocalization of P-protein itself. P-protein has been localized to both mature elements and their associated companion cells while P-protein RNA has been located solely in the companion cells. Additionally, the SUT1 sucrose transporter located in the mature sieve element plasmamembrane although its RNA is synthesized in the companion cell. Finally, phloem exudates obtained from **aphid stylets** or cut stems contain many hundreds of small (< 25KDa) proteins (**sieve tube exudate proteins; STEPS**) that continue to exude from the phloem for considerable sample periods. This continuous supply of newly-synthesized (data from labeling studies) proteins strongly implicates the companion cells as the site of synthesis, and the transport of the proteins through the plasmodesmata into the sieve tubes.

For those plants with secondary growth, both secondary xylem and phloem develop from the vascular cambium. Associated with secondary xylem are ray cells which, unlike the axially arranged xylem and phloem, are arranged radially. These rays can move metabolites laterally

through the bole of a tree, storing substances otherwise toxic to cells in the heartwood or outer bark which eventually die.

Phloem:

For phloem transport to be effective, all large organelles are degraded during development so as not to impede the flow of assimilate through the cell. Hence, the nucleus, vacuole, Golgi bodies, rough endoplasmic reticulum, and ribosomes are missing from mature phloem sieve elements. Additionally, the plasmodesmata of the sieve element are enlarged between adjacent sieve elements (**sieve pores**) to enhance flow of assimilate between elements. Despite the paucity of organelles, sieve elements are not dead and maintain a functional plasma membrane, continuous through the sieve pores, that is essential for the job they do. Thus, a series of sieve elements are bounded by a single plasma membrane forming a **syncytium**, essentially a single compartment.

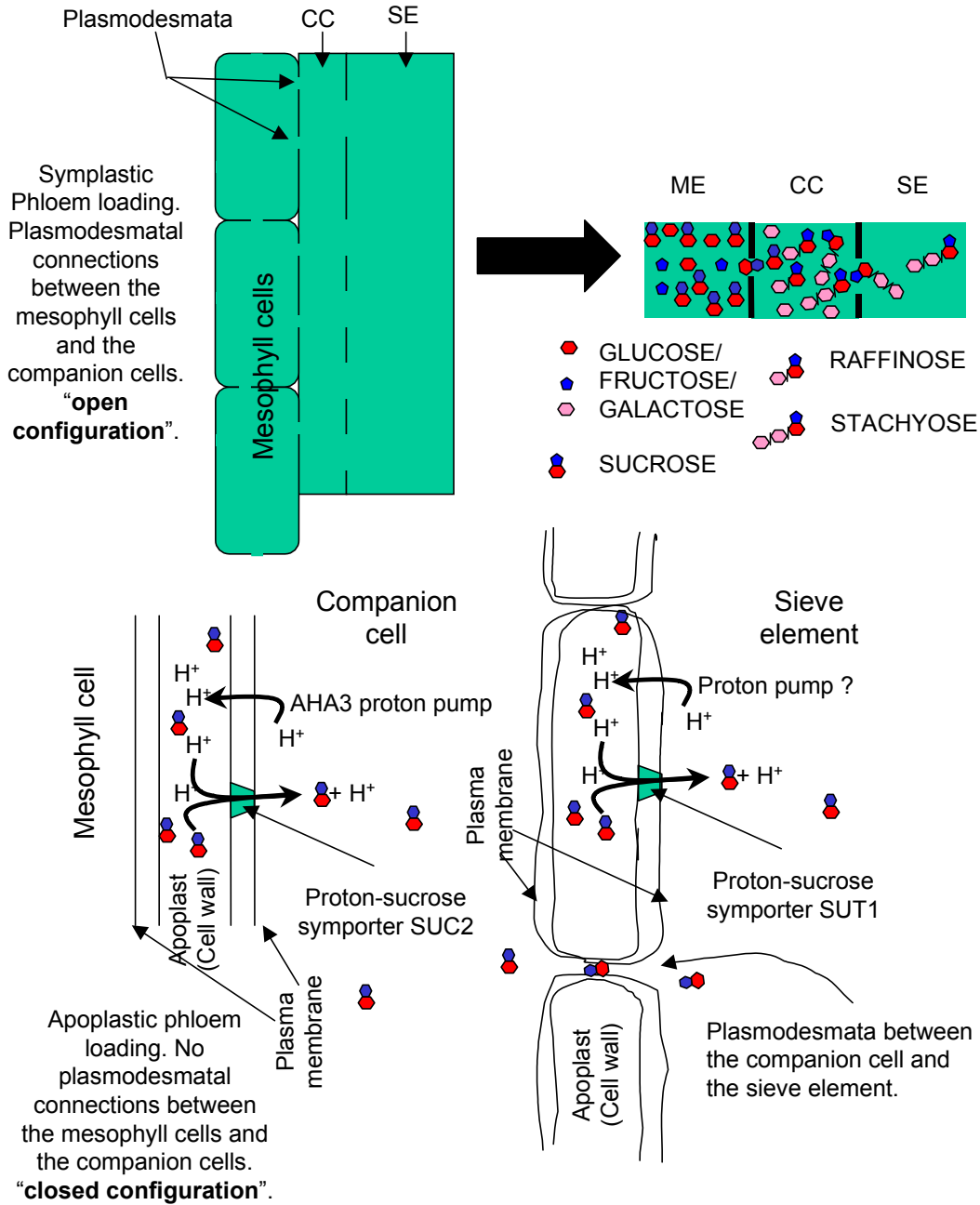
During phloem development, the **phloem mother cell** divides to produce a phloem cell precursor and the precursor to a **companion cell**. The housekeeping of the mature sieve element will be done by the companion cell that assumes the regulatory responsibilities for the neighboring, enucleate sieve element. In some plants, there are numerous plasmodesmatal connections, serving as a symplastic pathway, among the sieve element, its attendant companion cell, and the mesophyll. In others the sieve element and its companion cell have a paucity of plasmodesmatal connections with other cell types. This diversity has provided support for the contention that phloem loading can occur in two different methods depending on the species of plant. Plants are thought to load material into the phloem via either: 1) **symplastic-** or; 2) **apoplastic-phloem loading**. The **polymer-trapping hypothesis** functions in some species of plants and involves symplastic phloem loading. According to this hypothesis, mono- and disaccharides are small enough to be capable of diffusing from mesophyll cells into companion cells along a concentration gradient through plasmodesmata. In the companion cells these simple sugars are combined into larger oligomers, oligomers of sufficient size to prevent their diffusion back through the narrow plasmodesmata leading into the mesophyll cells. However, due to the large diameter of the branch plasmodesmata leading into the sieve element from the companion cell, these sugars can diffuse into the sieve tube and be transported.

In contrast to the symplastic route, some species have no plasmodesmatal connection between the mesophyll cells and the companion cells. There is direct evidence for phloem loading from an apoplastic pathway involving a **proton pump AHA3**, and a **proton-sucrose symporter SUC2**, located in the companion cells. Sucrose, produced by the mesophyll cells is dumped into the apoplast and then recovered into the companion cells via SUC2. Regardless of which pathway is used, companion cells are implicated in the delivery of material to the sieve elements. However, recent evidence has led to the belief that a second apoplastic loading mechanism exists in the sieve elements themselves. Immunolocalization experiments have demonstrated the presence of a **proton-sucrose symporter SUT1** in the plasma membrane of the phloem sieve element.

Phloem translocation has been estimated to be **40 cm/hr**, and, due to the proximity of the organelles to the flowing assimilate stream, it is possible that the organelles are subjected to considerable shear forces. The organelles must therefore, be anchored in place along the cell periphery. Additionally, any intra-phloem transport of molecules not abundant in the translocation stream must be compartmentalized, probably within the lumen of the sieve element reticulum (SER).

Sieve elements develop hydrostatic pressures in excess of **30 atmospheres!** The cell walls of sieve elements are therefore, modified to be able to contain this high pressure without bursting. One of the most fundamental modifications is the production of cellulose microfibrils at right angles to the axis of elongation of developing sieve elements. These microfibrils act like hoops around a barrel, assisting the cell to maintain its shape under the pressures developed within. Along with the obvious practical advantage of not bursting, this reinforced cell will not

undergo deformation (bulging) although considerable pressure is applied within, thereby propagating this pressure longitudinally along the phloem tissue.



Xylem:
Hormonal control of xylogenesis:

Endogenous auxin appears to be responsible for determining the initiation of **tracheary element (TE)** differentiation and the size of the resulting TEs. Cytokinin, apart from enhancing the sensitivity of tracheary initials to auxin, is also required for the induction of TE differentiation and its progression to completion. There is indirect evidence that ethylene is also involved in controlling TE development. Recently, brassinosteroids have been shown to be necessary for the transition from stage II to stage III of tracheary element differentiation (see below).

As mentioned above, much of what is known about xylem differentiation at the molecular level has been acquired using the inducible *Zinnia elegans* cell culture system. This system induces parenchymal cells in culture to first de-differentiate and then to re-differentiate into TEs (**transdifferentiation**). The molecular markers identified in this system reflect its artificial nature in that the de-differentiation phase is not usually present in normal TE differentiation from **protoxylem** or **cambial tissue**. Hence the system has more in common with wound-induced TE differentiation where pre-existing cells undergo **de-differentiation** prior to developing into TEs.

Stage I: De-differentiation:

Using the *Zinnia* mesophyll cell as a model, this stage commences with the cells losing the ability to conduct photosynthesis, the expression of wound-induced genes and the acquisition of the ability to elongate and differentiate. Three groups of genes are up-regulated during this stage, 1) wound-induced genes; 2) genes whose products are associated with the protein synthetic apparatus and; 3) the remainder.

Stage II: Restriction of developmental potential:

The accumulation of TED2, 3, and 4 (**Tracheary element differentiation-related genes**) gene products. This accumulation occurs between 12 and 24 hours prior to the synthesis of the secondary cell wall. These genes are also upregulated in vivo in procambial cells destined to become TEs (TED3) or TEs or phloem elements (TED4 and TED2).

Inhibitors of poly(ADP-ribose) polymerase, an enzyme necessary for DNA excision repair, also inhibit the development of TEs. These same inhibitors also repressed the expression of all TEDs.

There is a marked increase in the transcript abundance of a number of genes whose products are involved in the protein translational machinery which is correlated with a dramatic increase in protein and RNA amounts present in these differentiating cells. Additionally, tubulin gene expression increases, providing the means of orchestrating secondary cell wall synthesis in the third stage of development. Actin gene transcription increases as well, and large cables of actin form along which cytoplasmic streaming occurs.

Stage III: TE specific development:

Brassinosteroids are necessary for the transition from stage II to stage III of tracheary differentiation. In this last stage of tracheary element differentiation the secondary cell wall, necessary for the structural strength required to withstand the high negative pressures exerted by transpiration without implosion, is synthesized. The secondarily thickening of the cell wall occurs by the synthesis of cellulose microfibrils perpendicular to the direction of flow which, as in phloem, strengthen the element like hoops around a barrel. Additional structural support is provided by cell wall proteins. An extensin protein as well as an arabinogalactan protein are in high concentration in mature tracheary elements. A characteristic alteration to the cell wall of the tracheary elements at this stage is their heavy lignification. Programmed cell death (see below) is tightly coupled temporally with secondary cell wall thickening in this stage of xylogenesis. Finally, autolysis occurs culminating in the generation of a cell corpse...a mature xylem element.

Apoptosis (Programmed cell death (PCD)) vs necrosis:

All cells die. How they do so varies. Some are slated for death internally, genetically programmed to die a physiological death while others die due to injury. **Apoptosis** or **programmed cell death**, is a process of death from internal factors up-regulated in some cells during normal cellular differentiation and development of multicellular organisms. This process is also involved in tissue homeostasis, pathological conditions and aging. Cells undergoing apoptosis are characterized by cell volume loss, plasma membrane blebbing, nuclear condensation, and endonucleolytic degradation of DNA at discrete intervals.

Not all cells die through apoptosis. Dramatically traumatized cells such as those suffering severe wounding or other overwhelming stress undergo **necrosis**, a non-physiological death involving cell swelling, eventual lysis, and the leakage of the cell contents into the intercellular space. Necrosis does not usually play a role in differentiation and development and so will not be dealt with further.