Phloem development and function probed with a companion-cell marker

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The growth of a leaf from emergence to final size involves periods of cell division, cell and tissue differentiation, and cell expansion. Modern techniques involving the expression of green fluorescent protein (GFP) have enabled us to investigate the structural and functional development of the phloem, the pathway for photoassimilate movement within the leaf.

Transgenic tobacco plants were produced that express GFP specifically in the companion cells of the phloem using the promoter of the *Arabidopsis* sucrose transport protein, AtSUC2[1]. In *Arabidopsis,* the AtSUC2 promoter is switched on at the onset of sucrose export, and is only expressed in the functional phloem of source leaves. A similar pattern is seen when the AtSUC2-GFP gene is expressed in tobacco plants, suggesting that the expression of GFP occurs only in those veins that are exporting sucrose[2,3]. The AtSUC2-GFP plants were examined using the confocal laser scanning microscope to locate the expression of GFP, thus enabling us to identify the pattern of functional phloem development in leaves of different developmental stages. GFP expression was taken as evidence of the vein being functionally mature. Structural maturity preceeds functional maturity. Therefore, if GFP was not being expressed, the electron microscope was used to look at the structural maturity of the veins.

Basipetal maturation of phloem loading in veins of cotyledons Following germination, the first leaves to appear on a plant are the cotyledons, which provide a source of assimilate for the growing plant. Initially only one vein, the midrib (class I), is present and shows GFP expression (Figs. 1A and B). However, as the leaf grows additional vein classes are formed and these, once mature, also express GFP (Fig. 1C). The pattern of expression shows that phloem maturation occurs basipetally, with the class II veins near the tip of the leaf showing fluorescence before those at the base (Figs. 1C-E). Similarly, class III veins mature basipetally (Fig. 1F). Tobacco has five vein classes; classes I, II and III are called major veins and, in mature leaves, these are normally used for phloem unloading. Classes IV and V, the minor veins, mature once a leaf undergoes the sink-source transition and assumes source status, and these vein classes

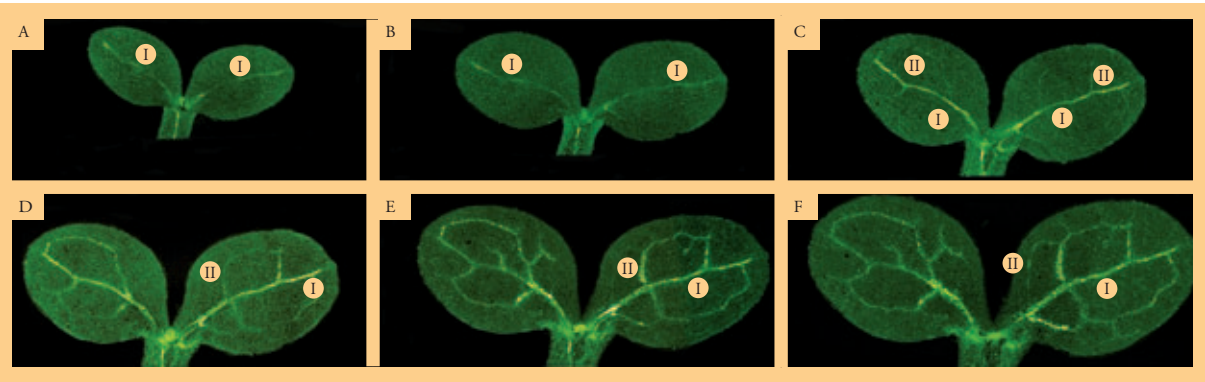

Figure 1 Basipetal development of major veins in cotyledons. AtSUC2-GFP coyledons imaged daily to show the progression of vein maturation. GFP is expressed first in the midrib (I) (A,B), then in class II veins (II) near the tip (C) and then towards the base (D,E,F) of the leaf.

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Figure 2 A mature AtSUC2-GFP cotyledon expressing GFP in all the major vein classes.

are involved in phloem loading and assimilate export. In cotyledons, which must immediately function as photosynthetic sources, prior to the structural maturation of the minor veins, phloem loading occurs not from the minor veins (which are absent during early development) but from the major veins (Fig. 2). In fact, in young cotyledons, phloem loading occurs from whichever vein classes are functionally mature at the time.

Acropetal maturation of phloem unloading in immature sink leaves In AtSUC2-GFP plants, the GFP synthesised in the companion cells is able to move into the sieve elements and be transported, along with photoassimilate, to developing leaves and other sink areas of the plant. When sink leaves first emerge they have only the midrib to facilitate the transport and unloading of assimilate (Figs. 3A and B). However, as the leaf grows other vein classes are formed and mature in an acropetal direction; the class II veins at the base of the leaf (Fig. 3D) maturing in advance of those at the tip. By the time the leaf is a complete sink, a network of class I, II and III veins has formed. Within this major vein network, phloem unloading of GFP takes place predominantly from the class III veins (Fig 4)[4].

AtSUC2-GFP-ER plants A second line of transgenic plants has been produced in which GFP, again expressed from the AtSUC2 promoter, is targeted to the endoplasmic reticulum. In these plants, GFP is unable to traffick into the sieve element, producing companion-cell autonomous GFP expression which is restricted only to source tissue undergoing phloem loading, In these AtSUC2-GFP-ER plants the cotyledons showed an identical pattern of phloem development to those plants expressing free GFP in the companion cells (Fig. 5A), but in this case the developing sink leaves showed no GFP expression until they commenced the transition from sink to source.

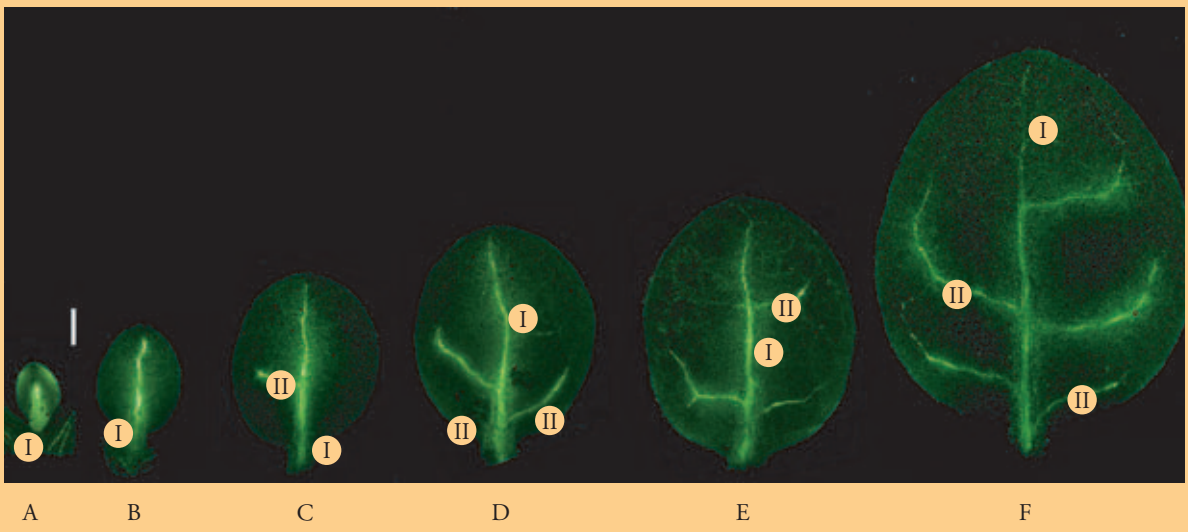

Figure 3 Acropetal development of major veins in sink leaves.
Unloading of GFP into developing AtSUC2-GFP sink leaves takes place initially from the class I vein (I) (A,B), the basal class II veins (II) (C,D) and finally the apical class II veins (E,F).

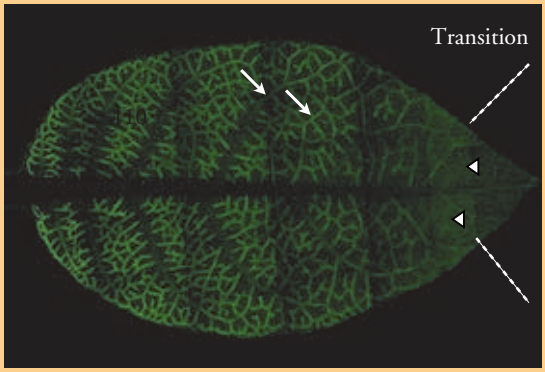

Figure 4 Unloading of GFP into an AtSUC2-GFP sink leaf from the major veins (arrows). The transition from sink to source has commenced at the tip of the leaf, as shown by the punctate GFP expression in the minor veins (darts).

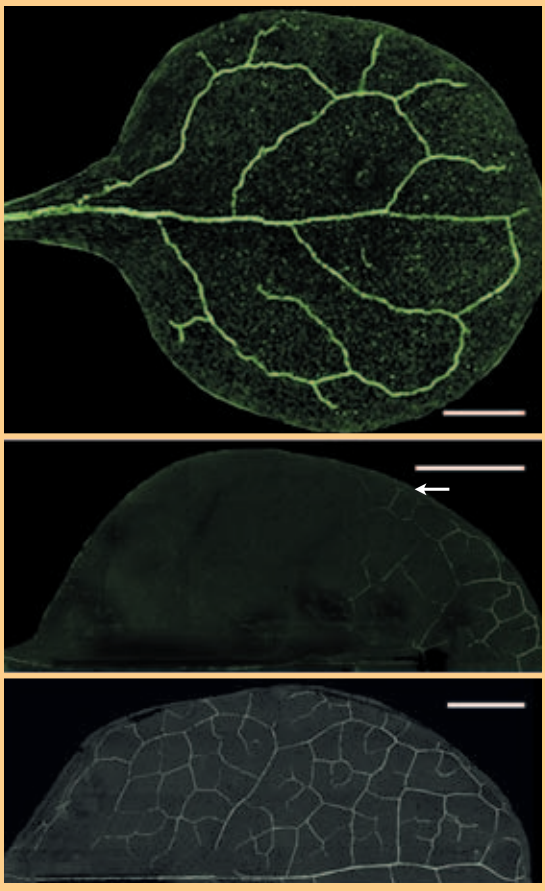

Figure 5 GFP expressed in the companion cells of AtSUC2-GFP-ER plants. (A) Cotyledon expressing GFP in the major veins. (B) Leaf undergoing the transition to source (arrow) expressing GFP in the major veins at the tip and (C) throughout the leaf. The minor veins in these small leaves have not yet formed.

In plants, the sink-source transition commences with the cessation of photoassimilate unloading from the major veins, accompanied by maturation of the minor veins[4]. Following the functional maturation of the minor veins, phloem loading, as shown by GFP expression (Fig. 5B) begins at the tip of the leaf and progresses basipetally, towards the petiole. It is interesting to contrast the acropetal development of the major veins and subsequent phloem unloading in an emerging leaf, with the basipetal development of minor veins and phloem loading that occurs later during the sink-source transition. However, in the case of cotyledons, there is no other source tissue to support them and so these leaves do not have an initial phase of phloem unloading. Rather, phloem loading must be initiated at the same time as structural and functional maturation of the major vein network proceeds. This makes it necessary for cotyledons to utilise the major veins for phloem loading; a task normally reserved for the minor vein network.

Figure 6 Shading treatment on plants at day 1 (top) and day 12 (bottom). The black disc is 19mm in diameter.

The effect of shading on the sink- source transition

In order to study the effect of light on the progression of the sink-source transition, areas of tissue near the tip of AtSUC2-GFP-ER sink leaves were sandwiched between discs of opaque plastic to produce localised

areas of shading (Fig. 6). The discs were left in place for 12 days, during which time the leaves passed through the sink-source transition. Following the period of shading, areas of leaf under the discs were examined for GFP expression and compared to neighbouring unshaded tissue. In the unshaded area, GFP was expressed in all the veins (Fig. 7A), both major and minor, as expected. However, in the shaded area, the major veins expressed GFP (Fig. 7B) but the minor veins, although they had matured structurally (data not shown), did not become fluorescent.

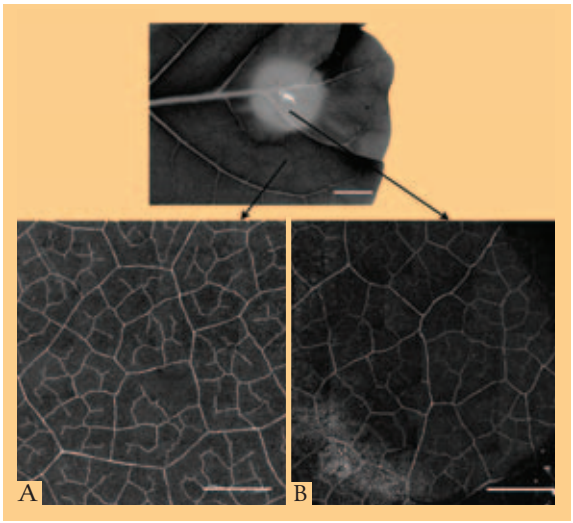

Figure 7 The effect of partial shading on an AtSUC2-GFP-ER leaf. GFP is expressed in all vein classes in the non-shaded area (A) but only in the major veins within the shaded region (B).

Does the signal for phloem maturation come from the unshaded area? In a parallel experiment, discs were excised from near the tip of sink leaves, floated on water and kept either in the light or dark for 12 days to mimic the unshaded and shaded treatments on intact leaves. However, in this case the leaf discs were isolated from neighbouring leaf tissues. In discs maintained in the light, GFP was expressed in the major veins but not the minor veins (Fig. 8A), although the latter had once again matured (Fig. 8C). In contrast, the minor veins in the shaded discs remained structurally immature (Fig. 8D), and in these discs GFP was not expressed by either the major or the minor veins (Fig. 8B). These data suggest that the signal to initiate expression of the *AtSUC2* promoter within the phloem of major veins does not require communication with the rest of the leaf, provided the tissue remains in the light. However, light is not the signal required to initiate *AtSUC2* expression in the minor veins. The signal to initiate minor vein development (structural maturation) is potentially light and this signal is different from the one that initiates *AtSUC2-GFP* expression (functional maturation) in companion cells.

By expressing GFP in tobacco plants, under the control of a companion-cell specific promoter, we have been able to investigate the patterns of vein development in maturing cotyledons and sink leaves. We have also been able to identify a variety of structural and functional control points that regulate the maturation of the minor veins and the expression of sucrose transport proteins within the major and minor veins.

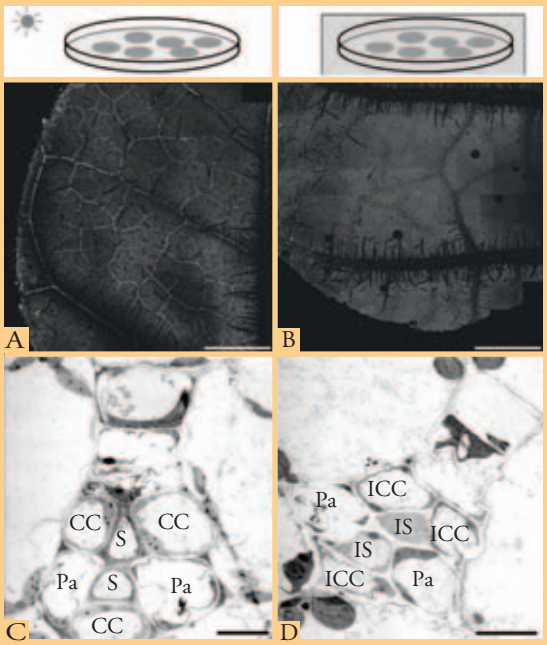

Figure 8 Leaf discs isolated from AtSUC2-GFP-ER plants. GFP is expressed in the major veins in the light (A) but not in the dark (B). The minor veins have matured in the light (C) but not in the dark (D). (Pa = phloem parenchyma, CC = companion cell, S = sieve element, ICC = immature companion cell, IS = immature sieve element)

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