

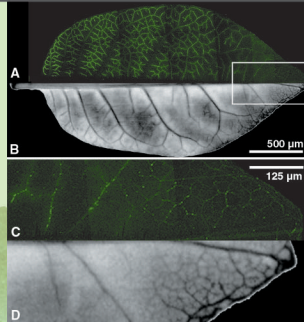
The Influence of Light on the Sink-to-Source Transition



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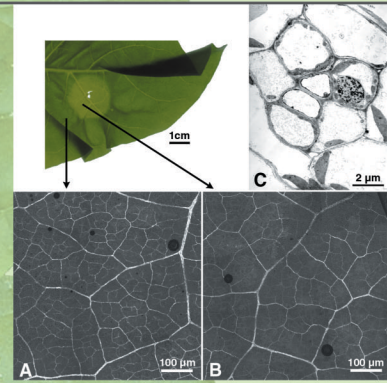
As a leaf develops it undergoes a transition from being a sink (or net importer of assimilate) to a source (or net exporter). Assimilate is imported into the sink leaf by the major veins (class I, II & III) and unloaded predominantly from the class III veins. As the transition proceeds unloading ceases, previously immature minor veins undergo structural maturation and sugar export commences.

In *Arabidopsis thaliana*, this export is mediated by the AtSUC2 sucrose-H⁺ symporter, located in the companion cells. The promoter for this symporter has been used to control the expression of GFP in tobacco plants. These AtSUC2-GFP plants express GFP within the companion cells of source leaves, the GFP moves into the sieve elements and is transported along with assimilate to sink leaves (A) where it is unloaded from the class III veins. In a leaf undergoing the transition to source this unloading ceases and GFP expression is restricted to the companion cells of the minor veins giving them a threadlike appearance (C). Radiolabelled sucrose accumulates in major veins in the sink area (B) and all vein classes in source areas of the leaf (D).



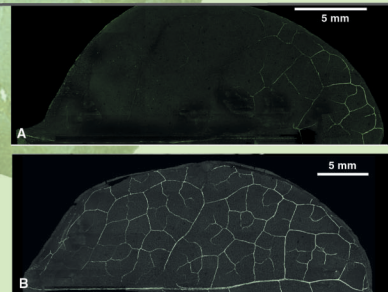
How does light affect the sink-source transition?

AtSUC2-GFP sink leaves were shaded with opaque discs for 12 days (see background) and then examined using the CLSM. During this time the leaf underwent the transition to a source. Within unshaded areas the veins had developed as expected, and GFP was expressed in the major and minor veins (A). In contrast, in the shaded area GFP was expressed in the major but not the minor veins (B) although these were structurally mature (C).

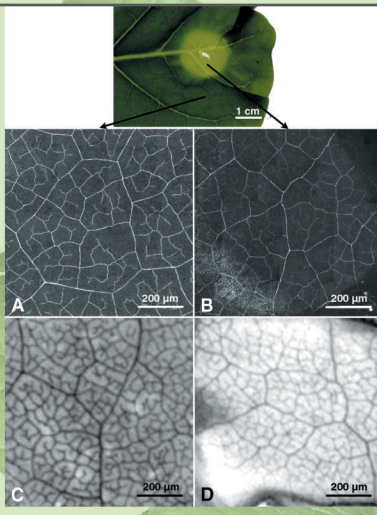


Was the presence of GFP in the major veins due to import into sink tissue?

Since free GFP is imported into the major veins in sink leaves of AtSUC2-GFP plants we attempted to identify whether it had been retained within these veins. A second line of transgenic plants was produced in which GFP, again expressed from the AtSUC2 promoter, was targeted to the endoplasmic reticulum. In these plants GFP is unable to traffic into the sieve elements, producing companion-cell autonomous GFP expression which is restricted to source tissue undergoing phloem loading. Thus a developing sink leaf shows no expression of GFP until the transition commences at the tip (A) and then progresses basipetally down the leaf (B)



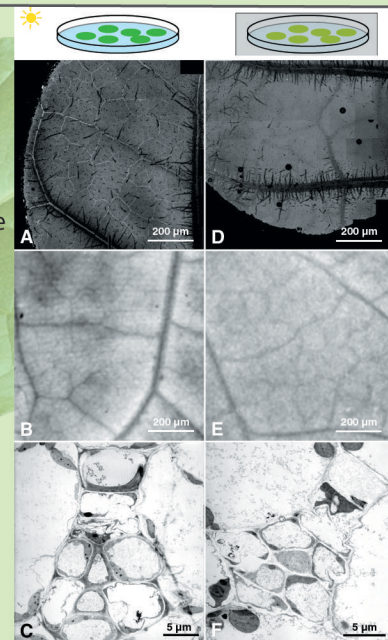
When shaded, AtSUC2-GFP-ER plants show the same pattern of expression as AtSUC2-GFP plants. Within the shaded area GFP is expressed in the major but not the minor veins (B), contrasting with unshaded areas (A). Therefore, the presence of GFP is not due to import into sink tissue. However, both the major & minor veins in unshaded (C) & shaded regions (D) of the leaf accumulate radiolabelled sucrose.



What is the signal to switch on the AtSUC2 promoter?

In isolated leaf discs the major, but not the minor veins express GFP in the light (A) but not the dark (D). Therefore AtSUC2 could be switched on by the light in the major but not the minor veins, and in intact leaves it is assumed that a signal, generated in the unshaded area moves into the shaded area of the leaf.

In contrast, the major but not the minor veins accumulate sucrose in the light (B) and the dark (E). Also, the minor veins mature in the light (C) but not the dark (F) suggesting that light could be required for structural maturation.



In conclusion, we have demonstrated that:

1. The expression of the AtSUC2 promoter in tobacco is influenced by light, or a product of light.
2. Expression within the major veins is regulated differently from that in the minor veins.
3. Maturation of the minor veins requires light, or a product of light.

We still do not know how the expression of the AtSUC2 promoter relates to the expression of endogenous tobacco sucrose transporters, and if these are regulated similarly by light. It is clear from radiolabelled sucrose experiments that sucrose is taken up when AtSUC2 is not being expressed indicating that the regulation of the tobacco sucrose transporters could be different from that of the AtSUC2 promoter expressed in tobacco.