Biosynthesis of vitamin C in plant phloem

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Vitamin C (L-ascorbic acid, AsA) is an essential human nutrient obtained primarily from plants. There is considerable interest in the elucidation of mechanisms responsible for the biosynthesis and accumulation of AsA in crop plants with a view to the development of strategies for optimising the vitamin C content of storage organs such as fruits and tubers. In general, green tissues are known to contain high levels of AsA which plays an important role in photosynthetic activity. On the other hand non-photosynthetic tissues show a much greater degree of variability with no obvious taxonomic explanation. For example, in fruits the variability extends from less than 3 µg gFW^{-1} in the medlar (*Mespilus germanica*) to over 27 mg gFW^{-1} in the camu camu (*Mirciara dubia*). Additionally, environmental conditions and agricultural practices such as fertilisation and water supply are also known to affect the AsA content of crops.

With the objective of optimising AsA accumulation in storage organs we sought to establish whether it is synthesised *in situ* or imported from other sites such as the foliage. Substantial levels of AsA were detected in the phloem of many crop plants by the use of aphid stylectomy, confirming earlier reports[1] and suggesting that phloem transport may be implicated in AsA accumulation in storage organs. Further investigations using courgette fruits, from which copious amounts of phloem exudates can be obtained, revealed the presence of all the known soluble enzymatic activities of the AsA biosynthetic pathway. Evidence of functional AsA biosynthesis in the phloem tissue was obtained with isolated phloem strands from celery petioles. Incubation with the precursors L-galactose and L-galactono-1,4-lactone resulted in up to a 10-fold increase in AsA concentration. Isolated phloem strands were also shown to convert distant ^{14}C-labelled precursors (e.g. [U-^{14}C]glucose or [U-^{14}C]mannose), to AsA more readily than in celery parenchyma.

The unexpected finding of high AsA biosynthetic capacity by the plant phloem raises the possibility that this process may be an important determinant of AsA

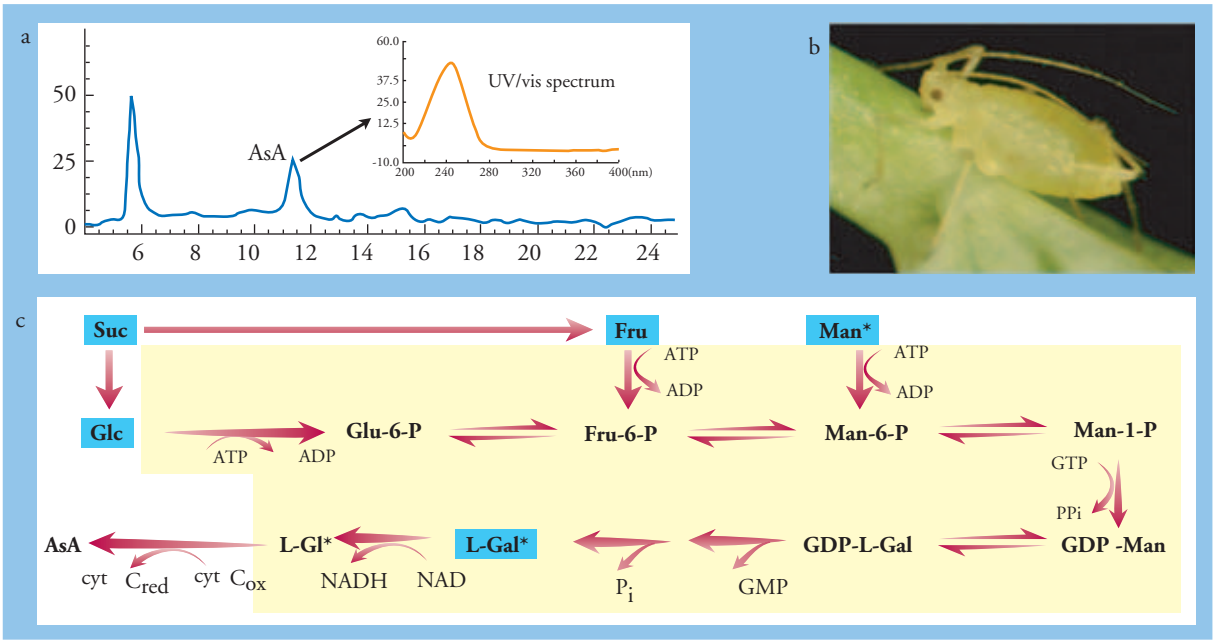

Figure 1 Proposed model of AsA biosynthesis in plant phloem.
High concentrations of AsA were demonstrated in plant phloem (HPLC trace a) by isolation of uncontaminated sieve tube content following stylectomy performed on feeding aphids (b). The inset in panel A confirms that the peak with identical retention time to authentic AsA also has an identical absorbance spectrum. Isolation of phloem exudate from courgette fruit allowed in vitro detection of all known soluble enzymes of the biosynthetic pathway (yellow boxed area in c) and unlabelled and ^{14}C-labelled precursor feeding demonstrated an operational pathway in isolated celery vascular bundles (marked * in panel c). Future work will concentrate on identification of AsA biosynthetic substrates in the phloem (potential substrates are highlighted in blue).

accumulation in storage organs. This hypothesis is at odds with a recent report showing direct AsA uptake and transport in Arabidopsis and Medicago[1]. We have also obtained evidence for L-[1-^{14}C]AsA uptake by source phloem of *Nicotiana benthamiana* but, intriguingly, we have also shown substantial uptake of the AsA biosynthetic intermediates D-[U-^{14}C]mannose and L-[1-^{14}C]galactose. Given the presence of the enzymic machinery for the conversion of these intermediates to AsA within the phloem, the possibility exists that AsA biosynthesis may occur within the phloem *en route* to sink-tissues. The challenge is now to establish which substrate is used for phloem AsA synthesis *in vivo*.

If functionally operational, phloem AsA biosynthesis may represent an important determinant of AsA accumulation in storage organs and a novel target for crop improvement. For example, modulation of phloem AsA biosynthesis would directly affect the AsA content in storage organs of those plants where assimilate unloading occurs via the symplast. Current work (supported by SEERAD, BBSRC, the Blackcurrant Growers Association and GlaxoSmithKline) aims at establishing the relative importance of endogenous synthesis, phloem transport and phloem synthesis to sink AsA content in important crops such as potato and blackcurrant.

References

[1] Franceschi, V.R. & Tarlyn, N.L. (2002). *Plant Physiology* **130**, 649-656.