Mechanism of vitamin C accumulation in blackcurrant fruit

Paul G. Walker, Simon D.A. Pont, Nicola Marquis & Robert D. Hancock

In recent years, there has been intense interest in the biosynthesis, metabolism and physiological functions of vitamin C in plants. Considerable advances have been made in understanding the biosynthesis of vitamin C with the publication of the *de novo* pathway in 1998 and the subsequent identification of three alternative pathways. In addition, breakthroughs have been achieved in understanding vitamin C catabolism with the identification of enzymes and pathway intermediates involved in the synthesis of oxalic, threonic and tartaric acids from vitamin C. Finally, significant strides have been made in understanding the role of vitamin C in various aspects of plant physiology and it has become clear that the vitamin plays an essential role in the modulation of reactive oxygen signalling which has implications for a range of plant responses to developmental, biotic and abiotic stimuli.

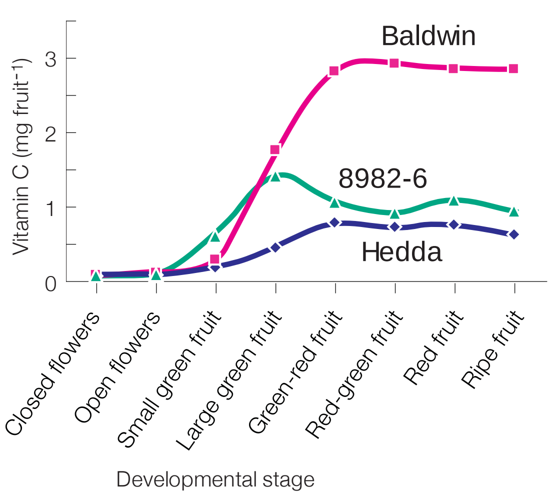

Figure 1 Numbers of nematodes developing on plants after exposure to double–stranded RNA targeting chorismate mutase (red bars) or a control gene (GFP – blue bars).

Despite these advances, very little attention has been paid to how vitamin C accumulates in the plant sink tissues (fruit, tubers) that form a major part of the human diet. Work undertaken at SCRI and elsewhere has shown that vitamin C synthesis can occur in heterotrophic (non-photosynthetic) tissues, however, it is also known that vitamin C can be transported from actively photosynthesising leaves to fruits and tubers (Tedone *et al.*, 2004). Uncertainty remains regarding the contribution of each source towards the accumulation of vitamin C in heterotrophic plant tissues.

Blackcurrants are exceptionally high in vitamin C with typical commercial cultivars containing 160 mg per 100 ml juice, approximately four times the amount found in orange juice. Historically, they were highly prized for their high vitamin C content and this trait remains an important target in modern breeding programmes. In order to help define molecular targets for accelerated breeding, a systematic analysis of the mechanism of vitamin C accumulation in blackcurrant fruit was undertaken (Hancock *et al.*, 2007). This is the first time that such an analysis has been performed for any crop.

Vitamin C accumulated early in fruit development during the expansion stage and prior to the development of red, blue or black colouration (Figure 1). In order to test the vitamin C transport hypothesis, radiolabelled vitamin C was supplied to leaves and translocation to fruit determined. Only 1% of the supplied radioactivity was recovered in fruit after 48h suggesting that transport was not an important mechanism for vitamin C accumulation in fruit. Furthermore, at the time of maximum fruit vitamin C accumulation leaf biosynthetic capacity halved contrary to what would be expected if leaves were supplying significant amounts of the vitamin to fruit.

In fruit, rates of both vitamin C biosynthesis and degradation varied with fruit maturity. In young fruit that were actively accumulating vitamin C, biosynthesis was high and degradation low and this situation was reversed in older fruit where vitamin C accumulation had ceased.

Taken together, these data support the contention that in blackcurrant fruit vitamin C accumulation occurs as a result of *in situ* biosynthesis and that transport from

distant organs does not provide a major contribution to fruit vitamin C levels. Instead, sugars are synthesised in leaves and transported to fruit providing the substrate for vitamin C synthesis. These findings allow the search for predictive markers of fruit vitamin C levels to be focused on biosynthetic genes and specific variations within one of the biosynthetic genes has already been associated with high vitamin C content. By testing for these genetic variants at the seedling stage, rather than waiting four years for plants to mature, selections can be made at a much earlier stage. This will result in the accelerated breeding of high vitamin C cultivars. Such an approach could be transferred to other fruits and vegetables that have a long generation time.

References

Tedone, L., Hancock, R.D., Alberino, S., Haupt, S. & Viola, R. 2004. Long-distance transport of L-ascorbic acid in potato. *BMC Plant Biology* **4**, 16.

Hancock, R.D., Walker, P.G., Pont, S.D.A., Marquis, N., Vivera, S., Gordon, S.L., Brennan, R.M. & Viola, R. 2007. L-Ascorbic acid accumulation in fruit of *Ribes nigrum* occurs by *in situ* biosynthesis via the L-galactose pathway. *Functional Plant Biology* **34**, 1080-1091.

Acknowledgments

This work was funded under the Defra Horticulture LINK scheme (MRS/003/02) with financial contributions from GlaxoSmithKline, the Blackcurrant Growers Association, the Horticultural Development Council, the Scottish Government and the Biotechnology and Biological Sciences Research Council.