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COMMENTS

A MYB-INDUCED MECHANISM FOR ENHANCED ASCORBATE IN RED-FLESHED APPLES

Richard V. ESPLEY (✉), Kui LIN-WANG

The New Zealand Institute for Plant and Food Research Limited, Mount Albert, Auckland, New Zealand.

ABSTRACT

The results presented in "The anthocyanin biosynthetic regulator MdMYB1 positively regulates ascorbic acid biosynthesis in apple" (An et al., this issue) provide evidence for a new mechanism for the elevation of ascorbate concentration in apple. Using a red-fleshed apple breeding population, the authors show how the anthocyanin-regulating MYB transcription factor, MdMYB1, also increases ascorbate concentrations by directly activating transcription of the dehydroascorbate reductase gene *MdDHAR*. This gene recycles oxidized ascorbate back to ascorbate, leading to elevated concentrations of vitamin C. These red-fleshed apples have enhanced concentrations of both anthocyanins and ascorbate, both of which are appealing traits for the development of healthier apples.

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Correspondence: Richard.Espley@plantandfood.co.nz

In plants, ascorbate (vitamin C) is an essential antioxidant and cofactor in redox reactions. Ascorbate is at the forefront of reactive oxygen species detoxification during pathogen infection and other stress conditions. It is also strongly associated with numerous important cellular processes and general plant development. Ascorbate is predominantly synthesized in the Smirnoff-Wheeler pathway and the amount of ascorbate is tightly regulated at the transcriptional and translational levels[1].

Humans have lost the ability to synthesize ascorbate and rely on vegetables and fruit for their dietary intake of this key molecule[2]. Thus breeding high-ascorbate plant cultivars, which will not only serve to enhance people's dietary intake, but also may help to protect the plant during growth and maintain fruit quality during postharvest storage.

In the paper by An et al.[3], the authors describe the analysis of progeny of a cross between the white-fleshed apple cv. Orin and the red-fleshed apple cv. Guanghui. There are a number of red-fleshed apple breeding programs around the world aimed at producing novel high-quality apple cultivars[4]. In that study, the authors show how the segregating red-fleshed fruit contain relatively higher concentrations of ascorbate. In apple, ascorbate concentrations are often relatively low[5], so efforts to increase this through selective breeding would be highly desirable. A number of QTLs have been identified, collocating with potential ascorbate candidates, including one of the key biosynthetic enzymes, GGP, and the dehydroascorbate reductase gene *DHAR*[2]. While some studies have linked the simultaneous production of anthocyanin and ascorbate, this is generally as a result of some stress condition, such as high light[6]. Red flesh in apple is derived from a mutation in the promoter sequence of the anthocyanin MYB regulator MdMYB10 (MYB1/A), creating a self-activating transcriptional loop[7]. It is known that MdMYB10 activates the anthocyanin pathway, but until now MdMYB10 has not been linked with either ascorbate biosynthesis or ascorbate recycling.

An and colleagues[3] show that not only do the red-fleshed fruit have a higher ascorbate concentration, but also a lower concentration of DHA (involved in ascorbate oxidation) as well as enhanced DHAR enzyme expression levels than the white-fleshed progeny. Furthermore, the transcript abundance of *MdMYB1* was positively correlated with the expression level of *MdDHAR*. These authors used overexpression of *MdMYB1* in apple callus to verify these findings experimentally. The callus showed increased ascorbate concentrations, again accompanied by lower DHA content, higher DHAR enzyme and higher expression levels of *MdDHAR*. Also, the overexpression of *MdMYB1* in apple callus promoted the expression of the ascorbate oxidase gene *MdAO*, the ascorbate peroxidase gene *MdAPX1* and the monodehydroascorbate reductase gene *MdMDHAR*, indicating that MdMYB1 may contribute to ascorbate homeostasis. To validate the mode of function the authors used both ChIP-PCR assays and EMSA to determine the binding affinity of MdMYB1 to the promoter region of *MdDHAR* and validated these results using the dual luciferase assay to show enhanced transcriptional activity of the *MdDHAR* promoter in the presence of MYB1.

These findings provide evidence for a new mechanism for increased ascorbate concentrations in apple via the MYB1-induced activation of *MdDHAR*, leading to increased ascorbate. Combining traits of red flesh and higher vitamin C would seem a useful future breeding target for apple improvement. From a consumer perspective, such an apple would not only be more visually attractive but would also potentially offer enhanced health benefits from both the higher anthocyanin and ascorbate concentrations.

REFERENCES

1. Bulley S, Laing W. The regulation of ascorbate biosynthesis. *Current Opinion in Plant Biology*, 2016, **33**: 15–22
2. Fenech M, Amaya I, Valpuesta V, Botella M A. Vitamin C content in fruits: biosynthesis and regulation. *Frontiers in Plant Science*, 2019, **9**: 2006
3. An J, Wang X, You C, Hao Y. The anthocyanin biosynthetic regulator MDMYB1 positively regulates ascorbate acid biosynthesis in apple. *Frontiers of Agricultural Science and Engineering*, 2021 [Published online] doi: 10.15302/J-FASE-2020367
4. Wang N, Jiang S, Zhang Z, Fang H, Xu H, Wang Y, Chen X. *Malus sieversii*: the origin, flavonoid synthesis mechanism, and breeding of red-skinned and red-fleshed apples. *Horticulture Research*, 2018, **5**(1): 70
5. Mellidou I, Chagné D, Laing W A, Keulemans J, Davey M W. Allelic variation in paralogs of GDP-L-galactose phosphorylase is a major determinant of vitamin C concentrations in apple fruit. *Plant Physiology*, 2012, **160**(3): 1613–1629
6. Page M, Sultana N, Paszkiewicz K, Florance H, Smirnoff N. The influence of ascorbate on anthocyanin accumulation during high light acclimation in *Arabidopsis thaliana*: further evidence for redox control of anthocyanin synthesis. *Plant, Cell & Environment*, 2012, **35**(2): 388–404
7. Espley R V, Brendolise C, Chagné D, Kutty-Amma S, Green S, Volz R, Putterill J, Schouten H J, Gardiner S E, Hellens R P, Allan A C. Multiple repeats of a promoter segment causes transcription factor autoregulation in red apples. *Plant Cell*, 2009, **21**(1): 168–183