Pucker et al. BMC Plant Biology (2021) 21:297
https://doi.org/10.1186/s12870-021-03080-9

BMC Plant Biology

CORRESPONDENCE Open Access

The report of anthocyanins in the betalain-pigmented genus *Hylocereus* is not well evidenced and is not a strong basis to refute the mutual exclusion paradigm

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Abstract

Here we respond to the paper entitled "*Contribution of anthocyanin pathways to fruit flesh coloration in pitayas*" (Fan et al., BMC Plant Biol 20:361, 2020). In this paper Fan et al. 2020 propose that the anthocyanins can be detected in the betalain-pigmented genus *Hylocereus*, and suggest they are responsible for the colouration of the fruit flesh. We are open to the idea that, given the evolutionary maintenance of fully functional anthocyanin synthesis genes in betalain-pigmented species, anthocyanin pigmentation might co-occur with betalain pigments, as yet undetected, in some species. However, in absence of the LC-MS/MS spectra and co-elution/fragmentation of the authentic standard comparison, the findings of Fan et al. 2020 are not credible. Furthermore, our close examination of the paper, and re-analysis of datasets that have been made available, indicate numerous additional problems. Namely, the failure to detect betalains in an untargeted metabolite analysis, accumulation of reported anthocyanins that does not correlate with the colour of the fruit, absence of key anthocyanin synthesis genes from qPCR data, likely mis-identification of key anthocyanin genes, unreproducible patterns of correlated RNAseq data, lack of gene expression correlation with pigmentation accumulation, and putative transcription factors that are weak candidates for transcriptional up-regulation of the anthocyanin pathway.

Background

In the plant kingdom, betalains occur only in the order Caryophyllales where they substitute the otherwise ubiquitous anthocyanin pigments [1, 2]. Although betalains are found in most families in Caryophyllales, several families have anthocyanin pigmentation and do not produce betalains. Betalains and anthocyanins have never been found in the same species and are widely held to be mutually exclusive at the organismal level [3, 4]. However, both pigments have been observed in a genetically engineered tomato plant [5], on transgenic heterologous production of betalains. The molecular basis of mutual exclusion is unclear, especially as betalain-pigmented species seem to retain all the genes encoding the necessary enzymatic machinery for anthocyanin synthesis. It remains a remarkable and largely unexplained biological conundrum that has been reinforced by repeated observations for over fifty years [6–8].

With this as context, Fan et al. [9] recently reported anthocyanins within the betalain-pigmented genus *Hylocereus* (Cactaceae), also commonly called Pitaya. Fan et al. [9] analysed the fruits of three closely related species – a red-fleshed *Hylocereus polyrhizus, a* white-fleshed *Hylocereus undatus*, and an intermediate pink-fleshed hybrid (*H. polyrhizus x H. undatus*). Based on the analysis, they reported to correlate the accumulation of anthocyanins with the colour of red and pink fruit pulps, and the expression

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