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MYBs affect the variation in the ratio of anthocyanin and flavanol in fruit peel and flesh in response to shade

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ABSTRACT

Fruit pigment accumulation, which represents an important indicator of nutrient quality and appearance value, is often affected by low light under rain, cloud, fog and haze conditions during the veraison period. It is not known whether continuous low light interferes with the production and accumulation of secondary metabolites in veraison fruit. In this paper, we measured pigments and the transcriptional level of genes related to secondary metabolites, i.e., flavonoid biosynthesis in the peel and flesh of *Malus* crabapple 'Radiant' fruit in response to normal light and shade from 10th July to 30th August. The results showed crosstalk between the flavonoid biosynthetic genes and the involvement of key transcription factors such as McMYB4, McMYB7, McMYB10, and McMYB16 in the regulation of the ratio of anthocyanins and flavanols, which accounted for the different colouration of the fruit peel and flesh under shade conditions. A model is proposed for the regulation of the flavonoid pathway in the peel and flesh of 'Radiant' fruit based on our study results. Moreover, the molecular mechanism for 'Radiant' fruit colouration provides reference information for understanding the light regulatory mechanism involved in the biosynthesis of flavonoids and for designing the next generation of apple breeding.

1. Introduction

Flavonoids, an important group of naturally occurring phenolic compounds, include flavonols, flavones, and proanthocyanidins (PAs), which are responsible for the colouration of leaves, flowers and fruits [1]. Anthocyanins are mainly present in purple coloured fruits, such as berries, blackcurrant, plum, and some indigenous fruits [2,3]. During the process of development and ripening, fruit changes colour as a result of changes in various compounds and the presence of flavonoids. Anthocyanin pigments and associated flavonoids in plants can attract insects for pollination and can protect fruit from UV damage as well as provide benefits for human health. Compared to most phenolic compounds, anthocyanin possesses stronger antioxidant capacity and antimutagenic and anticancer activities. Therefore, public attention is now being focused on how to modify flavonoid metabolism in plants.

As a multi-enzymatic pathway, there are numerous constructive genes involved in flavonoid biosynthesis [2–5], such as the early-stage genes phenylalanine ammonia lyase (PAL), and cinnamate-4-hydroxylase (C4H), 4-coumaroyl-CoA synthase (4CL), chalcone synthase (CHS), chalcone isomerase (CHI) and flavanone 3-hydroxylase (F3H). When flavonoid 3′-hydroxylase (F3′H) and dihydroflavonol 4-reductase (DFR) result in the production of leucoanthocyanidin from dihydroflavonol, a branching point is produced in the metabolic pathway. One of the steps is leucoanthocyanidin, which is transformed into modified anthocyanins by anthocyanin synthase (ANS) and uridinediphosphate-glucose (UFGT). Another step is flavonol synthase (FLS) acting on dihydroflavonols to generate flavonols, and the final step is the production of flavanols from leucoanthocyanidin and anthocyanidin in the presence of leucoanthocyanidin reductase (LAR) and anthocyanidin reductase (ANR), respectively. Based on the numerous studies reported, it is obvious that the flavonoid biosynthetic pathways appear to be mainly regulated at the transcriptional level. The direct regulation of the expression of the structural genes in the pathway has an effect on anthocyanin accumulation, which controls the development of the red fruit colour.

The MYB regulators, especially numerous R2R3-MYB proteins, which are reported to be one of the largest transcription factor (TF) families in plants, have been proved to be involved in the regulation of plant-specific processes, including primary and secondary metabolism, developmental processes, cell apoptosis and identification, and responses to biotic and abiotic stresses [6–8]. For example, ZmMYB31

Abbreviations: CHS, chalcone synthase; F3′H, flavonoid 3′-monooxygenase; DFR, dihydroflavonol 4-reductase; ANS, anthocyanidin synthase; LAR, leucoanthocyanidin reductase; ANR, anthocyanidin reductase.

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and ZmMYB42 are R2R3-MYB TFs implicated in the regulation of phenylpropanoid genes in maize [9], and PcMYB10 plays a critical role in anthocyanin biosynthesis in red purple fruit [10]. In many plant species, MYB proteins have been reported to be the key components that regulate flavonoid biosynthesis [10–17]. The flavonol regulators, MYB12/PFG1 (PRODUCTION OF FLAVONOL GLYCOSIDES1), MYB11/PFG2, and MYB111/PFG3, regulate the expression of the four early biosynthetic genes, chalcone synthase (CHS), chalcone isomerase (CHI), flavanone 3-hydroxylase (F3H), flavonoid 3′-hydroxylase (F3′H), and flavonol synthase (FLS), whereas the anthocyanin regulators, MYB75/PAP1 (PRODUCTION OF ANTHOCYANIN PIGMENT1), MYB90/PAP2, MYB113, and MYB114, control the expression of the late biosynthetic genes, dihydroflavonol reductase (DFR) and leucoanthocyanidin dioxygenase/anthocyanidin synthase (LDOX/ANS).

Light acts as a key environmental factor that not only controls plant primary functions such as photosynthesis and photorespiration but also plays an important role in flavonoid metabolism; light can regulate flavonoid biosynthesis and organ colouration in various plant species [18,

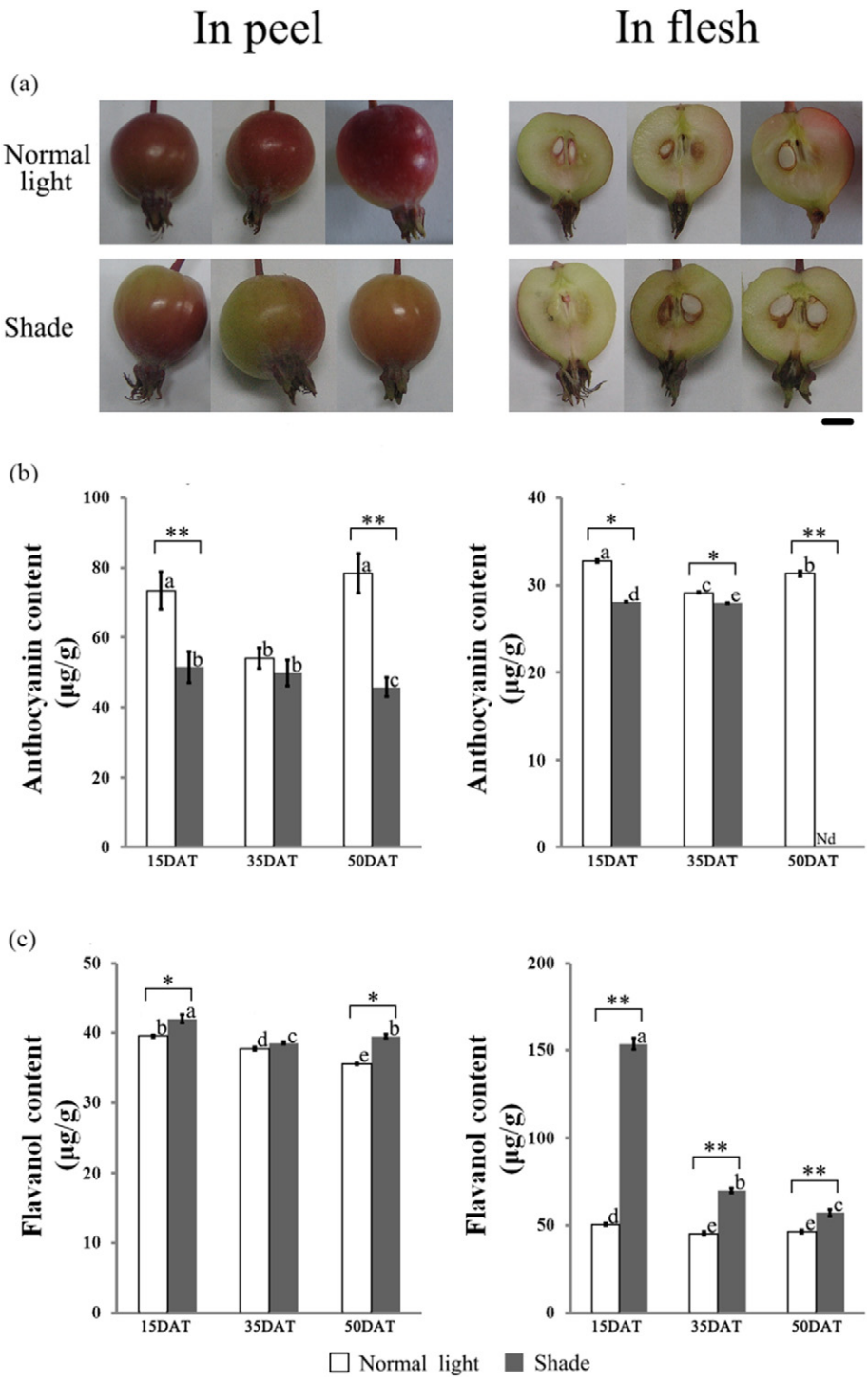

Fig. 1. The fruit colour phenotypes and the flavonoid contents in *Malus* sp. Radiant grown under natural light or shade. Scale bar = 0.5 cm. (a) The fruit peel and flesh colour phenotypes. (b) The anthocyanin content in the peel and flesh. (c) The flavanol content in the peel and flesh. a,b,c,d,e were calculated using one-way ANOVA followed by Duncan's SSR (shortest significant ranges) test. * and ** indicate significant linear correlations at the 0.05 and 0.01 levels, respectively, which were calculated using Duncan's new multiple range test. DAT, days after treatment. Nd means not detectable. The error bars represent the mean ± SD of at least three biological replicates.