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Review

The molecular network regulating the coloration in apple

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ABSTRACT

Preferences for the appearance of apples may vary from country to country and region to region, but polished redness is undeniably eye-catching and could easily win a good first impression. Anthocyanin is considered as the dominant pigment responsible for the red coloration in apple. Many reports have been published to elucidate the biosynthetic genes and the upstream regulatory genes involved in the anthocyanin accumulation. This paper reviews the recent studies on the biosynthetic and regulatory genes associated with the pigmentation as well as the external stimuli affecting anthocyanin production in apple.

Contents

1. Introduction

An apple a day keeps the doctor away. While apple (*Malus* × *domestica*) may not possess the magic power stated by the proverb, it is indeed one of the most widely cultivated fruit trees around the world, whose fruits are rich in the content of health-promoting antioxidants such as anthocyanins (Cliff et al., 2002; Allan et al., 2008). Anthocyanin serves not only as the reactive oxygen species scavenger, but also as an important secondary metabolites, i.e., glycosides of anthocyanidins are responsible for the red, purple (pH < 7.0) and blue (pH > 7.0) hues of various kinds of leaves, flowers and fruits (Lancaster, 1992; Konczak and Zhang, 2004; Takos et al., 2006a). Cyanidin 3-glycosides (cy3-gly) is the main form of anthocyanin in apple peel; cyanidin 3-galactoside (cy3-gal) covers 80% of the total cy3-gly, which is obviously higher in red cultivars like 'Jonathan' and in the bagging-treated non-red cultivar like 'Mutsu' (the cultivar that could turn red after the bag application) (Lancaster, 1992; Ban et al., 2009; Peng et al., 2013). Nowadays, the consumers not only appreciate the esthetic appeal, but also consider nutritional quality as an influential added value in the fruit purchasing criteria. Considering the appearance and nutritional benefit, red cultivars definitely occupy a larger market share than their non-red counterparts (Cliff et al., 2002; Ban et al., 2007a).

For centuries, apple breeders have been trying to improve the red coloration quality. As light is an indispensible element for

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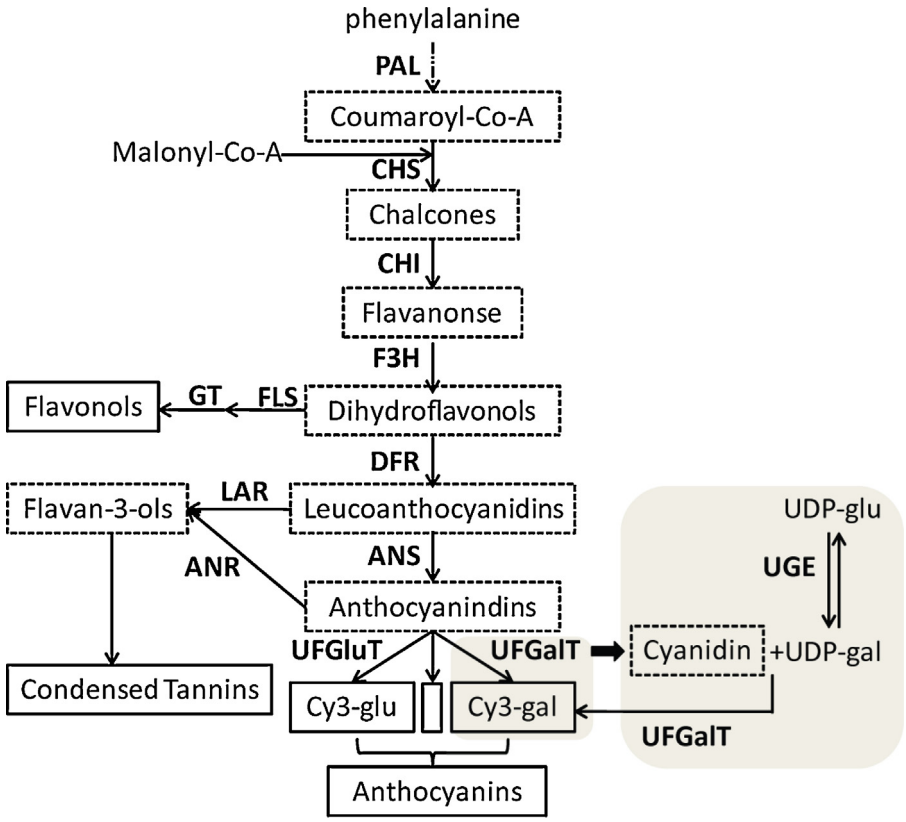

Fig. 1. Diagram for the flavonoid biosynthetic pathway in apple. The intermediates and end products of the pathway are indicated in dashed and solid line boxes, respectively. The empty box between cyanidin 3-glucoside (Cy3-glu) and cyanidin 3-galactoside (Cy3-gal) represents other forms of anthocyanins in apple; UDP-glu and UDP-gal stand for UDP-glucose and UDP-galactose, respectively. The bigger gray part is the supplementary to the smaller one. Bold uppercase abbreviations are the enzymes required for each step. PAL, phenylalanine ammonia lyase; CHS, chalcone synthase; CHI, chalconeisomerase; F3H, flavanone-3β-hydroxylase; FLS, flavonol synthase; GT, unidentified enzyme encoding a glycosyltransferase for flavonol glycone synthesis; DFR, dihydroflavonol-4-reductase; ANS, anthocyanidin synthase; LAR, leucoanthocyanidin reductase; ANR, anthocyanindin reductase; UFGluT, UDP-glucose:flaonoid-3-*O*-glycosyltransferase; UFGalT, UDP-galactoside:flaonoid-3-*O*-glycosyltransferase; UGE, UDP-glucose 4-epimerase.

anthocyanin accumulation in apple and enhanced light exposure can induce the red pigmentation, reflective film has long been used as an effective planting approach in orchard management to raise light volume and even light distribution in the canopy (Faragher, 1983; Elfving et al., 1990; Miller and Greene, 2003; Iglesias and Alegre, 2009). Besides film application, both ethephon and phosphorus–calcium mixed compounds are proposed to improve the red coloration in apple (Li et al., 2002). Nevertheless the advanced management means are confined to the ever-changing external environment. Meanwhile, traditional breeding methods like crossing are time-consuming and efficiency-limiting. Fortunately, the apple breeding processes could be greatly facilitated by various emerging biotechnologies, such as marker-assisted selection and genetic transformation (Allan et al., 2008). However, the application of these unconventional methods requires a thorough understanding of the molecular mechanism of red coloration in apple.

As stated above, anthocyanin accumulation leads to red pigmentation in apple. To date, the biosynthetic pathway for anthocyanin has been clearly deciphered (Honda et al., 2002; Ban et al., 2007a; Hichri et al., 2011). Anthocyanin accumulation is well accepted to be regulated at the transcriptional level, especially via the MYB/basic helix-loop-helix (bHLH)/WD40 protein complex (MBW) (Espley et al., 2007). Currently, this regulatory pathway has been expanded to cover some newly characterized components. Accordingly, both the functional and regulatory pathways pertinent to anthocyanin biosynthesis will be covered below.

2. Functional genes involved in anthocyanin biosynthesis

As shown in Fig. 1, flavonols, condensed tannins (CTs) and anthocyanin share several biosynthetic steps in the flavonoid pathway. So far, the enzymes involved in anthocyanin biosynthesis have been well characterized, not only in apple, but also in grape (*Vitis vinifera*), pear (*Pyrus communis* L.), petunia (*Petunia hybrida*) and rose (*Rosa hybrida*) (Dela et al., 2003; Steyn et al., 2005; Mori et al., 2007). The conversion of phenylalanine to *trans*-cinnamic acid, catalyzed by L-phenylalanine ammonia-lyase (PAL) via ammonia elimination reaction, is the initial step in the flavonoid pathway (Jones, 1984). The second peak of PAL activity is paralleled with anthocyanin accumulation in strawberry (Jones, 1984; Cheng and Breen, 1991). In the library of UV-B-treated 'Mutsu' by suppression subtractive hybridization (SSH), two ESTs annotated as PAL 1 precursor are identified (Peng et al., 2013), suggesting the importance of PAL in anthocyanin accumulation. However, since anthocyanin accumulation is independent on PAL activity, it is concluded that PAL is not the key regulatory enzyme in anthocyanin formation of apple peel (Ju et al., 1995).

Two peaks of anthocyanin biosynthesis are observed in apple fruits, one in un-matured fruitlets and the other in ripening fruits (Honda et al., 2002). It has been reported that the anthocyanin concentration in ripening apples is coordinately and positively related to the expressions of the genes encoding downstream required enzymes, such as *chalcone synthase* (*MdCHS*), *flavanone3-hydroxylase* (*MdF3H*), *dihydroflavonol 4-reductase* (*pDFR*), *anthocyanidin synthase* (*MdANS*) and *UDP-glucose:flavonoid*