



Review

Expression analysis of anthocyanin biosynthesis key regulatory genes involved in pomegranate (*Punica granatum* L.)



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ARTICLE INFO

Article history:

Received 3 October 2014

Received in revised form 11 February 2015

Accepted 13 February 2015

Keyword:

Pomegranate

Anthocyanin

AN1 (bHLH)

AN2 (MYB)

WD40

DFR and real time PCR

ABSTRACT

Pomegranate (*Punica granatum* L.) is one of the oldest and important horticultural plants in Iran. Pomegranate fruits are an important source of secondary metabolites such as phenolic compounds, tannins, colors and alkaloids. Anthocyanin accumulation in plant organs is associated with gene expression in anthocyanin biosynthesis pathway. Regulation of anthocyanin biosynthesis expression is controlled by a number of transcription factors including MYB, basic helix–loop–helix (bHLH) and WD-repeat protein (WD-Repeat) proteins. In order to study the expression level of some transcription factors/genes and their role in pomegranate skin color, real time PCR analysis was used to assess the expression patterns among different pomegranate genotypes. Results of this study showed that white and green skin genotypes had the highest AN1 (bHLH) expression, whereas bright red and black skin genotypes accumulated the lowest AN1 transcript level. Furthermore, green and bright red genotypes showed the highest and the lowest expression level with regard to AN2 (MYB) transcription factor, respectively. The DFR gene expression in samples with black skin was the highest while in white skin samples the lowest expression of this gene was shown. This result indicates the influence of WD40 transcription factor on DFR structural gene and its effective role on synthesis of skin color. With regard to expression of three transcription factors in all genotypes, it was evident that MYB–bHLH–WD40 complex confers the skin color in pomegranate. Total anthocyanin content measurement showed a positive correlation between the level of DFR gene expression and accumulation of anthocyanins in different genotypes.

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## 1. Introduction

Anthocyanins are the largest group of water-soluble pigments in many plants (Horbowicz et al., 2008; Oancea and Oprean, 2011). Anthocyanins pigments are accumulated in cell vacuoles (Oancea and Oprean, 2011) and produce a diverse pigmentation from orange to red, purple and blue in flowers, fruits and vegetables (Horbowicz et al., 2008). Anti-oxidant effects, protecting DNA and the photosynthetic machinery from high radiation fluxes, resistance to cold and drought stress, anti-aging and anti-cancer properties and recruitment of pollinators are some well known roles of anthocyanins (Horbowicz et al., 2008; Oancea and Oprean, 2011; Winkel-Shirley, 2001). Recently, a number of studies have focused on the importance of anthocyanins, especially their health-promoting properties and radical scavenging features (Oancea and Oprean, 2011; Lo Piero et al., 2005).

Pomegranate fruit is a rich source of phenolic compounds, especially anthocyanin pigments (Perez-Vicente et al., 2002). Although different types of anthocyanins are found in pomegranate juice, pomegranate skin contains few anthocyanins (Hernández et al., 1999).

Skin color in pomegranate exhibit a range of different colors, including yellow, green, or pink overlain with pink to deep red or indigo to fully red, pink or deep purple color and black (Holland et al., 2009). Generally, the color intensity is affected by chemical structure and concentration of each anthocyanin pigment (Hernández et al., 1999), light, various abiotic stresses and temperature (Feng et al., 2010; Zhang et al., 2012).

Anthocyanin biosynthetic pathway has been investigated in many plants such as petunia, snapdragon flowers, maize kernels (Holton and Cornish, 1995), orange and apple. The results of these studies have revealed that many enzymes, structural genes and regulatory elements play important roles in the biosynthesis pathway of anthocyanins (Chandler et al., 1989; Holton and Cornish, 1995; Yuan et al., 2013; Geekiyanage et al., 2007).

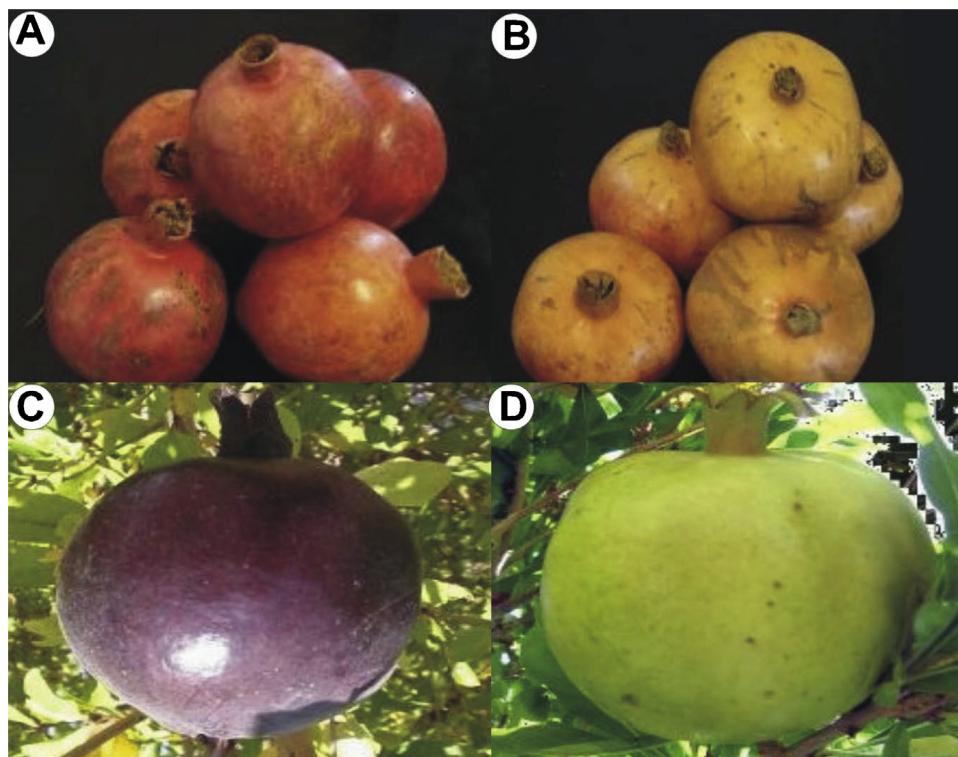
Enzymes/proteins involved in anthocyanin biosynthesis regulation are members of protein families containing R2R3-MYB domains, bHLH (basic helix–loop–helix) domains and WD40 repeat proteins (Laitinen et al., 2008; Feng et al., 2010). Each of these regulatory factors, separately or together regulate expression of structural genes (Laitinen et al., 2008; Feng et al., 2010). For example, MYB–bHLH–WD40 complex determines the red color in pear (Zhang et al., 2011). It has been shown that, *jaf13* gene encodes bHLH regulator of anthocyanin gene expression in petunia (Lu and Yang, 2006). It has been strongly suggested that MYBA transcription factors regulate *UFGT* structural gene in grape (Kobayashi et al., 2002; Bogs et al., 2007). The roles of such transcription factors have also been proven in apple (Ban et al., 2007; Vimolmangkang et al., 2013).

Real time PCR analysis in *Solanum pinnatisectum* has shown that the expression of *CHS*, *F3H*, and *DFR* genes has resulted in anthocyanin accumulation (Lu and Yang, 2006). Previous studies in maize have shown that MYB factor C1, together with its bHLH partner R, activate the accumulation of cyanidin derivatives as common pigments in differentiated maize tissues (Grotewold et al., 1998). In *Arabidopsis*, the WD40-repeat protein TTG1 regulates the expression of *DFR* gene (Shirley et al., 1995). Since pomegranate exhibits a range of different skin colors, the aim of this study was to investigate the expression level of key transcription factor/genes controlling anthocyanin biosynthesis in pomegranate in relation to skin color.

## 2. Materials and methods

### 2.1. Plant materials

Four pomegranate genotypes with Iranian names, including Poost Sefid-e-Shirin (PSS: white skin and sweet), Poost Sabz-e-Abarkoh (PSA; green skin from Abarkoh), Poost Siyah-e-Saveh (PSAV; black skin from Saveh) and Poost Geermez-e-AlakSaveh



**Fig. 1.** Pomegranate genotypes used in this study. (A) Poost Geermez-e-AlakSaveh (PGA), (B) Poost Sefid-e-Shirin (PSS), (C) Poost Siyah-e-Saveh (PSAV) and (D) Poost Sabz-e-Abarkoh (PSA).