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Analysis of structural genes and key transcription factors related to anthocyanin biosynthesis in potato tubers

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ABSTRACT

Anthocyanin is well-known plant colorant that accounts for the characteristic color of potato tubers and their excellent antioxidant properties. Anthocyanin synthesis involves several enzyme genes and transcription factors. In this paper, the expression of 10 enzyme genes and 11 transcription factors related to anthocyanin biosynthesis in 8 genotypes of colored potato tubers were examined. The results showed that chalcone synthase gene (*CHS*), flavanone 3-hydroxylase gene (*F3H*), dihydroflavonol 4-reductase gene (*DFR*), glutathione S-transferase gene (*GST*), flavonoid 3′,5′-hydroxylase gene (*F3′5′H*) and anthocyanidin synthase gene (*ANS*) play important roles in anthocyanin biosynthesis in colored potato tubers. Further analysis examining the expression correlation between transcription factors and enzyme genes indicated that 4 novel transcription factors, *MYB11207*, *MYB47415*, *MYB79714* and *bHLH31926*, may affect anthocyanin synthesis in tubers by regulating the expression of several enzyme genes, especially the MYB member *MYB47415*.

1. Introduction

Potato (*Solanum tuberosum* L.) is a major staple food and the fourth largest crop grown worldwide. The skin and flesh of potato can be pigmented white, red, purple, dark purple and so on. Anthocyanin is a well-known plant pigment that accounts for the characteristic color of potato tubers (Eichhorn and Winterhalter, 2005) and their excellent antioxidant properties (Dai et al., 2009; Zafra-Stone et al., 2007). The dietary intake of anthocyanin from colored potatoes has been promoted as a possible means of preventing human cancer and other chronic diseases (Stushnoff et al., 2008), because anthocyanin can promote antioxidant enzyme activities (Zhao et al., 2009).

The enzymes involved in the anthocyanin biosynthetic pathway are encoded by a series of genes shown in Fig. 1 (Holton and Cornish, 1995). In potato, some of the genes have been identified and their expression is tissue-specific (Lu and Yang, 2006). The potato *R* and *P* loci, which were first described by Salaman (1910), are required for the production of red and purple anthocyanin, respectively. *R* and *P* have been shown to code for the anthocyanin biosynthetic enzymes dihydroflavonol 4-reductase (DFR) (De Jong et al., 2003; Zhang et al., 2009) and flavonoid 3′,5′-hydroxylase (F3′5′H) (Jung et al., 2005), respectively. *DFR* was proven to be present in all red-colored potatoes and several white clones using restriction fragment length polymorphism (RFLP) (De Jong et al., 2003). *DFR* was introduced into the potato cultivar Prince Hairy, which has white tubers and pale blue flowers, under the control of the CaMV 35S promoter. In transgenic lines, the flower color changed to purple while the tubers remained white (Zhang et al., 2009). Also the potato *F3′5′H* gene was placed under the control of a doubled CaMV 35S promoter and introduced into the red-skinned cultivar 'Desiree'. The tuber and stem tissues that are colored red in Desiree were purple in transformed lines (Jung et al., 2005). The potato UDP-glucose: flavonoid-3-*O*-glucosyltransferase (*3GT*) gene was introduced into Desiree under the control of the GBSSI promoter. The tuber color and anthocyanin contents were noticeably enhanced in the transgenic plants compared to the wild-type control plants (Wei et al., 2012). However, the roles of other enzyme genes in anthocyanin biosynthesis in potato tubers are unclear.

The natural variation in potato tuber color results from tissue-specific differences in the accumulation of anthocyanin pigments. It has been known that there are three classes of transcriptional regulators

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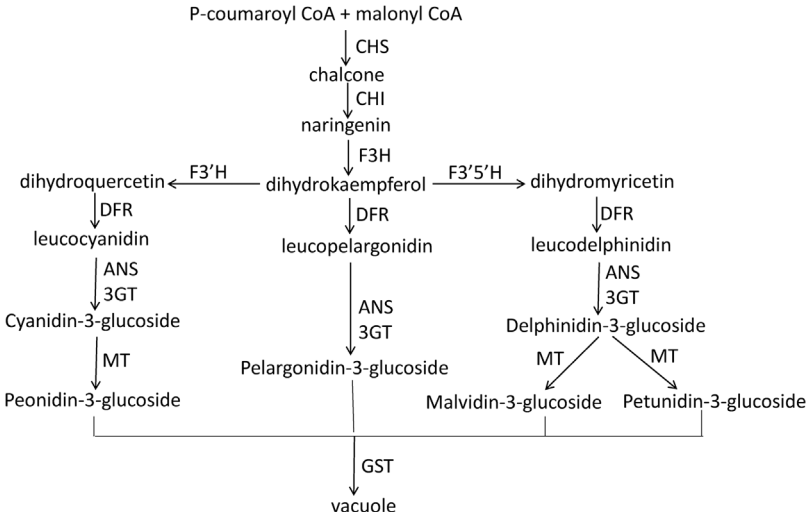

Fig. 1. Anthocyanin biosynthesis pathway. CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavanone 3-hydroxylase; F3′H, flavonoid 3′-hydroxylase; F3′5′H, flavonoid 3′5′-hydroxylase; DFR, dihydroflavonol 4-reductase; ANS, anthocyanidin synthase; 3GT, UDP-glucose: flavonoid-3-*O*-glucosyltransferase; MT, methyltransferase; and GST, glutathione S-transferase. Adapted from Holton and Cornish (1995).

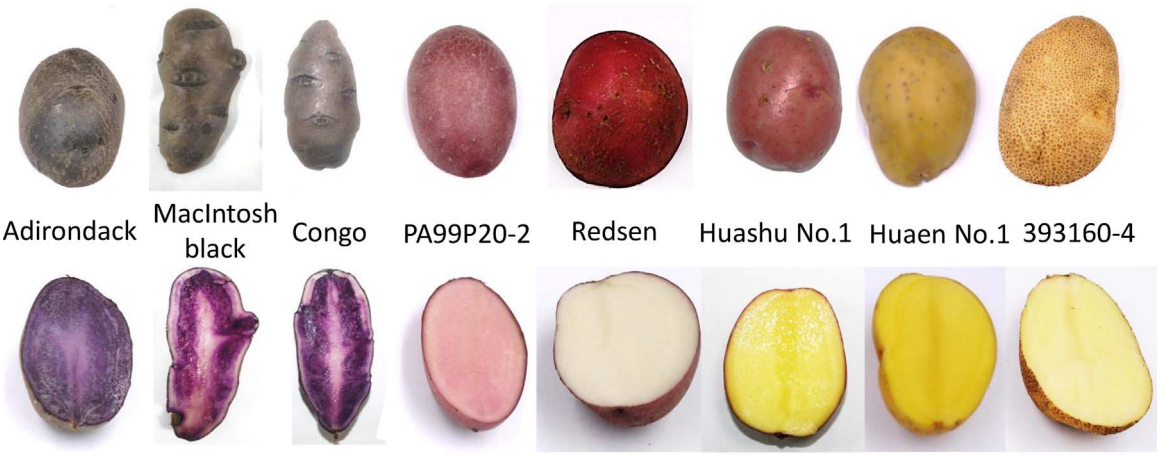

Fig. 2. Skin and flesh colors of the 8 genotypes used in this study.

involved in anthocyanin synthesis, which are R2R3-MYB, basic helix-loop-helix (bHLH), and WD40-repeat (WDR) proteins (Grotewold, 2006). Some of the transcriptional factors related to anthocyanin biosynthesis in potato have been identified in recent years. The *D* locus, which is equivalent to the *I* locus in diploid potato tubers, encodes an R2R3-MYB gene named *StAN2* that was cloned from the tuber skin of Y83-5, a red-colored tetraploid clone. The expression showed that *StAN2* was expressed in the skin of colored potatoes but not white-colored potato. *StAN2* was also transformed into three cultivars, which caused the transgenic lines to accumulated anthocyanin in the tubers as well as in other tissues (Jung et al., 2009). *StAN2* could significantly activate the *DFR* and *F3′5′H* promoters, which was confirmed by dualluciferase assays (Liu et al., 2016). Another two MYB genes from potato, *StMYBA1* and *StMYB113*, could promote the synthesis of anthocyanin in tobacco leaves via transient activation (Liu et al., 2016), but the roles of these genes in potato tubers requires further research. MYB proteins can also promote anthocyanin synthesis by interacting with bHLH proteins (Feller et al., 2011). Two bHLH transcription factors in potato, StJAF13 and StbHLH1 (Anthocyanin 1b in the Potato Genome Sequence Consortium (PGSC) database), interact with StAN2; this interaction was confirmed using yeast two-hybrid and bimolecular fluorescence complementation (BiFC) assays. Stable co-transformation of *StAN2* and either *StbHLH1* or *StJAF13* in tobacco (*Nicotiana tabacum*) produced a stronger pigmentation with compared with *StAN2* overexpression alone (D'amelia et al., 2014). *StAN11*, which is a WDR transcription factor, was cloned from the potato cultivar Chieftain (*Solanum tuberosum* L.) and introduced into the potato cultivar Desiree. Compared to the wild-type control, the transgenic tuber skin color was significantly deepened, which was highly consistent with the accumulation of anthocyanin (Li et al., 2014). However, whether there are novel MYB, bHLH and WDR transcription factors involved in potato tuber anthocyanin synthesis and how they regulate key enzyme genes remains unclear.

In this study, we selected 10 structural genes (Fig. 1) and 11 transcription factors related to anthocyanin biosynthesis and analyzed their expression patterns in colored potato tubers with 8 genotypes. We found that 6 structural genes and 4 novel transcription factors play important roles in anthocyanin biosynthesis.

2. Materials and methods

2.1. Plant materials

Three purple skin and purple flesh potato genotypes (Adirondack, MacIntosh black and Congo), one red skin and red flesh genotype (PA99P20-2), two red skin and white/yellow flesh genotypes (Redsen and Huashu No.1) and two yellow skin and yellow/white flesh genotypes (Huaen No.1 and 393160-4) were used in the current study (Fig. 2). The plants were grown at 20–25 °C in 24-cm diameter plastic pots with nursery substrates (Sheng Sheng Agriculture group, Foshan, China) in the greenhouse at Huazhong Agricultural University (Wuhan, China) under a 16 h: 8 h light: dark cycle. The mature tubers were harvested, frozen in liquid nitrogen, and then stored at −80 °C until further molecular and biochemical analyses. Huashu No.1 and