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Anthocyanin accumulation and expression of anthocyanin biosynthetic genes in tartary buckwheat cultivars

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Objectives

We used anthocyanin biosynthetic genes clones, along with previously isolated clones for phenylalanine ammonia lyase (PAL) and chalcone synthase (CHS), to compare the expression of anthocyanin biosynthetic genes in different organs of tartary buckwheat cultivars ‘Hokkai T10’ and its parent cultivar ‘Hokkai T8.’ In addition, we analyzed the anthocyanin and flavonol contents in seedlings of these 2 cultivars.

Materials and Methods

- Two cultivars of tartary buckwheat, ‘Hokkai T8’ and ‘Hokkai T10,’ were bred by the National Agricultural Research Center (Hokkaido, Japan).
- RNA extraction and polymerase chain reaction analyses
- Cloning of cDNAs encoding anthocyanin biosynthetic enzymes
- Flavonoid staining and Quantification of anthocyanin content

Results

Six genes involved in anthocyanin biosynthesis in tartary buckwheat have been cloned, namely *FtC4H*, *Ft4CL*, *FtCHI*, *FtF3H*, *FtF3´H*, and *FtANS*, which encode cinnamate 4-hydroxylase (C4H), 4-coumarate:CoA ligase (4CL), chalcone isomerase (CHI), flavones3-hydroxylase (F3H), flavonoid 3´-hydroxylase (F3´H), and anthocyanidin synthase (ANS), respectively. Then, these cDNAs were used, along with previously isolated clones for phenyl alanineammonia-lyase (PAL) and chalcone synthase (CHS), to compare gene expression in different organs, flowering stages, and maturing seeds of tartary buckwheat cultivars ‘HokkaiT8’ and ‘HokkaiT10.’ Quantitative real-time polymerase chain reaction analysis showed that these anthocyanin biosynthetic genes were most highly expressed in the stems and roots of HokkaiT10. *FtANS* gene was more highly expressed than other genes during flowering and maturing seeds. In addition, anthocyanin concentration was higher in ‘HokkaiT10’ than in ‘HokkaiT8’, however, naringenin chalcone, a flavonoid, was absent from ‘HokkaiT10’ seedlings based on fluorescence microscopy.

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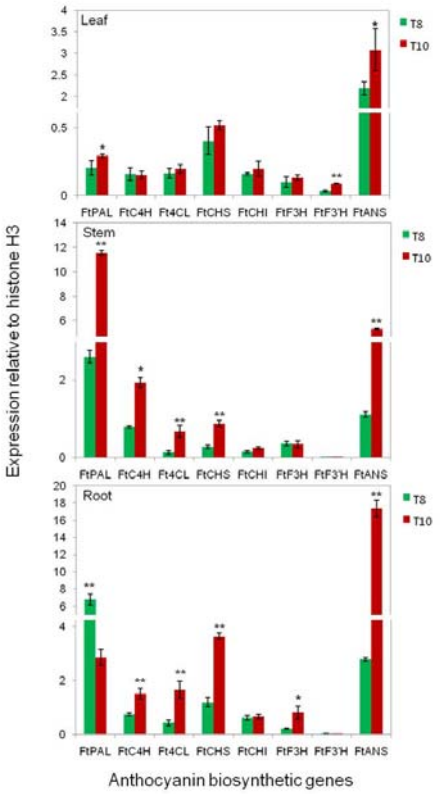

Figure 1. Differential expression of anthocyanin biosynthetic genes in different organs of tartary buckwheat cultivars 'Hokkai T8' and 'Hokkai T10.' The height of each bar represents the mean of 3 measurements from three different plants of each cultivar and error bars indicate the standard deviation. Asterisks indicate significant differences compared with between 'Hokkai T8' and 'Hokkai T10' cultivars by Student *t*test ($^{*}P < 0.05$, $^{**}P < 0.01$).

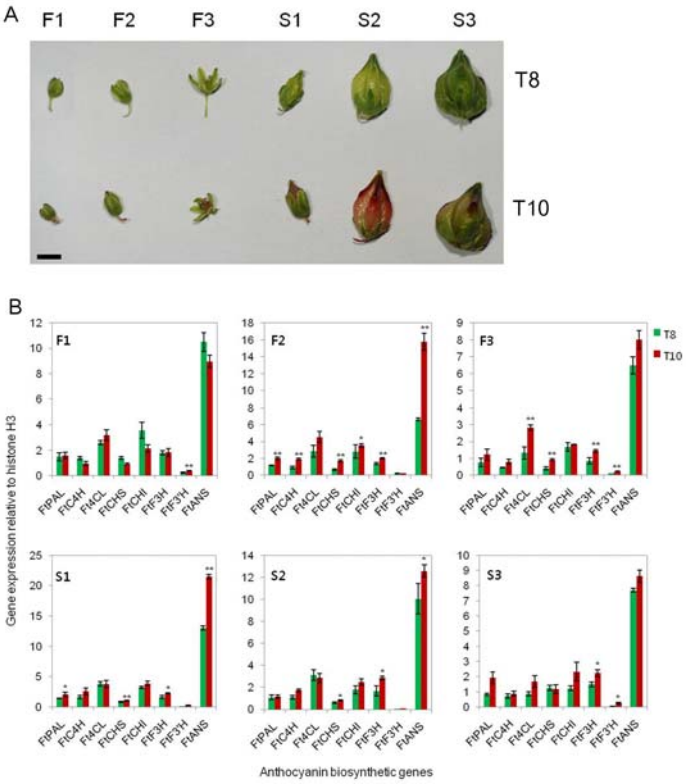

Figure 2. Anthocyanin biosynthetic genes in flowering stages and maturing seeds of tartary buckwheat cultivars 'Hokkai T8' and 'Hokkai T10.' (A) Picture of flowering stages (F1 - F3) and maturing seeds (S1 - S3). (B) Differential expression of anthocyanin biosynthetic genes in different flowering stages and maturing seeds of tartary buckwheat cultivars 'Hokkai T8' and 'Hokkai T10.' The height of each bar represents the mean of 3 measurements from three different plants of each cultivar and error bars indicate the standard deviation. Asterisks indicate significant differences compared with between 'Hokkai T8' and 'Hokkai T10' cultivars by Student *t*test ($^{*}P < 0.05$, $^{**}P < 0.01$).