Genetic and Epigenetic Mechanisms Controlling Flower Color and Pattern Diversity in *Dahlia*

-Abstract version-

Sho OHNO

2016

Abstract

Dahlias (*Dahlia variabilis*) are popular Asteraceae ornamental plants cultivated in many countries due to huge variation in flower shapes, sizes and colors. This wide variation is based onto complicated genetic background: namely dahlia is an autoallooctaploid with the chromosome number (2n = 8x = 64) having a large genome size. Dahlias exhibit a wide range of petal colors, such as ivory, yellow, pink, red, purple and black. In addition to huge color variation, there are flower color patterns such as variegation and bicolor. Pigments contributing to wide range of flower color in dahlia are flavonoids, mainly anthocyanin, butein, and flavone derivatives.

Polyploids are very common among plants. Polyploidy can be evolutionally advantageous in three points, i.e. heterosis, asexual reproduction and gene redundancy. In polyploids, complicated combination of alleles and/or loci can make for enormously huge variations in phenotype. However, it might be difficult that single loss of function mutation in biosynthetic pathway genes exhibit a phenotypic change, because of gene redundancy. Many horticultural plants are polyploids; therefore, how they diverse and regulate phenotypic characteristics is fascinating from the horticultural aspect.

In this study, I investigated the mechanisms controlling flower color and pattern diversity in *Dahlia*. Understanding the roles of genetic and epigenetic mechanisms controlling flower color and pattern diversity in dahlias provides useful information about not only the future breeding of dahlias, but also the regulation scheme of redundant genes in polyploid species.

In Chapter 1.1, the key factors for anthocyanin biosynthesis were analyzed using transposon tagging to MJOr and MJY, which are spontaneously occurring single-color bud mutants of ‘Michael J’ that has orange variegation patterns on yellow petals. MJOr

produced completely red petals with anthocyanins, butein, and flavones, whereas MJY produced completely yellow petals with butein and flavones but without anthocyanins. Gene expression analysis revealed that six structural genes (*DvCHS1*, *DvF3H*, *DvDFR*, *DvANS*, *Dv3MT* and *DvGST*) in the anthocyanin synthesis pathway and *DvIVS*, a basic helix-loop-helix transcription factor, were downregulated in MJY. A CACTA superfamily transposable element was found in the *DvIVS* genomic region of MJY and was named *Tdv1*. These findings demonstrated that *DvIVS* is involved in the regulation of anthocyanin synthesis in dahlia flowers, and transposition of the *Tdv1* contributes to flower variegation patterning.

In Chapter 1.2, relationship between flower color intensities and *DvIVS* was analyzed using 12 cyanic cultivars with the petals of different color intensities. Flower color intensity was determined by the anthocyanin content and these cultivars were classified into four color groups depending on their anthocyanin content: deep purple cultivars (high anthocyanin content), purple cultivars (moderate anthocyanin content), pink cultivars (low anthocyanin content), and ivory white cultivars (no anthocyanin). In all these cultivars, a positive correlation was observed between the anthocyanin content and expression of some structural genes in the anthocyanin synthesis pathway that are regulated by *DvIVS*. A positive correlation was also found between anthocyanin content and *DvIVS* expression (r = 0.96), suggesting that quantitative expression of *DvIVS* determines flower color intensities in dahlia. Furthermore, *DvIVS* alleles were classified into at least six types on the basis of polymorphisms in the promoter and coding region. Correlations were observed between flower color intensity and these *DvIVS* allelic types, indicating that *DvIVS* contributes to the diversity of flower color in dahlia by controlling the anthocyanin content.

In Chapter 1.3, the underlying mechanism controlling the formation of the white part in bicolor flowering dahlias was analyzed by comparing with ivory white cultivars. There were no flavonoids in the white part of bicolor petals, whereas flavone accumulated in petals of ivory white cultivars. Comparing expression levels of flavonoid biosynthetic genes between single-colored petals and the white part of bicolor petals, *DvCHS1* and *DvCHS2* were significantly lower in the white part of bicolor petals, while other flavonoid biosynthetic genes were almost the same. Comparing small RNA mapping profiles onto *CHS* genes between a single-colored petal and the white part of a bicolor petal, small RNAs from the white part of a bicolor petal were mapped onto two of four *CHSs*, *DvCHS1* and *DvCHS2*, while small RNAs from the single-colored red petal were not mapped onto all four *CHS*s. These results suggested that simultaneous post-transcriptional gene silencing (PTGS) of *CHS*s, mainly *DvCHS1* and *DvCHS2*, was a key factor for the white part formation in bicolor flowering dahlia.

In Chapter 2, petal color lability of bicolor flowering dahlias was analyzed. Bicolor flowering cultivars generally produce inflorescences with bicolor petals characterized by a colored basal part and a white tip; however, they frequently produce single-colored petals. This petal color lability prevents production of uniform cut or pot flowers of bicolor dahlias and reduces the economic value of bicolor cultivars. In this section, to reveal the underlying mechanism and to control color lability, the pattern of occurrence of single-colored petals was characterized in a red–white bicolor flowering cultivar ‘Yuino’. ‘Yuino’ produced inflorescences with bicolor petals, red petals, and both red and bicolor petals. Red petals occurred at the outer whorls or sectorally in a mixed inflorescence, similar to a chimera or a lateral mutant. There were strong relationships between the inflorescence color and the leaf phenotype; red petal-producing plants accumulated flavonoids in leaves, whereas only bicolor petal-producing plants tended not to accumulate flavonoids in leaves. This suggests that petal color of ‘Yuino’ is associated

with flavonoid synthesis in shoots. Therefore, a phenotypic difference is observed not only in petal colors but also at the whole plant level, and this phenotypic lability can be interpreted as flip of a switch of flavonoid biosynthesis in shoot apical meristem.

In flavonoid poor leaves, *DvCHS2* expression was lower than in flavonoid rich leaves. Small RNAs in a flavonoid poor leaf were mapped on *DvCHS2* genes indicating that the suppression of *DvCHS2* was post-transcriptional. Therefore, PTGS of *DvCHS2* is associated with flip of a switch of flavonoid biosynthesis in shoot apical meristem. The results that small RNAs in flavonoid poor leaves were poorly mapped on *DvCHS1*, and bicolor cultivars shared the specific *DvCHS2-1* allele, suggesting *DvCHS2-1* might be a trigger gene of *CHS* PTGS.

In conclusion, from genetic aspect, it was demonstrated that a bHLH transcription factor, *DvIVS* acts as a regulator of anthocyanin biosynthesis, and diversify flower color intensity in dahlia. From epigenetic aspect, it was demonstrated that simultaneous silencing of multiple CHS genes is associated with bicolor pattern and petal color lability in bicolor cultivars. Because dahlias are polyploids, quantitative regulation by multiple alleles of the transcription factor, and PTGS that avoid gene redundancy play important roles to extend flower color and patterning.