

Molecular studies on the chalcone synthase deficient unstable bicolored *Dahlia variabilis*

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Dahlia (*Dahlia variabilis*) is a popular octoploid garden plant with more than 50,000 varieties in different colors, shapes and sizes [1]. We investigated a variegated dahlia which spontaneously produces either pure white colored petals on otherwise red flowers, or completely white flowers and completely red flowers on the same plant. Previous enzymatic studies showed that the white petals of this variety lack chalcone synthase activity (CHS) [2]. *Dahlia variabilis* possess two types of CHS genes, *DvCHS1* (AB576660) and *DvCHS2* (AB591825), that show only 69% nucleotide similarity in their coding regions [3]. Whereas *DvCHS2* was reported to be constitutively present, *DvCHS1* is expressed only in cyanic flowers, together with dihydroflavonol synthase and anthocyanidin synthase [4].

The presence of flavonoids and anthochlors was analysed by HPLC. White colored parts generally showed very low concentrations of flavones (luteolin and apigenin), deoxy-chalcones and -aurones (isoliquiritigenin, butein and sulfuretin) and anthocyanins (pelargonidin and cyanidin), correlating well with the previously reported absence of CHS activity, which creates a bottleneck at the entrance to the flavonoid pathway. Red petals, in contrast, showed 10-50-fold higher accumulations of anthocyanins and 10-25 higher concentrations of other flavonoids and anthochlors.

We analyzed the expression of genes involved in flavonoid and anthochlor biosynthesis including transcription factors, using quantitative RT-PCR. Among these, the two CHS showed marked differences. We observed a high expression of *CHS2* in red colored petals and almost absent *CHS2* expression in white petals. *CHS1* expression was observed in both petal types, but was higher than *CHS2* expression in white petals and lower than *CHS2* expression in red petals. Cloning of full-size *CHS1* and *CHS2* from genomic DNA and cDNA correlated with the quantitative RT-PCR studies and did not indicate the presence of mutations in the *CHS* genes which would explain the absence of CHS activity in the white petals.

The sporadic presence of variegated petals may indicate the presence of transposable elements as shown by [3], who identified a *Tdv1* (transposable element of *Dahlia variabilis* 1) belonging to the CACTA superfamily of transposable elements in the fourth intron of the *IVS* gene. In addition, post-transcriptional *CHS* silencing could be involved [3]. Further experiments are in progress.

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[3] S. Ohno, M. Hosokawa, M. Kojima, Y. Kitamura, A. Hoshino, F. Tatsuzawa, M. Doi, S. Yazawa, Simultaneous post-transcriptional gene silencing of two different chalcone synthase genes resulting in pure white flowers in the octoploid dahlia, Planta, 234 (2011) 945-958.

[4] S. Ohno, M. Hosokawa, A. Hoshino, Y. Kitamura, Y. Morita, K.-I. Park, A. Nakashima, A. Deguchi, F. Tatsuzawa, M. Doi, A bHLH transcription factor, *DvIVS*, is involved in regulation of anthocyanin synthesis in dahlia (*Dahlia variabilis*), Journal of Experimental Botany, 62 (2011) 5105-5116.

We gratefully acknowledge funding by the Austrian Science Fund (FWF) P26468-B16 and from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No 675657.