

**Title** DEVELOPMENT OF A TRANSIENT RNA INTERFERENCE (RNAi) SYSTEM FOR ANALYZING AN EFFECT OF DFR SILENCING ON FLOWER COLOR OF DENDROBIUM SONIA CV. EARSAKUL

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#### ABSTRACT

*Dendrobium* Sonia cv. Earsakul is the important export orchids of Thailand. To improve its flower color using metabolic engineering, the molecular basis of genes involved in anthocyanin biosynthesis is required. The aims of this study were to determine the expression profile of *DFR* in *D. Sonia* cv. Earsakul flowers and to develop a transient RNA interference (RNAi) system for determining the effect of *DFR* silencing on flower color of *D. Sonia* cv. Earsakul. The expression patterns of *DFR* gene in sepals and petals at seven flower developmental stages were determined by semi-quantitative RT-PCR. The expression level of *DFR* gene were gradually increase with flower development to the maximum level in flower developmental stages 4 (the 3.3-3.5 cm flower bud) before declining to undetectable in flower developmental stages 6 (the opening flower) and 7 (the opened flower). The expression of the *DFR* gene in the sepals and petals was developmentally regulated and corresponded to anthocyanin accumulation. The regulation of the *DFR* expression was different between the sepals and petals at the early and late stages of flower development. Up-regulation of the *DFR* expression in the petals started earlier than in the sepals whereas down-regulation of *DFR* expression in the petals occurred later than in the sepals. In the white tissues of the petals of the flower buds, the expression of the *DFR* gene was repressed and low levels of anthocyanins were detected. This

indicates that the purple and white tissues of the *D. Sonia* cv. Earsakul petals are attributed to differential regulation of the *DFR* expression. To develop a transient RNAi system, two *DFR*-hairpin RNA binary vectors, pSTARGATE-*DFR* and pWATERGATE-*DFR*, were constructed and agroinfiltrated into the sepals and petals of *D. Sonia* cv. Earsakul flower bud stages 2 and 3. The sepals and petals infiltrated with *Agrobacterium tumefaciens* strain EHA105 containing either pSTARGATE-*DFR* or pWATERGATE-*DFR* exhibited colorless regions around the infiltrated sites within 3 days after infiltration. The expression level of endogenous *DFR* transcripts at colorless regions of infiltrated sepals and petals were significantly lower than the normal color regions. This transient RNAi system is a simple and effective technique that can be used to determine an effect of anthocyanin biosynthetic gene silencing on flower color of *D. Sonia* cv. Earsakul and other *Dendrobium* hybrids.

