

Transcriptome studies deliver multiple candidate sequences for chalcone reductase in the Asteraceae species

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Yellow flower coloration appeared as an adaption to the color sense of insects, in order to attract them as pollinators. Many Asteraceae species accumulate carotenoids, as well as chalcones and aurones, collectively known as anthochlor pigments or anthochlors. Deoxy type anthochlors are chemically stable and coexist with other yellow colored pigments, such as carotenoids, in different parts in the petals. Such flowers are monochromatic to the human eye, but can appear bicolored to pollinators because of the different UV absorbance of carotenoids and anthochlors. These patterns are known as honey guides or nectar guides [1]. Chalcone reductase (CHR) was identified as a key enzyme, inducing deoxy-chalcone formation when co-acting with chalcone synthase (CHS), and was first described in crude extracts of *Glycyrrhiza echinata* [2]. To date, CHR-like enzymes have been described in various plants of the legume species, such as alfalfa (*Medicago sativa*), soybean (*Glycine max*), Kudzu vine (*Pueraria lobata*) or *Lotus japonicus*. Although deoxychalcones are present in flowers of the Asteraceae species [3], cloning of functional CHRs derived from Asteraceae species through sequence homology was not successful, as yet.

We used bioinformatic studies with transcriptomes obtained from various plants of the Asteraceae family (*Cosmos sulphureus*, *Coreopsis grandiflora*, *Bidens ferulifolia* and *Dahlia variabilis*) to elucidate the sequences and structures of CHR from plants of the Asteraceae family. Four isoforms of CHR were found in the transcriptome from *Cosmos sulphureus*, sharing a sequence identity between 72 and 95%.

In all CHRs, regardless of the species of origin, we found highly conserved amino acids that are assigned to the 'catalytic tetrad' in CHRs from legumes [4]. Those amino acids were found in CHRs obtained from the transcriptome data the same distance from each other as in the legume CHRs. However, other amino acids, which are assigned to form the active site cavity, were partly missing in the Asteraceae CHRs. Consequently, a recombinant enzyme produced with such a cDNA clone was not functionally active. Cloning and sequencing of further potential candidates is currently ongoing.

[1] Scogin R, Zakar K (1976) Anthochlor pigments and floral UV patterns in the genus *Bidens*. Biochemical Systematics and Ecology 4: 165–167

[2] Ayabe, S.-I., Udagawa, A., and Furuya, T. (1988). NAD(P)H-dependent 6-deoxychalcone synthase activity in *Glycyrrhiza echinata* cells induced by yeast extract. Arch. Biochem. Biophys. 261, 458–462.

[3] Miosic S, Knop K, Holscher D, Greiner J, Gosch C, Thill J, Kai M, Shrestha BK, Schneider B, Crecelius AC, Schubert US, Svatos A, Stich K, Halbwirth H (2013) 4-Deoxyaurone formation in *Bidens ferulifolia* (Jacq.) DC. PLoS One 8:e61766

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