Structural analysis of two distinct dihydroflavonol 4-reductases in *Gerbera* Hybrids

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Dihydroflavonol 4-reductase (DFR) is a key enzyme within anthocyanin biosynthesis known for its distinct substrate specificity found in various plant species (1,2). Based in the findings of enzymatic studies and *in vivo* inhibition experiments in cyanidin accumulating genotype "Clivia" two DFRs with different substrate preference were postulated. Cloning approaches with petals of "Clivia" led to the identification of a second DFR sequence sharing around 96% identity to the previously cloned DFR from pelargonidin accumulating variety "Regina" (1,3). The obtained recombinant protein shows a higher preference for dihydroquercetin (DHQ) while dihydrokaempferol (DHK) was still converted to leucopelargondin (LPg) to a certain extend. However, the overall ratio showed a clear preference for the cyaniding branch of the pathway.

To shed light on this biochemical aspect of the anthocyanins biosynthetic pathway, we have attempted an *in silico* structure-based approach aiming to relate the specific amino acid differences in the DFRs of *Gerbera* "Regina" and "Clivia" varieties, with their molecular structures and enzyme selectivity data. The DFR models have been built using the crystal structure of the DFR from *Vitis vinifera*[4] (PDB ID 2C29) as a template. We suggest that a residue belonging to the "specificity loop" located near the substrate "binding site" confers the observed substrate-binding specificity. Namely, Gly135 (*Gerbera* "Regina" DFR) or Val135 (*Gerbera* "Clivia" DFR) respectively, is likely to unlock or lock the orientation of the conserved residue Asn134 that in turn is engaged in hydrogen bonding interactions with 4' OH' of ring B of DHK or both 3' OH' and 4' OH' of ring B of DHQ. Interestingly the side chain orientation of Val135 (*Gerbera* "Clivia" DFR) is restricted and stabilized by hydrophobic interactions with the conserved residues Ile154 and Phe165.

Tab. 1: substrate specificity of Gerbera DFRs *in-vitro* with DHK and DHQ as substrates.

	heterologous expression in yeast	
% conversion	*Gerbera* „Regina"	*Gerbera* „Clivia"
DHK to LPg	30,66	10,07
DHQ to LCy	49,41	62,92
ratio LPg/LCy	0,62	0,16

As substrate a mixture of 2000 dpm [^{14}C]-DHK and [^{14}C]-DHQ each was used

References

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