FUNCTIONALLY ACTIVE RECOMBINANT FLAVONOID 3'-HYDROXYLASE FROM A PELARGONIDIN ACCUMULATING POINSETTIA CULTIVAR

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MAIN CONCLUSION

A poinsettia cultivar was identified accumulating prevalently pelargonidin type anthocyanins in the bracts. An *F3'H* cDNA clone was isolated from this cultivar encoding functionally active protein as demonstrated with the heterologously expressed enzyme. Therefore, further factors must be involved in the establishment of the colour in this variety.

INTRODUCTION

Poinsettia (*Euphorbia pulcherrima*) is a prominent ornamental plant of particular seasonal interest because the bright red coloration of their bracts induced by short day is typically associated with Christmas time. In the most common red poinsettias, cyanidin type (two hydroxyl groups in B-ring) anthocyanidins are prevalent in the bracts, but pelargonidin type anthocyanins are also present (one hydroxyl group in B-ring) to some extent (Figure 1) [1].

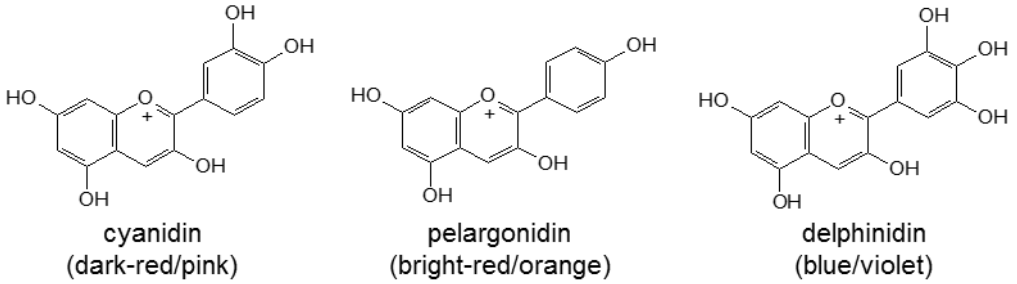

Figure 1: Chemical structures of the three main types of anthocyanins with respect to the hydroxylation pattern of the B-ring.

The biosynthesis of pelargonidin type anthocyanins requires the presence of a dihydroflavonol 4-reductase (DFR) accepting the monohydroxylated dihydroflavonol (dihydrokaempferol, DHK) as substrate and typically lack enzymes responsible for the introduction of the second and third hydroxyl group in the B-ring, flavonoid 3' hydroxylase (F3'H) and flavonoid 3'5' hydroxylase (F3'5'H), respectively [2]. Plants lacking pelargonidin type anthocyanins frequently possess a DFR with different substrate specificity that does not allow the conversion of DHK. We identified a cultivar with bright orange-red colour that accumulates prevalently pelargonidin type anthocyanins and analyzed the underlying flavonoid background at the level of selected enzymes and genes. An *F3'H* cDNA clone was isolated from this cultivar which encoded a functionally active enzyme.

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MATERIALS AND METHODS

The analysis was carried out with young bracts of commercially available *Euphorbia pulcherrima* cv. Harvest Orange (HO), Christmas Feelings (CF) and Christmas Beauty (CB). The plant material was collected in December 2015, frozen in liquid nitrogen and stored at -80 °C. Pigment composition was analyzed as described previously [3]. mRNA was extracted with the µMACS mRNA isolation Kit (Miltenyi Biotec, Germany). cDNA was synthetized using the SuperScript II Reverse Transcriptase (Invitrogen, Carlsbad, CA) and the oligodT SMART NUP primer. Based on specific sequence information of *F3'H* fragments from an unpublished *E. pulcherrima* transcriptome study, 5'-partial *F3'H* cDNA clones were isolated from the three poinsettia varieties and full size clones were generated by 3'RACE techniques using the *Taq/Pwo* Expend High Fidelity PCR System (Roche, Mannheim, Germany). Heterologous expression in yeast and enzyme assays were performed as described previously [4]. DFR assays were carried out as described earlier [2].

RESULTS AND DISCUSSION

Enzyme preparations from the 3 different poinsettia cultivars did not indicate the presence of a specific DFR which does not accept DHK as substrates. Highest conversion rates were observed with dihydromyricetin as substrate, but dihydroquercetin and DHK were accepted to a comparable extent. We isolated putative *F3`H* cDNA clones from HO, CB and CF, with open reading frames of 520 (HO), 510 (CB) and 511 (CF) amino acids, respectively. The cDNA clones showed sequence identities of 67 to 76 % to F3'H sequences from other species on amino acid level. The *F3'H* cDNA clones of CB and CF showed 98.6 % sequence identity with only 5 exchanges of amino acids. The *F3'H* cDNA clones from HO showed higher sequence identity to the cDNA clone from CF (97.5%) than to the one of CB (96.2%). There was a particularly variable area at the N-terminus starting from position 15 (numbering from HO) with an insert of 9 amino acids in the HO sequence and of one amino acid in the CF sequence compared to the CB sequence. We do, however, not expect a high impact of these insertions on functional activity as the N-terminus of F3'Hs usually encodes the membrane anchor. The recombinant enzymes obtained from the 3 cDNA clones showed functional activity, albeit the HO F3'H showed lower conversion rates than the others. Our investigations clearly demonstrated that the orange-red colour of cv. HO is not based on the lack of functional F3'H in the bracts. Future investigations will concentrate on analysis of quantitative gene expression and on the question if the differences in the amino acids of the F3'H cDNA clone of cv. HO is responsible for the lower conversion rates observed.

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