Cloning of Divergent Chalcone Synthase Sequences from *Pelargonium*

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Abstract
Chalcone synthase (CHS) is the key enzyme of the flavonoid-anthocyanin pathway. It leads to the production of a highly diverse group of compounds. Its members play different roles in the interaction of the plant with its environment. In most studied plants, not only one gene, but a small gene family codes for CHS-like enzymes. The cultivated *Pelargoniums* are of hybrid origin and in many cases polyploid as well. We cloned genomic fragments of CHS genes from three botanical species *Pelargonium frutetorum*, *P. inquinans* *P. lanceolatum* and three hybrids a *Pelargonium-Zonale-Hybrid*, a *Pelargonium-Peltatum-Hybrid*, and a *Pelargonium-Grandiflorum-Hybrid*. We compared the resulting sequences to investigate their heterogeneity and possible relatedness.

INTRODUCTION
Chalcone synthase (CHS), a member of the polyketide synthase multigene family, is the key enzyme in flavonoid biosynthesis. CHS catalyses the condensation of 3 malonyl-CoA with p-coumaroyl-CoA or caffeoyl-CoA, respectively, influencing together with the enzymes flavonoid 3'-hydrodylase (F3'H) and flavonoid 3',5'-hydroxylase (F3',5') the anthocyanidin type formed in the flowers (Fig. 1). We cloned partial CHS genes from several *Pelargonium* species to investigate their heterogeneity and relatedness in this horticulturally important genus.

MATERIALS AND METHODS

Plant Material
Young leaf material of three botanical species: *Pelargonium frutetorum* and *P. inquinans* both from section *Ciconium* and *Pelargonium lanceolatum* from section *Glaucophyllum* and three hybrids: *Pelargonium-Zonale-Hybrid ‘Robe’*, also placed into section *Ciconium*, *Pelargonium-Peltatum-Hybrid ‘Ville de Paris Red’* from section *Dibrachya* and a *Pelargonium-Grandiflorum-Hybrid ‘Schoko’*, from section *Eumorpha* was used for the investigations.

Molecular Techniques
Genomic DNA was isolated with Qiagen DNeasy Plant Mini Kit. Candidate chalcone synthase fragments were PCR amplified from ~50ng DNA using partial degenerated oligonucleotide primers designed against conserved amino acid motifs based on functional CHS sequences. Fragments with expected length were cloned into pcR®2.1-TOPO® vector from Invitrogen, transformed into *Escherichia coli* TOP 10 chemically competent cells and selected on kanamycin containing LB medium. Plasmids were isolated from overnight cultures with Quantum Prep Plasmid Isolation Kit from Biorad and analysed by EcoRI restriction endonuclease digestion and gel electrophoresis. Selected clones were sequenced by the Custom Sequencing service of MWG Biotech AG (Ebersberg, Germany). The sequences were compared by BLAST (Tatusova and Madden 1999).
RESULTS AND DISCUSSION
Although several hundreds of CHS genes have been cloned from other plants, up to now, only a genomic CHS fragment (468 bp) has been reported from Pelargonium-Peltatum-Hybrid ‘Ville de Paris’ (Pelargonium × hederaefolium, accession number X95801). As in other plant species, Southern blot analysis using this fragment indicated the presence of a CHS multigene family in the Pelargonium genus as well (Denis-Peixoto et al. 1997).

Now we successfully cloned one to two CHS fragments between 788 and 1334 bp from three Pelargonium species and three commercial hybrids. Comparison of the genomic sequences on the nucleotide and amino acid levels is shown in Table 1, together with a partial cDNA sequence of Pelargonium-Zonale-Hybrid ‘Robe’: P. × Zonale ‘Robe’ (CHS1).

We found one group of nucleotide sequences consisting of P. × Zonale ‘Robe’ CHS2 with 100 % identity to P. inquinans CHS1 and 99 % identity to P. frutetorum CHS1 and P. × Peltatum ‘Ville de Paris’ CHS3, respectively. Another group consists of P. × Zonale ‘Robe’ CHS1 and P. frutetorum CHS2 also with 99 % identity. The similarity between the two groups is only 80 % on the nucleotide level. The similarities between the remaining four nucleotide sequences ranges from 88 % between P. × Peltatum ‘Ville de Paris’ CHS2 and P. × Grandiflorum CHS1, to 41 % between P. × Peltatum ‘Ville de Paris’ CHS1 and P. lanceolatum CHS1.

Our results prove on the molecular level the close relatedness of the two species and the three hybrids in the Dibrachya and Ciconium sections and their further relationship with P. lanceolatum from the Glaucophyllum section. They also show that in Pelargoniums two or possibly more CHS genes exist with probably different characteristics and mode of expression.

Further work will be concerned with the isolation of full-length clones and the characterisation of the expression of the respective genes. Moreover, heterologous expression in Escherichia coli to investigate their substrate specificity.

ACKNOWLEDGEMENTS
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Literature Cited
Table 1. Comparison of *Pelargonium* CHS sequences (% identify).

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<td>64</td>
<td>67</td>
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<td>65</td>
<td>67</td>
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<td>87</td>
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<td>99</td>
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**NUCLEOTIDE**
Fig. 1. Chalcone synthase reaction its influence on the anthocyanidin type. F3´H: flavonoid 3´ hydroxylase, F3´5´H: flavonoid 3´5´ hydroxylase.