SUPPLEMENTAL METHODS

Heterologous expression of AtDCR in *E.Coli* cells and enzyme assays

For heterologous expression of *DCR* in bacteria under the control of a T7 inducible promoter, the *DCR* cDNA was amplified (without the stop codon) from a WT (ec. *Col*.) plants inflorescence library with the following oligonucleotides: sense- 5'- *BamHI*-GCGGATCCATGAAGATAAAGATTATGAG -3', antisense- 5'- *NotI*-CGCGGCCGCAACAAACCCATTGCCATTTCC -3' and cloned into the pET28a(+) vector (Novagen) in frame with the His-Tag at either the 5'- and 3'- termini. The pET28a(+) vector containing the DCR cDNA was transformed into pLysC cells (Novagen). Bacterial cultures were grown overnight at 37° C in Luria-Bertani medium supplemented with 100 µg ml^{-1} ampicillin and 30 µg ml^{-1} chloramphenicol. The next day, cultures were diluted with a Luria-Bertani medium (containing the appropriate antibiotics) to an OD^{600} of 0.06 and a final volume of 800 ml. This culture was grown at 37°C until the OD^{600} reached 0.4-0.6. After cooling down the culture to 22°C, 1mM IPTG was added to induce protein expression. After 6h incubation at 28°C, the cells were harvested by centrifugation. The His-tagged proteins were purified using the His Bind Quick 900 Cartridges (Novagen) as recommended by the manufacturer. For enzymatic assay, the crude and purified proteins were both used. The presence of the DCR recombinant protein in the crude and purified fraction was evaluated using an anti-HIS mouse monoclonal antibody (H1029, Sigma-Aldrich).

The following CoA donors were used for the enzymatic assay: feruloyl-CoA, coumaroyl-CoA, palmitoyl-CoA and stearoyl-CoA. The feruloyl and coumaroyl-CoAs were synthesized from ferulic and coumaric acids as described (Beuerle et al., 2002). Stearoyl- and palmitoyl-CoA were purchased from Sigma-Aldrich (USA). The acceptor compounds used for the enzymatic reactions were as following: ferulic acid, coumaric acid, octadecanol, eicosanol, glycerol, 16- hydroxy-hexadecanoic acid and 9(10), 16- dihydroxy-hexadecanoic acid. All donors except the 9(10), 16-dihydroxy-hexadecanoic acid (kind gift from Patrick Dussault) were purchased from Sigma-Aldrich (USA). Enzymatic assay was performed as described (Kalscheuer et al., 2004). The reaction was stopped by addition of 200ul of chlorophorm. The reaction products were identified using GC-MS analysis after derivatization with BSTFA (Franke et al., 2005).

Identification of T-DNA and Ds transposon insertions in the mutant lines

In order to genotype the mutant plants the following oligonucleotides were used:

For SALK_128228:

LP 5'- ATCCACGTGGCATTTTATGAG -3'

RP 5'- ACAATTCCAAACCAAACACAC -3'

LbB1 5'- GCGTGGACCGCTTGCTGCAACT -3'

For WiscDsLox245B03:

LP 5'- GTGAAAGTGTTTATCCCCACG -3'

RP 5'- TAGATTTTCAATCGGCGTGAC -3'

T-DNA p745 oligonucleotide 5'- AACGTCCGCAATGTGTTATTAAGTTGTC -3'

Riken Ds 12-1765-1:

LP 5'- GTCATTGTCTATGTTGGTCTC -3'

RP 5'- AGCTTCAAGAGTTATCTCCAC -3'

Transposon primer Ds3'-2a 5'- CGGATCGTATCGGTTTTC -3'

Supplemental Methods for Figure 1A

Full names and accession numbers of the proteins depicted in the phylogenetic tree are the following: AtDCR (*Arabidopsis thaliana*, At5g23940); HvDCR (*Hordeum vulgare*, TC161224); PhDCR (*Petunia hybrida*, TC1475); GhDCR (*Gossypium hirsutum*, TC80886); LaDCR (*Lavandula angustifolia*, ABI48361); PtDCR (*Populus trichocarpa*, TC90940); OsDCR (*Oryza sativa*, Os08g44840); PsDCR (*Picea sitchensis*, TC46575); GmDCR (*Glycine max*, TC247302); ZmDCR (*Zea mays*, TC435586); VvDCR (*Vitis vinifera*, TC76679); TaDCR (*Triticum aestivum*, TC265830 TC171356 TC207365); NtDCR *(Nicotiana tabacum*, TC5963); StDCR (*Solanum tuberosum*, TC146646); MtDCR (*Medicago truncatula*, TC102320); LeDCR (*Lycopersicon esculentum*, TC172671 TC6463 TC11619); SDT (*Arabidopsis thaliana* spermidine disinapoyl transferase, At2g23510); SCT (*Arabidopsis thaliana* spermidine dicoumaroyl transferase, At2g25150); Glossy2 (*Zea maize*, CAA61258); Pf5MaT (*Perilla frutescens* malonyl CoA:anthocyanin 5-O-glucoside-6'''-O-malonyltransferase, AAL50565); Dv3MaT (*Dahlia variabilis* malonyl CoA:anthocyanin 3-O-glucoside-6''-O-malonyltransferase, AAO12206); Sc3MaT (*Pericallis cruenta* malonyl-CoA: anthocyanidin 3-O-glucoside-6''-O-malonyltransferase, AAO38058); Dm3MAT1 (*Chrysanthemum x morifolium* anthocyanidin 3-O-glucoside-6''-O-malonyltransferase, AAQ63615); BEAT (*Clarkia breweri* acetyl CoA: benzylalcohol acetyltransferase, AAC18062); DAT (*Catharanthus roseus* deacetylvindoline 4-O-acetyltransferase, AAC99311); HCBT (*Dianthus caryophyllus* anthranilate N-hydroxycinnamoyl/benzoyltransferase, CAB06430); AHCT (*Gentiana triflora* anthocyanin 5-aromatic acyltransferase, BAA74428); AT5MAT (*Arabidopsis thaliana* anthocyanin O-malonyltransferase, At3g29590); transferase (*Arabidopsis thaliana*, At5g39050); CER2 (*Arabidopsis thaliana*, At4g24510); WSD1 (*Arabidopsis thaliana* bifunctional wax synthase, At5g37300).

SUPPLEMENTAL LITERATURE CITED:

Beuerle T, Pichersky E (2002) Purification and characterization of benzoate:coenzyme A ligase from *Clarkia breweri*. Arch Biochem Biophys **400**: 258-264

Franke R, Briesen I, Wojciechowski T, Faust A, Yephremov A, Nawrath C, Schreiber L (2005) Apoplastic polyesters in *Arabidopsis* surface tissues--a typical suberin and a particular cutin. Phytochemistry **66**: 2643-2658

Kalscheuer R, Luftmann H, Steinbüchel A (2004) Synthesis of novel lipids in *Saccharomyces cerevisiae* by heterologous expression of an unspecific bacterial acyltransferase. Appl Environ Microbiol **70**: 7119-7125