Supplementary information

Transcript profiling of two potato cultivars during glycoalkaloid-inducing treatments shows differential expression of genes in sterol and glycoalkaloid metabolism

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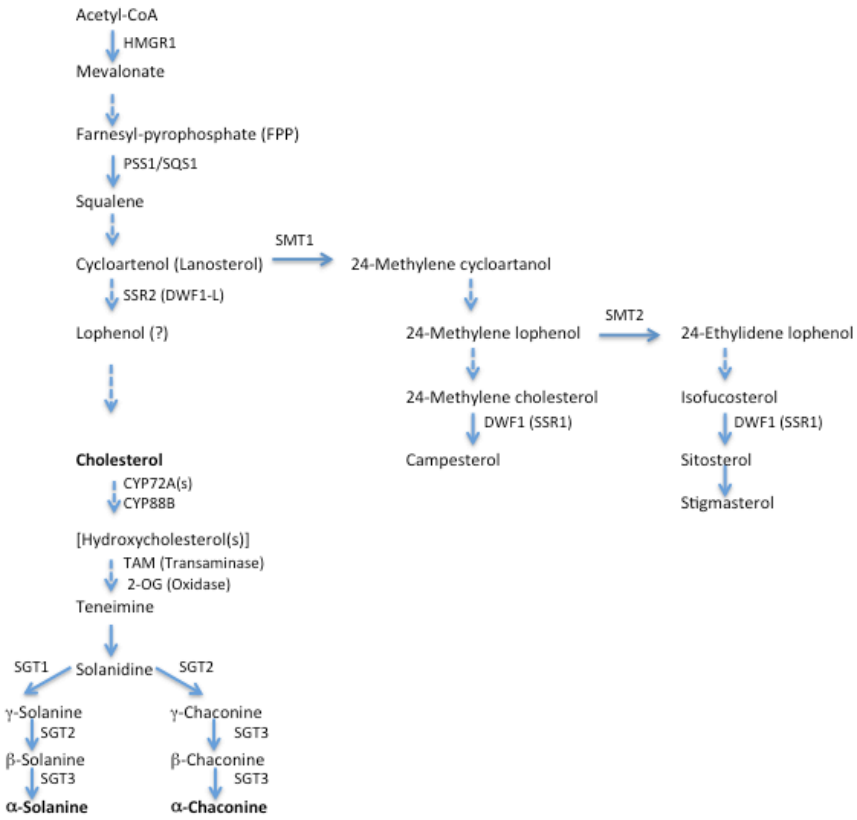

Figure S1. Working model for sterol and glycoalkaloid biosynthesis in the potato (*Solanum tuberosum*). The predominant glycoalkaloids in cultivated potato species, α-solanine and α-chaconine, are are derived from the C_{27} sterol cholesterol, and synthesized from the same aglycone, solanidine. Final conversions of solanidine to α-solanine and α-chaconine are catalyzed by three glycosyl transferases (SGT1, SGT2 and SGT3). Based on Cárdenas *et al.*[1].

Abbreviations: CYP, cytochrome P450; DWF1, sterol 24(28) reductase; HMGR, hydroxy-3-methylglutaryl CoA reductase; PSS1 (also denoted SQS1), squalene synthase; SGT1, solanidine galactosyl transferase; SGT2, solanidine glucosyl transferase; SGT3, β-solanine/β-chaconine rhamnosyl transferase; SMT1, sterol methyltransferase type 1, SMT2, sterol methyltransferase type 2; SSR2 (DWF1-L), sterol 24(25) reductase. Dashed lines indicate more than one enzymatic step.

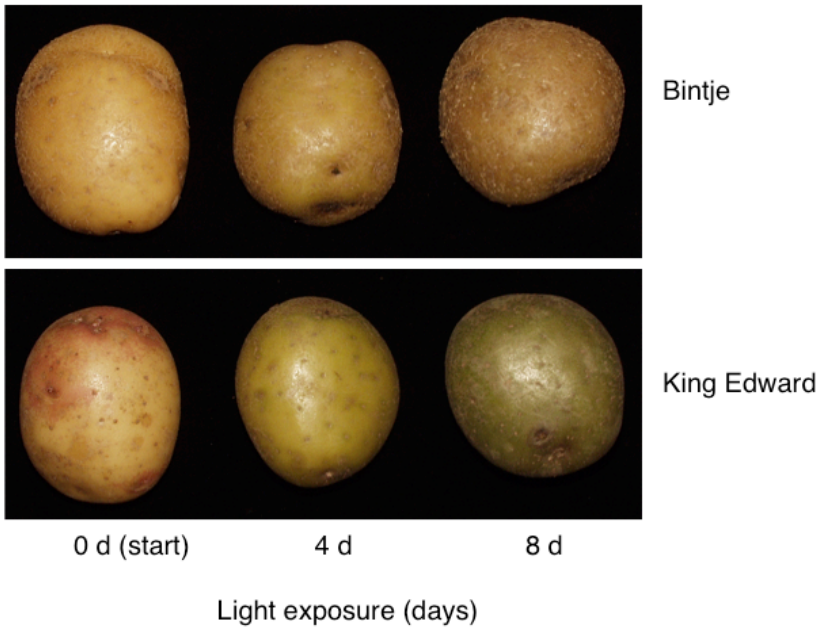

Figure S2. General appearance of Bintje and King Edward tubers subjected to light exposure. Tubers were exposed to constant white fluorescent light in a growth cabinet for the time points indicated. The transcriptional profiling and metabolic assays were performed during the initial 4 days. Spectral distribution of the light source and temperature conditions were as described[2].

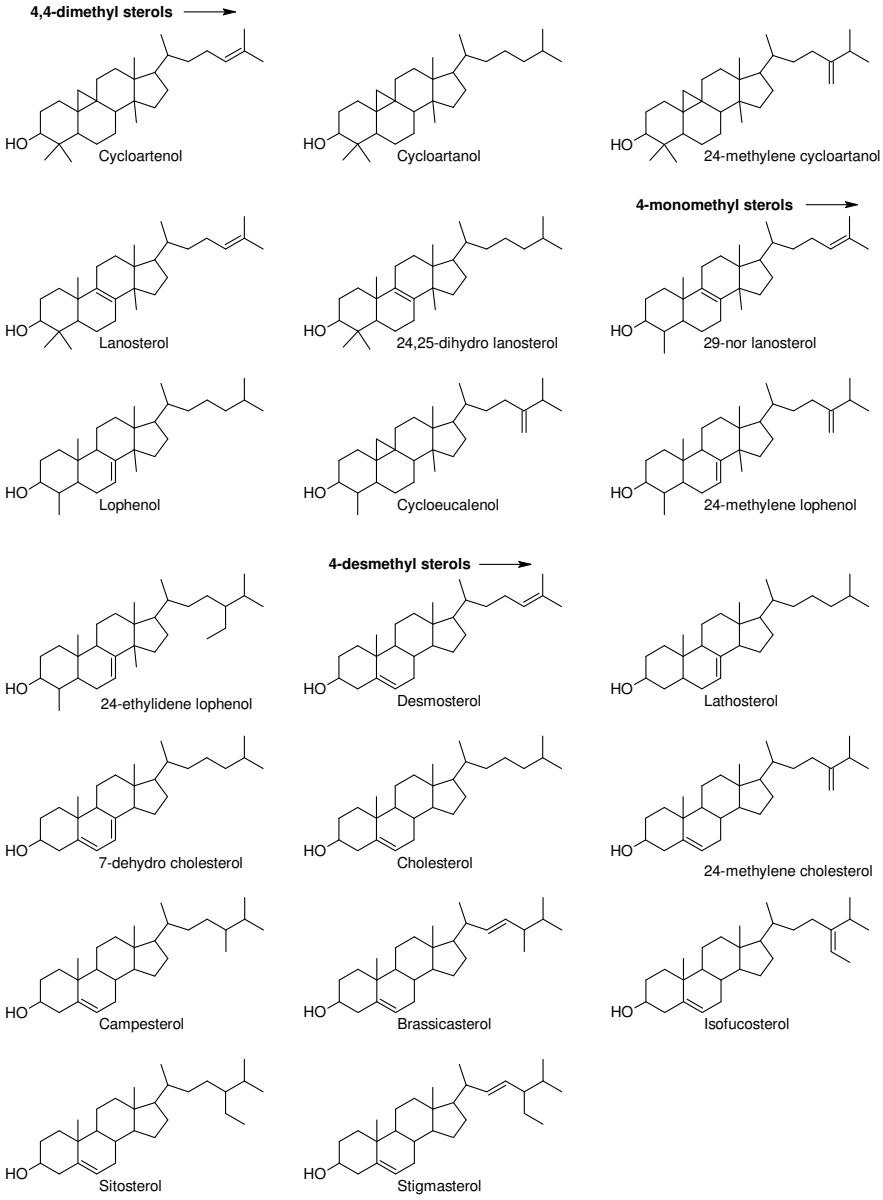

Figure S3. Chemical structure of sterols investigated. Mass to charge ratios (m/z) of molecular ions and main fragments are listed in Supplementary Table S13.

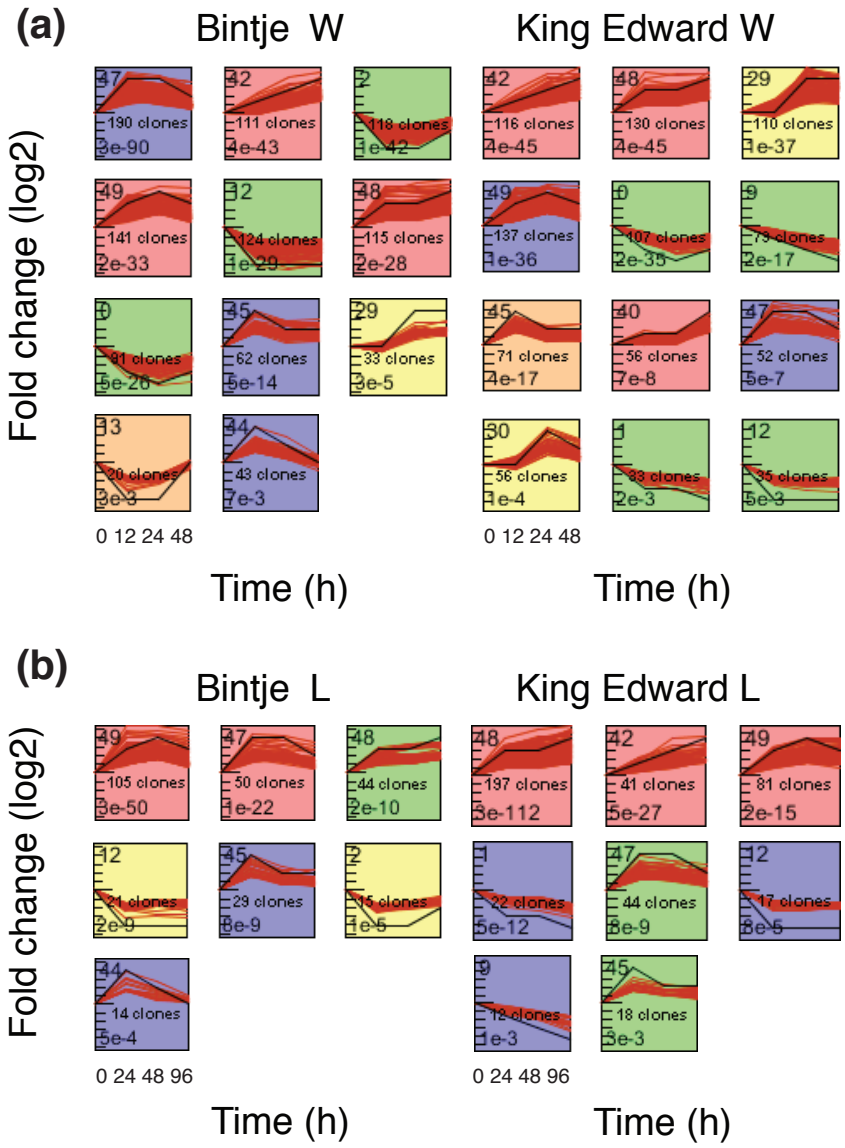

Figure S4. Temporal gene expression profiles in potato tubers subjected to a wounding stress (W) or a light exposure (L). Significant expression profiles identified by the STEM software[3] from datasets obtained by microarray analysis of potato tubers from cultivars Bintje and King Edward, subjected to wounding (**a**), or light exposure (**b**). Each profile corresponds to a specific model of temporal expression ($p<0.001$). Clusters with similar colour show similar expression pattern based on STEM correlation coefficients. The individual gene expression pattern in each cluster is indicated in red, and the specific model profile by a black bold line. For each temporal profile a profile ID number is given at the top, gene numbers in the middle, and p-values at the bottom. A full list of genes and temporal profiles is given in Supplementary Tables S6 to S9.

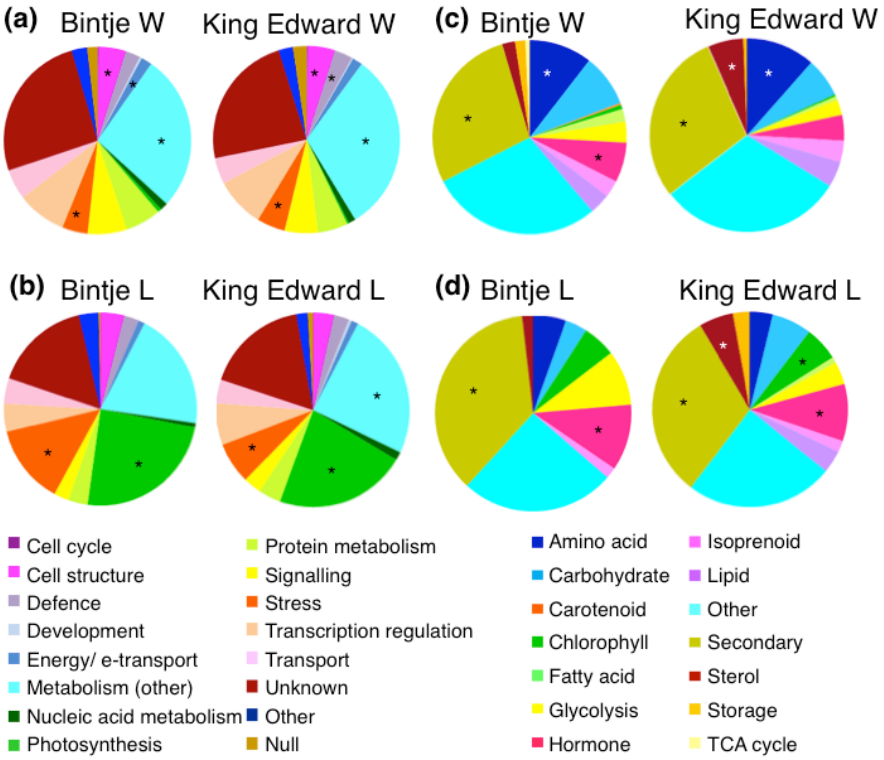

Figure S5. Functional classification of genes assigned significant temporal expression categories. (**a**) and (**b**), Gene ontology annotation categories of genes assigned significant STEM profiles (see Supplementary Fig. S4), in Bintje and King Edward tubers subjected to a wounding stress (W) or a light exposure (L). (**c**) and (**d**), The class "Metabolism" in wounded or light-exposed tubers was sub-classified into the processes indicated. Asterisks (*) indicate a statistically overrepresented class significant at $p<0.05$ (Chi-square test using Yate´s correction).

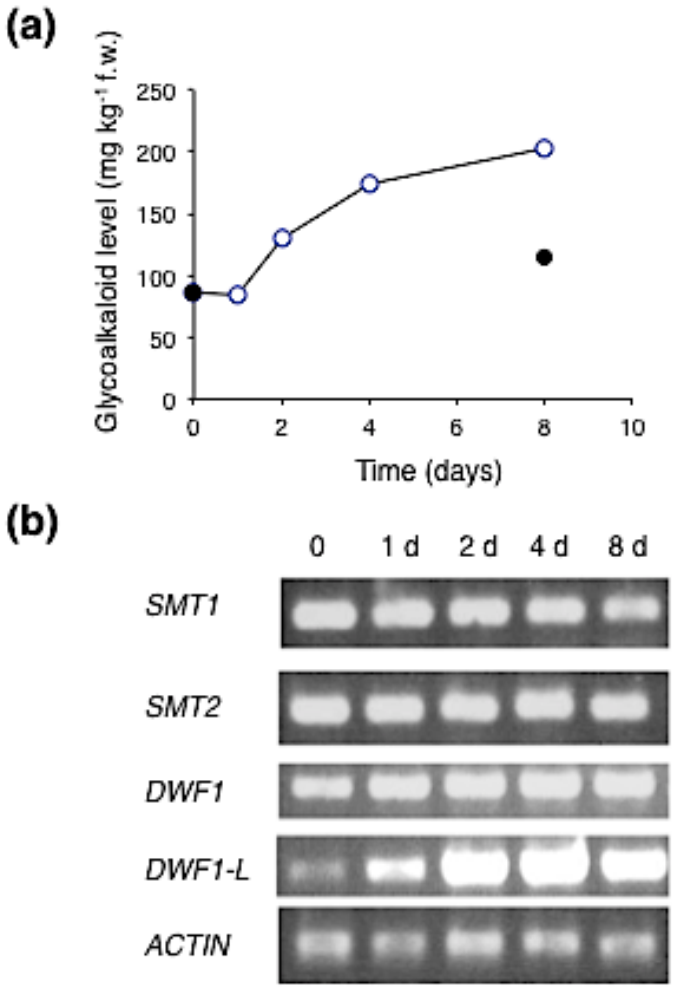

Figure S6. Glycoalkaloid levels and gene expression in potato tubers during light exposure. (**a**) Total glycoalkaloid levels were analysed by HPLC-UV in King Edward tubers exposed to white fluorescent light for the times indicated. Open symbols, light-treated; dark symbols, a parallel dark control by wrapping tubers in aluminium foil. (**b**) Semiquantitative RT-PCR analysis of RNA extracted from aliquots of the tubers analysed. Abbreviations: *SMT1*, sterol methyltransferase type 1; *SMT2*, sterol methyltransferase type 2; *DWF1*, sterol 24(28) reductase; *DWF1-L*, *DWF1*-like.

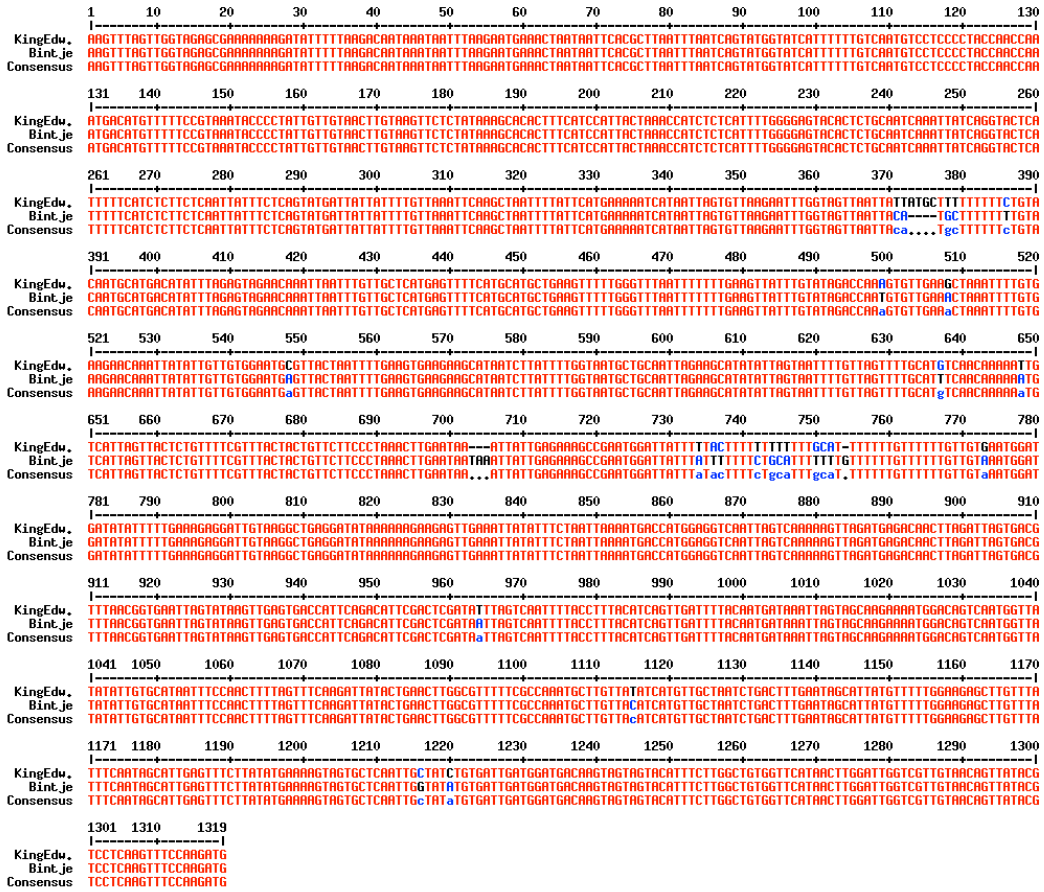

Figure S7. Comparison of upstream DNA sequences from the translational start codon in the *DWF1-L1* promoter. Promoter DNA (1300 bp) was amplified by nested PCR from genomic DNA from potato cultivars King Edward and Bintje, and sequenced in both directions. The comparison was made in MultAlin[4].

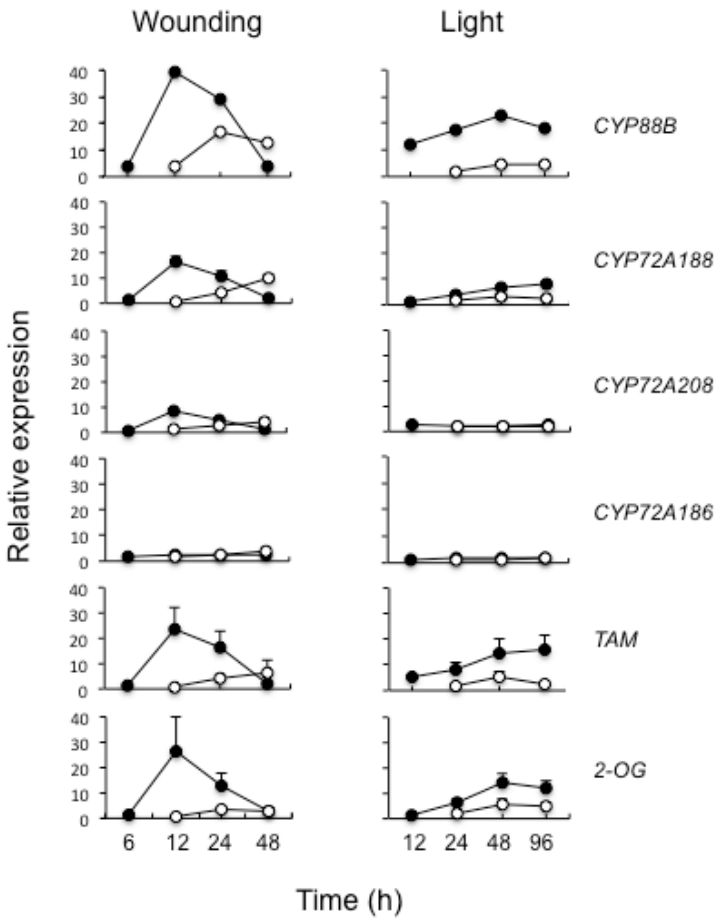

Figure S8. Temporal expression of genes coexpressed with *HMGR1/SMO1-L*/*DWF1-L*. Microarray analysis of potato tubers from cultivars Bintje (white circles) and King Edward (dark circles), subjected to a wounding stress (left), or a light exposure (right). Results for two additional *CYP72A* genes (*CYP72A208* and *CYP72A186*) that were not coexpressed are included for comparison. Average expression values from duplicate measurements of two biological replicates ± range or s.d. of homologous EST clones where applicable. Coexpression was judged from STEM analysis (Supplementary Fig S3 and Table S11).

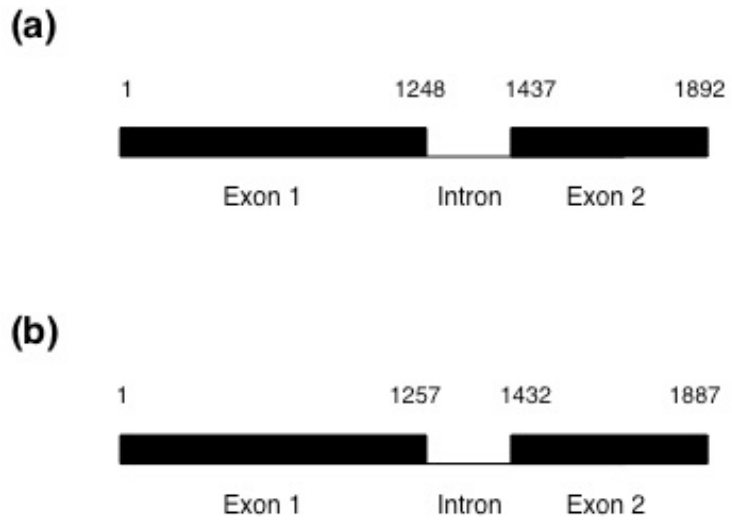

Figure S9. Genomic structure of *DWF1* and *DWF1-L*. Genomic sequences of the single *DWF1* (**a**), and *DWF1-L* (**b**), gene were retrieved from the fully sequenced genome of the wild potato species *Solanum phureja*. Intron/exon borders were deduced by comparison to fullength cDNA sequences.

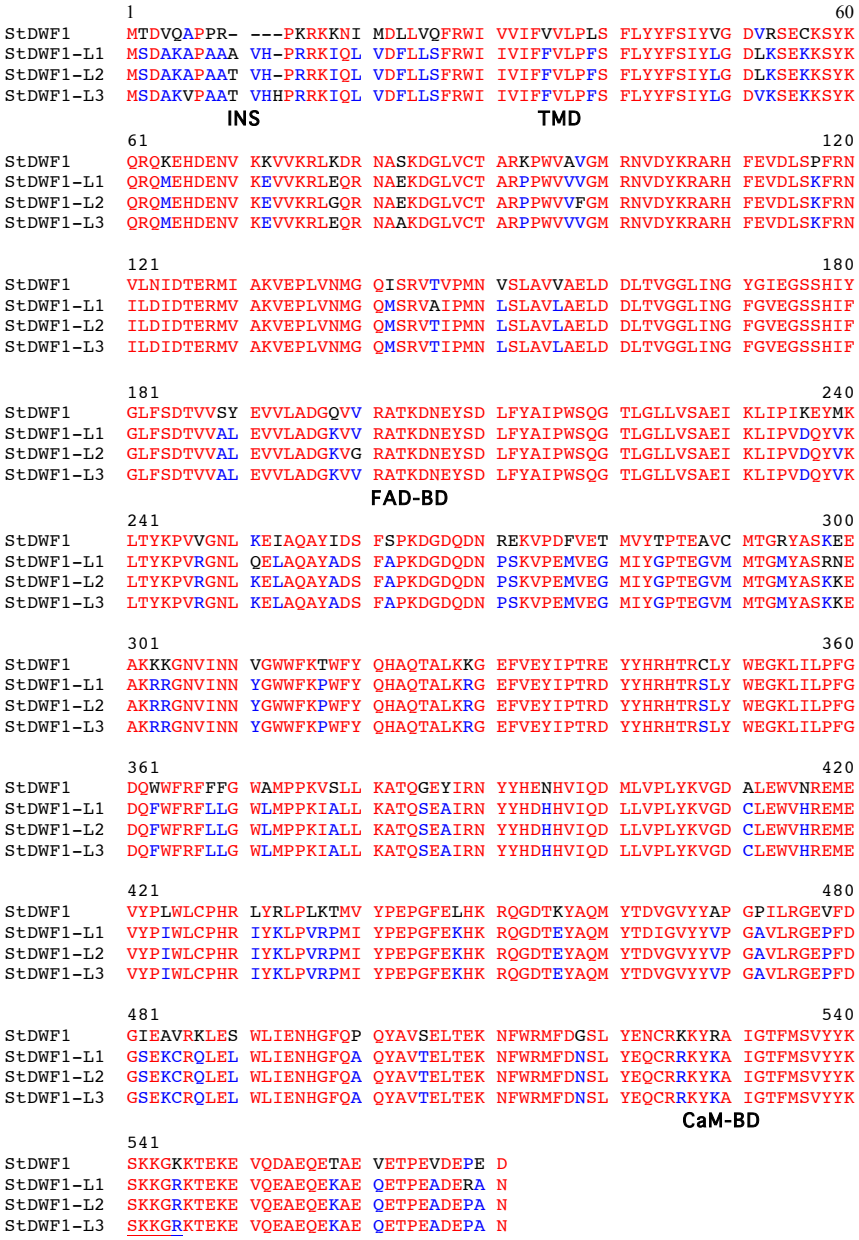

Figure S10. Alignment of deduced potato DWF1 and DWF1-L proteins. Nucleotide sequences were obtained from Kennebec cDNA clones, and alignments of deduced aa sequences were made using MultAlin[4]. Amino acid residues with a high degree of conservation (>90 %) are labelled in red, and residues with a low conservation are labelled in blue/black. Indicated in the alignment is a stretch of inserted hydrophobic residues (INS) that is characteristic for the DWF1-like (DWF1-L) type of proteins. Also displayed are conserved domains in plant DWF1 proteins, including a suggested transmembrane domain (TMD)[5], a FAD-binding domain (FAD-BD)[6], and a Calmodulin-binding domain (CaM-BD)[7]. EMBL nucleotide database acc. no: StDWF1 (FN995649), StDWF1-L1 (FN995650), StDWF1-L2 (FN995651), StDWF1-L3 (FN995652).

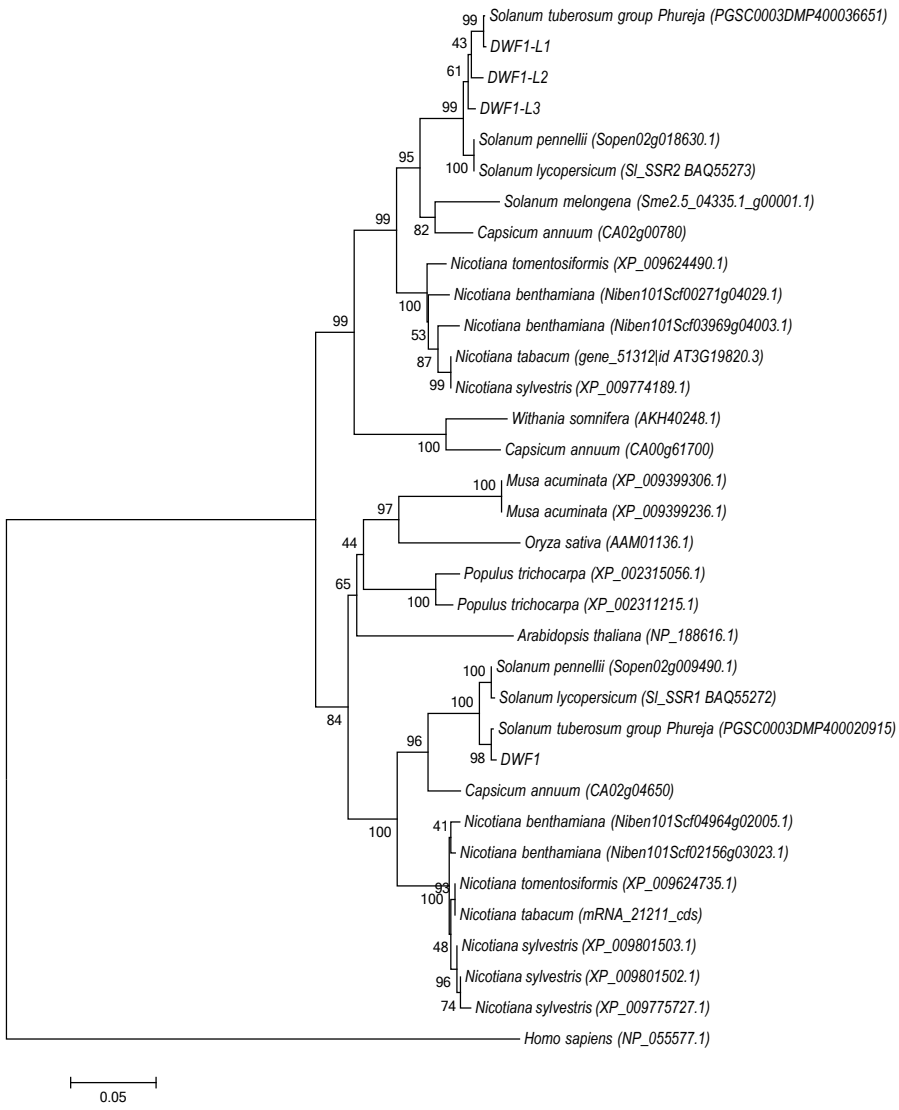

Figure S11. **Phylogenetic relationships of potato DWF1 and DWF-L proteins.** A bootstrap consensus neighbour-joining tree was constructed using ClustalW and MEGA6[8], with default settings and a number of 1000 replications. The human (*Homo sapiens*) orthologue DHCR24 was included as an outgroup. The tree was made using 34 amino acid sequences deduced from available full-length cDNA sequence information (NCBI and Sol Genomic Network databases), and with an emphasis on Solanaceous plant species. All positions containing gaps and missing data were eliminated. There were a total of 504 positions in the final dataset. Numbers at branches indicate bootstrap support.

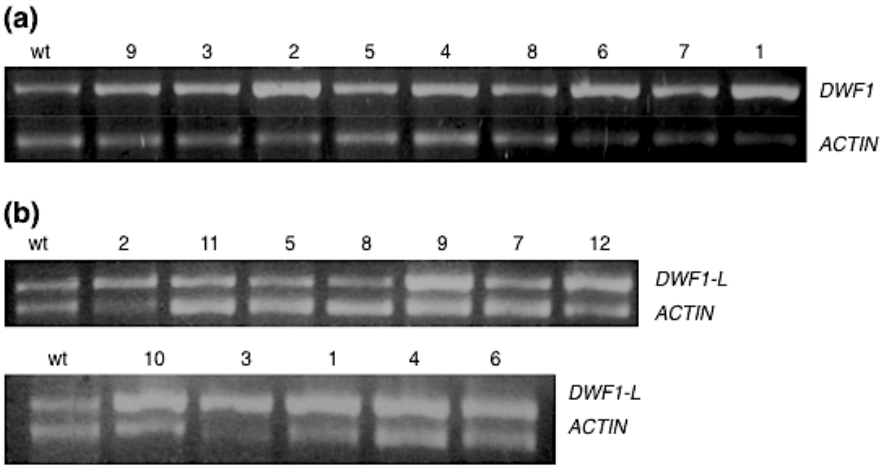

Figure S12. Multiplex RT-PCR analysis of transgene expression. Expression of *DWF1* and *DWF1-L* was assayed using multiplex RT-PCR in leaves from wild-type potato Désirée plants, and derived 35S:*DWF1* (**a**) and 35S:*DWF1-L* (**b**) transformants. An *ACTIN-101* primer pair was used as an internal control for amplification. Transformant clone numbers correspond to those in Supplementary Table S12.

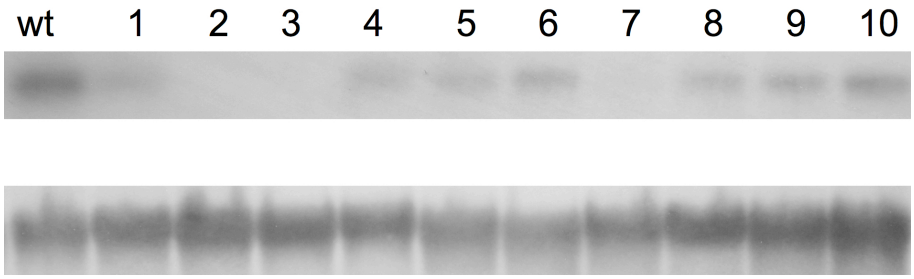

Figure S13. RNA gel blot analysis of as*DWF1* potato transformants. Total RNA was extracted from young leaves of a wild-type Désirée plant and ten derived antisense as*DWF1* transformants, and 20 µg was transferred onto a nylon membrane and hybridized under stringent conditions with a 500 bp 5′-terminal *DWF1* probe (upper), and as a loading control washed and re-hybridized with a *16S rRNA* probe (lower).

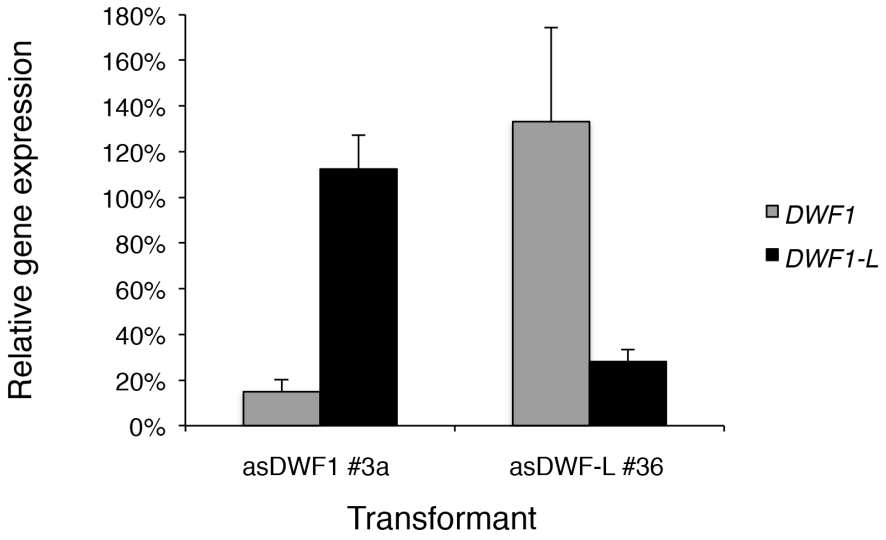

Figure S14. Expression of *DWF1* and *DWF1-L* genes in *asDWF1* and *asDWF1-L* potato transformants. Gene expression was monitored by QPCR analyses of RNA extracted from young leaves of wild-type and antisense transgenic plants that were grown at three separate occasions during a period of one year. Expression values were normalised to the expression of the *β-TUBULIN* gene, and to the corresponding normalised expression in wild-type plants grown in parallel. For each genotype, a similar expression profile was obtained from at least one independent transformant. Mean value ± s.d. of three plants each analysed in technical triplicates.

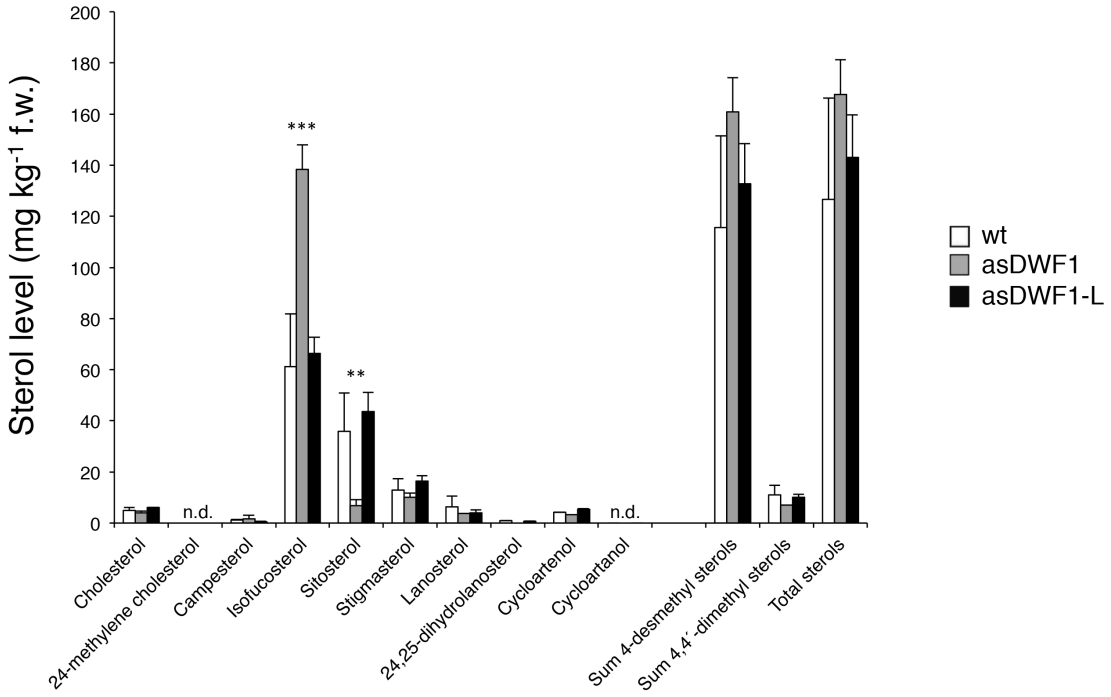

Figure S15. Sterol profile in tubers from wild-type potato plants and antisense *asDWF1* and *asDWF1-L* transformants. The 4-desmethyl and 4,4 dimethylsterol levels were analysed by GC-MS. A difference from wild-type significant at $p<0.01$ (**) or $p<0.001$ (***) is indicated by asterisks. Mean value ± s.d. for plants grown at separate occasions during a period of at least one year. Wild-type (n=5), *asDWF1* line #3a (n=4) and *asDWF1-L* #36 (n=2). For all genotypes, the sum of 4-monomethyl sterols was below 5 mg kg^{-1}, and are not shown for clarity.

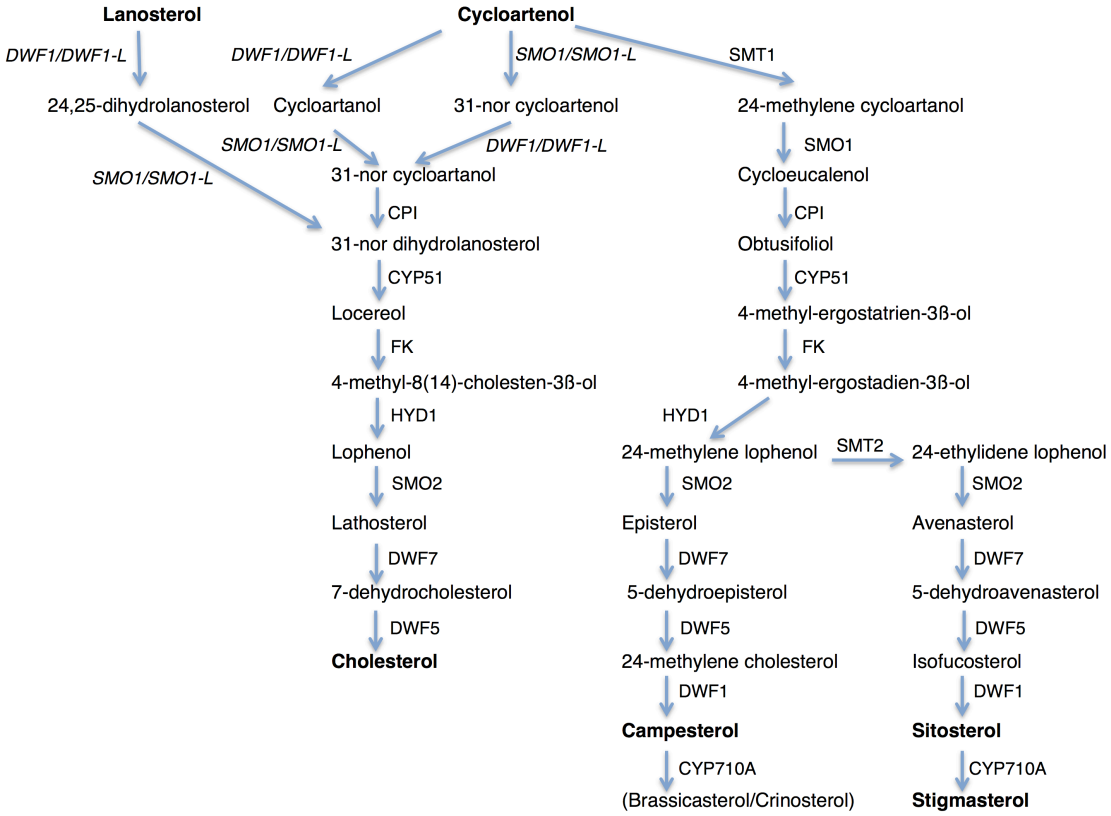

Figure S16. Model for the biosynthesis of cholesterol and other sterols in plants. Each arrow indicates a catalytic step. The metabolic steps at the dimetylsterol level, putatively catalysed by SMO1/SMO1-L and DWF1/DWF1-L, are indicated in italics. Occurrence of DWF1-L is seemingly specific to plant species containing steroidal glycoalkaloids, other plant species likely utilise DWF1 also at the 4,4-dimethyl sterol level. Major endpoint 4-desmethyl sterols in potato are indicated in bold. Abbreviations: DWF1-L, sterol 24(28) reductase-like; SMT1, sterol methyltransferase type 1; SMO1-L, sterol C4-methyl oxidase type 1-like; SMO1, sterol C4-methyl oxidase type 1; CPI, cyclopropyl sterol isomerase; CYP51, sterol 14-demethylase; FK, sterol Δ^{14}-reductase; HYD1, Δ^{8}-Δ^{7} sterol isomerase; SMT2, sterol methyltransferase type 2; SMO2, sterol C4-methyl oxidase type 2; DWF5, sterol Δ^{7}-reductase; DWF7, sterol Δ5-reductase; DWF1, sterol 24(28) reductase; CYP710A, sterol C-22 desaturase.

Supplementary References

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