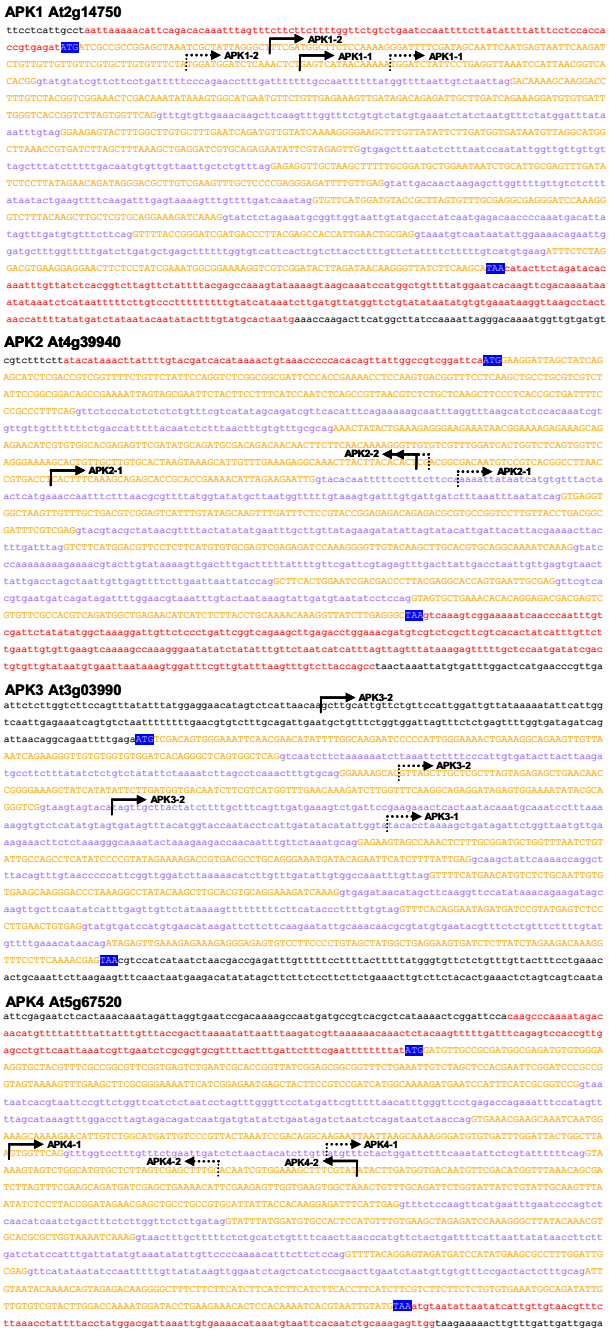
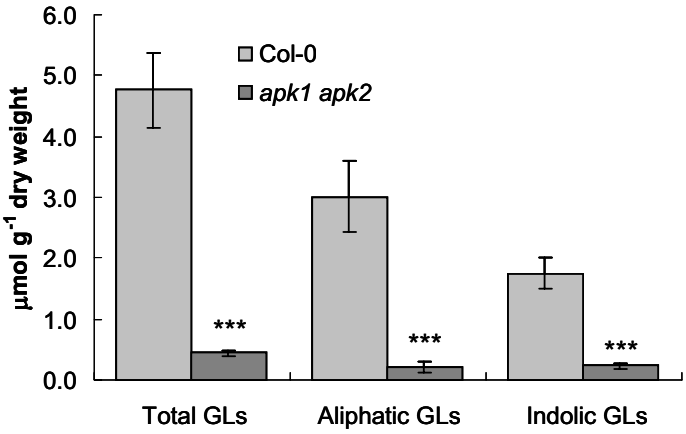
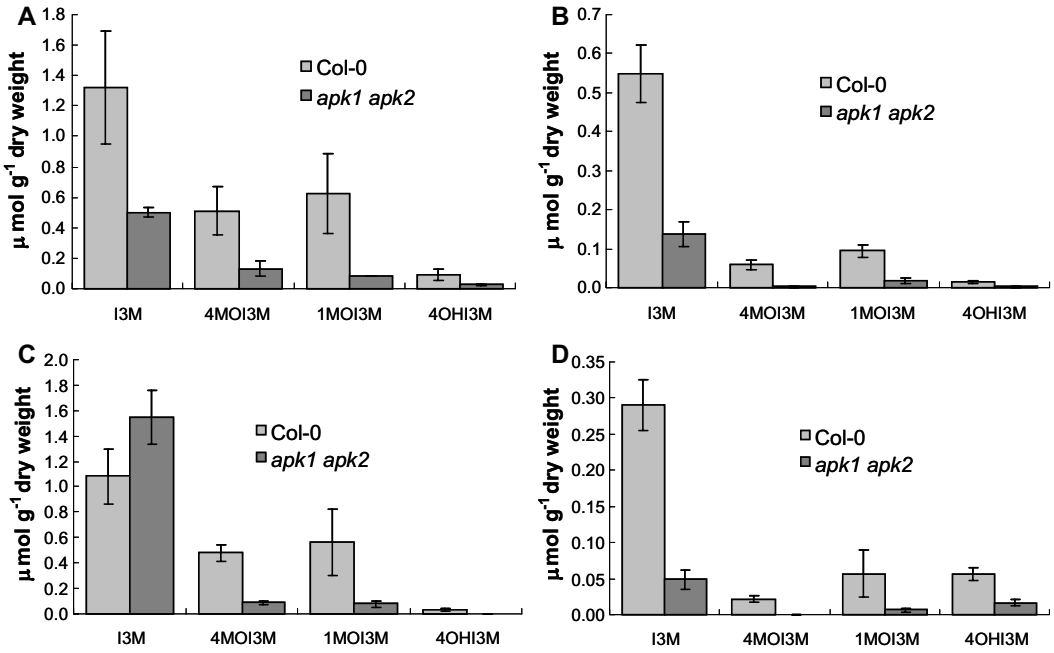
Supplemental Data. Mugford et al. (2009). Disruption of Adenosine-5'-Phosphosulphate Kinase in *Arabidopsis* Reduces Levels of Sulphated Secondary Metabolites.



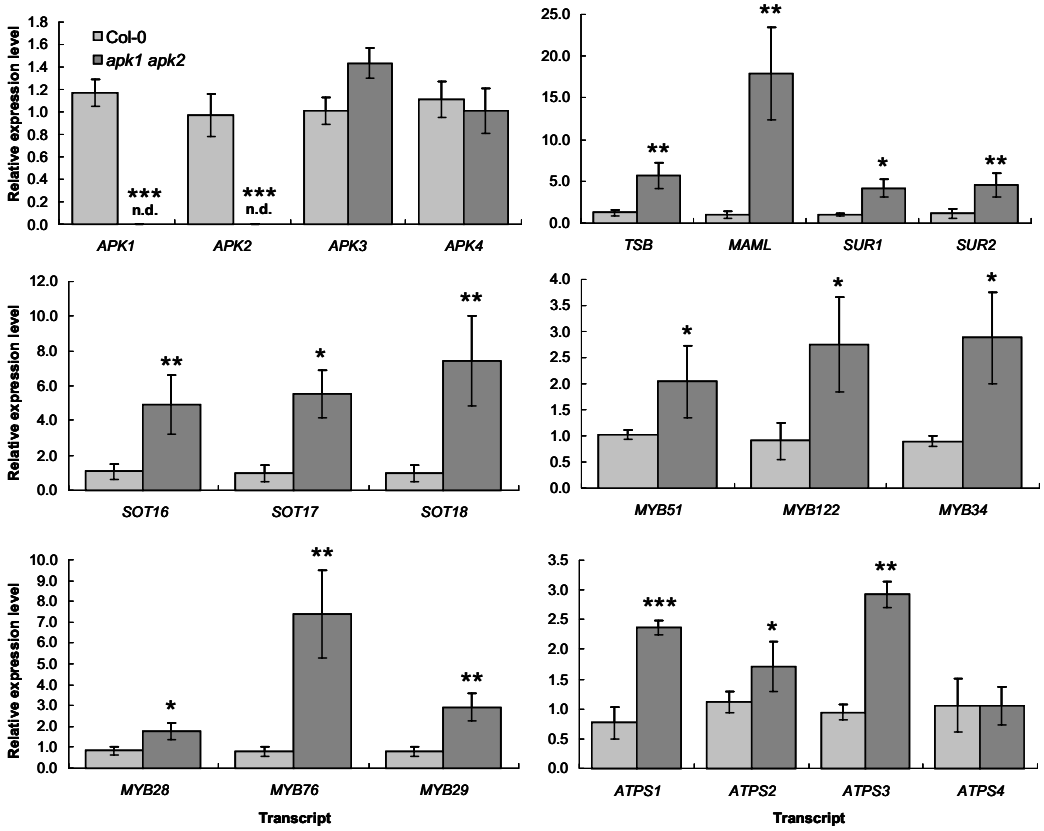

**Supplemental Figure 1. T-DNA insertion sites of APK mutant alleles.**
Insertion sites were determined by sequencing left border PCR products amplified from genomic DNA from homozygous insertion lines. Dashed arrows indicate insertion sites annotated in TAIR database, solid arrows indicate the actual insertion sites determined by sequencing. The direction of the arrows denotes the orientation of the T-DNA within the genome. Red letters indicate non-translated regions, exons and introns are marked in yellow and blue, respectively.



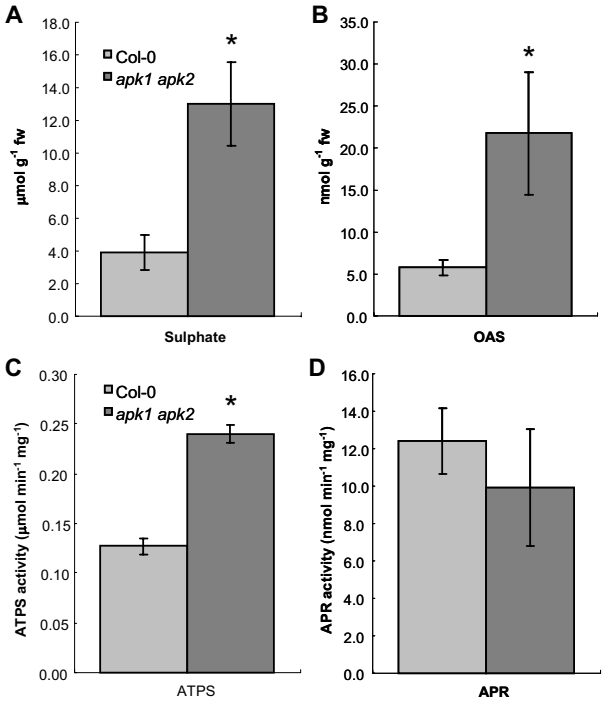

**Supplemental Figure 2. Accumulation of glucosinolates in 2-week old plants grown in hydroculture.** Sterilised seeds of WT and *apk1 apk2* were placed in 250 ml flasks containing 50 ml of 0.5 x MS The flasks were placed in a growth chamber under constant light conditions for two weeks. Data are presented as mean ± SD from three biological replicates. Asterisks denote values significantly different from WT at $P<0.001$.



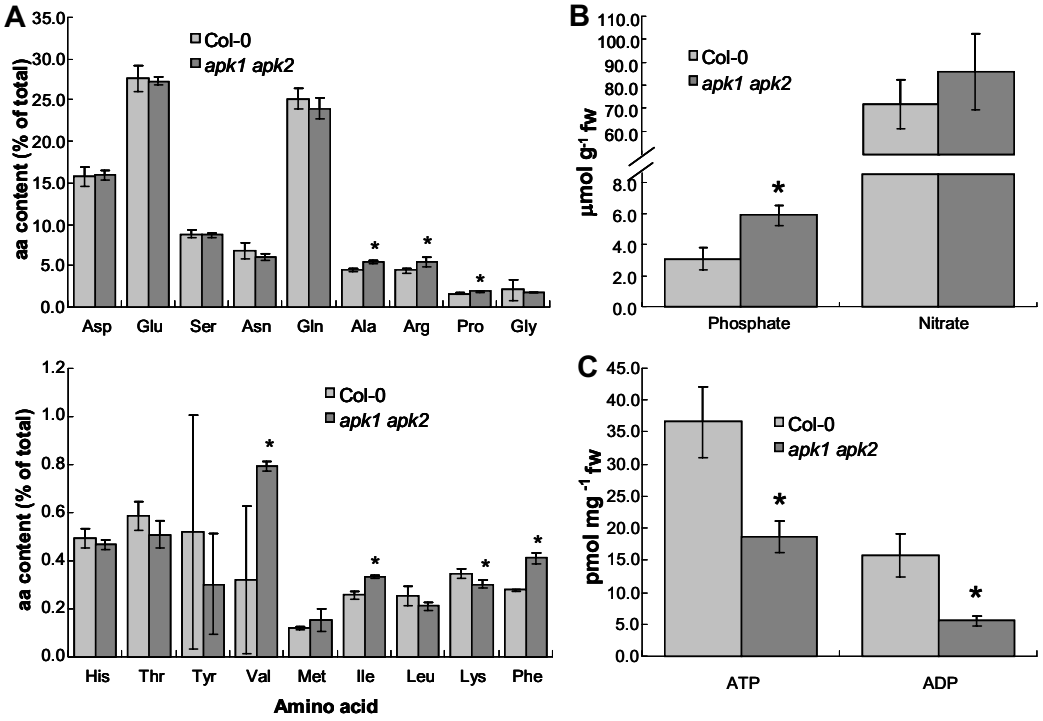

**Supplemental Figure 3. Variation in levels of indolic glucosinolates.**
Four independent measurements of indolic GLs in leaves of WT and *apk1 apk2* plants. Plants for each experiment were grown separately. Data are presented as means ± SD from five (C) or four (A,B,D) biological replicates. (A) represents the dataset used for Figure 6 in the paper.



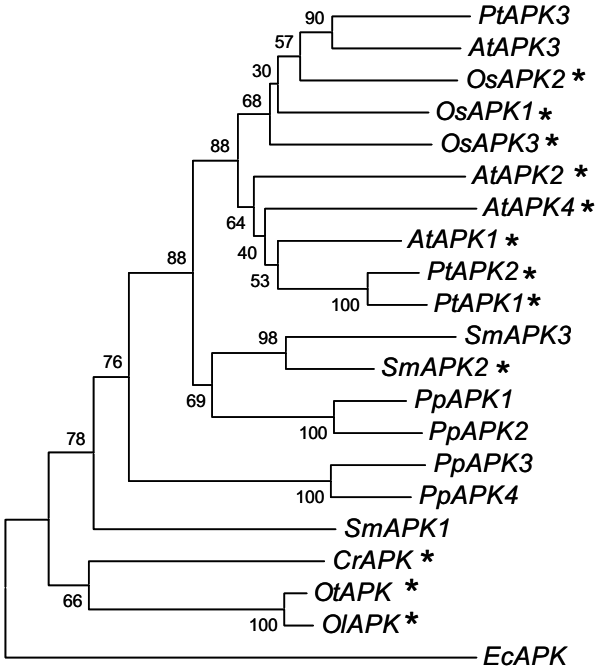

**Supplemental Figure 4. Quantitative Real-Time RT-PCR analysis of glucosinolate biosynthetic genes and ATPS gene family in Col-0 and *apk1 apk2* knockout mutant.**
PCR was performed on cDNA synthesised from total RNA extracted from the rosette leaves of 5-week-old plants. Data are presented as means ± SD from four biological replicates. Asterisks denote values significantly different from Col-0 at P<0.05 (*), P<0.01 (**) and P<0.001 (***).




**Supplemental Figure 5. Analysis of sulphate assimilation in *apk1 apk2* plants.** Concentration of (A) sulphate and (B) *O*-acetylserine were determined in rosette leaves of 5-week old plants by HPLC. Enzyme activity of (C) ATPS and (D) APR was measured in the same rosette leaves. Data are presented as means ± SD from four biological replicates. Asterisks denote values significantly different from Col-0 at $P<0.05$.




**Supplemental Figure 6. Metabolite analysis of *apk1 apk2* plants.**
Concentration of (A) amino acids, (B) nitrate and phosphate, and (C) ATP and ADP were determined in rosette leaves of 5-week old plants by HPLC. Data are presented as means ± SD from three to four biological replicates. Asterisks denote values significantly ($P<0.05$) different from WT values.




**Supplemental Figure 7. Phylogenetic analysis of APS kinases from plants and green algae.** Neighbor-joining tree was constructed from alignment of amino acid sequences of APS kinases from *A. thaliana* (At), *Oryza sativa* (Os), *Populus tremuloides* (Pt), *Selaginella moellendorffii* (Sm), *Physcomitrella patens* (Pp), the green algae *Chlamydomonas reinhardtii* (Cr), *Ostreococcus tauri* (Ot) and *Ostreococcus lucimarinus* (Ol), and *Escherichia coli* (Ec) as an outgroup. Numbers represent results of bootstrap analysis with 1000 replicates. Asterisks mark sequences possessing putative plastid targeting peptides.

**Supplemental Table 1.** Sequence identity in percent identical residues of Arabidopsis APS kinase isoforms and the APK from *E. coli*. The numbers in top right part of the table represent identity of the mature proteins on amino acid level, while the bottom left part denotes the identity at nucleotide level. The alignments were performed with the program ALIGN of Biology WorkBench 3.2 using the default parameters.

| | APK1 | APK2 | APK3 | APK4 | EcAPK |
|---|---|---|---|---|---|
| APK1 | | 72.5 | 71.6 | 68.8 | 46.6 |
| APK2 | 60.4 | | 67 | 65.5 | 44.9 |
| APK3 | 51.6 | 48.3 | | 62 | 45.2 |
| APK4 | 58 | 58.6 | 48.9 | | 47 |
| EcAPK | 44.7 | 47.2 | 39.8 | 44.2 | |

**Supplemental Table 2.** Fold-changes of transcription levels of genes of primary sulphur metabolism and metabolism of sulphated compounds in *apk1 apk2* mutant. n/e = not expressed, n/r = not represented on Affymetrix chip. Transcripts highlighted in bold are significantly altered in the *apk1 apk2* mutant at $q < 0.05$ or as determined by qRT-PCR at $p < 0.05$ (compare Fig. 8 and Supplemental Figure 4).

| Gene | AGI code | Description | Fold-change | p value | q value |
|---|---|---|---|---|---|
| SULTR1,1 | At4g08620 | Group 1 sulphate transporters | n/e | - | - |
| SULTR1,2 | At1g78000 | | 1.922 | 0.374 | 0.450 |
| SULTR1,3 | At1g22150 | | n/e | - | - |
| **SULTR2,1** | **At5g10180** | Group 2 sulphate transporters | **3.317** | **0.00013** | **0.0123** |
| SULTR2,2 | At1g77990 | | 1.049 | 0.981 | 0.680 |
| SULTR3,1 | At3g51900 | Group 3 sulphate transporters | n/r | - | - |
| SULTR3,2 | At4g02700 | | n/e | - | - |
| SULTR3,3 | At1g23090 | | 0.612 | 0.0100 | 0.113 |
| SULTR3,4 | At3g15990 | | 3.258 | 0.116 | 0.312 |
| SULTR3,5 | At5g19600 | | 0.354 | 0.374 | 0.450 |
| SULTR4,1 | At5g13550 | Group 4 sulphate transporters | 1.370 | 0.0244 | 0.168 |
| SULTR4,2 | At3g12520 | | 1.219 | 0.273 | 0.446 |
| SULTR5,1 | At1g80310 | Group 5 sulphate transporters | 1.140 | 0.134 | 0.323 |
| SULTR5,2 | At2g25680 | | 1.806 | 0.374 | 0.450 |
| **ATPS1** | **At3g22890** | ATP sulphurylases | **1.639** | **0.00207** | **0.0552** |
| ATPS2 | At1g19920 | | 0.950 | 0.554 | 0.526 |
| **ATPS3** | **At4g14680** | | **1.969** | **0.000687** | **0.0312** |
| ATPS4 | At5g43780 | | 0.673 | 0.0156 | 0.137 |
| APR1 | At4g04610 | APS reductases | 1.846 | 0.00541 | 0.0825 |
| APR2 | At1g62180 | | 0.793 | 0.0772 | 0.270 |
| APR3 | At4g21990 | | 0.652 | 0.0240 | 0.166 |
| SiR | At5g04590 | Sulphite reductase | 1.379 | 0.00219 | 0.0582 |
| SERAT1,1 | At5g56760 | Serine acetyltransferases | 1.165 | 0.120 | 0.312 |
| SERAT2,1 | At1g55920 | | 1.310 | 0.00251 | 0.0601 |
| SERAT2,2 | At3g13110 | | 1.134 | 0.0277 | 0.178 |
| SERAT3,1 | At2g17640 | | 0.894 | 0.0950 | 0.296 |
| SERAT3,2 | At4g35640 | | n/r | - | - |
| **OASA1** | **At4g14880** | O-acetylserine (thiol)lyases | **1.546** | **0.000177** | **0.0151** |
| OASTL | At1g55880 | | n/e | - | - |
| OASB | At2g43750 | | 0.835 | 0.0235 | 0.164 |
| OASTL | At3g22460 | | 0.646 | 0.0385 | 0.205 |

| | | | | | |
|---|---|---|---|---|---|
| CS26 | At3g03630 | Cysteine synthases | 1.008 | 0.981 | 0.686 |
| CYSD1 | At3g04940 | | 0.913 | 0.438 | 0.478 |
| CYSC1 | At3g61440 | | 1.082 | 0.0568 | 0.241 |
| CYSD2 | At5g28030 | | 0.990 | 0.973 | 0.679 |
| GSH1 | At4g23100 | Glutathione synthesis | 1.398 | 0.00449 | 0.0768 |
| GSH2 | At5g27380 | | 1.411 | 0.00233 | 0.0585 |
| SLIM1/EIL3 | At1g73730 | Sulphate Limitation transcription factor | 0.880 | 0.320 | 0.450 |
| SOT1 | At5g43690 | Sulphotransferases | n/e | - | - |
| SOT2a | At3g51210 | | n/e | - | - |
| SOT2b | At1g62650 | | n/r | - | - |
| SOT3 | At4g26280 | | n/e | - | - |
| SOT4 | At2g27570 | | n/e | - | - |
| SOT5 | At3g45070 | | n/r | - | - |
| SOT6 | At3g45080 | | n/r | - | - |
| SOT7 | At1g28170 | | n/e | - | - |
| SOT8 | At1g13420 | | n/e | - | - |
| SOT9 | At1g13430 | | n/r | - | - |
| SOT10 | At2g14920 | | n/r | - | - |
| SOT11 | At2g03750 | | 0.979 | 0.725 | 0.602 |
| SOT12 | At2g03760 | | 1.495 | 0.363 | 0.450 |
| SOT13 | At2g03770 | | n/e | - | - |
| **SOT14** | **At5g07000** | | **0.580** | **0.000415** | **0.0243** |
| SOT15 | At5g07010 | | 0.495 | 0.0193 | 0.149 |
| **SOT16** | **At1g74100** | | **2.097** | **0.00489** | **0.0805** |
| **SOT17** | **At1g18590** | | **4.186** | **0.000146** | **0.0130** |
| **SOT18** | **At1g74090** | | **2.291** | **0.000278** | **0.0239** |
| SOT19 | At3g50620 | | 0.970 | 0.753 | 0.614 |
| SOT20 | At2g15730 | | n/r | - | - |
| SOT21 | At4g34420 | | n/r | - | - |
| AtPSK1 | At1g13590 | PSK precursors | n/e | - | - |
| **AtPSK2** | **At2g22860** | | **4.678** | **0.00164** | **0.0487** |
| AtPSK3 | At3g44735 | | 0.994 | 0.923 | 0.670 |
| **AtPSK4** | **At3g49780** | | **2.051** | **0.0156** | **0.137** |
| AtPSK5 | At5g65870 | | 1.203 | 0.219 | 0.408 |
| AtPSY1 | At5g58650 | PSY precursors | 0.896 | 0.282 | 0.450 |
| AtPSY2 | At3g47295 | | 0.913 | 0.617 | 0.553 |
| AtPSY3 | At2g29995 | | 1.671 | 0.021 | 0.153 |

**Supplemental Table 3.** Metabolic pathways over-represented among the altered transcripts - pathways more highly expressed in the *apk1 apk2* mutant at p<0.005. The pathways were identified by iterative group analysis using Aracyc metabolic pathways.

| **Group name** | **Members** | **Members changed** | **p-value** |
|---|---|---|---|
| Glucosinolate biosynthesis from tryptophan | 3 | 3 | 5.08E-05 |
| Glucosinolate biosynthesis from phenylalanine | 4 | 3 | 0.000198 |
| IAA biosynthesis | 8 | 2 | 0.000508 |
| Leucine biosynthesis | 18 | 4 | 0.000984 |
| Glucosinolate biosynthesis from homomethionine | 3 | 2 | 0.00129 |
| NAD biosynthesis (from aspartate) | 6 | 6 | 0.00226 |
| Cytokinin-glucoside biosynthesis | 77 | 7 | 0.00237 |
| Jasmonic acid biosynthesis | 14 | 4 | 0.00382 |
| Lipoxygenase pathway | 11 | 6 | 0.00403 |

**Supplemental Table 4.** Metabolic pathways over-represented among the altered transcripts- pathways more highly expressed in the WT at p<0.005. The pathways were identified by iterative group analysis using Aracyc metabolic pathways.

| **Group name** | **Members** | **Members changed** | **p-value** |
|---|---|---|---|
| Cellulose biosynthesis | 41 | 9 | 0.000926 |
| Cytokinins degredation | 6 | 3 | 0.000937 |
| Cytokinin-glucoside biosynthesis | 77 | 5 | 0.00115 |
| Triacylglycerol biosynthesis | 5 | 1 | 0.00331 |
| Glycine biosynthesis | 8 | 8 | 0.00341 |
| Glycine degredation | 8 | 8 | 0.00370 |

**Supplemental Table 5.** Primer sequences for identification of T-DNA insertions in *apk* mutants

| Mutant | Mutant ID | 5' Primer | 3' Primer |
|---|---|---|---|
| *apk1-1* | SALK_053427 | CCACAACTGCATATAGACTATGC | GACCCAAATCACACATCC |
| *apk1-2* | SALK_034586 | CCACAACTGCATATAGACTATGC | GACCCAAATCACACATCC |
| *apk2-1* | SALK_093072 | TAACGTCTCTGCTCAAGC | ATGTTTTCGGTGAGGTGC |
| *apk2-2* | SALK_077590 | TAACGTCTCTGCTCAAGC | ATGTTTTCGGTGAGGTGC |
| *apk3-1* | SALK_115182 | GCGCATATGTCGACAGTGGGAAATTC | CTATCACTACATATGAGACACC |
| *apk3-2* | SALK_115507 | CCGTGATTATCTCTGTCAGC | TTCCACTCTATCCTCTGC |
| *apk4-1* | SALK_035815 | ACTTCCGTCCGATCATGG | GCTCGATCATCTGCTTCG |
| *apk4-2* | SALK_068072 | ACTTCCGTCCGATCATGG | GCTCGATCATCTGCTTCG |
| LBb1 | all | GCGTGGACCGCTTGCTGCAACTC | |

**Supplemental Table 6.** Primer sequences for RT-PCR analysis of APK transcripts in T-DNA lines

| Gene | 5' Primer | 3' Primer |
|---|---|---|
| *APK1* | GCTTTCGATGGCTTCTTC | GACCCAAATCACACATCC |
| *APK2* | TAACGTCTCTGCTCAAGC | ATGTTTTCGGTGAGGTGC |
| *APK3* | AACTGAAAGGCAGAAGTTG | TTCCACTCTATCCTCTGC |
| *APK4* | ACTTCCGTCCGATCATGG | GCTCGATCATCTGCTTCG |

**Supplemental Table 7.** Primer sequences for expression analysis by qPCR

| gene | 5' primer | 3' primer |
|---|---|---|
| actin2 | ATGGAAGCTGCTGGAATCCAC | TTGCTCATACGGTCAGCGATG |
| PSK2 | ATGGTAGACGAAAACTGTGGTGC | TCAGTATGAGCGACCAAAGTCCT |
| PSK3 | CCTGGCAGTTCTCTTCCTCATTTT | TCTCTTGATCTTCTTTTCCTCTTCG |
| PSK4 | AGAAGAAAGCTGCAACGGAATT | AATCGGTGTGAAGAACAAGGCT |
| APK1 | CCTTACGAGCCACCATTGAACTG | GCCATTTCGATAGGAGAAGTTCCT |
| APK2 | CAAAATCAAAGGCTTCACTGGAATC | TGTTTCAGCACTACCTCGCAATT |
| APK3 | GTTGAAAGAGAAAGAGGGAGAGTGTC | AGAGATCACTTCCTCAGCCATAGC |
| APK4 | TCTTCTTCTCTGTGTGAAATGGCAG | TTCTTCAGGTATCCATTTTGGTCC |
| TSB | CGTTGACACTCTTCTTCTCTAACCG | AGCCTCAGGCACCTCTGCTACTT |
| MAM-L | TTCGTTACTTCTCACATCGTCGAG | GGAGCAACATGAGACGAACAGAGT |
| CYP83B1 | GGCAACAAACCATGTCGTATCAAG | CGTTGACACTCTTCTTCTCTAACCG |
| SUR2 | CAAGACGAGCCGTTGCTGAT | GGACGTGGGAGCAAGATGTTT |
| SOT16 | CGAAGTCGTCGAACTCACAGAGTT | AAAGACCTTCGAGGAGACATTCTTG |
| SOT17 | GGAATCCAAAACCATAAACGACG | CGGATCTTTTGGTCTCCAGCC |
| SOT18 | CCCTACCGAGTCACGACGAGA | GGTAGCCACCAGTAACCACCATACT |
| MYB51 | CTACAAGTGTTTCCGTTGACTCTGAA | ACGAAATTATCGCAGTACATTAGAGGA |
| MYB122 | GTCCGTTGAGTCTTGTTTGGAGAATA | TGTCAATCCCTTCACAGGAGTCAT |
| MYB34 | CACGACTGTCGATAATTTTGGGTTT | CATATTGTCATCTTCGTTCCAGGAA |
| MYB28 | TCCCTGACAAATACTCTTGCTGAAT | CATTGTGGTTATCTCCTCCGAATT |
| MYB76 | ACGTTTTGGACGATCGAGCTCTAC | TGATTGAGAGAACGAGTCTGGGAGT |
| MYB29 | GAAATATGATGCTTCCTTGAGCTCC | ACGGTGTAGAGCTGATCAAGGTTC |
| ATPS1 | CCCGCCAGACGGTTTTATGT | ACTTCTGGTAGTCTACCATTACCCGC |
| ATPS2 | CAGATCCTCTTACGTTTCTCACATCA | GGTTGGTGAAAGAGGATGGTTTTG |
| ATPS3 | GAGAGAACCCACCAGATGGATTTA | TCCGGTTAGTGTCAAACTGTCGTAG |
| ATPS4 | TTCTTCAGCAGCAGCCATCG | ACGAGAAGCATGATGGTTATGGA |

**Supplemental Table 8.** Primer sequences for the pET14b constructs for heterologous expression of recombinant APKs

| Gene | Cloning site | 5'- Primer[a] | 3'- Primer |
|---|---|---|---|
| *APK1* | NdeI / XhoI | GCGCATATGGTTTCTATGGATGGATCTC | CGCTCGAGTTATGCTTGAAGATAACC |
| *APK2* | NdeI / BamHI | GCGCATATGGCCGTTAACGTCTCTGCTC | GCGGATCCTTAGCCCTCAAGATAACC |
| *APK3* | NdeI / BamHI | GCGCATATGTCGACAGTGGGAAATTC | GCGGATCCTTACTCGTTTTGAAGGAAACC |
| *APK4* | XhoI / BamHI | GCGCTCGAGTCCGGTGAAACGAAGCAAATC | GCGGATCCTTACATACAATTACGTGATTTTG |

[a] Underlined sequences correspond to the indicated restriction sites used for cloning into the pET14b vector

**Supplemental Table 9.** Primer sequences for the chimeric gene constructs of CaMV35S promoter-APK cDNA-GFP

| Construct | 5' Primer[a] | 3' Primer |
|---|---|---|
| *APK1*(N-term) | ACGCGTCGACATGATCGCCGCCGGAGC | CATGCCATGGCCTGTCTATCAACTTTCTCAAC |
| *APK1*(full) | ACGCGTCGACATGATCGCCGCCGGAGC | CATGCCATGGCTGCTTGAAGATAACCCTTG |
| *APK2*(N-term) | ACGCGTCGACATGGAAGGATTAGCTATCAG | CATGCCATGGCTTGTCTGTCGCATCTGC |
| *APK2*(full) | ACGCGTCGACATGGAAGGATTAGCTATCAG | CATGCCATGGCGCCCTCAAGATAACCTTTG |
| *APK3*(full) | CCGCTCGAGATGTCGACAGTGGGAAA | CATGCCATGGCCTCGTTTTGAAGGAAAC |
| *APK4*(N-term) | ACGCGTCGACATGGATGTTGCCGCGATG | CATGCCATGGCTTGCCTGTCGGATTTAG |
| *APK4*(full) | ACGCGTCGACATGGATGTTGCCGCGATG | CATGCCATGGCCATACAATTACGTGATTTTG |

[a] Underlined sequences correspond to the *Sal*I and *Nco*I restriction sites used for fusion construction.

**Supplemental Table 10.** Primer sequences for creation of transgenic plants expressing APK promoter-coding sequence-GFP

| **Promoter/ gene** | **5' Primer**[a] | **3' Primer** |
|---|---|---|
| APK1 | ACTAGTTTCAGTAGCAATCTAAGTATGG | GAATTCTGCTTGAAGATAACCCTTGTTATC |
| APK2 | CTCGAGTTTGGAGGAGGAGACTAACAAC | TCTAGAGCCCTCAAGATAACCTTTGTTTTG |
| APK3 | ACTAGTAAATCTGGTTTACTTCATGC | GGATCCCTCGTTTTGAAGGAAACCTTTG |
| APK4 | GTCGACCCTTACAGGTTTTGACAACAGTC | GTCGACCATACAATTACGTGATTTTGTGG |
| GFP | GAATTCATGGTGAGCAAGGGCGAGGAGCTG | GAGCTCTTACTTGTACAGCTCGTC |

[a] The underlined sequences correspond to the restriction sites used for fusion construction.