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A gossypol biosynthetic intermediate disturbs plant defense response

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**1. Supplementary Figures**

**S c234 content.emf**

**Figure S1.** The 8-hydroxy-7-keto-δ-cadinene (C234) content in the VIGS-*CYP71BE79* cotton plants. The content was determined by HPLC at 254nm. Plant tissues were harested two weeks post the VIGS treatment on the 10-day old cotton seedlings and data were derived from six biological replicates, and the mean ± SD are shown.

**figS1.tif**

**Figure S2.** The compound 8-hydroxy-7-keto-δ-cadinene (C234, at 200 μM) did not affect the phytopathogenic bacterium (*Pseudomonas syringae* pv. *maculicola* (*Psm*) ES4326) growth in culture condition. Data were derived from six biological replicates, and the mean ± SD are shown.

**fig S2.tif**

**Figure S3.** Proportions of C234+ (left) and *Psm*+ (right) genes in main MapMan functional categories. The total number (#) of genes in each category is indicated on the left. The absolute number of C234+ and *Psm*+ genes within a particular gene category is indicated on the horizontal bars. Asterisks indicate significant enrichment (or depletion) of gene categories in C234+ or *Psm*+ genes (\*\*p < 0.001; \*p < 0.05; Fisher’s exact test).

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**Figure S4.** Proportions of *Psm*+ and PC+-P genes in defined gene groups representing MapMan functional categories and *Arabidopsis* main transcription factor families. Asterisks indicate significant enrichment (or depletion) of gene categories in *Psm*+ or PC+-P genes (\*\*p < 0.001; \*p < 0.05; Fisher’s exact test).

**1. Supplementary Tables**

**Table S1.** Primers used in this investigation.

|  |  |  |
| --- | --- | --- |
| **Primer name** | **Primer sequence (5'-3')** | **Target gene** |
| GRXS3-qrtF | GCAAGACAGTTTTCAAATATCTATCAC | AT4G15700 |
| GRXS3-qrtR | CAAGCTGCCCTCCTATGAAC | AT4G15700 |
| GRXS4-qrtF | CGCTCTTCGAACACATTTCA | AT4G15680 |
| GRXS4-qrtR | GCCCTCCAATAAACACCACA | AT4G15680 |
| GRXS5-qrtF | AACCGCTCCCTTGTTCCTAT | AT4G15690 |
| GRXS5-qrtR | TTCTTGTGTATGTTTTCTTTGTTCA | AT4G15690 |
| GRXS7-qrtF | TAGACCTTGGCGTAAATCC | AT4G15670 |
| GRXS7-qrtR | TGCCCTCCTATGAACACC | AT4G15670 |
| GRXS8-qrtF | TGCATGTCACACACAATCAAG | AT4G15660 |
| GRXS8-qrtR | AAAGCCATAAAGCCCCAAA | AT4G15660 |
| ROXY10-qrtF | CAAATCCAGCGGTTTACGA | AT5G18600 |
| ROXY10-qrtR | TGGCTCCACCGACCAACTC | AT5G18600 |
| PR1-qrtF | CTCGAAAGCTCAAGATAGCCC | AT2G14610 |
| PR1-qrtR | TTCCACCATTGTTACACCTCAC | AT2G14610 |
| UBQ5-qrtF | TTGAATCATCCGACACCATC | AT3G62250 |
| UBQ5-qrtR | GCTCCACAGGTTGCGTTAG | AT3G62250 |

**Table S2.** Comparison of the fold of change of the C234+, *Psm*+ and (*Psm+*C234)+ genes. Numbers show log2-fold change in expression in C234-treated, *Psm*-inoculated and *Psm*C234-inoculated samples referred to the CK samples. The genes listed are responsive to all the three treatments.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| gene\_name | C234/CK | *Psm*/CK | *Psm*C234/CK | (*Psm+*C234)/*Psm* | FDR | Description |
| AT2G37760 | 1.26 | 1.71 | 2.94 | 1.23 | <0.05 | AKR4C8 |
| AT5G24200 | 1.39 | 4.64 | 5.48 | 0.84 | <0.05 | alpha/beta-Hydrolases superfamily protein; |
| AT4G12490 | 1.73 | 1.46 | 3.97 | 2.50 | <0.05 | AZI3 |
| AT1G05710 | 1.48 | 1.95 | 2.88 | 0.93 | <0.05 | basic helix-loop-helix (bHLH) DNA-binding superfamily protein |
| AT5G20230 | 1.24 | 3.56 | 4.90 | 1.34 | <0.05 | BCB |
| AT3G50930 | 0.87 | 2.99 | 3.50 | 0.51 | <0.05 | BCS1 |
| AT3G57260 | 1.90 | 5.82 | 6.27 | 0.45 | <0.05 | beta 1,3-glucanase |
| AT3G13380 | 1.10 | 1.88 | 2.71 | 0.82 | <0.05 | BRL3 |
| AT2G41410 | 0.68 | 1.75 | 2.17 | 0.42 | <0.05 | Calcium-binding EF-hand family protein |
| AT2G46600 | 0.63 | 1.31 | 1.57 | 0.26 | <0.05 | Calcium-binding EF-hand family protein |
| AT1G19020 | 1.52 | 2.15 | 3.41 | 1.26 | <0.05 | CDP-diacylglycerol-glycerol-3-phosphate 3-phosphatidyltransferase; |
| AT2G43570 | 2.02 | 5.56 | 6.84 | 1.27 | <0.05 | CHI |
| AT2G17120 | 1.13 | 1.31 | 1.91 | 0.60 | <0.05 | CL-1 |
| AT5G27420 | 1.43 | 2.68 | 3.97 | 1.29 | <0.05 | CNI1 |
| AT5G60950 | 1.26 | 2.81 | 2.65 | -0.16 | <0.05 | COBL5 |
| AT4G04490 | 1.37 | 3.53 | 4.70 | 1.17 | <0.05 | CRK36 |
| AT4G11890 | 1.24 | 2.98 | 4.22 | 1.23 | <0.05 | CRK45 |
| AT3G26210 | 0.88 | 2.71 | 4.04 | 1.33 | <0.05 | CYP71B23 |
| AT3G14620 | 0.69 | 1.46 | 2.46 | 1.00 | <0.05 | CYP72A8 |
| AT3G25180 | 4.94 | 2.84 | 5.65 | 2.80 | <0.05 | CYP82G1 |
| AT3G49340 | 5.15 | 3.70 | 4.63 | 0.93 | <0.05 | Cysteine proteinases superfamily protein |
| AT4G23210 | 1.08 | 2.97 | 3.71 | 0.73 | <0.05 | cysteine-rich RLK (RECEPTOR-like protein kinase) 13; |
| AT4G10500 | 1.48 | 5.50 | 7.03 | 1.53 | <0.05 | DLO1 |
| AT1G03820 | 3.53 | 0.96 | 3.53 | 2.57 | <0.05 | E6-like protein |
| AT4G37990 | 4.47 | 2.45 | 6.29 | 3.83 | <0.05 | ELI3 |
| AT3G27400 | 4.30 | 0.59 | 4.17 | 3.57 | <0.05 | Encodes a pectate lyase involved in response to nematodes. |
| AT1G29330 | 0.97 | 2.11 | 2.67 | 0.56 | <0.05 | ER lumen protein retaining receptor family protein |
| AT5G09440 | 1.32 | 2.03 | 3.31 | 1.28 | <0.05 | EXL4 |
| AT1G79530 | 1.19 | 0.67 | 1.35 | 0.68 | <0.05 | GAPCP-1 |
| AT5G55050 | 0.82 | 1.23 | 1.44 | 0.21 | <0.05 | GDSL-motif esterase/acyltransferase/lipase |
| AT1G54010 | 3.46 | 7.58 | 6.28 | -1.30 | <0.05 | GLL23 |
| AT1G02920 | 1.43 | 3.49 | 4.09 | 0.60 | <0.05 | GST11 |
| AT1G02930 | 2.32 | 3.91 | 4.69 | 0.78 | <0.05 | GSTF6 |
| AT2G46680 | 1.00 | 2.00 | 2.57 | 0.57 | <0.05 | HB-7 |
| AT5G42020 | 0.75 | 1.11 | 1.75 | 0.64 | <0.05 | Heat shock protein 70 (Hsp 70) family protein |
| AT3G50480 | 1.47 | 3.58 | 4.14 | 0.55 | <0.05 | HR4 |
| AT5G49280 | 1.04 | 1.50 | 1.86 | 0.36 | <0.05 | hydroxyproline-rich glycoprotein family protein |
| AT4G18630 | 3.21 | 1.68 | 3.97 | 2.29 | <0.05 | hypothetical protein (DUF688); |
| AT3G14060 | 2.25 | 3.04 | 4.89 | 1.85 | <0.05 | hypothetical protein; |
| AT1G21100 | 3.02 | 3.39 | 4.99 | 1.60 | <0.05 | IGMT1 |
| AT5G23020 | 0.85 | 1.22 | 1.98 | 0.75 | <0.05 | IMS2 |
| AT5G12930 | 1.12 | 1.86 | 2.68 | 0.82 | <0.05 | inactive rhomboid protein; |
| AT2G39330 | 1.11 | 4.04 | 4.22 | 0.17 | <0.05 | jacalin-related lectin 23; |
| AT2G28500 | 2.62 | 1.26 | 3.46 | 2.20 | <0.05 | LBD11 |
| AT1G19610 | 2.09 | 1.54 | 2.94 | 1.40 | <0.05 | LCR78 |
| AT3G22600 | 1.82 | 3.67 | 5.35 | 1.67 | <0.05 | LTPG5 |
| AT3G23560 | 0.89 | 1.59 | 2.35 | 0.76 | <0.05 | MATE efflux family protein |
| AT3G45290 | 0.87 | 1.47 | 2.27 | 0.79 | <0.05 | MLO3 |
| AT2G47800 | 0.81 | 2.09 | 2.69 | 0.60 | <0.05 | MRP4 |
| AT5G09590 | 0.60 | 0.69 | 1.13 | 0.44 | <0.05 | MTHSC70-2 |
| AT3G29250 | 2.31 | 3.47 | 5.24 | 1.77 | <0.05 | NAD(P)-binding Rossmann-fold superfamily protein |
| AT3G22550 | 0.86 | 1.34 | 1.42 | 0.08 | <0.05 | NAD(P)H-quinone oxidoreductase subunit, putative (DUF581) |
| AT1G60730 | 0.98 | 2.32 | 3.50 | 1.19 | <0.05 | NAD(P)-linked oxidoreductase superfamily protein |
| AT4G05020 | 0.87 | 1.83 | 2.37 | 0.54 | <0.05 | NDB2 |
| AT3G50910 | 0.69 | 1.41 | 1.78 | 0.37 | <0.05 | netrin receptor DCC |
| AT5G42050 | 0.77 | 1.73 | 2.86 | 1.13 | <0.05 | NRP |
| AT4G29520 | 1.00 | 1.66 | 1.91 | 0.25 | <0.05 | Nucleophosmin |
| AT1G76930 | 1.73 | 1.79 | 2.81 | 1.02 | <0.05 | ORG5 |
| AT4G36648 | 0.64 | 2.37 | 3.22 | 0.85 | <0.05 | other\_RNA |
| AT1G04980 | 1.88 | 3.25 | 3.48 | 0.23 | <0.05 | PDI10 |
| AT1G21750 | 0.59 | 1.20 | 1.65 | 0.45 | <0.05 | PDIL1-1 |
| AT1G17745 | 0.81 | 2.39 | 3.23 | 0.84 | <0.05 | PGDH |
| AT1G43910 | 1.24 | 3.37 | 3.88 | 0.50 | <0.05 | P-loop containing nucleoside triphosphate hydrolases superfamily protein |
| AT4G02330 | 3.11 | 0.74 | 3.31 | 2.56 | <0.05 | PME41 |
| AT2G18660 | 1.67 | 5.78 | 6.73 | 0.95 | <0.05 | PNP-A |
| AT4G23680 | 3.13 | 3.36 | 6.75 | 3.39 | <0.05 | Polyketide cyclase/dehydrase and lipid transport superfamily protein |
| AT2G14610 | 2.39 | 7.55 | 8.81 | 1.26 | <0.05 | PR1 |
| AT1G75040 | 1.87 | 3.88 | 4.48 | 0.61 | <0.05 | PR5 |
| AT1G48210 | 0.93 | 2.24 | 3.37 | 1.12 | <0.05 | Protein kinase superfamily protein |
| AT3G49120 | 0.98 | 1.97 | 3.70 | 1.73 | <0.05 | PRX34 |
| AT3G25010 | 0.79 | 3.45 | 4.61 | 1.16 | <0.05 | receptor like protein 41 |
| AT5G27060 | 3.50 | 4.62 | 6.91 | 2.29 | <0.05 | receptor like protein 53 |
| AT1G63840 | 0.88 | 1.40 | 3.46 | 2.06 | <0.05 | RING/U-box superfamily protein |
| AT5G10380 | 0.85 | 3.10 | 3.61 | 0.51 | <0.05 | RING1 |
| AT2G32680 | 0.99 | 3.06 | 3.79 | 0.72 | <0.05 | RLP23 |
| AT2G29350 | 2.47 | 4.88 | 6.99 | 2.11 | <0.05 | SAG13 |
| AT2G41800 | 4.58 | 0.95 | 4.93 | 3.97 | <0.05 | TEB |
| AT5G38900 | 4.14 | 5.37 | 6.94 | 1.57 | <0.05 | Thioredoxin superfamily protein; |
| AT5G44572 | 1.53 | 1.81 | 2.91 | 1.10 | <0.05 | transmembrane protein |
| AT2G18690 | 1.21 | 3.89 | 5.24 | 1.35 | <0.05 | transmembrane protein |
| AT5G44570 | 2.61 | 4.26 | 5.42 | 1.15 | <0.05 | transmembrane protein |
| AT5G08240 | 1.55 | 4.12 | 5.05 | 0.93 | <0.05 | transmembrane protein; |
| AT5G46350 | 2.55 | 2.29 | 4.12 | 1.82 | <0.05 | WRKY8 |
| AT5G17000 | 0.73 | 0.63 | 1.55 | 0.92 | <0.05 | Zinc-binding dehydrogenase family protein |

**Table S3.** GO-term enrichments of C234-downregulated (C234-) genes.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| GO term | Ontology | Description | Number in input list | Number in BG/Ref | FDR |
| GO:0045454 | P | cell redox homeostasis | 8 \* | 136 | 3.80E-07 |
| GO:0019725 | P | cellular homeostasis | 8 \* | 328 | 0.00014 |
| GO:0008794 | F | arsenate reductase (glutaredoxin) activity | 6 \* | 14 | 1.20E-10 |
| GO:0030611 | F | arsenate reductase activity | 6 \* | 15 | 1.30E-10 |
| GO:0015035 | F | protein disulfide oxidoreductase activity | 8 \* | 94 | 1.40E-09 |
| GO:0015036 | F | disulfide oxidoreductase activity | 8 \* | 102 | 2.20E-09 |
| GO:0016667 | F | oxidoreductase activity, acting on sulfur group of donors | 8 \* | 283 | 3.90E-06 |
| GO:0009055 | F | electron carrier activity | 9 \* | 525 | 3.40E-05 |

\*All the six glutaredoxin genes, *AtGRXS3/4/5/7/8* and *ROXY10* are included. P, biological process; F, molecular function.