SUPPLEMENTAL DATA

NMR and MS data for fenclorim metabolites

S-(4-Chloro-2-phenylpyrimidin-6-yl)-cysteine (FC) was recovered as a white amorphous solid (13.3 mg, 0.043 mmol, 43%); mp >140 °C dec. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.45-8.43 (2H, m, *H*-2′,6′ on Ph), 7.68 (1H, s, *H*-5), 7.60-7.51 (3H, m, *H*-3′,4′,5′ on Ph), 4.06 (1H, dd, J = 14.1, 4.0, 1H of the Cys*H*-*β*), 3.63 (1H, dd, J = 8.7, 4.0, Cys*H*-*α*), 3.38 (1H, dd, J = 14.1, 8.7, 1H of the Cys*H*-*β*); ^{13}C NMR (125 MHz, DMSO-d_6): δ 171.7 (C4), 167.9 (C=O), 163.0 (C2), 158.9 (C6), 135.3 (C1′), 131.8 (C4′), 128.7 (C3′,5′), 128.3 (C2′,6′), 116.1 (C5), 53.2 (CysC-*α*), 31.0 (CysC-*β*); MS-ES$^+$ (*m/z*) 312 ([M + H]$^+$, ^{37}Cl, 42), 310 ([M + H]$^+$, ^{35}Cl, 100), 223 ([M – SCH_2CHNH_2COOH]$^+$, ^{35}Cl, 6); HRMS-ES$^+$ (*m/z*) Calcd for $C_{13}H_{13}O_2N_3{}^{35}ClS$ [M + H]$^+$: 310.0412, found 310.0414.

S-(4-Chloro-2-phenylpyrimidin-6-yl)-glutathione (FG) was collected as a white fluffy solid (36.5 mg, 73.7 nmol, 74%); mp >200 °C dec. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.79 (1H, br s, GlyN*H*), 8.71 (1H, d, *J*= 7.6, GluN*H*), 8.42 (2H, d, J = 7.5, *H*-2′,6′ on Ph), 7.62 (1H, s, *H*-5), 7.59-7.51 (3H, m, *H*-3′,4′,5′ on Ph), 4.69-4.65 (1H, m, Cys*H*-*α*), 3.97 (1H, dd, J = 13.5, 3.8, 1H of the Cys*H*-*β*), 3.77-3.68 (2H, m, Gly*H*-*α*), 3.33-3.28 (2H, m, 1H of the Cys*H*-*β* and Glu*H*-*α*), 2.38-2.30 (2H, m, Glu*H*-*γ*), 1.99-1.79 (2H, m, Glu*H*-*β*); ^{13}C NMR (125 MHz, DMSO-d_6): δ 172.6 (C=O), 172.3 (C=O), 171.6 (C=O), 171.2 (C4), 170.9 (C=O), 163.9 (C2), 159.7 (C6), 136.0 (C1), 132.5 (C4′), 129.5 (C3′,5′), 129.0 (C2′,6′), 116.7 (C5), 53.7 (GluC-*α*), 52.6 (CysC-*α*), 42.0 (GlyC-*α*), 32.1 (GluC-*γ*), 31.8 (CysC-*β*), 27.4 (GluC-*β*); MS-ES$^+$ (*m/z*) 537 ([M + H + MeCN]$^+$, ^{35}Cl, 14%), 498 ([M + H]$^+$, ^{37}Cl, 36), 496 ([M + H]$^+$, ^{35}Cl, 100), 367 (20); HRMS-ES$^+$ (*m/z*) Calcd for $C_{20}H_{23}O_6N_5{}^{35}ClS$ [M + H]$^+$: 496.1052, found 496.1059.

S-(4-Chloro-2-phenylpyrimidin-6-yl)-γ-glutamylcysteine (FγEC) was collected as a white fluffy solid (12.8 mg, 0.12 mmol, 55 %); ^{1}H NMR (700 MHz, DMSO-d_6): δ 8.59 (1H, br d, J = 7.7, N*H*), 8.39-8.37 (1H, m, *H*-2′,6′ on Ph), 7.61 (1H, s, *H*-5), 7.56-7.54 (1H, m, *H*-4′ on Ph), 7.51-7.48 (2H, m, *H*-3′,5′ on Ph), 4.56-4.53 (1H, m, Cys*H*-*α*), 3.96 (1H, dd, J = 13.8, 4.6, 1H of the Cys*H*-*β*), 3.34-3.28 (2H, m, 1H of the Cys*H*-*β* and Glu*H*-*α*), 2.26 (2H, t, J = 7.7, Glu*H*-*γ*), 1.90-1.79 (2H, m, Glu*H*-*β*); ^{13}C NMR (176 MHz, DMSO-d_6): δ 172.4 (Cquat), 172.2 (Cquat), 172.1 (Cquat), 170.2 (Cquat), 163.6 (C2), 159.5 (C6), 135.8 (C1′), 132.3 (C4′), 129.2 (C3′,5′), 128.7 (C2′,6′), 116.5 (C5), 53.6 (GluC-*α*), 52.2 (CysC-*α*), 32.2 (GluC-*γ*), 31.5 (CysC-*β*), 27.5 (GluC-*β*); MS-ES$^+$ (*m/z*) 441 ([M + H]$^+$, ^{37}Cl, 40%), 439 ([M + H]$^+$, ^{35}Cl, 100); HRMS-ES$^+$ (*m/z*) Calcd for $C_{18}H_{20}N_4O_5{}^{35}Cl^{32}S$ [M + H]$^+$: 439.0838, found 439.0832.

S-(4-Chloro-2-phenylpyrimidin-6-yl)-N-malonylcysteine (FMC). The putative *N*-malonyl-cysteine conjugate (FMC) was purified from Arabidopsis cells fed with 300 µM fenclorim for 24 h, by preparative HPLC using the conditions described for the synthesis of conjugates. MS-ES$^+$ (*m/z*) 398 ([M + H]$^+$, ^{37}Cl, 43%), 396 ([M + H]$^+$, ^{35}Cl, 100); HRMS-ES$^+$ (*m/z*) Calcd for $C_{16}H_{14}N_3O_5{}^{35}Cl^{32}SNa$ [M + Na]$^+$: 418.0235, found 418.0236.

Methyl-esterification of FMC. FMC (0.32 mg, 8.08 nmol) in anhydrous methanol (80 µl) was reacted for 1 h with 120 µL of a freshly prepared ethereal solution of diazomethane [S1]. Analysis by MS-ES$^+$ (*m/z*) 426 ([M + H]$^+$, ^{37}Cl, 39%), 424 ([M + H]$^+$, ^{35}Cl, 100), 223 (10), 202 (15); HRMS-ES$^+$ (*m/z*) Calcd for $C_{18}H_{18}N_3O_5{}^{35}Cl^{32}SNa$ [M + Na]$^+$: 446.0548, found 446.0548.

S-(4-N-Acetylcysteine-2-phenylpyrimidin-6-yl)-glutathione (FACG). Prepared according to the standard procedure using FG (1.0 mg, 2.1 μmol) and *N*-acetyl-cysteine (0.7 mg, 4.2 μmol). MS-ES$^+$ (*m/z*) 623 ([M + H]$^+$, 27%), 494 (12), 365 (11), 350 (25), 298 (25), 221 (100), 199 (35), 145 (59), 130 (35), 121 (25); HRMS-ES$^+$ (*m/z*) Calcd for $C_{25}H_{31}N_6O_9{}^{32}S_2$ [M + H]$^+$: 623.1589, found 623.1591.

S-(4-N-Acetylcysteine-2-phenylpyrimidin-6-yl)-cysteine (FACC). Prepared according to the standard procedure using FC (0.9 mg, 3.0 μmol) and *N*-acetyl-cysteine (1.0 mg, 6.0 μmol); MS-ES$^+$ (*m/z*) 437 ([M + H]$^+$, 100%); HRMS-ES$^+$ (*m/z*) Calcd for $C_{18}H_{21}N_4O_5{}^{32}S_2$ [M + H]$^+$: 437.0948, found 437.0952.

4-Chloro-6-(methylthio)-2-phenylpyrimidine (CMTP). To a solution of fenclorim (1.35 g, 6.00 mmol) in anhydrous DMF (12 m1) was added sodium thiomethoxide (0.082 g, 1.17 mmol) with stirring under an atmosphere of argon for 30 min. Distilled water (12 m1) was added followed by ethyl acetate (10 m1) and the resulting solution washed with brine (2 × 10 m1). The combined aqueous phases were washed with ethyl acetate (2 × 10 ml). The organic layers were dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by flash column chromatography on silica gel (chloroform: petroleum ether 40-60 °C 1:19) gave CMTP as a white solid (0.088 g, 0.37 mmol, 32%); mp 78-79 °C (lit. 80-81 °C petroleum ether [S2]), together with 4,6-bis(methylthio)-2-phenylpyrimidine as a white solid (0.060 g, 0.24 mmol, 21%). The CMTP was analyzed by ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.46-8.44 (2H, m, *H*-2′,6′ on Ph), 7.51-7.46 (3H, m, *H*-3′,4′,5′ on Ph), 7.10 (1H, s, *H*-5), 2.68 (3H, s, C*H*$_3$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 172.3 (C6), 164.1 (C2), 159.5 (C4), 136.1 (C1′), 131.5 (C4′), 128.6 (ArC), 128.5 (ArC), 115.4 (C5), 12.8 (CH_3); MS-ES$^+$ (*m/z*) 239 ([M + H]$^+$, ^{37}Cl, 38%), 237 ([M + H]$^+$, ^{35}Cl, 100); HRMS-ES$^+$ (*m/z*) Calcd for $C_{11}H_{10}N_2{}^{35}Cl^{32}S$ [M + H]$^+$: 237.0248, found 237.0248.

S-(4-(methylthio)-2-phenylpyrimidin-6-yl)-N-malonylcysteine (MPMC) HRMS-ES$^+$ (*m/z*) Calcd for $C_{17}H_{18}N_3O_5{}^{32}S_2$ [M + H]$^+$: 408.0682, found 408.0688.

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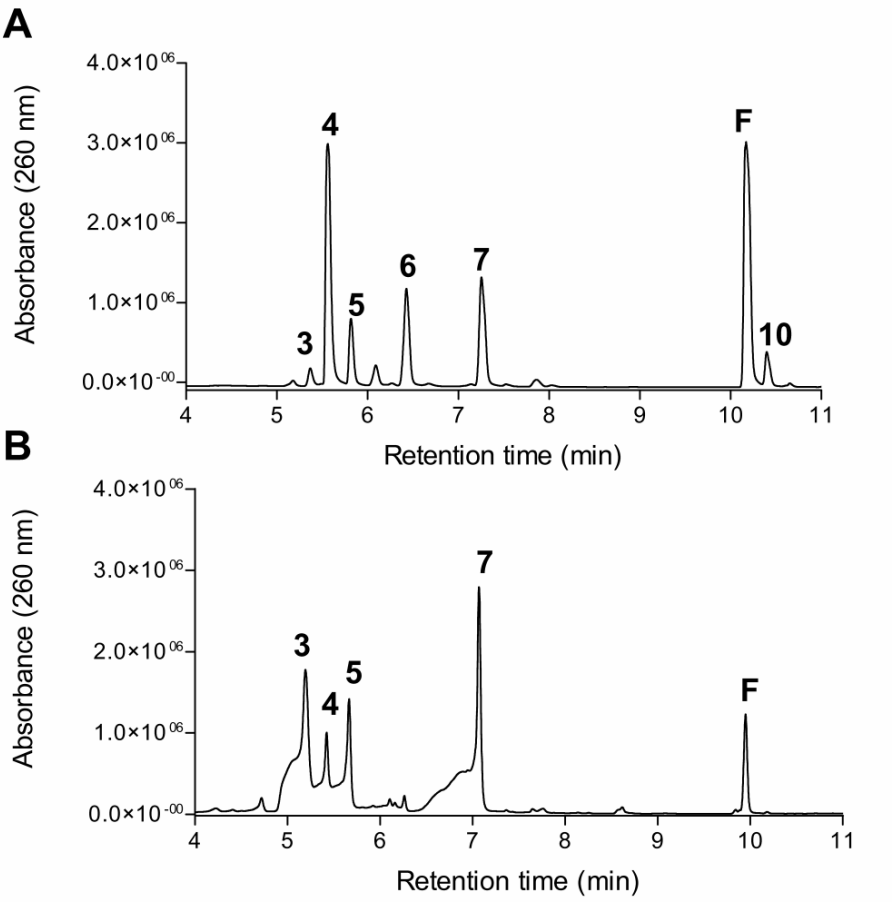
SUPPLEMENTAL FIGURE LEGEND

Supplemental fig. Profile of metabolites (A) in Arabidopsis root cultures 24 h after treatment with 100 μM fenclorim and (B) in rice shoots grown on agar containing 100 μM fenclorim for 4 days. Numbered peaks correspond to metabolites identified in fig. 3B.


A
Absorbance (260 nm)
4.0×10^06
3.0×10^06
2.0×10^06
1.0×10^06
0.0×10^-00
3
4
5
6
7
F
10
4
5
6
7
8
9
10
11
Retention time (min)
B
Absorbance (260 nm)
4.0×10^06
3.0×10^06
2.0×10^06
1.0×10^06
0.0×10^-00
3
4
5
7
F
4
5
6
7
8
9
10
11
Retention time (min)