**The Asian corn borer *Ostrinia furnacalis* feeding increases the direct and indirect defense of mid-whorl stage commercial maize in the field**

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**Figure S1** Volcano plots of differential expression genes (DEGs) in maize induced by *O. furnacalis* attack for 2, 4, 12 and 24 h compared with control. The horizontal axis shows the log2 fold change between the two samples. The log10 (*P*-value) is plotted on the vertical axis. Each gene is represented by one point on the graph. Red and green points are DEGs that pass the screening threshold; those red and green points represent up- and down-regulated genes, respectively. Black points represent genes with no differences.

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**Figure S2** Correlations between RNA-seq and qRT-PCR gene expression data. A linear regression line (green), a liner regression model, correlation coefficients and the y = x line (black, dotted) are also shown in each panel. Shading represents the 95% confidence interval for each regression line. For RNA-seq data, fold-changes of gene expression level (fragments per kilobase of transcript per million mapped reads, FPKMs) were normalized to the FPKM of the first replicate of control. For qRT-PCR data, fold-changes of gene expression level were calculated using the 2−ΔΔCT method. Fold change were log2 transformed.

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**Figure S3** Effects of *O. furnacalis* feeding on salicylic acid (SA) and abscisic acid (ABA) biosynthesis. (a) SA and (b) ABA concentration (ng g-1 FW) in maize leaves. (c) Relative expression changes of the gene involved in SA and ABA biosynthesis pathway. Values are presented as fold change relative to control (0 h). Mean±SE, n=3. ICS, isochorismate synthase; PAL, phenylalanine ammonia lyase; NCED, 9-cis-epoxycarotenoid dioxygenaseinvolved; SDR,short chain dehydrogenase/reductase; AO, aldehyde oxidase.

**Table S1** Summary of RNA sequencing and mapping using the maize genome as the reference. 0, 2, 4, 12 and 24 indicate the time (h) of *O. furnacalis* feeding. The numerical values 1, 2, 3 indicate the different biological replicates.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Sample | Total clean reads | Total nucleotides  （nt） | Q20 percentage（%） | GC percentage（%） | Unmapped reads with RNA | Unique  mapped  reads | Multiple  Mapped  reads | Mapping  ratio |
| 0h-1 | 31162658 | 4674398700 | 94.73% | 58.87% | 29997742 | 21393592（71.32%） | 375904 （1.25%） | 72.57% |
| 0h-2 | 29540938 | 4431140700 | 94.63% | 59.03% | 27720276 | 19662731（70.93%） | 342562 （1.24%） | 72.17% |
| 0h-3 | 29055922 | 4358388300 | 94.51% | 58.20% | 26643750 | 18811646（70.60%） | 327600 （1.23%） | 71.83% |
| 2h-1 | 29848828 | 4477324200 | 94.90% | 57.49% | 28424550 | 19809077（69.69%） | 377962 （1.33%） | 71.02% |
| 2h-2 | 28311586 | 4246737900 | 94.62% | 57.95% | 26966968 | 19100530（70.83%） | 355988 （1.32%） | 72.15% |
| 2h-3 | 26006430 | 3900964500 | 94.61% | 57.86% | 24426180 | 17226166（70.52%） | 361324 （1.48%） | 72.00% |
| 4h-1 | 32547838 | 4882175700 | 94.55% | 57.89% | 31136650 | 21978741（70.59%） | 428960 （1.38%） | 71.97% |
| 4h-2 | 33787044 | 5068056600 | 94.31% | 58.13% | 32097408 | 22136586（68.97%） | 522770 （1.63%） | 70.60% |
| 4h-3 | 27342562 | 4101384300 | 94.84% | 57.83% | 26034902 | 18363767（70.54%） | 380640 （1.46%） | 72.00% |
| 12h-1 | 26729432 | 4009414800 | 94.00% | 57.51% | 25362750 | 17332306（68.34%） | 405792 （1.60%） | 69.94% |
| 12h-2 | 28602978 | 4290446700 | 94.44% | 57.75% | 27407692 | 18972961（69.22%） | 470754 （1.72%） | 70.94% |
| 12h-3 | 25831022 | 3874653300 | 94.54% | 57.25% | 24595036 | 15832590（64.37%） | 367326 （1.49%） | 65.87% |
| 24h-1 | 28060930 | 4209139500 | 94.82% | 58.04% | 26472406 | 18478298（69.80%） | 451352 （1.70%） | 71.51% |
| 24h-2 | 17888922 | 2683338300 | 94.72% | 58.11% | 16763888 | 11514708 （68.69%） | 258724 （1.54%） | 70.23% |
| 24h-3 | 27994842 | 4199226300 | 94.67% | 58.10% | 26081366 | 18169453（69.66%） | 461538 （1.77%） | 71.43% |

**Table S2** The common pathways of DEGs in the transcriptome of maize induced by *O. furnacalis* infestation for different period of time.

|  |  |
| --- | --- |
| **Pathway ID** | **Pathway** |
| ko00010 | Glycolysis / Gluconeogenesis |
| ko00030 | Pentose phosphate pathway |
| ko00051 | Fructose and mannose metabolism |
| ko00071 | Fatty acid degradation |
| ko00195 | Photosynthesis |
| ko00196 | Photosynthesis - antenna proteins |
| ko00350 | Tyrosine metabolism |
| ko00360 | Phenylalanine metabolism |
| ko00400 | Phenylalanine, tyrosine and tryptophan biosynthesis |
| ko00402 | Benzoxazinoid biosynthesis |
| ko00480 | Glutathione metabolism |
| ko00500 | Starch and sucrose metabolism |
| ko00520 | Amino sugar and nucleotide sugar metabolism |
| ko00592 | alpha-Linolenic acid metabolism |
| ko00620 | Pyruvate metabolism |
| ko00630 | Glyoxylate and dicarboxylate metabolism |
| ko00710 | Carbon fixation in photosynthetic organisms |
| ko00860 | Porphyrin and chlorophyll metabolism |
| ko00940 | Phenylpropanoid biosynthesis |
| ko00941 | Flavonoid biosynthesis |
| ko00945 | Stilbenoid, diarylheptanoid and gingerol biosynthesis |
| ko01200 | Carbon metabolism |

**Table S3** The developmental time and fecundity of *O. furnacalis* reared on the maize leaves previously infested by *O. furnacalis* for 0 and 24 h.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Stage or sex | 0 h | |  | 24 h | | *P* |
| n | Mean±SE |  | n | Mean±SE |
| Egg (d) | 119 | 4.00±0.00 |  | 117 | 4.00±0.00 | - |
| L1+L2 (d) | 116 | 4.56±0.05 b |  | 108 | 5.24±0.08 a | **<0.0001** |
| L3 (d) | 110 | 3.06±0.13 |  | 97 | 3.18±0.16 | 0.5658 |
| L4 (d) | 106 | 4.45±0.12 |  | 91 | 4.47±0.15 | 0.9262 |
| L5 (d) | 95 | 7.29±0.16 b |  | 77 | 10.38±0.37 a | **< 0.0001** |
| Larva (d) | 95 | 19.26±0.23 b |  | 77 | 22.95±0.39 a | **< 0.0001** |
| Pupa (d) | 92 | 6.18±0.08 b |  | 74 | 6.70±0.07 a | **< 0.0001** |
| Female (d) | 47 | 4.28±0.14 |  | 38 | 4.23±0.24 | 0.8605 |
| Male (d) | 45 | 4.27±0.14 |  | 36 | 3.94±0.16 | 0.1321 |
| TPOP (d) | 49 | 30.47±0.36 b |  | 34 | 35.90±0.58 a | <0.0001 |
| Fecundity (eggs/female) | 47 | 107.43±5.76 |  | 38 | 94.01±8.42 | 0.1873 |

L1, 1st instar; L2, 2nd instar; L3, 3rd instar; L4, 4th instar; L5, 5th instar; TPOP, total pre-oviposition period. Means in the same row followed by different letters are significantly different (*P* < 0.05) using the Tukey–Kramer procedure.

**Table S4** The meteorological parameters during the maize growth period.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Date | Maximum  temperature (℃) | Minimum temperature (℃) | Average temperature (℃) | Average humidity (%) | Atmospheric pressure (mbar) | Solar  radiation (KWpm2) | Wind speed (mps) |
| 2016/7/21 | 26.37 | 22.77 | 24.35 | 96.75 | 998.95 | 0.06 | 0.28 |
| 2016/7/22 | 30.54 | 23.03 | 26.45 | 88.36 | 999.49 | 0.20 | 0.34 |
| 2016/7/23 | 32.58 | 25.96 | 28.87 | 88.65 | 999.48 | 0.15 | 0.60 |
| 2016/7/24 | 32.99 | 24.86 | 27.50 | 87.89 | 1002.47 | 0.14 | 0.66 |
| 2016/7/25 | 31.01 | 24.45 | 27.07 | 91.79 | 1000.60 | 0.10 | 0.45 |
| 2016/7/26 | 31.53 | 23.33 | 27.32 | 71.83 | 1002.27 | 0.27 | 0.85 |
| 2016/7/27 | 33.01 | 22.84 | 27.80 | 80.82 | 1000.32 | 0.23 | 0.98 |
| 2016/7/28 | 32.47 | 21.64 | 27.29 | 81.80 | 999.94 | 0.24 | 0.62 |
| 2016/7/29 | 33.77 | 26.04 | 29.43 | 81.67 | 1002.53 | 0.20 | 0.98 |
| 2016/7/30 | 32.15 | 23.94 | 27.60 | 86.04 | 1006.01 | 0.12 | 1.38 |
| 2016/7/31 | 29.15 | 22.60 | 25.87 | 86.24 | 1007.09 | 0.13 | 0.96 |
| 2016/8/1 | 30.92 | 24.82 | 27.12 | 86.60 | 1007.25 | 0.12 | 0.66 |
| 2016/8/2 | 32.02 | 22.38 | 27.18 | 80.02 | 1007.93 | 0.21 | 0.78 |
| 2016/8/3 | 33.54 | 23.15 | 28.58 | 78.58 | 1007.96 | 0.23 | 0.40 |
| 2016/8/4 | 32.44 | 25.04 | 28.49 | 80.45 | 1008.11 | 0.20 | 0.69 |
| 2016/8/5 | 32.73 | 23.88 | 28.09 | 81.89 | 1007.70 | 0.18 | 0.44 |
| 2016/8/6 | 31.06 | 25.04 | 28.25 | 83.76 | 1004.27 | 0.13 | 0.45 |
| 2016/8/7 | 29.97 | 22.84 | 26.10 | 84.33 | 1005.96 | 0.15 | 0.96 |
| 2016/8/8 | 30.42 | 21.81 | 25.99 | 74.67 | 1007.93 | 0.23 | 1.18 |
| 2016/8/9 | 30.49 | 19.88 | 25.60 | 82.70 | 1006.58 | 0.17 | 0.49 |
| 2016/8/10 | 32.61 | 24.31 | 28.54 | 82.84 | 1005.21 | 0.20 | 0.95 |
| 2016/8/11 | 34.22 | 25.82 | 29.89 | 80.95 | 1004.73 | 0.19 | 0.68 |
| 2016/8/12 | 33.39 | 24.32 | 27.93 | 90.77 | 1003.58 | 0.11 | 0.69 |
| 2016/8/13 | 29.90 | 23.03 | 26.22 | 88.96 | 1003.84 | 0.07 | 0.36 |
| 2016/8/14 | 30.09 | 21.43 | 25.65 | 79.66 | 1004.45 | 0.14 | 0.38 |
| 2016/8/15 | 25.43 | 19.87 | 23.34 | 87.96 | 1006.45 | 0.08 | 0.50 |
| 2016/8/16 | 30.63 | 17.71 | 24.29 | 80.33 | 1004.03 | 0.24 | 0.58 |
| 2016/8/17 | 27.94 | 21.99 | 25.03 | 90.03 | 1002.28 | 0.07 | 0.21 |
| 2016/8/18 | 24.68 | 22.68 | 23.97 | 98.65 | 999.88 | 0.01 | 0.20 |
| 2016/8/19 | 29.85 | 22.39 | 25.55 | 84.26 | 1003.93 | 0.20 | 0.84 |
| 2016/8/20 | 30.91 | 20.89 | 25.51 | 81.37 | 1007.19 | 0.19 | 0.40 |
| 2016/8/21 | 33.18 | 20.53 | 26.93 | 76.15 | 1007.81 | 0.23 | 0.46 |
| 2016/8/22 | 31.95 | 21.90 | 26.74 | 76.02 | 1009.77 | 0.19 | 0.39 |
| 2016/8/23 | 31.09 | 21.79 | 26.04 | 79.48 | 1005.30 | 0.14 | 0.41 |
| 2016/8/24 | 30.02 | 24.48 | 26.92 | 83.98 | 1002.69 | 0.09 | 0.25 |

**Table S5** Primers used for qRT-PCR.

|  |  |  |
| --- | --- | --- |
| Gene name | Forward primer (5′- 3′) | Reverse primer(5′- 3′) |
| *Actin* | TACCATGTTCCCTGGGATTG | GTGGCGCAATCACTTTAACC |
| *ACO31* | CGGCGTCATCCTCCTGTT | GCTTCGTTGGCTGGGTTG |
| *Bx2* | AGGCGATGCTGTCCAACC | CGAGACCGTGATCCCAAAC |
| *OPR2* | GGGAGGAAGGCAACAAGGT | GAAAGGGTAATCTGTGTAGCCAAC |
| *TPS26* | AAAATGGCGTGAGCAAGGA | CATCTCCCATTCCAACAAACG |

**Data S1** Genes detected in all samples. Gene expression levels were shown by Fragments Per Kilobase of transcript per Million mapped reads (FPKMs). All samples harvest 0, 2, 4, 12 and 24 h after *O. furnacalis* feeding; NA: no annotation.

**Data S2** All up-regulated DEGs in maize leaves induced by *O. furnacali*s feeding for 2, 4, 12 and 24 h with a cutoff of 2-fold change relative to the control. FC: Fold change. NA: no annotation. BD: numerator or denominator is below detection. Notation is the same as for Data S3

**Data S3** All down-regulated DEGs in maize leaves induced by *O. furnacali*s infestation for 2, 4, 12 and 24 h with a cutoff of 2-fold change relative to the control.

**Data S4** KEGG pathway enrichment analysis of DEGs in the transcriptome of maize induced by *O. furnacalis* infestation for different periods of time.

**Data S5** Overrepresentation analysis of each profile using the Short Time-series Expression Miner (STEM) analysis tool to identify metabolic pathways that are being regulated.