

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ijms232415796/s1>

Table S1: The primers and genes selected for the RT-qPCR analysis

| Gene | Citrus ID | Description | Forward (5' - 3') | Reverse (5' - 3') |
|-----------------|----------------------------|--|------------------------|-----------------------------|
| <i>CsPeL</i> | <i>orange1.1g009075m.g</i> | pectin lyase-like superfamily protein | GTGACCCATTTACAGGTATT | ACAGGCTCTTATTTCTCAG |
| <i>CsCLH1</i> | <i>orange1.1g020188m.g</i> | chlorophyllase 1 | TCAAGACAGTGAATAAACCA | AGATTGGAATAGCTTGTGTT |
| <i>CsACA7</i> | <i>orange1.1g039445m.g</i> | alpha carbonic anhydrase 7 | TGGAAGACCTGATTCTCTAT | CGTAAGTTTATTGGTTGTTG |
| <i>CsCAMS1</i> | <i>orange1.1g006042m.g</i> | camelliol C synthase 1 | ATCTCTACTATCCCCATCCT | GTCCTCGTAATGAATGAACT |
| <i>CsANNAT4</i> | <i>orange1.1g021607m</i> | annexin 4 | GCTTACCAATTCTCTTTGA | GCTTGCTCCTTAGATAGT |
| <i>CsGLOX</i> | <i>orange1.1g048017m</i> | glyoxal oxidase-related protein | TGTTACCTTCTGGTGATGTA | TACTCCCACCCACTAATATC |
| <i>CsCSLB04</i> | <i>orange1.1g038469m.g</i> | cellulose synthase-like B4 | TCAGACTACATTCTGATTGG | CATTTTATCATCGTCTTCG |
| <i>CsCYS1</i> | <i>orange1.1g021775m.g</i> | Cysteine Synthase C1 (β-Cyanoalanine Synthase) | GCAGCGAACCCATGTTGATGAG | GGCCACTGCTGGTCTGTCC |
| <i>CsACT</i> | <i>orange1.1g037845m.g</i> | <i>Actin</i> | TTAACCCCAAGGCCAACAGA | TCCCTCATAGATTGGTACAGTATGAGA |
| <i>CsTUB</i> | <i>orange1.1g036688m.g</i> | <i>Tubulin</i> | GCATCTTGAACCCGGTAC | ATCAATTGCGCGCCTTCAG |

Table S2: The identification of the DEGs included in each biological process (BP) and molecular function (MF) (see Table 1) belonging to clusters 12 and 14.

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Table S3: The global data obtained by RNA-Seq analysis.

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Table S4: The gene ontology (GO) analysis ($P \leq 0.05$) of the molecular function (MF) processes enriched (Up) or under-represented (Down) in the flavedo of the freshly harvested (FH) Pinalate fruit in relation to the FH parental fruit. Only the DEGs ($p \leq 0.05$) that met a cutoff of $|\text{Log}_2 \text{FoldChange}| \geq 1$ were considered in the analysis. Three biological replicates from each condition were used

| | GO.ID | Term | pgo Fisher |
|------|------------|---|---------------|
| UP | GO:0004672 | protein kinase activity | 1.20E-18 |
| | GO:0003700 | DNA binding transcription factor activit... | 2.50E-10 |
| | GO:0030247 | polysaccharide binding | 6.30E-06 |
| | GO:0043565 | sequence-specific DNA binding | 3.20E-05 |
| | GO:0005524 | ATP binding | 3.50E-05 |
| | GO:0004842 | ubiquitin-protein transferase activity | 9.90E-05 |
| | GO:0003849 | 3-deoxy-7-phosphoheptulonate synthase ac... | 1.10E-03 |
| | GO:0016702 | oxidoreductase activity, acting on singl... | 4.10E-03 |
| | GO:0016762 | xyloglucan:xyloglucosyl transferase acti... | 7.60E-03 |
| | GO:0005516 | calmodulin binding | 1.09E-02 |
| | GO:0004674 | protein serine/threonine kinase activity | 1.11E-02 |
| | GO:0042132 | fructose 1,6-bisphosphate 1-phosphatase ... | 1.27E-02 |
| | GO:0004012 | phospholipid-translocating ATPase activi... | 1.83E-02 |
| | GO:0017025 | TBP-class protein binding | 2.43E-02 |
| | GO:0008519 | ammonium transmembrane transporter activ... | 3.25E-02 |
| | GO:0031683 | G-protein beta/gamma-subunit complex bin... | 3.88E-02 |
| | GO:0019001 | guanyl nucleotide binding | 3.97E-02 |
| | GO:0004970 | ionotropic glutamate receptor activity | 4.73E-02 |
| Down | GO:0016758 | transferase activity, transferring hexos... | 4.50E-11 |
| | GO:0005506 | iron ion binding | 6.60E-09 |
| | GO:0016705 | oxidoreductase activity, acting on paire... | 7.30E-08 |
| | GO:0020037 | heme binding | 2.10E-07 |
| | GO:0004568 | chitinase activity | 5.10E-05 |
| | GO:0016747 | transferase activity, transferring acyl ... | 1.00E-04 |
| | GO:0004144 | diacylglycerol O-acyltransferase activit... | 4.60E-03 |
| | GO:0008429 | phosphatidylethanolamine binding | 5.00E-03 |
| | GO:0003700 | DNA binding transcription factor activit... | 1.25E-02 |
| | GO:0046873 | metal ion transmembrane transporter acti... | 1.53E-02 |
| | GO:0045300 | acyl-[acyl-carrier-protein] desaturase a... | 1.64E-02 |
| | GO:0004867 | serine-type endopeptidase inhibitor acti... | 2.15E-02 |
| | GO:0005544 | calcium-dependent phospholipid binding | 2.71E-02 |
| | GO:0008171 | O-methyltransferase activity | 3.03E-02 |
| | GO:0004722 | protein serine/threonine phosphatase act... | 3.54E-02 |
| | GO:0050660 | flavin adenine dinucleotide binding | 4.50E-02 |
| | GO:0016491 | oxidoreductase activity | 4.69E-02 |
| UP/ | GO:0005215 | transporter activity | 1.10E-02 |
| Down | | | 1.65E-02 |

Table S5: The constitutive differences between Navelate and Pinalate fruit. The DEGs that met a p -adjusted value ≤ 0.05 and a cut-off of $|\text{Log}_2 \text{ Fold Change}| \geq 1$ in the comparison of the freshly harvested (FH) Pinalate and Navelate fruit. The fold change expression (FC) and $\text{Log}_2 \text{ FC}$ values of the compared conditions are included. Positive numbers denote higher expression values in the first term of the comparison (Pinalate). Negative values mean higher expression values in the second term (Navelate).

See excel

Table S6: The DEGs that met a p -adjusted value ≤ 0.05 and a cut-off of $|\text{Log}_2 \text{ Fold Change}| \geq 1$ in the specific comparison indicated and highlighted in yellow (IP vs. WP, infection Pinalate vs. wounding Pinalate), green (IPA vs. WPA, infection ABA-treated Pinalate vs. wounding ABA-treated Pinalate) and orange (IN vs. WN, infection Navelate vs. wounding Navelate). The fold change expression (FC) and $\text{Log}_2 \text{ FC}$ values of the compared conditions are included. Positive numbers denote higher expression values in the first term of the comparison. Negative values mean higher expression values in the second term.

See excel

Table S7: Identification of the DEGs (p -adjusted value ≤ 0.05 and a cut-off of $|\text{Log}_2 \text{ Fold Change}| \geq 1$) included in each induced or repressed biological process (BP), molecular function (MF) and cellular component (CC) (see Table 4) in the flavedo of the infected Pinalate oranges samples (treated or not with ABA) and of the infected fruit of its parental Navelate in relation to their control wounded samples.

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Figure S1: Multiple linear regression analysis (R^2) for the comparisons made between the RT-qPCR gene expression and the RNA-Seq data for the DEGs included in Table S1. The expression of these DEGs was quantified in the freshly harvested Navelate and Pinalate fruit, and in the wounded and infected Navelate, Pinalate and ABA-treated Pinalte fruit taken from fruit at 1 dpi.

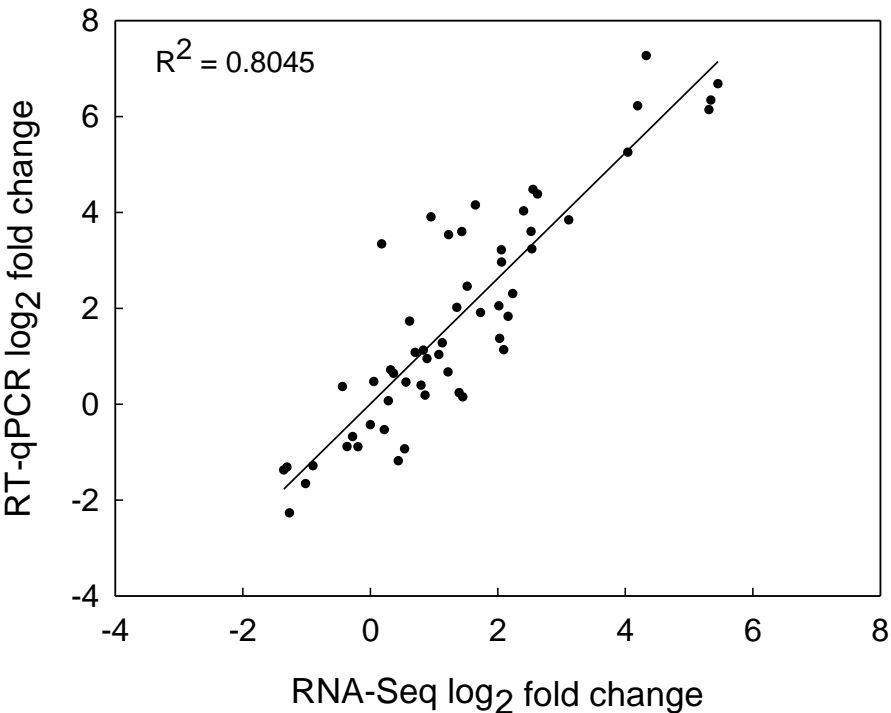


Figure S2: The 19 gene clusters identified after the gene clustering analysis of the 615 DEGs found in all the comparisons represented on the heatmap (see Figure 2C).

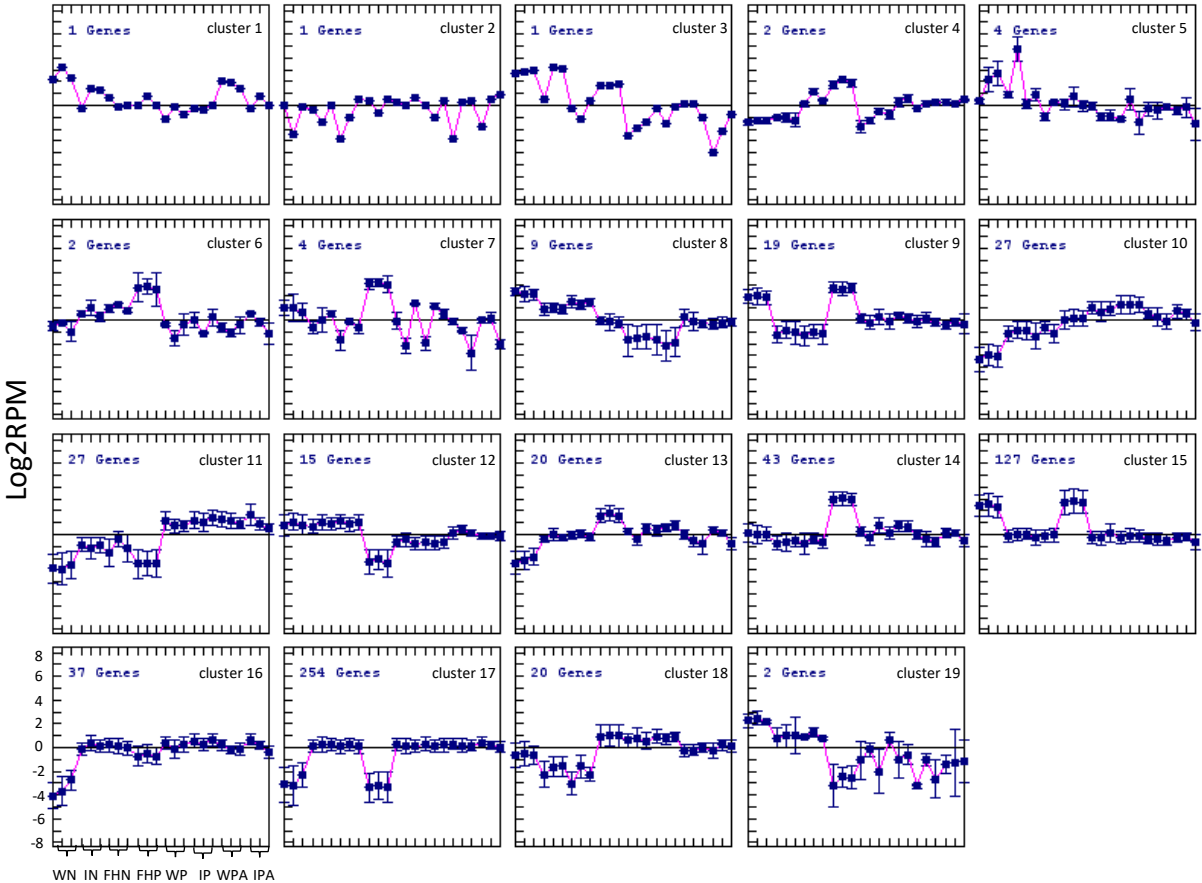


Figure S3: Relative gene expression of the selected DEGs (see Table S1) in the flavedo of the wounded (○) and infected (●) Pinalate fruit, treated or not with ABA, and of the fruit of its parental, in relation to the freshly harvested Pinalate or Navelate fruit. Changes in expression were determined in the fruit inoculated with *P. digitatum* (10^4 conidia mL⁻¹) or water (control), and left in the dark for up to 3 d at 20 °C. The error interval indicates the standard error of the determined mean value. * denotes significant differences ($p \leq 0.05$) between the infected and the control wounded samples for the same storage time.

