Supporting Information

**Elevated atmospheric CO2 concentrations alter grapevine (*Vitis vinifera*) systemic transcriptional response to European grapevine (*Lobesia botrana*) herbivory**

**Annette Reineke\*, Moustafa Selim**

Geisenheim University, Department of Crop Protection, Von-Lade-Str. 1, D-65366 Geisenheim, Germany

**\*Corresponding author:**

Annette Reineke

Geisenheim University, Department of Crop Protection

Von-Lade-Str. 1, D-65366 Geisenheim, Germany

Tel.: ++49-6722-502413

Fax.: ++49-6722-502410

email: annette.reineke@hs-gm.de

Supplementary Table S1. RNA-Seq data statistics of 24 grapevine leaf samples obtained from (1) two different levels of CO2 concentration (ambient: a; elevated: e) and (2) exposed (Lb) or not exposed (C) to *L. botrana* herbivory at (3) two grapevine growth stages (fruit development: –f; berry ripening: –b). Three RNA pools (indicated by 1, 2, 3) representing 3 biological replicates (each from one FACE ring) for each CO2 concentration, treatment and growth stage were sequenced.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sample ID** | **SRA accession** | **Total reads (raw)** | **Total reads (trimmed)** | **GC (%)** | **Overall read mapping ratio (%)** |
| aC-f1 | SAMN08093445  | 15,861,402 | 15,555,428 | 49.51 | 72.4 |
| aC-f2 | SAMN08093447  | 15,578,870 | 15,213,498 | 46.61 | 84.9 |
| aC-f3 | SAMN08093449  | 14,067,862 | 13,770,790 | 46.64 | 85.6 |
| eC-f1 | SAMN08093451  | 18,598,222 | 18,114,812 | 46.76 | 85.6 |
| eC-f2 | SAMN08093453  | 4,958,804 | 4,864,628 | 47.6 | 79.9 |
| eC-f3 | SAMN08093455  | 16,849,062 | 16,500,380 | 48.89 | 78.0 |
| aLb-f1 | SAMN08093457  | 17,016,456 | 16,702,712 | 48.78 | 70.0 |
| aLb-f2 | SAMN08093459  | 17,117,838 | 16,760,802 | 48.41 | 74.7 |
| aLb-f3 | SAMN08093461  | 14,647,170 | 14,334,176 | 46.96 | 83.3 |
| eLb-f1 | SAMN08093463  | 17,451,360 | 17,030,680 | 46.19 | 85.7 |
| eLb-f2 | SAMN08093465  | 15,676,426 | 15,342,104 | 47.24 | 83.8 |
| eLb-f3 | SAMN08093467  | 17,509,502 | 17,154,298 | 48.82 | 73.0 |
| aC-b1 | SAMN08093469  | 18,747,522 | 18,354,022 | 51.76 | 52.1 |
| aC-b2 | SAMN08093471  | 17,046,064 | 16,665,326 | 48.91 | 77.0 |
| aC-b3 | SAMN08093473  | 16,464,372 | 16,105,806 | 50.95 | 62.8 |
| eC-b1 | SAMN08093475  | 16,614,502 | 16,312,410 | 51.17 | 55.0 |
| eC-b2 | SAMN08093477  | 13,706,066 | 13,444,896 | 48.35 | 75.7 |
| eC-b3 | SAMN08093479  | 16,668,752 | 16,269,546 | 46.97 | 77.7 |
| aLb-b1 | SAMN08093481  | 17,324,706 | 16,978,112 | 50.32 | 60.6 |
| aLb-b2 | SAMN08093483  | 15,903,092 | 15,561,132 | 50.37 | 65.8 |
| aLb-b3 | SAMN08093485  | 14,584,348 | 14,217,714 | 49.73 | 68.4 |
| eLb-b1 | SAMN08093487  | 15,679,026 | 15,338,534 | 51.14 | 56.7 |
| eLb-b2 | SAMN08093489  | 12,970,936 | 12,690,658 | 48.85 | 71.9 |
| eLb-b3 | SAMN08093491  | 17,171,906 | 16,781,556 | 46.63 | 80.8 |

Supplementary Table S2. Genes involved in “plant-pathogen interaction”, “defence response” or “response to biotic stimuli” as identified by GO enrichment analysis or via KEGG database classification in grapevine plants (growth stage fruit development) as a response to *L. botrana* herbivory at elevated CO2 and ambient CO2 concentrations. IDs are according to GenBank entries, fold change of expression levels of genes in three pairwise comparisons is shown only for significant genes (*p* < 0.05). For sample codes see Supplementary Table S6. Expression of genes printed in bold letters was validated by RT-qPCR.

|  |  |  |  |
| --- | --- | --- | --- |
| **Gene\_ID** | **Protein\_ID** | **Description** | **Fold change** |
| **aLb-f vs. aC-f** | **eLb-f vs. eC-f** | **eLb-f vs. aLb-f** |
| **100266543** | **XP\_010657614.1** | **brassinosteroid insensitive 1-associated receptor kinase 1-like** | **2.627** | **2.996** |  |
| 100243573 | XP\_002278179.1 | calcium-dependent protein kinase 2 | **2.088** | **2.596** |  |
| 100248850 | XP\_002267099.1 | calcium-dependent protein kinase 28 |  | **2.365** |  |
| **100253496** | **XP\_002274476.1** | **calcium-binding allergen Ole e 8-like** | **2.533** | **4.695** |  |
| 100244152 | XP\_002283755.1 | calmodulin-like protein 11 | **2.166** |  |  |
| 100255889 | XP\_002282351.2 | caltractin | **2.062** |  |  |
| 100263254 | XP\_010649201.1 | probable calcium-binding protein CML41 | **2.974** |  |  |
| 100267648 | XP\_002273785.1 | endoplasmin homolog | **2.222** | **2.222** |  |
| **100233033** | **NP\_001267967.1** | **enhanced disease susceptibility 1 (EDS1)** | **2.494** | **3.091** |  |
| 100250236 | XP\_002283967.2 | lipase-like PAD4 | **6.804** | **6.970** |  |
| 100253457 | XP\_002280786.1 | lipase-like PAD4 | **6.463** | **11.268** |  |
| 100257078 | XP\_002280729.1 | lipase-like PAD4 | **7.493** | **10.771** |  |
| 100241155 | XP\_002281195.1 | probable disease resistance protein At1g12280 | **2.109** | **2.195** |  |
| 104881039 | XP\_010657950.1 | probable disease resistance protein At4g27220 | **2.376** | **2.924** |  |
| 100853252 | XP\_010652660.1 | probable disease resistance protein At5g66900 |  | **3.037** |  |
| 100854398 | XP\_010658280.1 | putative disease resistance protein At1g50180 |  | **-2.161** |  |
| 100244083 | XP\_002276170.1 | protein SGT1 homolog |  | **2.107** |  |
| 100252764 | XP\_002268128.2 | uncharacterized aarF domain-containing protein kinase At1g79600, chloroplastic-like |  | **-2.037** |  |
| 100854803 | XP\_003633333.1 | disease resistance protein RPM1-like |  | **2.044** |  |
| **100256051** | **XP\_002275317.3** | **disease resistance protein RPM1-like** | **3.582** | **3.055** |  |
| 100852949 | XP\_003632405.1 | disease resistance protein RPM1-like | **2.058** | **2.540** |  |
| 100267151 | XP\_010657373.1 | G-type lectin S-receptor-like serine/threonine-protein kinase At4g03230 | **2.6289** | **2.225** |  |
| 100246525 | XP\_002274785.1 | major allergen Pru av 1 | **3.414** |  | **-2.045** |
| 100261887 | XP\_002274535.1 | major allergen Pru av 1 | **2.661** |  | **-2.814** |
| 100256795 | XP\_002274617.1 | major allergen Pru av 1 |  |  | **-2.796** |
| 100233053 | NP\_001267997.1 | mildew resistance locus o 15 (MLO15)  | **2.362** | **2.524** |  |
| 100233063 | NP\_001268013.1 | MLO11 protein | **3.274** | **3.051** |  |
| **100233061** | **XP\_002276608.1** | **MLO-like protein 10** | **2.225** | **2.362** |  |
| **100267074** | **NP\_001267956.1** | **pathogenesis-related protein 10.3** | **3.891** |  |  |
| **100258426** | **XP\_002273815.2** | **pathogenesis-related protein 10.8** |  | **-3.257** |  |
| **100267750** | **XP\_002283780.1** | **allene oxide synthase** |  | **2.707** |  |

Supplementary Table S3. Genes identified by GO enrichment analysis in grapevine plants (growth stage berry ripening) as a response to *L. botrana* herbivory at elevated CO2 and ambient CO2 concentrations. IDs are according to GenBank entries, fold change of expression levels of genes in two pairwise comparisons is shown only for significant genes (*p* < 0.05). For sample codes see Supplementary Table S6.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **GO Term(s)** | **Gene\_ID** | **Protein\_ID** | **Description** | **Fold change** |
| **eLb-b vs. eC-b** | **eLb-b vs. aLb-b** |
| GO:0003700 | 100247374 | XP\_002276500.1 | transcription factor BEE 3 | **2.272** |  |
| GO:0003700 | 100264451 | XP\_002279570.1 | zinc finger protein ZAT10 | **-2.186** |  |
| GO:0003677 | 100265533 | XP\_002284384.1 | zinc finger protein ZAT11 |  | **-2.636** |
| GO:0003700 | 100241331 | XP\_010653659.1 | transcription elongation factor SPT6 |  | **2.133** |
| GO:0003700; GO:0003677 | 100242144 | XP\_002276215.1 | ethylene-responsive transcription factor 4 |  | **-2.254** |
| GO:0003700; GO:0003677 | 100243417 | XP\_002279760.1 | ethylene-responsive transcription factor 12 |  | **-2.164** |
| GO:0003700; GO:0003677 | 100254640 | XP\_002270581.2 | ethylene-responsive transcription factor 2 |  | **-2.031** |

Supplementary Table S4. Expression of eight genes involved in grapevine’s response (growth stage fruit development) to *L. botrana* herbivory at elevated CO2 and ambient CO2 concentrations. Expressions were assessed by RT-qPCR in three biological replicates per treatment (herbivory and control) and CO2 concentration. The normalized relative fold change of expression levels in three pairwise comparisons is shown with the 95% confidence interval. Significant differences in expression ratios at *p* < 0.05 are indicated by an asterisk. Significant differences in expression ratios after Bonferroni corrections are indicated by two asterisks. For sample codes see Supplementary Table S6.

|  |  |  |
| --- | --- | --- |
|  |  | **Ratio** |
| **Gene ID** | **Gene**  | **aLb-f vs. aC-f (95% CI)** | **eLb-f vs. eC-f (95% CI)** | **aLb-f vs. eLb-f (95% CI)** |
| 100266543 | brassinosteroid insensitive 1-associated receptor kinase 1-like (cdpk1) | 1.68 (0.5; 5.7) | 2.40 (1.6; 3.6) \* | 0.78 (0.3; 2.5) |
| 100253496 | calcium-binding allergen Ole e 8-like (cba8) | 2.91 (0.9; 9.4) | 5.36 (2.8; 1.0) \*\* | 0.73 (0.2; 2.4) |
| 100233033 | enhanced disease susceptibility 1 (eds1) | 2.48 (0.8; 7.3) | 3.46 (1.7; 7.2) \* | 0.90 (0.3; 2.7) |
| 100256051 | disease resistance protein RPM1-like (drp1) | 1.87 (0.4; 8.1) | 2.59 (2.1; 3.2) \*\* | 0.82 (0.2; 3.5) |
| 100233061 | mildew resistance locus o 10 (mlo10)  | 3.06 (1.8; 5.3) \* | 3.16 (2.2; 4.6) \*\* | 0.94 (0.7; 1.3) |
| 100267074 | pathogenesis-related protein 10.3 (pr10.3) | 1.02 (2.4; 4.3) \* | 3.95 (5.8; 2.7) |  1.8 (0.3; 1.2) |
| 100258426 | pathogenesis-related protein 10.8 (pr10.8) | 0.60 (1.2; 2.9) | 4.7 (3.6; 6.1) \*\* | 1.2 (0.2; 6.8) |
| 100267750 | allene oxide synthase (aos) | 6.82 (2.9; 1.6) \* | 4.09 (2.9; 5.8) \*\* | 1.57 (0.9; 2.8) |

Supplementary Table S5. Expression of eight genes involved in grapevine’s response (growth stage berry ripe for harvest) to *L. botrana* herbivory at elevated CO2 and ambient CO2 concentrations. Expressions were assessed by RT-qPCR in three biological replicates per treatment (herbivory and control) and CO2 concentration. The normalized relative fold change of expression levels in three pairwise comparisons is shown with the 95% confidence interval. No significant differences in expression ratios at *p* < 0.05 were evident. For sample codes see Supplementary Table S6.

|  |  |  |
| --- | --- | --- |
|  |  | **Ratio** |
| **Gene ID** | **Gene**  | **aLb-b vs. aC-b (95% CI)** | **eLb-b vs. eC-b (95% CI)** | **aLb-b vs. eLb-b (95% CI)** |
| 100266543 | brassinosteroid insensitive 1-associated receptor kinase 1-like (cdpk1) | 1.01 (0.7; 1.5) | 1.14 (0.7; 1,8) | 0.76 (0.5; 1.1) |
| 100253496 | calcium-binding allergen Ole e 8-like (cba8) | 1.08 (0.4; 2.9) | 1.54 (0.7; 3.2) | 0.63 (0.3; 1.3) |
| 100233033 | enhanced disease susceptibility 1 (eds1) | 0.85 (0.4; 1.8) | 1.29 (0.6; 2.7) | 0.61 (0.2; 1.3) |
| 100256051 | disease resistance protein RPM1-like (drp1) | 0.79 (0.2; 2.5) | 1.10 (0.6; 1.9) | 0.59 (0.2; 1.7) |
| 100233061 | mildew resistance locus o 10 (mlo10)  | 0.78 (0.4; 1.5) | 1,11 (0.9; 1.4) | 0.84 (0.6; 1.1) |
| 100267074 | pathogenesis-related protein 10.3 (pr10.3) | 1.0 (0.3; 3.9) | 1.07 (0.3; 3.6) | 0.99 (0.2; 4.0) |
| 100258426 | pathogenesis-related protein 10.8 (pr10.8) | 0.5 (0.05; 5.6) | 0.90 (0.2; 3.3) | 1.44 (0.5; 3.7) |
| 100267750 | allene oxide synthase (aos) | 0.80 (0.3; 1.8) | 1.54 (0.8; 2.9) | 0.47 (0.2; 1.1) |

Supplementary Table S6. Weather conditions during the experimental periods conducted in the Geisenheim VineyardFACE facility in July and September 2015.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Date 2015** |  **Air temperature [°C]**  | **Soil temperature [°C]** | **Precipitation** | **Evaporation** | **Sunshine** |
| Daily average | Max. | Min. | Daily average5 cm | Daily average10 cm | Daily average20 cm | Daily average50 cm | Daily average100 cm | Daily sum[mm] | Haude - Gras [mm] | Daily sum[hours] |
| 15. Jul | 22.6 | 29.4 | 16.1 | 26.7 | 26.1 | 24.6 | 22.6 | 21.1 | 0.0 | 5.8 | 9.6 |
| 16. Jul | 25.4 | 32.6 | 18.0 | 29.5 | 28.6 | 26.8 | 23.7 | 21.1 | 0.0 | 8.3 | 13.7 |
| 17. Jul | 27.3 | 35.3 | 20.0 | 29.4 | 28.7 | 27.5 | 24.7 | 21.4 | 0.8 | 11.2 | 8.9 |
| 18. Jul | 25.0 | 31.1 | 19.2 | 29.2 | 28.8 | 27.8 | 25.3 | 21.8 | 0.0 | 7.5 | 8.6 |
|   |  |  |  |  |  |  |  |  |  |  |  |
|   |  |  |  |  |  |  |  |  |  |  |  |
| 27. Sep | 13.9 | 18.8 | 9.2 | 13.6 | 14.0 | 14.7 | 15.5 | 16.3 | 0.0 | 3.0 | 9.0 |
| 28. Sep | 12.8 | 18.3 | 6.5 | 12.6 | 13.0 | 13.8 | 15.1 | 16.2 | 0.0 | 2.8 | 10.2 |
| 29. Sep | 13.4 | 18.9 | 6.9 | 12.6 | 12.9 | 13.7 | 14.8 | 16.1 | 0.0 | 2.9 | 8.0 |
| 30. Sep | 13.3 | 17.9 | 9.6 | 13.2 | 13.5 | 14.0 | 14.8 | 15.9 | 0.0 | 2.8 | 10.5 |

Supplementary Table S7. Description of grapevine treatments (two CO2 concentrations, with and without *L. botrana* herbivory, two different grapevine phenological stages), and the resulting pairs of comparison with respective research questions.

|  |  |  |  |
| --- | --- | --- | --- |
| **Code** | **CO2 concentration** | **Herbivory** | **Grapevine phenological stage** |
| aC-f | ambient (ca. 400 ppm) | no | fruit development |
| aC-b | berries ripe for harvest |
| eC-f | elevated (ca. 450 ppm) | no | fruit development |
| eC-b | berries ripe for harvest |
| aLb-f | ambient (ca. 400 ppm) | yes | fruit development |
| aLb-b | berries ripe for harvest |
| eLB-f | elevated (ca. 450 ppm) | yes | fruit development |
| eLb-b | berries ripe for harvest |
|  |  |
| **Pair** | **Question** |
| aC-f vs. aLb-f/aC-b vs. aLb-b | How do grapevine plants respond to *L. botrana* herbivory under ambient CO2 concentration (at growth stages fruit development or berry ripening)? |
| eC-f vs. eLb-f/eC-b vs. eLb-b | How do grapevine plants respond to *L. botrana* herbivory under elevated CO2 concentration (at growth stages fruit development or berry ripening)? |
| aC-f vs. eC-f/aC-b vs. eC-b | How do grapevine plants respond to elevated CO2 concentration (at growth stages fruit development or berry ripening)? |
| aLb-f vs. eLb-f/aLb-b vs. eLb-b | How do grapevine plants respond to elevated CO2 concentration under *L. botrana* herbivory (at growth stages fruit development or berry ripening)? |

Supplementary Table S8. Primer information for grapevine *L. botrana* herbivory responsive genes and two housekeeping genes (GADPH and cyclophilin) used for validation of RNA-Seq data by RT-qPCR.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Gene ID** | **Gene**  | **Primer Sequence Forward** | **Primer Sequence Reverse** | **Amplicon (bp)** | **Efficiency (%)** | **R2** |
| 100266543 | brassinosteroid insen-sitive 1-associated receptor kinase 1-like (cdpk1) | 5’-AGGGGGCTTGCTTATTTGCATG-3’ | 5’-ATGCTTGCAGCCTTCACATCAC-3’ | 71 | 104.6 | 0.995 |
| 100253496 | calcium-binding aller-gen Ole e 8-like (cba8) | 5’-AGTTGCACGCGGTGTTCAAG-3’ | 5’-ACTCCTCGAAATTGACGCAACC-3’ | 111 | 108.5 | 0.966 |
| 100233033 | enhanced disease susceptibility 1 (eds1) | 5’-ACCCGTGGCTTTGCTATT-3’ | 5’-GGAAAGGTACTAGCATCCATCTT-3’ | 104 | 95.7 | 0.998 |
| 100256051 | disease resistance protein RPM1 (drp1) | 5’-AGTTCGCACAGAAGCTTATGCC-3’ | 5’-TGCTTAATGATCGCGAGCCTTG-3’ | 150 | 100.5 | 0.994 |
| 100233061 | mildew resistance locus o 10 (mlo10)  | 5’-GGGCATCCATAATTCCCTTAGT-3’ | 5’-GGACCACTGCATGTCTTTCT-3’ | 105 | 85.4 | 0.986 |
| 100267074 | pathogenesis-related protein 10.3 (pr10.3) | 5’-GATGTTTTGACGAGCGGCATTG-3’ | 5’-TCTTTGCCGCCCTTAACGTG-3’ | 149 | 114.0 | 0.986 |
| 100258426 | pathogenesis-related protein 10.8 (pr10.8) | 5’-ATGGGTGTCACCAGTTTCACAC-3’ | 5’-AGCTTTGGGATCAGGTTGTTGG-3’ | 101 | 80.1 | 0.950 |
| 100267750 | allene oxide synthase (aos) | 5’-TTGCCACAACCTCCTTTTCGC-3’ | 5’-ATTGGGTGTGCAGTTTCACTCC-3’ | 113 | 108.9 | 0.966 |
| 100233024 | GAPDH | 5’-TCAAGGTCAAGGACTCTAACACC-3’ | 5’-CCAACAACGAACATAGGAGCA-3’ | 226 | 104.3 | 0.999 |
| EC969926 | cyclophilin | 5’-GGAGCCTGAGCCTACCTTCTC-3’ | 5’-GTGTTCGGCCAGGTGGTAGA-3’ | 66 | 99.7 | 0.989 |



Supplementary Fig. S1: (A) Aerial view (picture by Winfried Schönbach) of the Geisenheim VineyardFACE experimental site showing localization of the three ambient CO2 (designated as a1, a2 and a3) and three elevated CO2 (designated as e1, e2 and e3) grapevine FACE rings. One ring has a diameter of 12 m. (B) Schematic illustration of one VineyardFACE ring planted with two different grapevine varieties (green dots: cv. Riesling and red dots: cv. Cabernet Sauvignon). Distance between rows is 1.80 m and between vines 0.90 m. (C) Detail of the Geisenheim VineyardFACE facility (picture by Winfried Schönbach).

Supplementary Fig. S2a: Daily CO2 concentration measured in July 2015 at a height of 1.7 m in Geisenheim VineyardFACE rings with ambient (blue dots) and elevated (red dots) CO2 concentration including standard error. CO2 concentrations were measured in 20 min intervals between 4:20 and 20:00.

Supplementary Fig. S2b: Daily CO2 concentration measured from mid-August to end of September 2015 at a height of 1.7 m in Geisenheim VineyardFACE rings with ambient (blue dots) and elevated (red dots) CO2 concentration including standard error. CO2 concentration was measured in 20 min intervals between 5:20 and 19:00.