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Data Article

Plant and fungus transcriptomic data from grapevine berries undergoing artificially-induced noble rot caused by *Botrytis cinerea*Arianna Lovato, Sara Zenoni, Giovanni Battista Torielli, Teresa Colombo¹, Elodie Vandelle*, Annalisa Polverari**

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ABSTRACT

Noble rot is a latent infection of grape berries caused by the necrotrophic fungus *Botrytis cinerea*, which develops under specific climatic conditions. The infected berries undergo biochemical and metabolic changes, associated with a rapid withering, which altogether offer interesting organoleptic features to sweet white wines. In this paper, we provide RNAseq datasets (raw and normalized counts as well as differentially expressed genes lists) of the transcriptome profiles of both grapevine berries (*Vitis vinifera* cv. Garganega) and *B. cinerea* during the establishment of noble rot, artificially induced in controlled conditions. The sequencing data are available in the NCBI GEO database under accession number GSE116741. These data were exploited in a comprehensive meta-analysis of gene expression during noble rot infection, gray mold and post-harvest withering. This highlighted an important common transcriptional reprogramming in different botrytized grape berry varieties and led to the identification of key genes specifically modulated during noble rot infection, which are described in the article entitled "Specific molecular interactions between *Vitis vinifera* and *Botrytis cinerea* are required for noble rot development in grape berries" Lovato et al., 2019.

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Specifications table

Subject area	Plant Biology
More specific subject area	Plant-pathogen interaction
Type of data	Raw and normalized counts; differentially expressed genes
How data was acquired	Sequencing data were acquired through Illumina HiSeq500 sequencing of TruSeq libraries prepared from RNA extracted from <i>Vitis vinifera</i> cv. Garganega berries artificially botrytized <i>in vitro</i>
Data format	Tables
Experimental factors	RNA used for library preparation and sequencing was isolated from mature <i>Vitis vinifera</i> cv. Garganega berries infiltrated <i>in vitro</i> with <i>Botrytis cinerea</i> to induce noble rot in controlled environmental conditions
Experimental features	RNA-seq data was obtained from 3′mRNA sequencing to estimate gene abundance in count per million (CPM) represent the expression level of each grapevine or <i>B. cinerea</i> transcript
Data source location	Not applicable
Data accessibility	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE116741
Related research article	Lovato A., Zenoni S., Tornielli GB., Colombo T., Vandelle E., Polverari A., Specific molecular interactions between <i>Vitis vinifera</i> and <i>Botrytis cinerea</i> are required for noble rot development in grape berries”, <i>Postharvest Biology and Technology</i>

Value of the data

- The reproducibility of the data among samples of different biological replicates demonstrates the reliability of the experimental design, consisting in the artificial *in vitro* botrytisation of grape berries to study noble rot in controlled conditions.
- The RNA-Seq data allow the readers to access to the transcriptomic profiles of both grapevine berries and *B. cinerea* in conditions of noble rot development from the same samples.
- The analysis of differentially expressed genes in both plant and fungus during noble rot provide new data for further meta-analyses using publically available datasets

1. Data

Data reported here describe the sequencing results obtained from grapevine (*V. vinifera* cv. Garganega) berries artificially botrytized *in vitro* and harvested 12 days after infection [1]. Water-infiltrated berries and *B. cinerea* mycelium grown *in vitro* were used as controls for plant and fungus differential gene expression, respectively. Each set of samples includes three biological replicates. Data are available at the NCBI GEO database under accession number GSE116741.

From RNAseq analysis, around 50 million raw reads were generated for each sample (Table 1).

The reproducibility attested by the correlation across samples of the same condition and unsupervised partitioning of samples in the correct subgroups, when based on either *V. vinifera* normalized gene expression levels across samples (Fig. 1) or *B. cinerea* normalized counts (Fig. 2) argue that the relative changes in expression levels between conditions are due to the underlying biology, rather than a side effect related to differences in the biomass of the fungus respect to the plant, and support the relevance of the results from the differential expression analysis.

2. Experimental design, materials and methods**2.1. Garganega berry withering**

Bunches of *V. vinifera* cv. Garganega berries were harvested in October 2014 in Monteforte d'Alpone (Verona, Italy) and were transferred to the Pasqua Vigneti e Cantine winery (Verona, Italy) for withering. Healthy grapes were collected at the commercial ripening stage (soluble solids content = $18.5 \pm 0.25\%$) and were placed in perforated plastic boxes known as *plateaux* (~5 kg in each) in a ventilated withering facility under natural conditions (17–20 °C, 78–82% relative humidity).

Table 1Statistics on *Vitis vinifera* and *Botrytis cinerea* TruSeq sequencing.

Samples	# of sequences in fastq files	# of sequences in filtered fastq files [lluQC.pl - NGS QC Toolkit]	% of High Quality reads [i.e.: passing filtering]	# of Aligned Pairs	% of Aligned Pairs
1) <i>Botrytis cinerea</i> biol. repl. 1	51.596.498	48.464.674	93,93%	44.728.327	92,30%
2) <i>Botrytis cinerea</i> biol. repl. 2	48.796.078	45.945.913	94,16%	42.621.946	92,80%
3) <i>Botrytis cinerea</i> biol. repl. 3	57.657.830	54.115.696	93,86%	50.145.232	92,70%
4) <i>B. cinerea</i> -infected berries biol. repl. 1	47.398.134	44.179.114	93,21%	30.345.824	68,50%
5) <i>B. cinerea</i> -infected berries biol. repl. 2	58.054.526	54.322.693	93,57%	35.755.979	65,60%
6) <i>B. cinerea</i> -infected berries biol. repl. 3	68.936.519	64.588.098	93,69%	45.693.676	70,50%
7) Water-injected berries biol. repl. 1	65.333.494	60.514.894	92,62%	44.998.907	74,20%
8) Water-injected berries biol. repl. 2	65.244.803	61.257.338	93,89%	46.173.353	75,20%
9) Water-injected berries biol. repl. 3	70.761.848	65.929.242	93,17%	48.576.393	73,50%

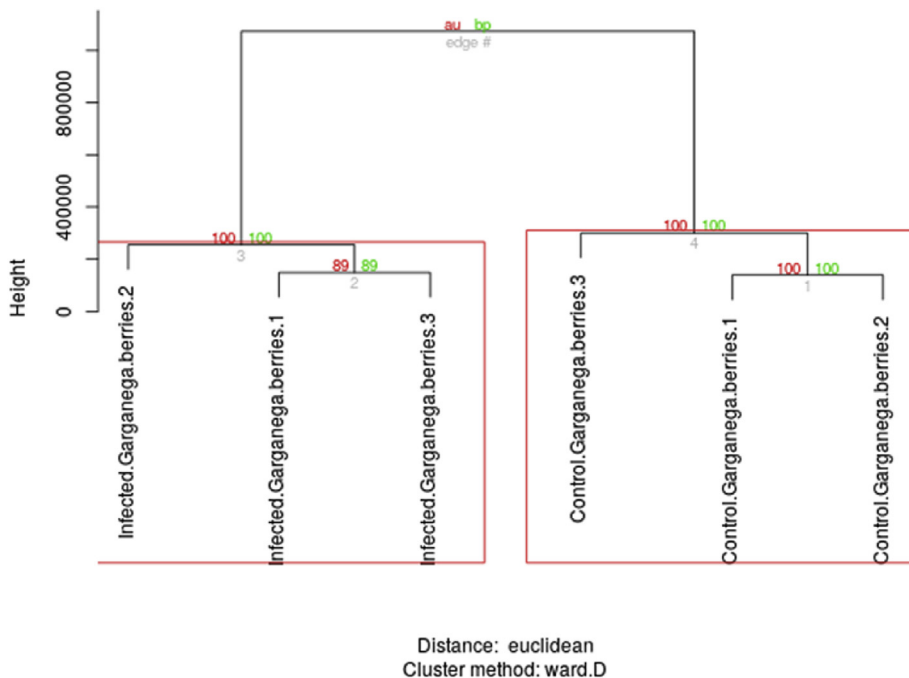


Fig. 1. Unsupervised clustering of samples based on *V. vinifera* cv. Garganega normalized gene counts. Infected.Garganega.berries, grape berries artificially infected *in vitro* with *B. cinerea*; Control.Garganega.berries, grape berries water-infiltrated. Numbers 1 to 3 refer to biological replicates.

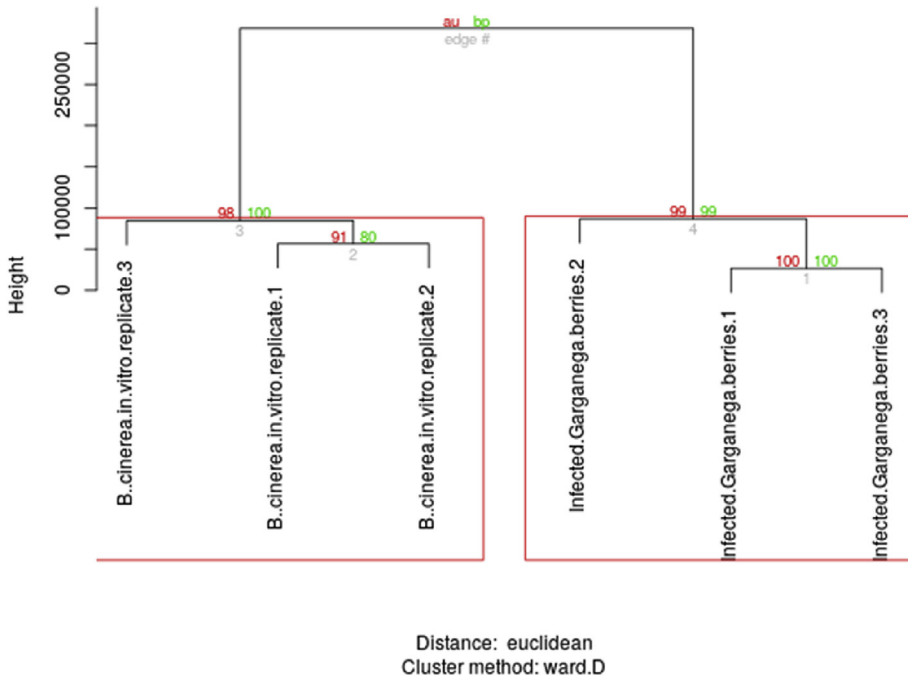


Fig. 2. Unsupervised clustering of samples based on *B. cinerea* normalized gene counts. *B.cinerea.in.vitro*, *B. cinerea* mycelium grown *in vitro* on synthetic medium; infected.Garganega.berries, grape berries artificially infected *in vitro* with *B. cinerea*. Numbers 1 to 3 refer to biological replicates.

Randomly selected replicate berries were analyzed weekly to determine the soluble solids content using a DBR35 digital refractometer (Giorgio Bormac, Carpi, Italy). Three dedicated *plateaux* were weighed weekly using a CH50K50 electronic balance (Kern, Balingen, Germany) to determine the sampling time corresponding to a berry soluble solids content of 26.6% and a percentage weight loss of ~30%.

2.2. *B. cinerea* inoculum preparation and *in vitro* growth conditions

The B05.10 strain of *B. cinerea* Pers.: Fr. (teleomorph *Botryotinia fuckeliana* (de Bary) Whetz.), isolated in 1999 from grape berries [2] and later sequenced [3,4], was cultured on solid potato dextrose agar (Formedium, Hunstanton, UK) in the dark for 7 d at room temperature followed by 5 d light exposure to promote fungal sporulation. Conidia were collected from sporulating cultures in 0.1% (v/v) water-diluted Tween-20, counted using the Fast-Read 102 microscope counting chamber (Biosigma, Cona, Italy) and diluted to 1×10^5 conidia mL^{-1} .

Liquid *B. cinerea* B05.10 cultures were incubated in flasks by inoculating 125 mL of potato dextrose broth (Formedium) with 7×10^6 conidia mL^{-1} . After 7 d of incubation at 22 °C shaking at 120 rpm, mycelia were recovered by filtration and subsequently frozen.

2.3. Artificial noble rot induction

Samples of 1000 withered Garganega (with pedicels) were surface-sterilized for 5 min in 70% ethanol and dried in sterility. Of the 1000 sterilized Garganega berries, 500 berries were injected, until berry saturation (i.e. appearance of the first drop on berry surface), with ~0.1 mL of a *B. cinerea* conidial

suspension (1×10^5 conidia mL⁻¹) using a 1-mL syringe with a needle and reaching berry mesocarp (almost the centre of the berry). In the same way, the remaining 500 berries were injected with sterile water as negative controls.

Garganega berries were incubated in sterile 24-well plates under controlled conditions (15 °C/15 h dark and 18 °C/9 h light) to induce noble rot. *B. cinerea* colonization was monitored daily in the infected berries.

Three biological replicates (~100 berries each) of infected and uninfected Garganega berries were collected, deseeded and frozen at the same *pourri plein*/withering stage 12 d after injection.

2.4. RNA extraction

Infected or uninfected berry pericarps representing each grapevine variety as well as *B. cinerea* mycelia from *in vitro* cultures were ground under liquid nitrogen and total RNA was isolated from 200 mg of powdered sample using the Spectrum Plant Total RNA Kit (Sigma-Aldrich, St. Louis, MO, USA). The RNA quantity, integrity and purity were confirmed using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and a Bioanalyzer Chip RNA 7500 series II (Agilent Technologies, Santa Clara, CA, USA).

2.5. RNA-Seq analysis

For each sample, an unstranded library was prepared from 2.5 µg of total RNA using the TruSeq Library Prep Kit v2 (Illumina, San Diego, CA, USA). Sequencing on an Illumina HiSeq500 device (101 bp, paired-end) was carried out at the Functional Genomics Centre (FGC), Department of Biotechnology, University of Verona, Verona, Italy.

The preprocessing and analysis of RNA-Seq data were carried out using high-performance computing resources made available by CINECA (Class C ISCR project: IsC33_NobleRot). Due to demultiplexing, the raw reads for each individual sample were represented by multiple FASTQ files in the RNA-Seq dataset (**GEO ID: GSE116741**). As the first data preprocessing step, all raw reads related to the same sample were merged in a unique FASTQ file. Because the reads were obtained by paired-end sequencing, the same merging procedure was applied to both sets of raw reads (R1 and R2) on each sample. Raw reads were quality controlled using IlluQC software as part of the NGS QC Toolkit [5] with default parameters (quality cutoff = 20; required percentage of nucleotides in reads with quality score at least equal to quality cutoff = 70%). The detailed description of individual parameters can be found in the NGS QC Toolkit reference manual (http://59.163.192.90:8080/ngsqctoolkit/NGSQCToolkitv2.3.3_manual.pdf).

Filtered reads were mapped against a mixed reference genome (*Botrytis cinerea* + *Vitis vinifera*) using TopHat v2.0.11 [6]. Mapped reads were summarized at the gene level into a count matrix using the htseq-count tool from the HTSeq library [7]. Gene models provided to the htseq-count tool to score reads mapping unambiguously to a single gene were obtained from the Grape Genome Database release V1 (<http://genomes.cribi.unipd.it/DATA/>) for *Vitis vinifera* [8] and from the Ensembl database release 28 (genome assembly: ASM15095v2) for *B. cinerea* [9]. Genomic data were downloaded on August 5, 2015.

The gene-level count matrix was used for differential expression analysis with edgeR [10]. Only genes showing at least three mapped reads in at least three samples of the count matrix were tested for differential expression (**GEO ID: GSE116741**). Modulated transcripts among the different conditions were defined using the following criteria: (i) $|\log_2 \text{FC}| > 1$; and (ii) Benjamini and Hochberg adjusted *p*-value < 0.05.

Following the summarization of mapped reads into a count-matrix, where counts represent the total number of reads aligning to each gene, two subsets of read counts, relative to *V. vinifera* genes (Supplemental File 1) or *B. cinerea* genes (Supplemental File 2), were separately normalized across samples before proceeding to the analysis of differential gene expression among the different conditions. In particular, library sizes, used to measure the relative abundance of each gene in each RNA sample and thus to express it as normalized gene counts suitable for the comparison across samples, were estimated based only on *V. vinifera* gene counts on one hand or only on *B. cinerea* gene counts on

the other hand, yielding two tables of normalized gene counts (Supplemental Files 3 and 4). Each of those two tables served as input to assay separately the differential expression across conditions for genes of *V. vinifera* (Supplemental File 5) or *B. cinerea* (Supplemental File 6).

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.dib.2019.104150>.

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