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Data Article

Transcriptomic dataset of *Malus domestica* young leaves in response to acibenzolar-S-methyl (ASM) and/or nitrogen nutrition

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ABSTRACT

Plant resistance inducers (PRIs) and nitrogen (N) nutrition are both known to affect plant defence but their interaction has not been well described. We addressed this question in apple (*Malus domestica*) by generating a transcriptomic data set of young leaves from seedlings grown in subirrigation systems allowing variations in nitrate supply as the sole nitrogen source. Plants under three contrasting N status (high; limited for 10 days; or just resupplied after a 12 days limitation) received foliar applications of the chemical elicitor acibenzolar-S-methyl (ASM), a functional analog of salicylic acid, or water. Two days later, the youngest developed leaves were sampled for total RNA extraction and sequencing analysis (RNAseq). The current dataset includes 1) a detailed protocol of plant sample production and 2) transcriptomic profile description of young leaves as normalized counts obtained from sequence mapping against the *Malus domestica* GDDH13v1.1 reference transcriptome. The raw data files and processed data are available at the Gene Expression Omnibus (GEO) repository under the accession number GSE264541. This dataset is a valuable resource to investigate

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further the molecular mechanisms underlying the role of nitrogen and/or ASM treatment in *Malus domestica*.

Specifications Table

Subject	Plant science: Plant physiology
Specific subject area	Transcriptome of apple seedlings in response to nitrogen status combined with defence stimulation with acibenzolar-S-methyl (ASM).
Type of data	Filtered raw reads (fastq), analysed RNA-seq data files (raw_counts.sf), total counts table (xls), total normalized count table (Supplemental file 1.xls) and figures (pptx)
Data collection	Data were obtained from young leaves of apple seedlings grown in greenhouse-controlled conditions under contrasting nitrogen regimes and ASM treatments. For each sample, total RNA was extracted from young leaves collected 2 days after ASM or water treatment. RNAseq was performed by Genome Quebec (Canada, https://genomequebec.com/), using Illumina NovaSeq 6000 PE100 technology (Illumina, San Diego, CA, USA) to generate 100 bp pair-end sequences. Processing of RNA-seq data included (1) raw reads filtering, (2) paired-reads mapping onto the *Malus domestica* GDDH13v1.1 reference transcriptome [1] and (3) bioinformatic analyses for counts normalization and data quality.
Data source location	• Institution: Research Institute for Horticulture and Seeds (IRHS), University of Angers, Institut Agro, INRAE • City/Town/Region: 49071 Beaucouzé • Country: France • Latitude and longitude: 47.478770912720876; −0.6121719283912697
Data accessibility	Repository name: Gene Expression Omnibus (GEO) database Data identification number: GSE264541 Direct URL to data: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE264541 Instructions for accessing these data: Go to https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE264541

1. Value of the Data

- This data set is original as it describes for the first time at a transcriptomic level the effect of the well-known chemical elicitor ASM on young leaves from apple seedlings grown under 3 different nitrogen regimes.
- Using this informations, researchers can identify leaf genes regulated by PRI treatment and/or different nitrogen status.
- Researchers will benefit from this data set to decipher molecular mechanisms underlying the combination of plant nitrogen status and induced resistance in order to investigate the unreliable efficacy of PRIs in fields.
- The reproducibility of the data among the different biological replicates demonstrates the reliability of the experimental design, which can be useful for the scientific communities working in the field of PRIs and nitrogen nutrition.

2. Background

Plant resistance inducers (PRIs) and nitrogen (N) nutrition are both known to affect plant defence [2,3] but their interaction has been little studied and needs further research [4]. The present work generated a transcriptomic data set to characterize further this interaction in ap-

Table 1
Results of sequencing and mapping.

RNA sample	N regime	N status at sampling time	Treatment*	Replicate	Total bases (Mb)	Mapping rate (%)
QU007	HN	High	Water	1	33 412 662	87.58 %
QU008	HN	High	ASM	1	34 096 110	86.53 %
QU011	Ante12	Limited	Water	1	30 632 757	87.83 %
QU012	Ante12	Limited	ASM	1	36 255 456	87.40 %
QU037	Post12	Resupplied	ASM	1	41 335 109	89.15 %
QU038	Post12	Resupplied	Water	1	37 546 958	88.02 %
RE007	HN	High	Water	2	32 870 012	88.27 %
RE008	HN	High	ASM	2	34 171 566	87.63 %
RE011	Ante12	Limited	Water	2	35 156 780	87.78 %
RE012	Ante12	Limited	ASM	2	34 224 442	87.81 %
RE037	Post12	Resupplied	ASM	2	33 917 839	87.87 %
RE038	Post12	Resupplied	Water	2	42 106 397	89.48 %
RS007	HN	High	Water	3	34 373 018	88.96 %
RS008	HN	High	ASM	3	34 566 473	87.24 %
RS011	Ante12	Limited	Water	3	40 967 365	88.20 %
RS012	Ante12	Limited	ASM	3	44 305,616	86.31 %
RS037	Post12	Resupplied	ASM	3	39 349 780	87.37 %
RS038	Post12	Resupplied	Water	3	35 725 864	88.22 %

* Treated 2 days before sampling.

ple (*Malus domestica*) seedlings. The well-known chemical PRI ASM [5] was chosen because of its performance in controlling several apple pests in greenhouse assays [6–8] and its ability to strongly reprogram apple transcriptome in greenhouse standard growing conditions [8]. The use of i) an automatic subirrigation system and ii) nutrient solutions with contrasting nitrate concentrations as the sole nitrogen source both adapted from literature [9,10] allowed the generation of a new dataset to compare transcriptome profiles of apple young leaves with 3 different N status (high; limited; and limited with subsequent N resupply) in combination with ASM or water treatments.

3. Data Description

Transcriptomic data for each combination of nitrogen status and ASM treatment were obtained by RNA sequencing using an Illumina NovaSeq 6000 S4 PE100 platform. Output reads ranged from 30.63 to 44.30 million bases per sample and mapping rates from 86.63 to 89.47 % (Table 1). The original sequencing datasets is available at the Gene Expression Omnibus (GEO) under the accession number GSE264541.

Total library sizes after normalization and log2 normalized counts per sample are represented Fig. 1A and B. A Heatmap of sample distance matrix between normalized counts was generated to illustrate the correlation among samples and biological replicates Fig 1C. A principal component analysis (PCA) was generated after scaling and centering normalized counts (Fig 1.D). The first component explains 44.7 % of total variation and corresponds to the treatment effect (ASM *vs* water). The second component explains 15.9 % of the total variation and corresponds to the nitrogen status of the samples.

4. Experimental Design Materials and Methods

4.1. Plant material and growth conditions

Apple seeds from open-pollinated *M. domestica* cv Gala were sown in standard potting mix. At a 3-leaf developmental stage, seedlings were individually transplanted into small pots filled

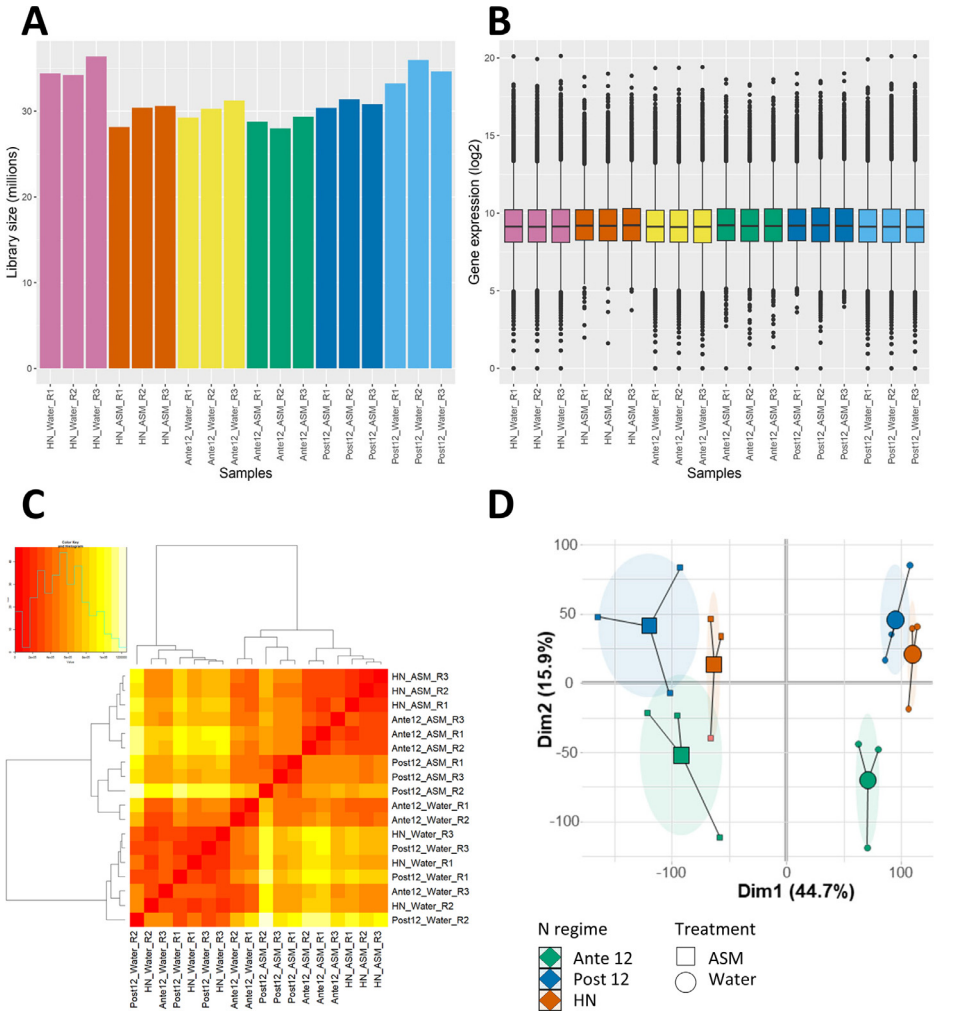

Fig. 1. Quality control of the RNA-seq data after filtering and TMM normalization. A) Library sizes for each sample (total normalized counts). B) Boxplot distribution of normalized counts (log2 normalized counts per gene per sample). C) Heatmap of Euclidean distance between samples clustered using Ward distance. D) PCA plot of normalized counts with samples coloured and shaped according to nitrogen modalities and treatment.

with expanded perlite (an inert siliceous substrate), and were grown in sub-irrigation devices (Fig. 2A) in a temperature-regulated greenhouse (21 ± 3 °C day; 18 ± 3 °C night) with 16 hours of daylight (natural photo-period supplemented with an additional lighting compensation if solar radiation was under 200 W on the greenhouse roof) and a relative humidity set at 70 %. Each sub-irrigation device consisted of 40 plants in an upper reservoir sub-irrigated for 20 min twice a day (at 9 a.m. and 6 p.m.) with 40 L of a nutrient solution pumped from a lower reservoir (Fig. 2A). The solution was renewed every 7 days in order to avoid fluctuations in nutrient concentrations. Two nutrient solutions with calcium nitrate as the sole source of nitrogen were used: a high nitrogen (HN) solution with 7 mM nitrate and a low nitrogen (LN) solution with 0.5 mM nitrate (Table 2). The low concentration of calcium in the LN solution was compensated with an addition of calcium sulfate. For each device, nutrient solutions were made by adding mineral salts to 40 L of tap water with a base level nitrogen concentration estimated at 0.3 mM.

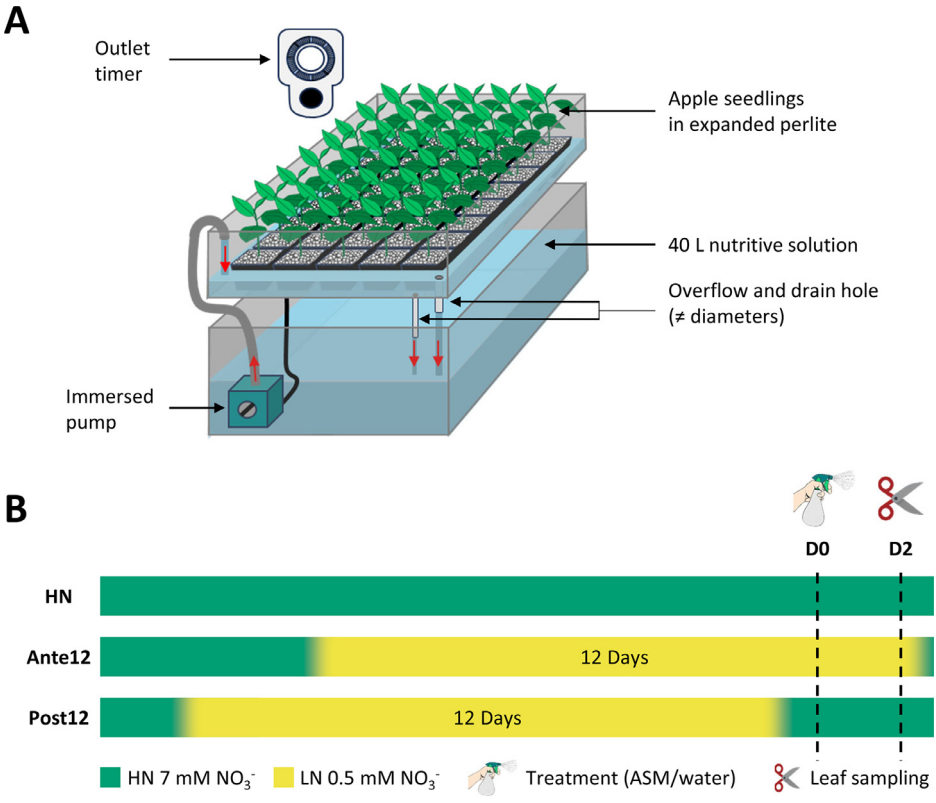

Fig. 2. Schematic representation of plant growth conditions. A) a sub-irrigation device, B) the protocol for plant growth according to 3 different nitrogen regimes, ASM or water treatment and sample collection 2 days post treatment. C) Picture of the "Post12" plants on their 12th day under limited N supply on the right, and the HN controlled plants on the left.

Table 2
Composition of sub-irrigation solutions.

	HN 7 mM	LN 0.5 mM
$Ca(NO_3)_2$ (mM)	3.5	0.25
KH_2PO_4 (mM)	1	1
$MgSO_4$ (mM)	1.5	1.5
K_2SO_4 (mM)	1	1
EDTA-Fe (mM)	0.043	0.043
$CaSO_4$ (mM)	–	3.25
Oligo DRIP6 (Haifa, France) (dilution of the commercial solution)	1.10^{-4}	1.10^{-4}

Apple seedlings were grown until the 6-leaf stage with the HN solution. Plants were then transferred on the LN solution for 12 days, or left on the HN solution. After 12 days, plants subjected to the LN solution were transferred back to HN to be resupplied with nitrogen (Fig. 2B). N limited plants were then only slighthly paler than the high N control plants (Fig. 2C).

4.2. Plant treatment

Plants were sprayed to run-off (with a pressurized hand sprayer) with the commercial product Bion® 50WG (Syngenta, Basel, Switzerland; 50 % of ASM) prepared in reverse osmosis water at a final concentration of 0.4 $g.L^{-1}$. The same water was used as control. All seedlings were sprayed on the same day irrespective of the nitrogen regime (Fig. 2B). The N-limited "Ante12" seedlings were ASM treated after 10 days on LN and sampled 2 days later, before N resupply. The N-resupplied "Post12" seedlings were ASM treated 1 day after N resupply on HN. The combination of 3 different N regime (HN, Ante12 and Post12) and 2 treatments (Water and ASM) led to 6 experimental conditions: 'HN_Water', 'HN_ASM', 'Ante12_Water', 'Ante12_ASM', 'Post12_Water' and 'Post12_ASM' (Fig. 2B). The experiment was repeated three times independently throughout the year.

4.3. Leaf sampling and RNA extraction

Two days after ASM or water treatment, two thirds of the youngest developed leaf at the time of treatment, were collected from 30 plants, pooled, directly frozen in liquid nitrogen and stored at - 80 °C. Frozen leaves were ground in liquid nitrogen and total RNA extractions were performed on approximately 50 mg FW using the kit Nucleospin RNA Plant (Macherey-Nagel GmbH & Co, Düren, Germany) according to the manufacturer's procedure. RNA quality was checked using the kit RNA 6000 Nano and a BioAnalyzer 2100 (Agilent technologies. Santa Clara. CA. USA) according to the manufacturer's instructions.

4.4. RNA sequencing and data processing

Libraries were generated using the Illumina mRNA Stranded protocol and sequenced with the Illumina NovaSeq 6000 S4 PE100 reads technology (Génome Québec. Canada) for 25 million pair-end reads. The sequenced reads were mapped on the reference transcriptional units from GDDH13v1.1 [1] using Salmon software [10]. Transcript levels were calculated in CPM and filtered with a CPM cutoff set at 5. RNA-seq libraries were normalized using the TMM (Trimmed Mean of the M-values) method implemented in the R package DeSeq2 [11] (https://cran.r-project.org/). PCA was perform using FactoMineR R package [12] and graphical outputs were generated using R packages gplots and ggplot2 (https://cran.r-project.org/web/packages/gplots) [13].

Limitation

None.

Ethics Statement

The authors have read and followed the ethical requirements for publication in Data in Brief and confirm that the current work does not involve human subjects, animal experiments, or any data collected from social media platforms.

CRediT Author Statement

Térance Mobarak: Data acquisition. data curation. Writing original draft. **Mathilde Orsel**: Conceptualization. Data curation. Writing original draft/review/editing. **Mickaël Delaire:** Conceptualization. Writing - review/editing. **Marie-Noëlle Brisset:** Funding acquisition. Writing - review/editing.

Data Availability

Transcriptomic data from Malus domestica response to Acibenzolar-S-Methyl (ASM) treatment and nitrogen limitation or resupply (Original data) (GEOmnibus).

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Declaration of Competing 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.

Supplementary Materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.dib.2024.110727.

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