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## Data in Brief

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## Data Article

# Dataset for the metabolic and physiological characterization of seeds from oilseed rape (*Brassica napus* L.) plants grown under single or combined effects of drought and clubroot pathogen *Plasmodiophora brassicae*

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## ABSTRACT

Faced with the challenges of adapting agriculture to climate change, seed production should have increased resilience to abiotic stress factors and the expected proliferation of pathogens. This concerns both the nutritional quality and seed vigor, two crucial factors in seedling establishment and yield. Both qualities are acquired during seed development, but how environment influences the genetic and physiological determinisms of these qualities remains to be elucidated. With a world production of 71 Mt of seeds per year, oilseed rape (*Brassica napus*) is the third largest oleaginous crop. But its productivity must cope with several abiotic stresses, among which drought is one of the main constraints in current and future climate scenarios. In addition, clubroot disease, caused by the pathogen *Plasmodiophora brassicae*, leads

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to severe yield losses for the Brassica crops worldwide. Clu-broot provokes the formation of galls on the infected roots that can restrict the flow of water and nutrients within the plant throughout the growth cycle. In order to get new insights into the impact of single or combined constraints on seed qualities, metabolic profiling assays were run for a collection of 330 seed samples (including developing, mature and imbibed seeds) harvested from plants of two *B. napus* cultivars (“Express” and “Montego”) that were grown under either drought conditions, the presence of *P. brassicae*, or a combination of both stresses. Metabolites were identified and quantified by UPLC or GC. In addition, monitoring germination traits was conducted for 60 mature seed lots under *in vitro* conditions using an automated phenotyping platform. The present dataset contains the raw contents for 42 metabolites (nmol.mg<sup>-1</sup> of seed dry weight) filtered and analyzed with statistical tests as well as germination speed and percentages. This dataset is available under accession at Data IN-RAE. These data will contribute to a better understanding of the crosstalk between the plant responses to water deprivation and/or pathogen attack and how it compromises seed quality. A better understanding of the molecular and physiological responses of the seed to (a)biotic stress on a molecular and physiological will be a first step to meet scientific and technological challenges of adapting seeds to their environment.

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## Specifications Table

Subject	Biological sciences
Specific subject area	Omics: Metabolomics Plant Science: Plant Physiology
Type of data	Table Figure
How data were acquired	Metabolite data were acquired on the Metabolic Profiling and Metabolomic Platform P2M2 ( <a href="https://www6.inrae.fr/p2m2/">https://www6.inrae.fr/p2m2/</a> ) <u>Amino acids:</u> Method: ultraperformance liquid chromatography (UPLC) Instruments: Acquity UPLC-DAD system, Waters Corp. Software for data processing: Empower 2 <u>Sugars and organic acids :</u> Method: gas chromatography (GC) Instrument: Trace 1300 GC-FID, Thermo-Fisher Scientific, Waltham, CA, USA Software for data processing: Chromeleon software (V7.2.10, Thermo-Fisher Scientific) Germination data were acquired on the PHENOTIC platform ( <a href="https://www6.inrae.fr/phenotic/">https://www6.inrae.fr/phenotic/</a> ).
Data format	Metabolite contents (nmol.mg <sup>-1</sup> of seed dry weight) filtered and analyzed with statistical tests. Percentages of germination, time to reach a given percentage of germination. All data are provided in a single xlsx sheet.

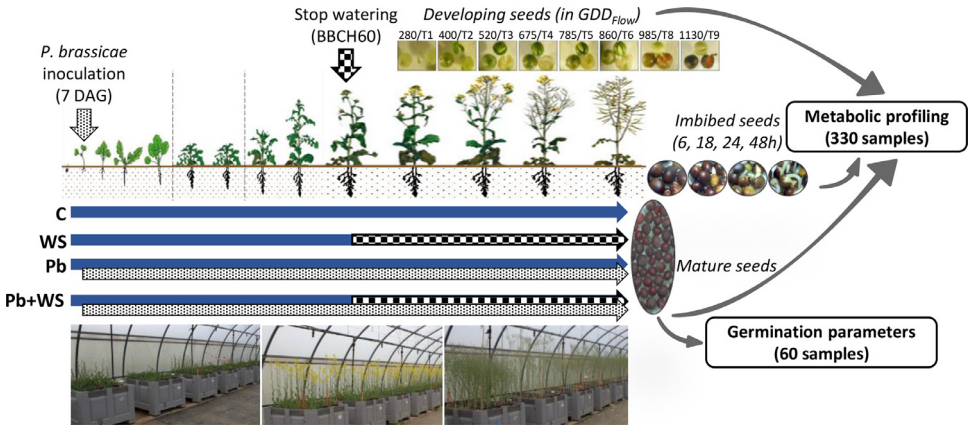
Parameters for data collection	Metabolites were extracted from seeds of <i>Brassica napus</i> (cv. “Express” and “Montego”). Seed-bearing mother plants were grown under 1) standard conditions, 2) water shortage (reduction of watering from the start of flowering onwards), 3) clubroot infection after inoculation by <i>Plasmiodiophora brassicae</i> at 7 days after germination, or 4) combination of both clubroot infection and water shortage. Immature seeds were collected from mother plants at 9 timepoints during seed development, mature dry seeds were harvested when the plants were fully senescent and imbibed seeds were soaked in water on a filter paper for 6, 18, 24 or 48 h at 20 °C in darkness. The experimental design was run over three cropping seasons with clubroot infection applied only during the last season. Germination was monitored under <i>in vitro</i> conditions at 20 °C in darkness.
Description of data collection	Metabolites were extracted with a methanol–chloroform–water mixture and derivatized prior to quantification by UPLC or GC. Compounds were quantified with respect to internal standards that were added to the samples. Monitoring speed of germination was conducted under <i>in vitro</i> conditions using the automated phenotyping platform PHENOTIC (INRAE-IRHS, Angers University, AgroCampus Ouest, GEVES). Image acquisition, image analysis and data analysis methods are described in detail by [1].
Data source location	Institution: Institute of Genetics, Environment and Plant Protection (IGEPP), INRAE, Institut Agro, Univ. Rennes 1 City/Town/Region: 35,650 Le Rheu Country: France Latitude and longitude (and GPS coordinates, if possible) for collected samples/data: 48°06′37.1″N 1°47′46.3″W
Data accessibility	Repository name: Data INRAE ( <a href="https://data.inrae.fr/">https://data.inrae.fr/</a> ) Data identification number: <a href="https://doi.org/10.15454/SNRK0V">https://doi.org/10.15454/SNRK0V</a> Direct URL to data: <a href="https://data.inrae.fr/dataset.xhtml?persistentId=doi:10.15454/SNRK0V">https://data.inrae.fr/dataset.xhtml?persistentId=doi:10.15454/SNRK0V</a>

## Value of the Data

- The present study describes a metabolic dataset from developing, mature and imbibing seeds of *B. napus* harvested from plants grown under four environmental conditions (standard watering/drought with or without inoculation by *P. brassicae*) and its relationship with germination behavior.
- These data are useful for the scientific community working on the impact of single or combined abiotic and biotic constraints on seed quality.
- These data provide clues to better understand the metabolic changes that occur in seeds when produced under various environmental conditions. They point out some metabolites that can be considered as potential biomarkers of stress response in seeds of oilseed rape. In addition, they also highlight correlations between the metabolic composition of the seeds and their germination behavior.

## 1. Data Description

This paper describes a metabolic profiling dataset from seeds of two cultivars of *Brassica napus*, “Express” and “Montego”, that were cultivated under single or combination of drought stress and/or *Plasmiodiophora brassicae* infection. Drought during seed filling has been shown to have a significant impact on oilseed rape productivity [2] and is one of the prime abiotic constraints



**Fig. 1.** Experimental design. Seeds were collected from oilseed rape plants grown under a sheltered tunnel and exposed to four different treatments. Developing, mature and imbibed seeds were sampled for the metabolic profiling assays and only the mature seed lots were used for the germination tests (refer to Table 1 for a full description of the samples used in this study). C, control; WS, water shortage; Pb, *P. brassicae* inoculation; Pb+WS, *P. brassicae* inoculation and water shortage; DAG, days after germination; BBCH60, start of flowering; GDD<sub>Flow</sub>, growing degree-days calculated in base 0 °C from BBCH60 (GDD<sub>Flow</sub> are given for Express here).

under current and future climate scenarios. On the other hand, clubroot, caused by the obligate telluric biotroph protist *Plasmodiophora brassicae*, is a disease that leads to severe yield losses for the Brassica crops worldwide [3]. Clubroot leads to the formation of galls on the roots of infected plants that can restrict the flow of water and nutrients within the plant. Above-ground symptoms including wilting, stunting, yellowing and premature senescence have been observed that can eventually lead to the plant death. In addition, the plant response to clubroot can be modified by water or nutrient availability [4,5]. Information on the experimental designs used to generate the data is provided in Fig. 1. Four different treatments were applied to the mother plants: 1) standard watering and no *P. brassicae* inoculation, hereafter referred to as the control (C) treatment; 2) water shortage (WS) by reducing watering from the onset of flowering and keeping the soil water potential around -600 mbar until the end of the crop cycle; 3) clubroot infection by inoculating 7-day old seedlings with resting spores *P. brassicae*, hereafter referred to as the Pb treatment; and 4) a combination of clubroot and water shortage, hereafter referred to as the Pb+WS treatment. Table 1 summarizes all the samples used in this study, according to the growing season (2015–2016, 2016–2017 or 2017–2018), the genotype (“Express” or “Montego”), the treatment (C, WS, Pb or Pb+WS) and the developmental stage (immature seeds from T1-T9 stages, mature dry seeds or imbibed seeds at 4 timepoints of soaking). A total of 330 samples were analyzed for their contents in 42 compounds derived from the primary metabolism (23 amino acids, 6 organic acids and 13 sugars) and 60 mature dry seed lots were phenotyped for a number of germination parameters, including the mean germination time (MGT), the time required to reach 10 or 90% of germination (T10 and T90 respectively), the time difference between times required to achieved 20 and 80% of germination ( $\Delta_{T80-T20}$ ) and the germination percentage at 30 or 96 h (GP30 and GP96 respectively) with all germination assays run at 20°C. Fig. 2 displays the score plot of the first two principal components following an unsupervised multivariate data analysis method PCA run on the metabolic profiles generated from the 330 seed samples. The effects of drought and/or *P. brassicae* infection on final seed metabolic composition and germination traits are shown for both genotypes in Table 2. Finally, Fig. 3. highlights the correlations between metabolites and germination parameters for seeds of Express and Montego cultivated under water shortage or standard conditions.

**Table 1**

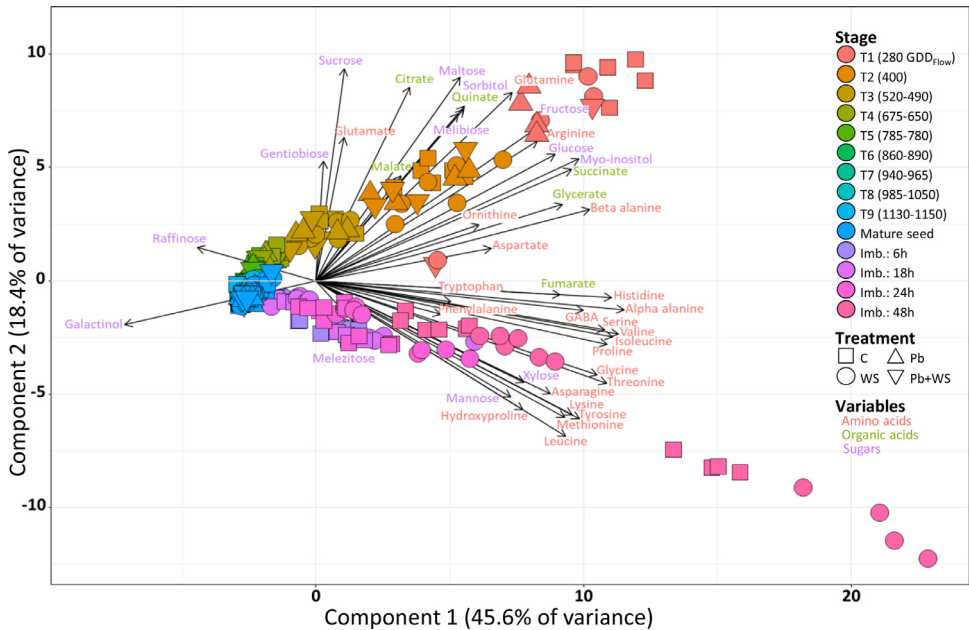
List of the samples used for the metabolic profiling assays (330 samples) as well as the germination tests (60 samples). T1-T9 refer to the timepoints when immature seeds were collected from the mother plants. C, control; WS, water shortage; Pb, *P. brassicae* inoculation; Pb+WS, *P. brassicae* inoculation and water shortage; rep, biological replicate.

Year	Genotype	Treatment	METABOLIC PROFILING				GERMINATION
			Developing seeds (T1-T9)	Mature seeds	Imbibition (6, 18, 24, 48 h)	TOTAL	Mature seeds
2015–2016	Express	C	–	2 rep	8: 4 × 2 rep	10	2 rep
2015–2016	Express	WS	–	2 rep	8: 4 × 2 rep	10	2 rep
2015–2016	Montego	C	–	2 rep	8: 4 × 2 rep	10	2 rep
2015–2016	Montego	WS	–	2 rep	8: 4 × 2 rep	10	2 rep
2016–2017	Express	C	–	5 rep	–	5	5 rep
2016–2017	Express	WS	–	5 rep	–	5	5 rep
2016–2017	Montego	C	–	5 rep	–	5	5 rep
2016–2017	Montego	WS	–	5 rep	–	5	5 rep
2017–2018	Express	C	27: (T1-T9) × 3 rep	8 rep	12: 4 × 3 rep	47	8 rep
2017–2018	Express	WS	25: (T1-T9) × 1 rep; (T2-T9) × 2 rep	8 rep	12: 4 × 3 rep	45	8 rep
2017–2018	Express	Pb	17: (T1-T9) × 1 rep; (T1-T7;T9) × 1 rep	4 rep	–	21	–
2017–2018	Express	Pb+WS	15: (T2-T8) × 1 rep; (T2-T9) × 1 rep	4 rep	–	19	–
2017–2018	Montego	C	27: (T1-T9) × 3 rep	8 rep	12: 4 × 3 rep	47	8 rep
2017–2018	Montego	WS	27: (T1-T9) × 3 rep	8 rep	12: 4 × 3 rep	47	8 rep
2017–2018	Montego	Pb	18: (T1-T9) × 2 rep	4 rep	–	22	–
2017–2018	Montego	Pb+WS	18: (T1-T9) × 2 rep	4 rep	–	22	–
						330	60

**Table 2**

Variation in metabolites and germination parameters of mature seeds harvested from plants grown under various conditions. Data are the mean values with standard errors resulting from 15 (C and WS) or 4 (Pb and Pb+WS) biological replicates of each 'genotype × treatment' combination. The metabolite contents are expressed in nmol.mg<sup>-1</sup> of seed dry weight. MGT (h), mean germination time; T10 and T90 (h), time required to reach respectively 10 or 90% of germination; Δ<sub>T80-T20</sub> (h), time difference between the times required to reach 20 and 80% of germination; GP30 and GP96 (%), germination percentages at 30 or 96 h respectively. All germination assays were run at 20 °C. C, control; WS, water shortage; Pb, *P. brassicae* inoculation; Pb+WS, *P. brassicae* inoculation and water shortage.

	Express				Montego			
	C	WS	Pb	Pb+WS	C	WS	Pb	Pb+WS
<b>Isoleucine</b>	0.06 ± 0.02	0.06 ± 0.02	0.05 ± 0.01	0.05 ± 0.00	0.05 ± 0.02	0.05 ± 0.01	0.06 ± 0.02	0.03 ± 0.00
<b>Leucine</b>	0.05 ± 0.01	0.06 ± 0.02	0.05 ± 0.01	0.05 ± 0.00	0.06 ± 0.02	0.05 ± 0.01	0.06 ± 0.02	0.04 ± 0.01
<b>Valine</b>	0.30 ± 0.06	0.31 ± 0.07	0.27 ± 0.02	0.26 ± 0.04	0.38 ± 0.07	0.32 ± 0.05	0.39 ± 0.06	0.28 ± 0.03
<b>Histidine</b>	0.05 ± 0.02	0.06 ± 0.04	0.06 ± 0.03	0.06 ± 0.01	0.05 ± 0.03	0.05 ± 0.03	0.07 ± 0.01	0.05 ± 0.01
<b>Lysine</b>	0.07 ± 0.02	0.08 ± 0.02	0.07 ± 0.01	0.07 ± 0.01	0.08 ± 0.03	0.09 ± 0.02	0.07 ± 0.01	0.07 ± 0.01
<b>Methionine</b>	0.04 ± 0.01	0.05 ± 0.02	0.04 ± 0.01	0.04 ± 0.00	0.04 ± 0.01	0.04 ± 0.02	0.05 ± 0.01	0.05 ± 0.00
<b>Phenylalanine</b>	0.18 ± 0.06	0.21 ± 0.05	0.14 ± 0.02	0.15 ± 0.01	0.13 ± 0.04	0.13 ± 0.05	0.13 ± 0.02	0.10 ± 0.01
<b>Threonine</b>	0.16 ± 0.02	0.17 ± 0.03	0.16 ± 0.02	0.15 ± 0.02	0.20 ± 0.03	0.18 ± 0.03	0.22 ± 0.03	0.15 ± 0.01
<b>Tryptophan</b>	0.21 ± 0.06	0.20 ± 0.05	0.18 ± 0.04	0.19 ± 0.02	0.14 ± 0.03	0.18 ± 0.07	0.14 ± 0.03	0.15 ± 0.01
<b>Asparagine</b>	1.97 ± 0.65	2.27 ± 0.70	1.58 ± 0.17	1.72 ± 0.13	2.40 ± 0.62	2.31 ± 0.80	2.30 ± 0.43	2.01 ± 0.14
<b>Aspartate</b>	2.07 ± 1.05	2.42 ± 0.90	1.27 ± 0.14	1.55 ± 0.14	2.18 ± 0.72	2.83 ± 0.62	1.76 ± 0.24	2.20 ± 0.16
<b>Alpha alanine</b>	0.51 ± 0.11	0.60 ± 0.10	0.43 ± 0.09	0.55 ± 0.13	0.43 ± 0.07	0.51 ± 0.13	0.45 ± 0.08	0.42 ± 0.09
<b>Beta alanine</b>	0.05 ± 0.02	0.05 ± 0.03	0.03 ± 0.00	0.03 ± 0.01	0.05 ± 0.02	0.05 ± 0.02	0.04 ± 0.00	0.04 ± 0.01
<b>Arginine</b>	0.10 ± 0.06	0.12 ± 0.05	0.07 ± 0.02	0.05 ± 0.04	0.08 ± 0.03	0.09 ± 0.04	0.07 ± 0.02	0.06 ± 0.00
<b>Glutamine</b>	0.14 ± 0.05	0.16 ± 0.15	0.13 ± 0.04	0.10 ± 0.05	0.15 ± 0.05	0.16 ± 0.05	0.18 ± 0.07	0.09 ± 0.02
<b>Glutamate</b>	6.44 ± 1.16	6.95 ± 0.88	5.77 ± 0.31	5.62 ± 1.09	7.30 ± 1.27	7.66 ± 1.41	6.27 ± 0.62	6.27 ± 0.49
<b>Glycine</b>	0.20 ± 0.15	0.27 ± 0.20	0.11 ± 0.01	0.11 ± 0.03	0.18 ± 0.07	0.29 ± 0.30	0.13 ± 0.01	0.13 ± 0.01
<b>Proline</b>	0.18 ± 0.16	0.21 ± 0.14	0.19 ± 0.15	0.12 ± 0.01	0.23 ± 0.12	0.17 ± 0.06	0.77 ± 0.83	0.13 ± 0.01
<b>Serine</b>	0.20 ± 0.05	0.22 ± 0.07	0.16 ± 0.01	0.17 ± 0.02	0.20 ± 0.04	0.17 ± 0.07	0.21 ± 0.05	0.12 ± 0.01
<b>Tyrosine</b>	0.09 ± 0.04	0.09 ± 0.04	0.07 ± 0.01	0.07 ± 0.00	0.09 ± 0.02	0.09 ± 0.02	0.09 ± 0.02	0.08 ± 0.00
<b>GABA</b>	0.04 ± 0.03	0.14 ± 0.38	0.04 ± 0.01	0.72 ± 1.4	0.11 ± 0.11	0.12 ± 0.18	0.09 ± 0.08	0.07 ± 0.08
<b>Citrate</b>	3.18 ± 0.90	4.39 ± 1.38	4.59 ± 0.37	4.01 ± 1.80	4.34 ± 0.78	4.95 ± 1.37	4.43 ± 1.28	5.18 ± 1.05
<b>Malate</b>	3.13 ± 0.89	5.85 ± 1.69	3.36 ± 0.23	3.90 ± 0.83	4.22 ± 0.73	6.02 ± 1.91	3.80 ± 0.72	5.17 ± 0.72
<b>Galactinol</b>	1.81 ± 0.51	2.12 ± 0.49	1.79 ± 0.10	1.74 ± 0.23	1.40 ± 0.30	1.81 ± 0.46	1.13 ± 0.26	1.58 ± 0.34
<b>Myo-inositol</b>	0.14 ± 0.13	0.27 ± 0.14	0.28 ± 0.03	0.29 ± 0.08	0.23 ± 0.15	0.37 ± 0.17	0.38 ± 0.07	0.46 ± 0.06
<b>Raffinose</b>	1.42 ± 0.28	1.43 ± 0.34	1.41 ± 0.14	1.37 ± 0.24	0.93 ± 0.09	1.09 ± 0.18	0.93 ± 0.20	1.06 ± 0.19
<b>Sucrose</b>	46.63 ± 10.65	54.34 ± 12.64	48.75 ± 0.66	43.66 ± 7.87	44.8 ± 6.70	49.65 ± 8.78	38.16 ± 6.26	43.19 ± 6.31
<b>MGT</b>	38.12 ± 2.81	33.61 ± 4.28	NA	NA	38.87 ± 3.38	36.02 ± 4.61	NA	NA
<b>T10</b>	28.72 ± 2.98	25.81 ± 3.17	NA	NA	29.41 ± 2.87	27.69 ± 3.56	NA	NA
<b>T90</b>	47.72 ± 3.51	41.26 ± 4.69	NA	NA	48.16 ± 3.88	44.39 ± 5.73	NA	NA
<b>D<sub>T80-T20</sub></b>	12.79 ± 3.09	9.95 ± 2.14	NA	NA	11.72 ± 2.66	10.76 ± 1.96	NA	NA
<b>GP30</b>	24.37 ± 12.56	42.03 ± 23.23	NA	NA	23.70 ± 15.23	34.23 ± 23.76	NA	NA
<b>GP96</b>	99.88 ± 0.32	99.95 ± 0.21	NA	NA	99.73 ± 0.46	99.75 ± 0.44	NA	NA



**Fig. 2.** Principal component analysis (PCA) score plot of metabolic profiles throughout seed development and imbibition in *Brassica napus*. Samples are represented according to their stage (immature seeds from T1-T9 with developmental stages given in GDD<sub>Flow</sub> into parentheses for Express and Montego respectively, mature dry seeds, imbibed seeds) and treatments (C, WS, Pb or Pb+WS). The metabolic variables are written in red (amino acids), green (organic acids) and purple (sugars).

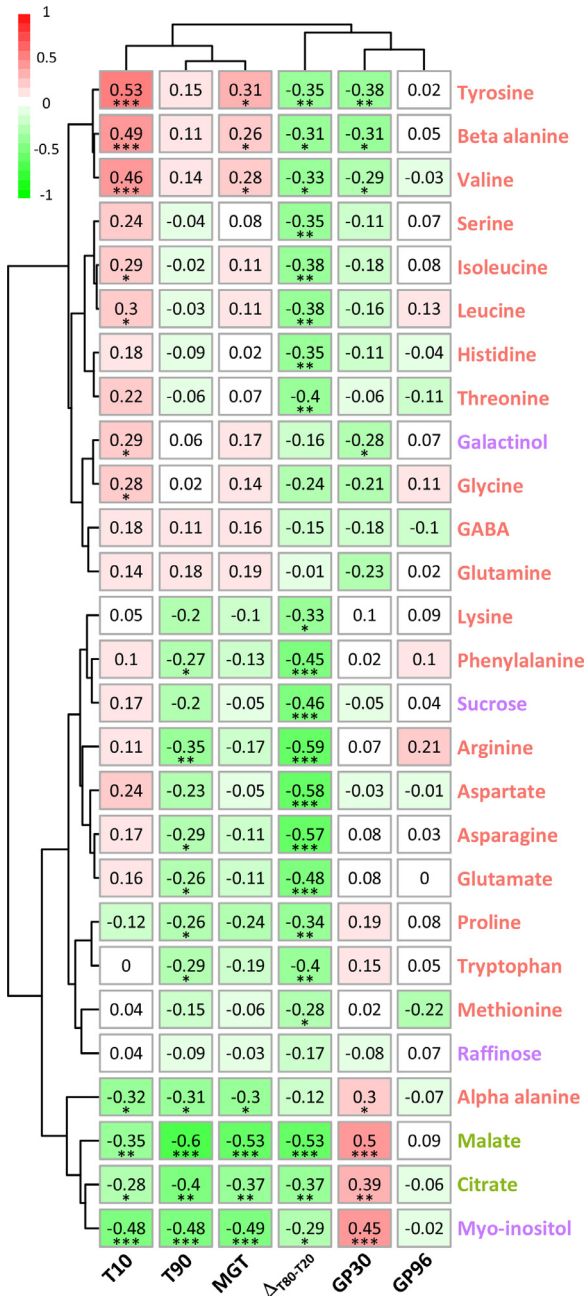
## 2. Experimental Design, Materials and Methods

### 2.1. Plant material and growth conditions

Plants of winter oilseed rape (*Brassica napus* L.) cv. “Express” and “Montego” were cultivated over three cropping seasons, namely 2015–2016 (Y1), 2016–2017 (Y2) and 2017–2018 (Y3) at INRAE Le Rheu, France. These genotypes were selected on the basis of similar phenology (mean flowering dates over a network of 7 year × location combinations were 1901 (± 190) GDD<sub>sow</sub> for both accessions; with GDD<sub>sow</sub>, growing degree-days calculated here from sowings with a base temperature of 0 °C) and seed yield performances (Express: 31 q/ha (± 6,2); Montego: 32,6 q/ha (± 5,3)) when conducted under optimal growth conditions (G. Bianchetti and N. Nesi, unpublished results). In addition, both genotypes were shown to be partially resistant to *Plasmodiophora brassicae* (M.J. Manzaneres-Dauleux, personal communication). The plants were grown using a semi-controlled system mimicking field conditions while allowing fine tuning of water shortage and/or early inoculation by *P. brassicae*. The system relies on tanks of ~1 m<sup>3</sup> filled with a mixture of sand, topsoil and Irish peat (5/3.4/1.6:v/v/v) to facilitate water drain where pre-germinated seeds were transferred at a density of 42 plants/m<sup>2</sup> with uniform spacing. Plants were irrigated individually by a drip system. The water potential of the substrate was monitored regularly throughout the growth cycle using a ceramic tensiometer (Tensionic®, SDEC, France; Vigouroux, 1997) placed in each tank at 25 cm depth. Similar growing conditions were applied during the three seasons with  $n = 2, 5, \text{ or } 8$  replicates for each combination ‘genotype × treatment’ in Y1, Y2, or Y3 respectively.

For each sample, seeds were harvested only from the main inflorescences and were collected as a mix from the 20 central plants of each tank to increase the plot representativeness and limit





**Fig. 3.** Correlations between metabolites from mature seeds of *B. napus* and germination traits. Data are the Pearson's correlation coefficients ( $r$ ) computed for the 27 metabolites and 6 germination variables from  $n = 60$  samples of mature seeds (2 genotypes  $\times$  2 treatments [C or WS]  $\times$  15 biological replicates) with  $p$ -values levels of significance as follows:  $p < 0.05^*$ ;  $p < 0.01^{**}$ ;  $p < 0.001^{***}$ ; none, non-significant. The colors refer to the pair-wise correlation coefficient ranging from  $-1$  (green) to  $1$  (red). The variables are written in red (amino acids), green (organic acids), purple (sugars) and black (germination). Refer to Table 2 for a description of the germination parameters.

the border effects. Developing seeds were collected from pods that were tagged on the day of flowering (*i.e.*, BBCH60 developmental stage according to [6]). A total of 9 timepoints, T1-T9, were considered as following (given for Express/Montego in  $GDD_{Flow}$ , growing degree-days calculated from the start of flowering with a base temperature of 0 °C): T1, 280/280; T2, 400/400; T3, 520/490; T4, 675/650; T5, 785/780; T6, 860/890; T7, 940/965; T8, 985/1050; T9, 1130/1150). Immature seeds were carefully removed from siliques, instantly frozen in liquid nitrogen and stored at -80 °C before analyses. Mature dry seeds were harvested when the vegetative parts were fully senescent and the seeds were dark and hard (*i.e.*, BBCH90 onwards).

## 2.2. Plant inoculation by *Plasmodiophora brassicae*

Seedlings were inoculated 7 days after germination as described by [7]. Briefly, 1 ml of a resting spore suspension ( $10^6$  spores.ml<sup>-1</sup>) of the eH isolate of *P. brassicae* [8] was applied at the bottom of the stem base of each seedling. Isolate eH was derived from a field-isolated population selected from turnip stubble (*B. rapa*) in Germany. It is a P1 pathotype according to the differential host set described by [8,9]. Clubroot symptoms were evaluated at 8 and 30 weeks after *P. brassicae* inoculation and at the end of the crop cycle. Plant roots were thoroughly removed from soil, washed and recorded according to [10] scale with one supplementary quotation (2+): 0, no visible swelling; 1, very slight swelling usually confined to lateral roots; 2, moderate swelling on lateral roots and taproot; 2+, severe clubs on all roots but some roots remain safe; 3, no root left, only one big gall. A disease index (D.I.) was calculated by summation of the coefficients (0, 25, 50, 75, 100) affecting each plant class frequency [10].

## 2.3. Application of water shortage

Water shortage (WS) was applied starting from the onset of flowering (*i.e.*, BBCH60). Before WS, all the tanks were supplied equally with a nutritive solution (Liquoplant™ FD 134 Hiver; 4 ‰ v/v) that was replaced by water after WS application in order to maintain the water potential of the substrate above -200 mbar for the control tanks and around -600 mbar for the treated ones.

## 2.4. Quantitative metabolite profiling of seed samples

For metabolite analysis, approximately 200 seeds were ground in liquid nitrogen and 10–15 mg of the freeze-dried powder were sampled for a methanol–chloroform–water-based extraction adapted from [11]. Samples were suspended in 500 µL of methanol containing 200 µM DL-3-aminobutyric acid (BABA) and 400 µM adonitol as internal standards for UPLC and GC quantification respectively. Samples were gently agitated for 15 min at room temperature. Then, 250 µL of chloroform was added followed by 10 min of agitation and a final addition of 500 µL of water. The samples were vortexed vigorously and centrifuged 5 min at 12 000 g. The supernatant (500–700 µL) was collected and two aliquots of 50 µL were independently vacuum-dried for 2 h at 35 °C before metabolite profiling using service provided by the Metabolic Profiling and Metabolomic Platform (P2M2, IGEPP, Le Rheu, France). For analysis of free amino acids, dry residues were dissolved in 50 µL of ultrapure water and 5 µL were used for the derivatization with the AccQ-Tag Ultra Derivatization Kit according to the supplier's recommendations (Waters Corp., Milford, MA, USA). Derivatized amino acids were analyzed using an ultraperformance liquid chromatography system with diode array detection (Acquity UPLC-DAD system, Waters Corp.) as described previously [11,12]. Empower 2 software was used for data processing. Methods for analysis of sugars and organic acids were adapted from [11,13]. Briefly, dry residues were resuspended in 50 µL of 20 mg.mL<sup>-1</sup> methoxyamine hydrochloride in pyridine at

40 °C for 60 min. Then, 50 µL of MSTFA (N-methyl-trimethylsilyltrifluoroacetamide) were added for the derivatization (30 min at 40 °C). One microliter of the mixture was injected into a gas chromatography-flame ionization detector system (Trace 1300 GC-FID, Thermo-Fisher Scientific, Waltham, CA, USA) equipped with TriPlus RSH autosampler (Thermo-Fisher Scientific), a split/splitless injector (split mode set to 1:25) at 260 °C, a TG 5MS 30 m × 0.32 mm × 0.25 mm column and a flame ionization detector at 310 °C. The temperature gradient was as follows: 4 min at 70 °C, 10 °C.min<sup>-1</sup> until 198 °C followed by 2 min at 198 °C, 1 °C.min<sup>-1</sup> until 202 °C, 15 °C.min<sup>-1</sup> until 268 °C followed by 3 min at 268 °C, 1 °C.min<sup>-1</sup> until 272 °C, 10 °C.min<sup>-1</sup> until 310 °C and finally 7 min at 310 °C. Chromeleon software (V7.2.10, Thermo-Fisher Scientific) was used for data processing. For both analyses, external standards of known concentration were run every 10 samples to calibrate the system. Compounds were quantified with respect to the internal standard signals, using BABA for amino acids and adonitol for sugars and organic acids. All metabolite contents were expressed in nmol.mg<sup>-1</sup> of seed dry matter (DM).

## 2.5. Germination parameters

Germination performances were monitored under *in vitro* conditions at 20 °C in darkness (except during LED flash lightening for imaging), using the automated high-throughput phenotyping facilities for seed quality analysis developed by the French National Seed Testing Station of the Variety and Seed Study Control Group (PHENOTIC platform, GEVES, Beaucouzé, France). Detailed information about the phenotyping system is given by [1]. Seed germination assays were carried out using 4 replicates of 25 seeds per seed lot. Numerous germination parameters were acquired by image analysis of each single seed over 96 h of imbibition at 20 °C as no further increase in germination was observed beyond this time point. The data were then aggregated to provide accurate germination time progress curves for each seed set.

## CRedit Author Statement

**Grégoire Bianchetti:** Investigation, Data curation, Visualization, Writing- Original draft preparation; **Cécile Baron:** Investigation; **Aurélien Carrillo:** Investigation; **Solenn Berardocco:** Investigation, Data curation, Visualization; **Nathalie Marnet:** Investigation, Data curation, Visualization; **Marie-Hélène Wagner:** Investigation, Data curation, Visualization; **Didier Demilly:** Software, Validation; **Sylvie Ducournau:** Validation; **Maria Manzaneres-Dauleux:** Methodology; **Françoise Le Cahérec:** Methodology; **Julia Buitink:** Supervision, Conceptualization, Writing- Reviewing and Editing; **Nathalie Nesi:** Supervision, Conceptualization, Writing- Reviewing and Editing.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships which have or could be perceived to have influenced the work reported in this article.

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