

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- | | |
|-------------------------------------|--|
| n/a | Confirmed |
| ☐ | ☒ The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☐ | ☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☐ | ☒ The statistical test(s) used AND whether they are one- or two-sided
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☐ | ☒ A description of all covariates tested |
| ☐ | ☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☐ | ☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☐ | ☒ For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☒ | ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☐ | ☒ Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated |

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	No software was used
Data analysis	All original code used throughout the data analysis is publicly available at the following GitHub repository: https://github.com/Guannan-Wang/BrassicaceaeAtlas. And the following software and packages were used during the data analysis (the version information is included whenever applicable): OrthoFinder v2.5.4 (https://github.com/davidemms/OrthoFinder); CLfinder (https://github.com/ohdongha/OrthNet); MMSeq2 (https://github.com/soedinglab/MMseqs2); Cell Ranger v5.0.1 (https://www.10xgenomics.com/support/software/cell-ranger/latest); Seurat v4.0.5 (https://satijalab.org/seurat/); SEMITONES (https://github.com/ohlerlab/SEMITONES); Symphony (https://github.com/immunogenomics/symphony); scArches (https://github.com/theislab/scarches); Mfuzz v. 2.54.0 (DOI: 10.18129/B9.bioc.Mfuzz); Augur (https://github.com/neurorestore/Augur); aerie (http://www.eisenlab.com/FuzzyK/FuzzyK.html); Monocle3 (https://cole-trapnell-lab.github.io/monocle3/); DESeq2 (DOI: 10.18129/B9.bioc.DESeq2); CODEML (http://abacus.gene.ucl.ac.uk/software/paml.html); FastQC v0.11.5 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc); HISAT2 v2.2.1 (https://daehwankimlab.github.io/hisat2/); featureCounts v2.0.3 (https://subread.sourceforge.net/); edgeR v3.36.0 (DOI: 10.18129/B9.bioc.edgeR); BiNGO (https://apps.cytoscape.org/apps/bingo); GOMCL (https://github.com/Guannan-Wang/GOMCL); Fiji (https://imagej.net/software/fiji)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

All data are available in the main text, the Supplemental Information, referenced databases and public databases. All raw sequence data generated in the current study have been deposited at NCBI under BioProject PRJNA1113801. All processed data can be found at NCBI GEO database under accession code GEO: GSE268881.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	No human related research have been used in this study
Reporting on race, ethnicity, or other socially relevant groupings	No human related research have been used in this study
Population characteristics	No human related research have been used in this study
Recruitment	No human related research have been used in this study
Ethics oversight	No human related research have been used in this study

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No sample size calculation was performed. Each sampling was performed on a new batch of seedlings from each species and sample size was based on the amount of individual plants which gave sufficient materials for each experiment.
Data exclusions	No data were excluded from the analysis.
Replication	For each species under each condition, data was collected from 2 biological replicates for single cell RNA-seq. For bulk RNA-seq, there were three replicates for each species. No technical repetitions were made in this study.
Randomization	Plants were grown on individual plates or hydroponic trays and different replicates for each treatment were allocated randomly in the growth chamber.
Blinding	Not applicable since no group allocation was conducted in this study.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☐	☒ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Plants

Seed stocks

Arabidopsis thaliana (ecotype Col-0): ABRC CS70000; Eutrema salsugenum (ecotype Shandong): ABRC CS22504; Schrenkiella parvula (ecotype Lake Tuz): ABRC CS22663; Camelina sativa (ecotype DH55): from Isobel Parkin, Univeristy of Saskatchewan; Sisymbrium irio: original accession from Chris Pires, University of Missouri).

Novel plant genotypes

We do not report any novel plant genotypes.

Authentication

Not applicable for this study.