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Pangenomics and machine learning for disease resistance

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2023

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- Illumina
- Good, cheap and short

- PacBio Sequel
- Long HiFi reads

- Oxford nanopore
- Long reads

Sequence genomes

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The genome of the mesopolyploid crop species *Brassica rapa*

A reference genome for pea provides insight into legume genome evolution.

A chromosome-based draft sequence of the hexaploid bread wheat (*Triticum aestivum*) genome

Studying the genetic diversity of yam bean using a new draft genome assembly.

Early allopolyploid evolution in the post-Neolithic *Brassica napus* oilseed genome

The *Brassica oleracea* genome reveals the asymmetrical evolution of polyploid genomes

Assembly of the non-heading pak choi genome and comparison with the genomes of heading Chinese cabbage and the oilseed yellow sarson.

Dispersion and domestication shaped the genome of bread wheat

Assembly and comparison of two closely related *Brassica napus* genomes.

Shifting the limits in wheat research and breeding using a fully annotated reference genome.

Draft genome sequence of chickpea (*Cicer arietinum*) provides a resource for trait improvement

The genome of a southern hemisphere seagrass species (*Zostera muelleri*)[1]

Sequencing and assembly of low copy and genic regions of isolated *Triticum aestivum* chromosome arm 7DS

A comprehensive draft genome sequence for lupin (*Lupinus angustifolius*), an emerging health food: Insights into plant-microbe interactions and legume evolution.

The improved assembly of 7DL chromosome provides insight into the structure and evolution of bread wheat.

Draft genome assembly and transcriptome dataset for European turnip (*Brassica rapa* L. ssp. rapifera), ECD4 carrying clubroot resistance

The genome sequence of the Antarctic bullhead notothen reveals evolutionary adaptations to a cold environment

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Pangenomes are the new reference

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- A single reference genome does not represent the diversity of a species
- PAV genes are responsible for important agronomic traits
- Pangenomes capture heritability missing in single genomes
- Need to know gene content for genome editing

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Building a pangenome

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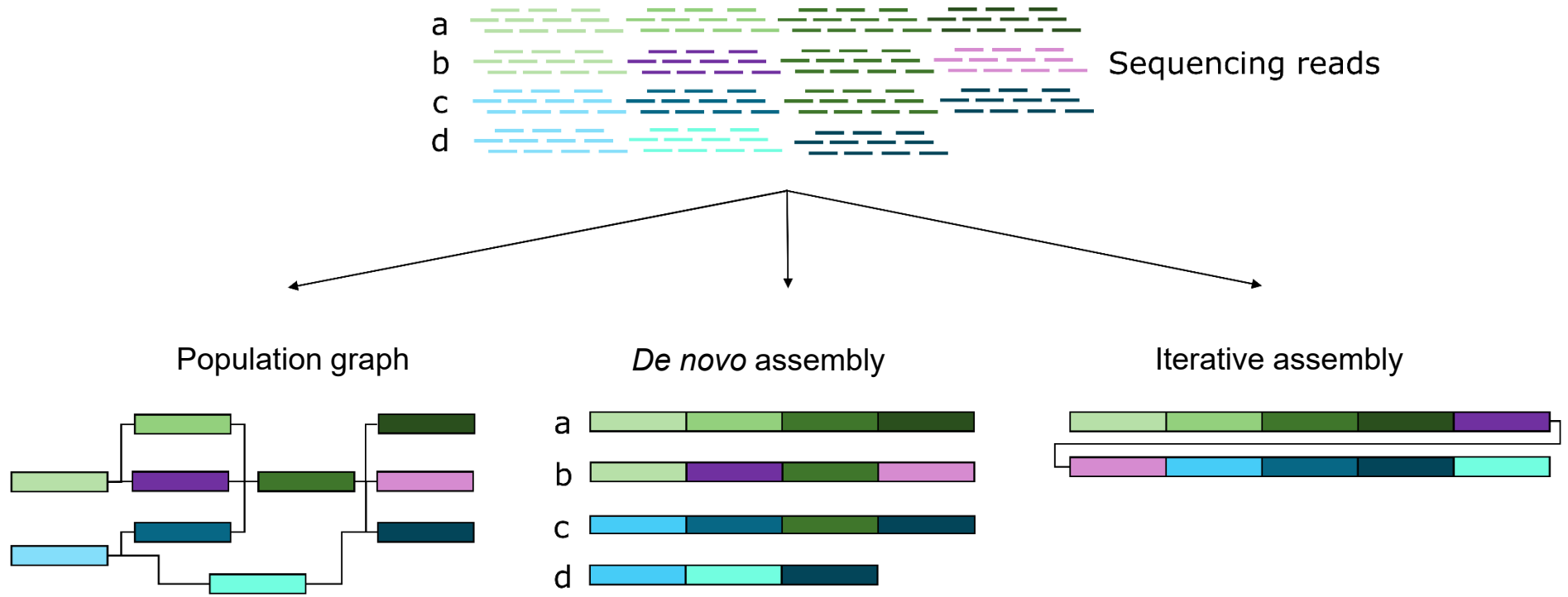

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Brassica oleracea pangenome

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ARTICLE

Received 22 Aug 2016 | Accepted 28 Sep 2016 | Published 11 Nov 2016

DOI: 10.1038/ncomms13390 OPEN

The pangenome of an agronomically important crop plant *Brassica oleracea*

Agnieszka A. Golicz[1], Philipp E. Bayer[2], Guy C. Barker[3], Patrick P. Edger[4], HyeRan Kim[5], Paula A. Martinez[1], Chon Kit Kenneth Chan[2], Anita Severn-Ellis[2], W. Richard McCombie[6], Isobel A.P. Parkin[7], Andrew H. Paterson[8], J. Chris Pires[9], Andrew G. Sharpe[10], Haibao Tang[11], Graham R. Teakle[3], Christopher D. Town[12], Jacqueline Batley[2] & David Edwards[2]

Diverse morphotypes

- Cabbage (2)
- Cauliflower (2)
- Broccoli
- Kale
- Brussels sprout
- Rapid cycler TO1000
- *B. macrocarpa*

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Brassica oleracea pangenome

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Previous reference genomes:

B. oleracea TO1000	54,458 genes, 488 Mbp
B. oleracea var. capitata	45,758 genes, 535 Mbp

Pangenome	61,379 genes, 587 Mbp,

18.7% of genes are variable

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Brassica oleracea pangenome

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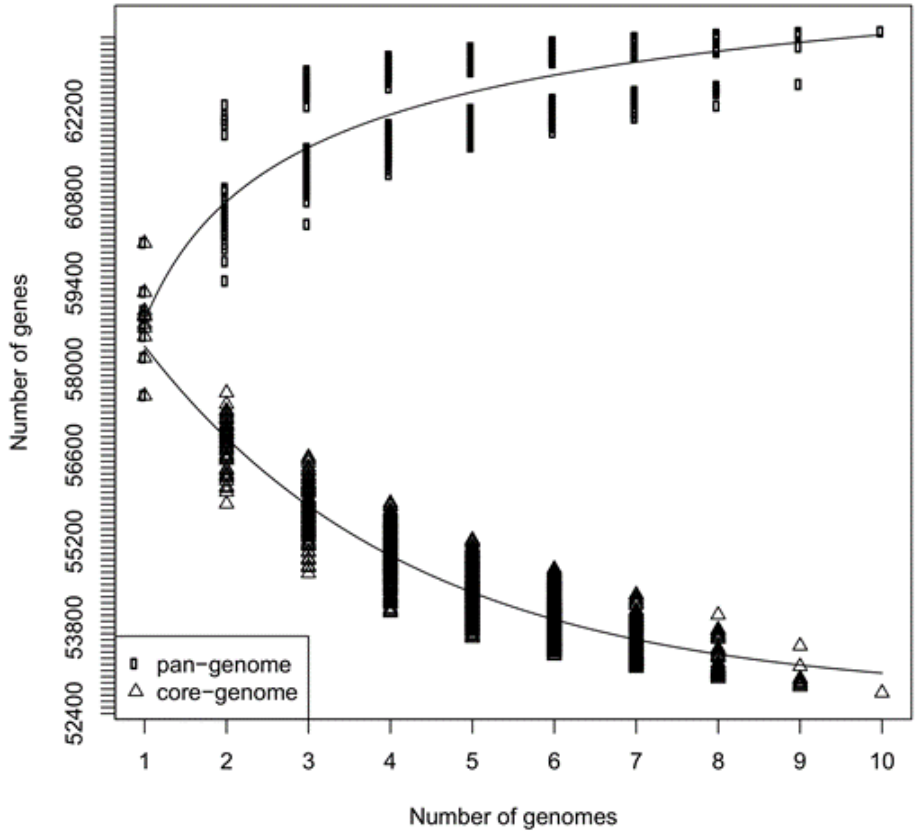
Adding more genomes captures more genes and defined the core and variable genome



Brassica oleracea

Predicted pangenome of 61,198 ± 394 genes (35,462 ± 250 gene families)

Predicted core genome size of 49,676 ± 96 genes (28,489 ± 51 gene families)

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Brassica oleracea pangenome

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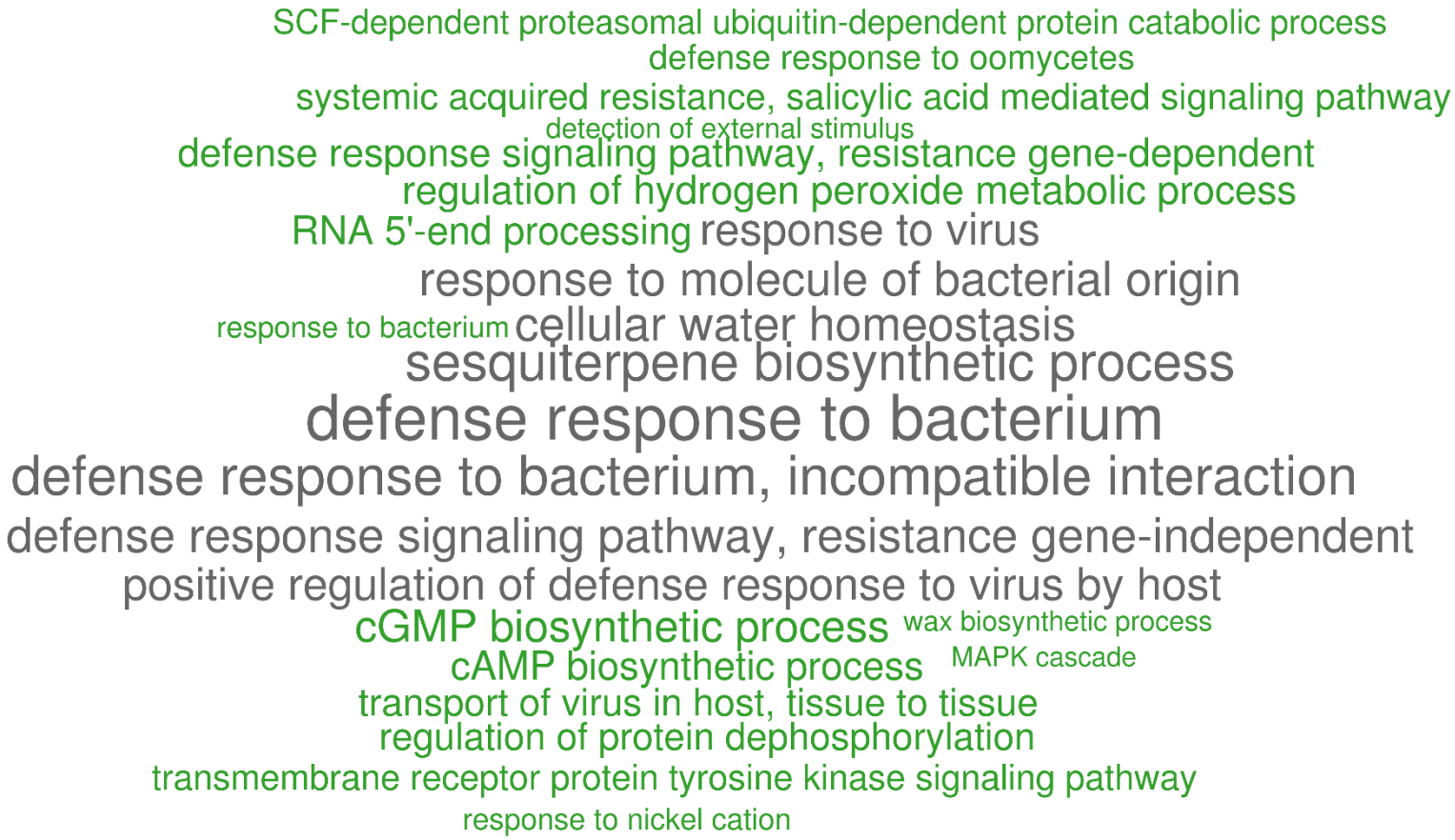

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Disease resistance genes

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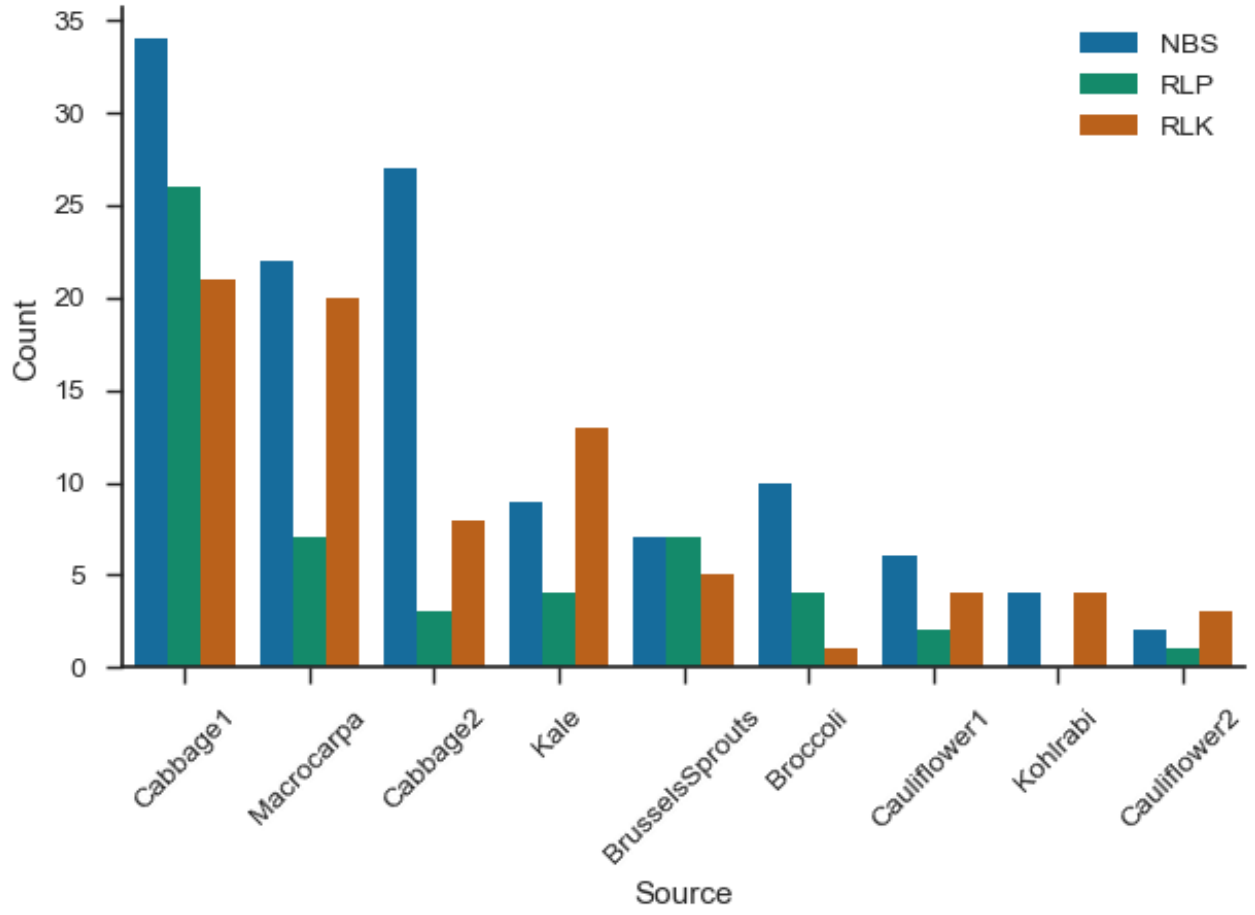

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Brassica napus

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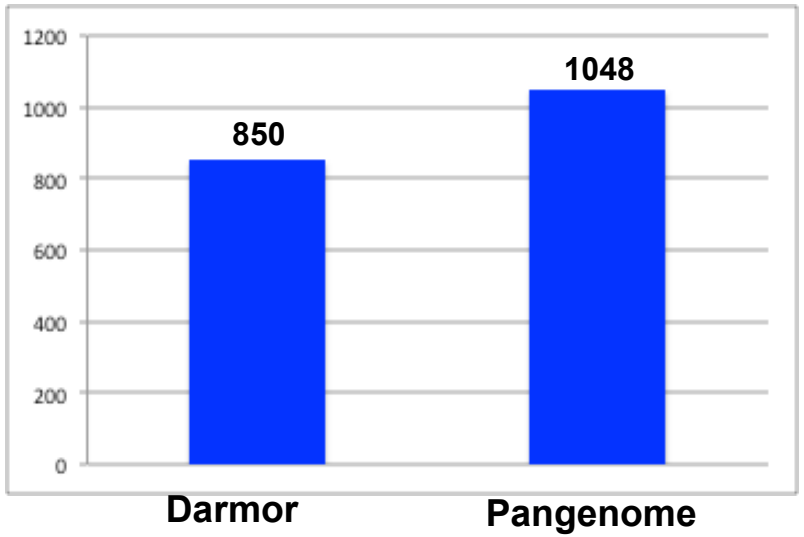
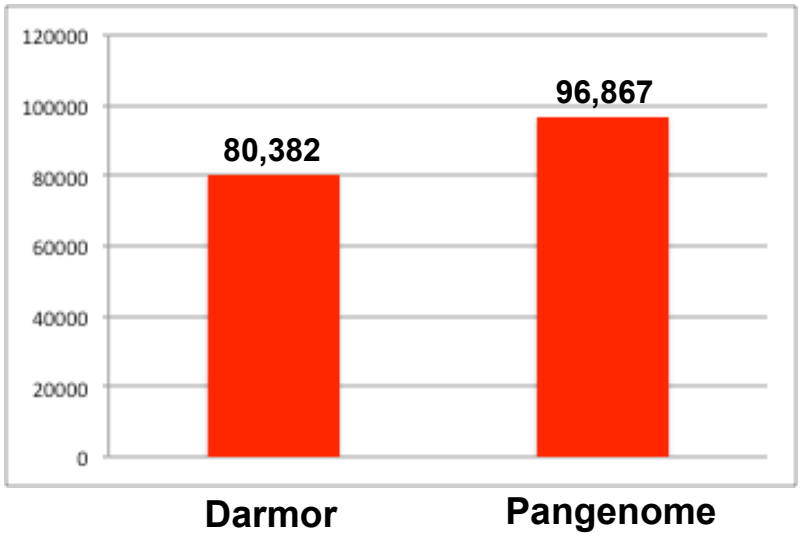
- 33 *B. napus*
- 20 resynthesised *B. napus*

Assembly size (Mb)



No. of genes



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Brassica napus

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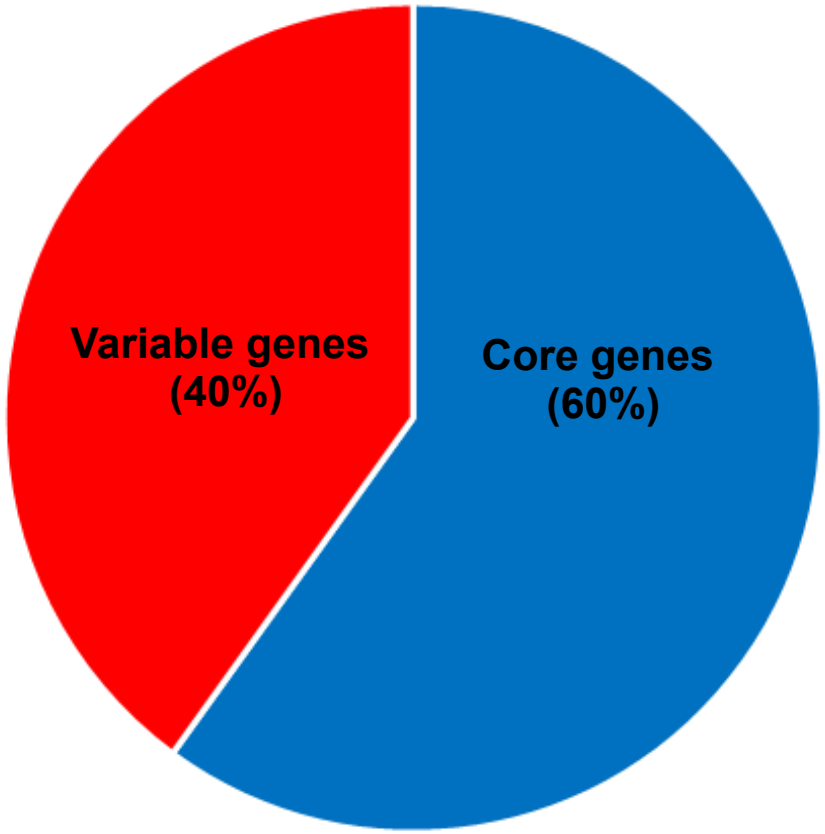

- Variable genes are shorter and have fewer exons than core genes
- Number of variable genes indicates how much diversity is present

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Brassica napus

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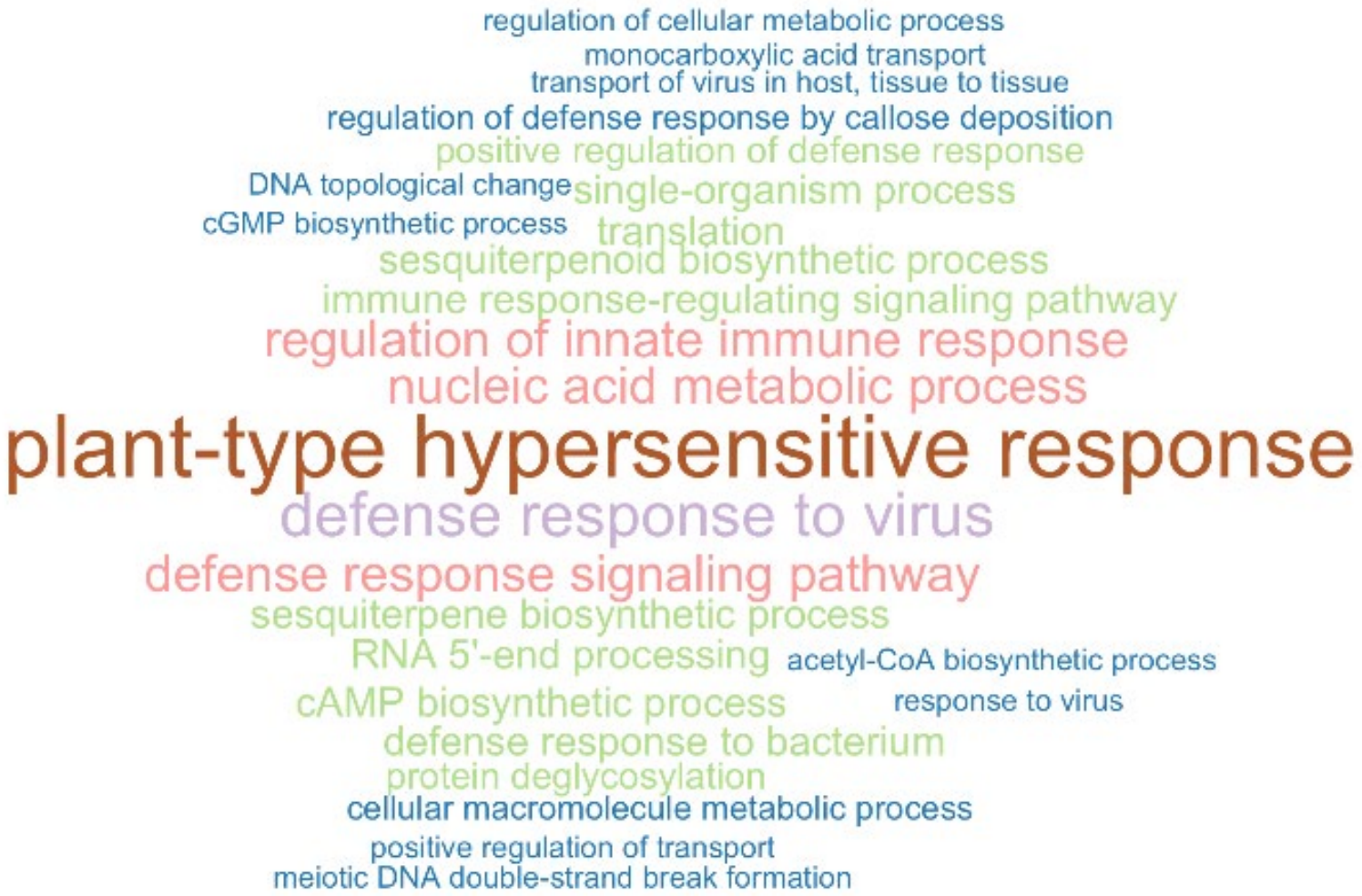

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Mechanism of PAV

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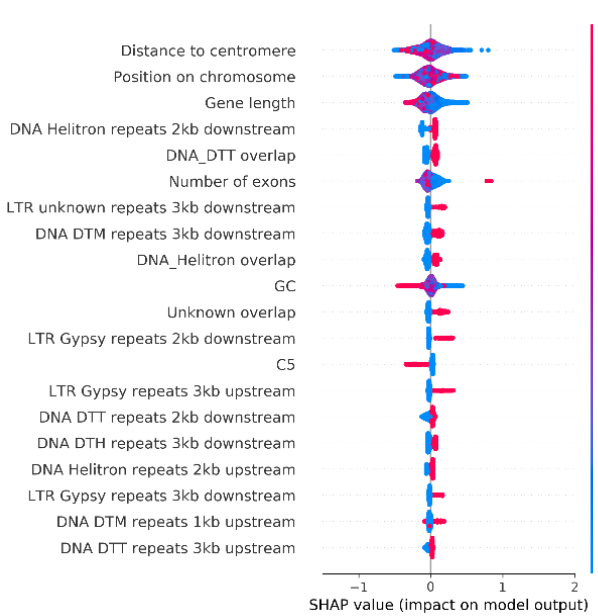
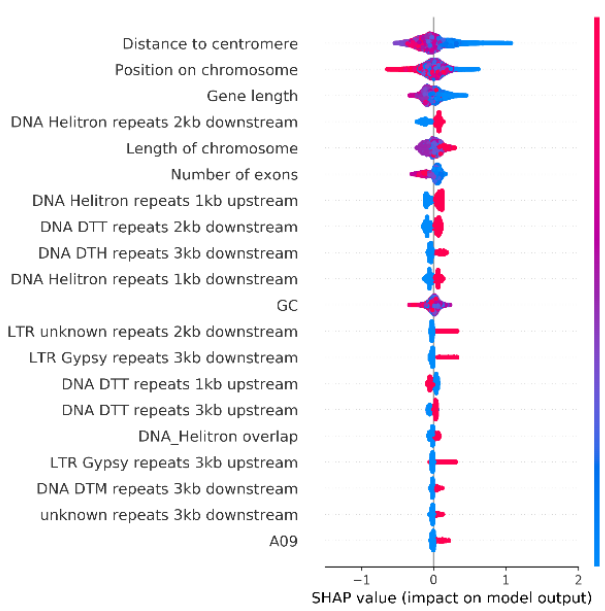
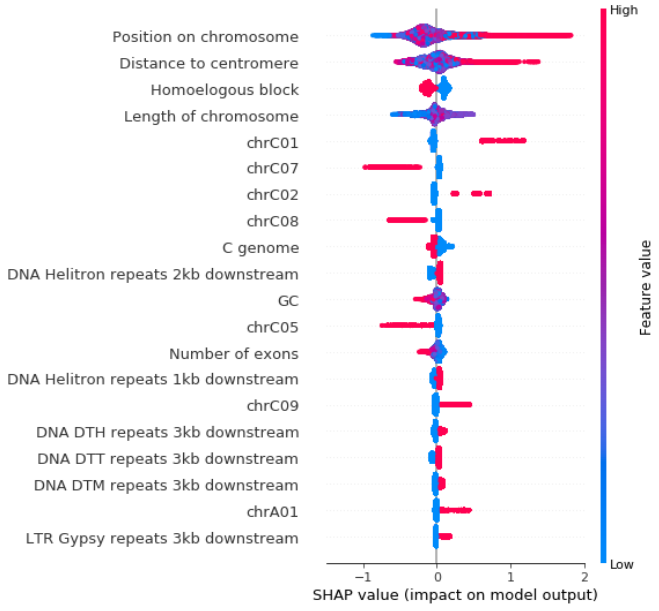


B. oleracea



B. rapa



B. napus

Bayer et al. (2021) Modelling of gene loss propensity in the pangenomes of three Brassica species suggests different mechanisms between polyploids and diploids. Plant Biotechnology Journal. 19 (12): 2488-2500

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Brassica napus

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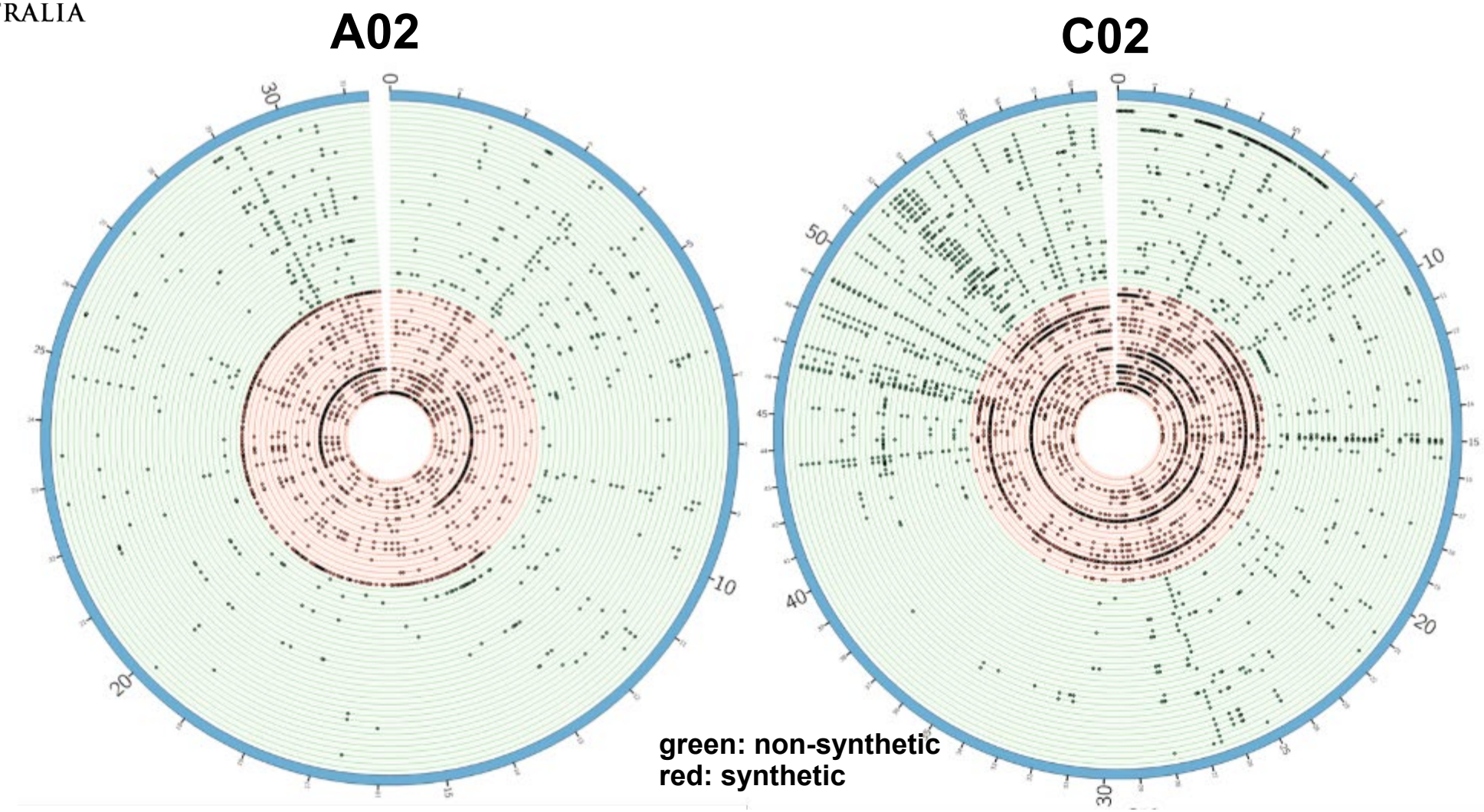

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Bayer PE, Petereit J, Durant E, Monat C, Rouard M, Hu H, Chapman B, Li C, Cheng S, Batley J, Edwards D. (2022) Wheat Panache: A pangenome graph database representing presence–absence variation across sixteen bread wheat genomes. The Plant Genome.

Golicz et al. (2020) Pangenomics comes of age: From bacteria to plant and animal applications. Trends in Genetics 36(2): 132-145

Zanini et al. (2021) Pangenomics in crop improvement – from coding SVs to finding regulatory variants with pangenome graphs. Plant Genome. 15 (1): e20177

Bayer et al. (2021) Modelling of gene loss propensity in the pangenomes of three Brassica species suggests different mechanisms between polyploids and diploids. Plant Biotechnology Journal. Plant Biotechnology Journal. 19 (12): 2488-2500

Varshney et al. (2021) A chickpea genetic variation map based on the sequencing of 3,366 genomes. Nature. 599: 622–627 https://doi.org/10.1038/s41586-021-04066-1

Hurgobin et al. (2018) Homoeologous exchange is a major cause of gene presence/absence variation in the amphidiploid Brassica napus. Plant Biotechnology Journal. 16 (7), 1265-1274

Bayer et al. (2022) Sequencing the USDA core soybean collection reveals gene loss during domestication and breeding. The Plant Genome. 15: e20109

Khan et al. (2020) Super-pangenome by integrating the wild side of a species for accelerated crop improvement. Trends in Plant Science. 25 (2): 148-158

Dolatabadian A, Bayer P, Tirnaz S, Hurgobin B, Edwards D, Batley J. (2020) Characterisation of disease resistance genes in the Brassica napus pangenome reveals significant structural variation. Plant Biotechnology Journal. 18 (4): 969-982

Ruperao et al. (2021) Sorghum pan-genome explores the functional utility for genomic- assisted breeding to accelerate the genetic gain. Frontiers in Plant Science. 12:963

Zhao et al. (2020) Trait associations in the pangenome of pigeon pea (Cajanus cajan) Plant Biotechnology Journal. 18: 1946-1954

Golicz et al. (2016) The pangenome of an agronomically important crop Brassica oleracea. Nature Communications 7:13390

Yu et al. (2019) Insight into the evolution and functional characteristics of the pan-genome assembly from sesame landraces and modern cultivars. Plant Biotechnology Journal. 17 (5): 881-892

Rijzaani H, Bayer PE, Rouard M, Doležel J, Batley J, Edwards D. (2022) The pangenome of banana highlights differences between genera and genomes. Plant Genome. 15: e20100

Bayer et al. (2017) Assembly and comparison of two closely related Brassica napus genomes. Plant Biotechnology Journal. 15 (12):1602-1610

Bayer et al. (2020) Plant pan-genomes are the new reference. Nature Plants. 6 (8): 1-7

Bayer et al. (2021) The application of pangenomics and machine learning in genomic selection. Plant Genome. e20112.

Wang et al. (2021). The chicken pan-genome reveals gene content variation and a regulatory region deletion in IGF2BP1 affecting body size. Molecular Biology and Evolution. 38 (11): 5066–5081

Golicz A, Batley J and Edwards D. (2016) Towards plant pangenomics. Plant Biotechnology Journal. 14 (4):1099-105

Montenegro JDM, Golicz AA, Bayer PE, Hurgobin B, Lee HT, Chan CKK, Visendi P, Lai K, Doležel J, Batley J, Edwards D. (2017) The pangenome of modern hexaploid bread wheat. Plant Journal. 90 (5): 1007-1013

Danilevicz et al. (2020) Plant Pangenomics: Approaches, Applications and Advancements. Current Opinion in Plant Biology. 54: 15-25

Hurgobin H and Edwards D. (2017) SNP discovery using a pangenome: has the single reference approach become obsolete? Biology 6 (1): E21

Tay Fernandez et al. (2022) Expanding gene-editing potential in crop improvement with pangenomes. International Journal of Molecular Sciences 23(4): 2276.

Hu H, Scheben A, Verpaalen B, Tirnaz S, Bayer PE, Hodel R, Batley J, Soltis D, Soltis P, Edwards D. (2022) Amborella gene presence/absence variation is associated with abiotic stress responses that may contribute to environmental adaptation. New Phytologist. 233 (4): 1548-1555

Tay Fernandez et al. (2022) Pangenomes as a resource to accelerate breeding of under-utilised crop species. International Journal of Molecular Sciences 23 (5): 2671

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Graph pangenome visualisation

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PLANT GENOMICS

Graph pangenomes find missing heritability

The use of association studies to identify candidate genes for complex biological traits in plants has been challenging due to a reliance on single reference genomes, leading to missing heritability. Graphical pangenomes and the identification of causal variants help overcome this and provide an important advance for crop breeding.

David Edwards and Jacqueline Batley

Edwards and Batley (2022) Nature Genetics 54: 919-920

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Link phenotypes to genome variation

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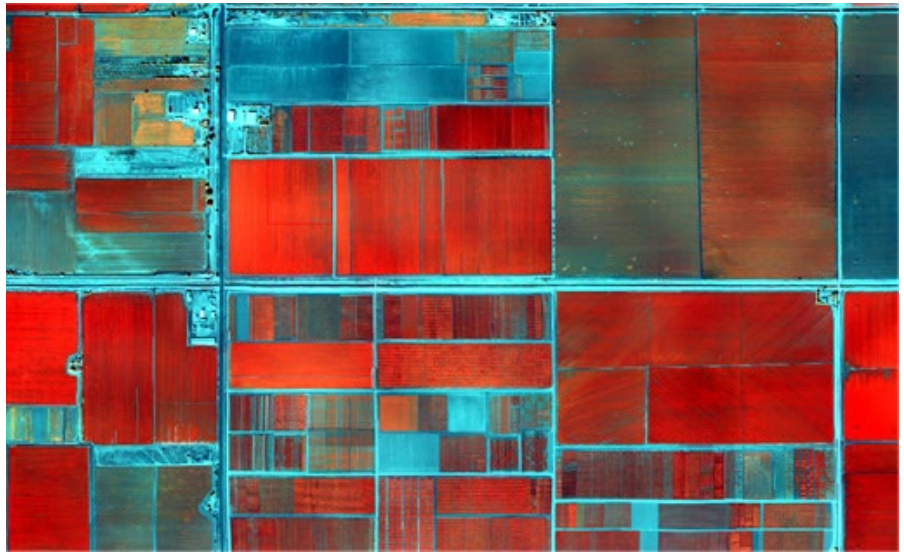
Next generation phenotyping



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The need for machine learning

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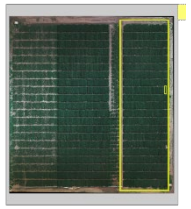
Types of Inputs

Image data

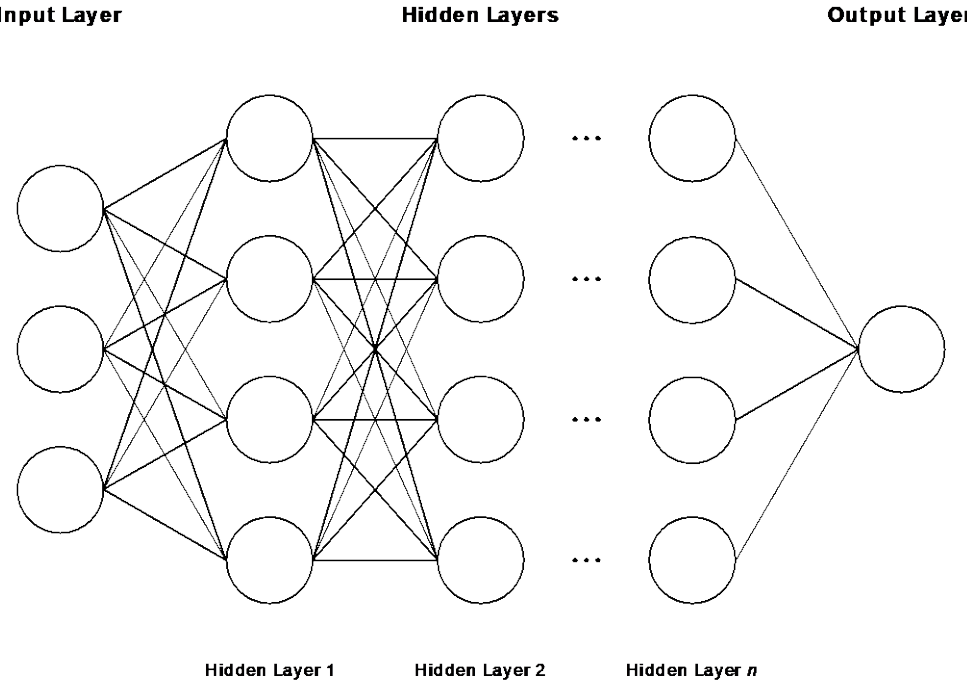


Text data

```
ACGTCACGTACTAG
CATGCATCGTAGCT
GTACGTACGTAGCT
```

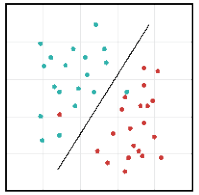
Tabular data

Contig	FDR	Control LB	Treatment MG
ptsG	5.10e-10	0.0	-3.96
setA	6.85e-8	0.0	4.24
sucB	3.10e-6	0.0	2.21
sucD	3.10e-6	0.0	2.46
deoC	3.10e-6	0.0	2.53

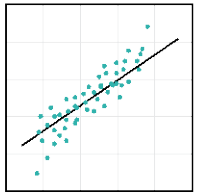


Types of Outputs

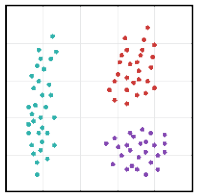
Classi cation



Regression



Clustering



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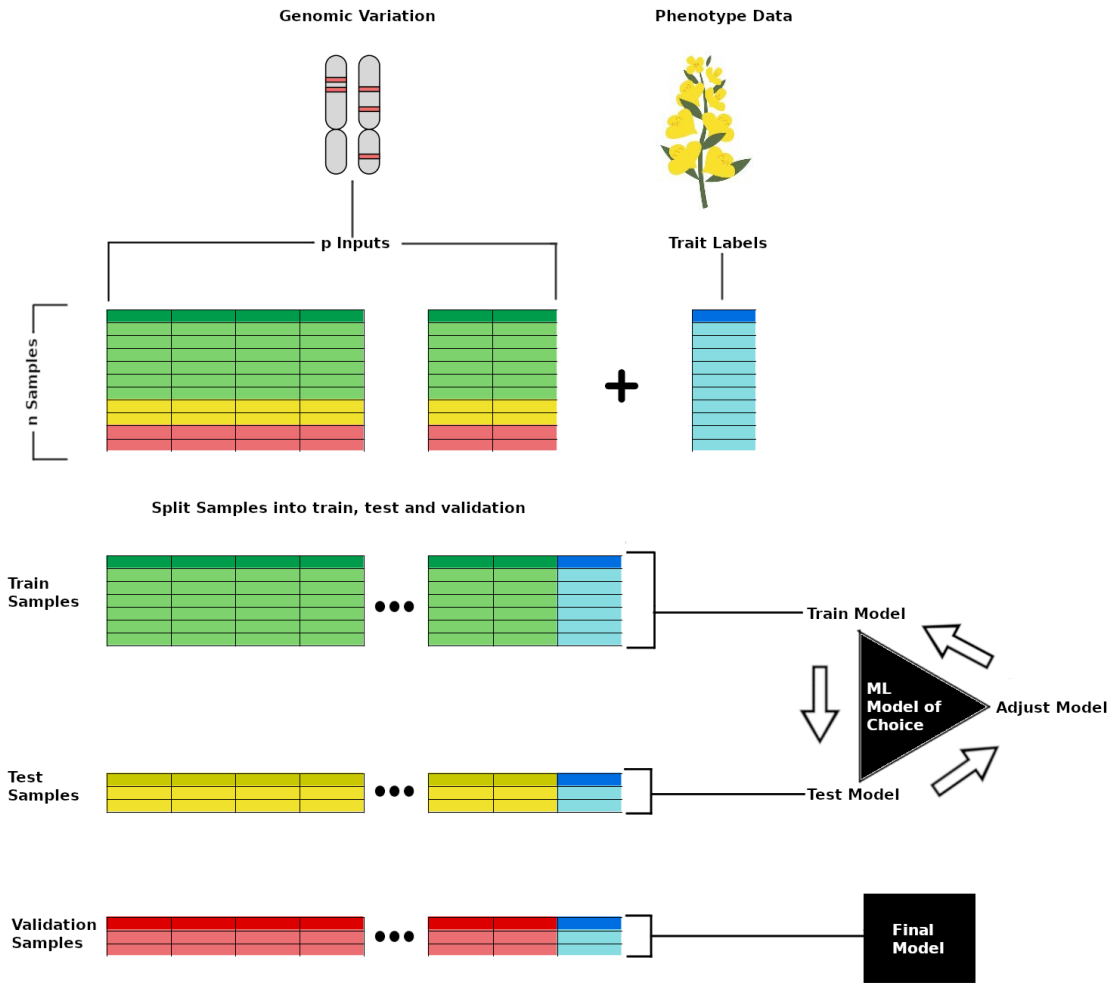
The need for machine learning



Danilevicz MF, Anderson R, Gill M, Batley J, Bennamoun M, Bayer PE, Edwards D. (2022) Plant genotype to phenotype prediction using machine learning. Frontiers in Genetics. 13: 822173

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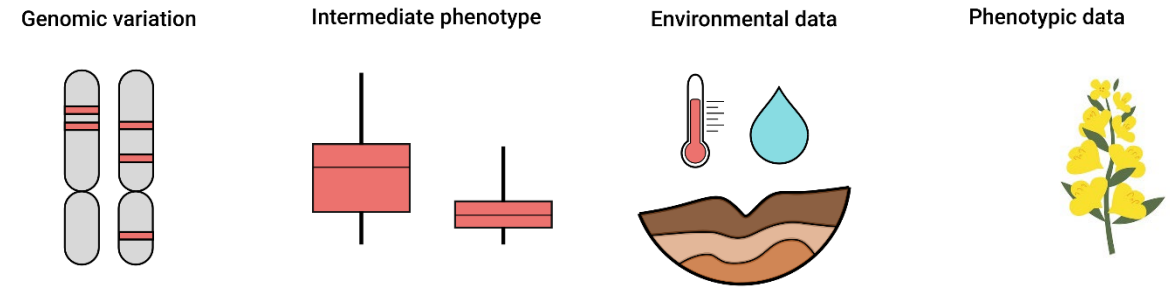
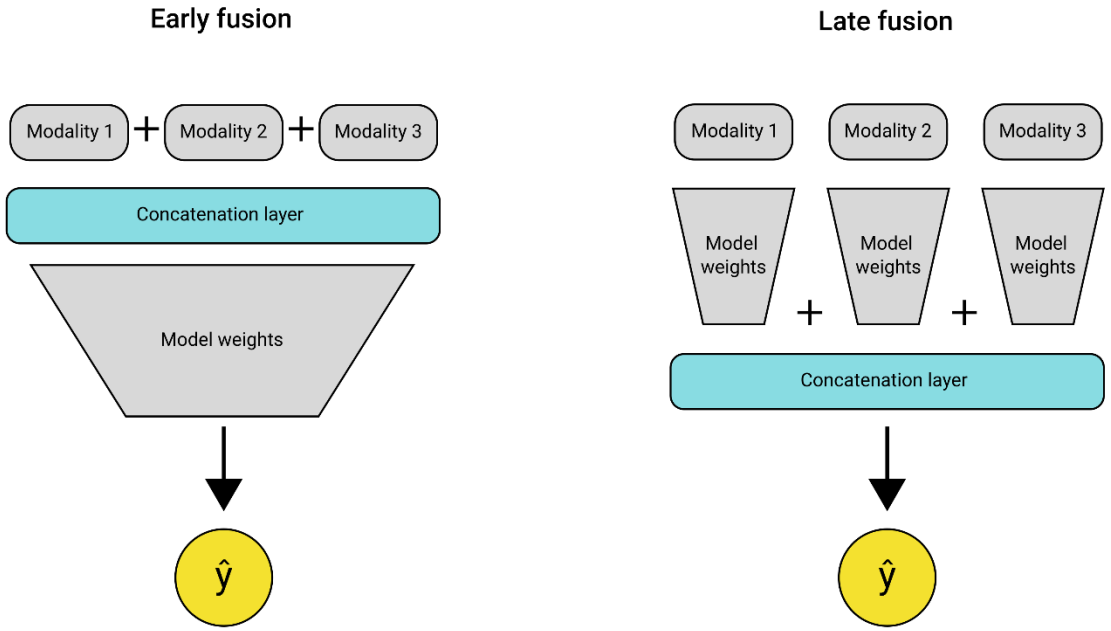
Data modalities



Multimodal deep learning



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Trait prediction from SNP data

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5.5 million SNPs, 700-1000 individuals per trait

Evaluation Metric	Trait	Learning Algorithm			
		XGB†	RF†	CNN†	DNN†
Accuracy	Flower Colour	**96.79%**	95.51%	87.82%	81.41%
	Pod Colour	**84.52%**	76.13%	70.32%	70.32%
	Pubescence Density	**91.56%**	81.17%	83.77%	80.52%
	Seed Coat Colour	**89.68%**	85.16%	84.52%	83.87%
RMSE % of Mean	Seed Oil Percentage	11.14%	**10.67%**	13.44%	10.74%
	Seed Protein Percentage	6.41%	**6.32%**	6.33%	6.91%
	Seed Weight	19.03%	21.63%	**17.77%**	18.91%

Identified genomic loci overlap with GWAS

Machine Learning outperforms Deep Learning

Gill M, Anderson R, Hu H, Bennamoun M, Petereit J, Valliyodan B, Nguyen HT, Batley J, Bayer PE, Edwards D. (2022) Machine learning models outperform deep learning models, provide interpretation and facilitate feature selection for soybean trait prediction. BMC Plant Biology 2, 180

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Yield prediction from image data

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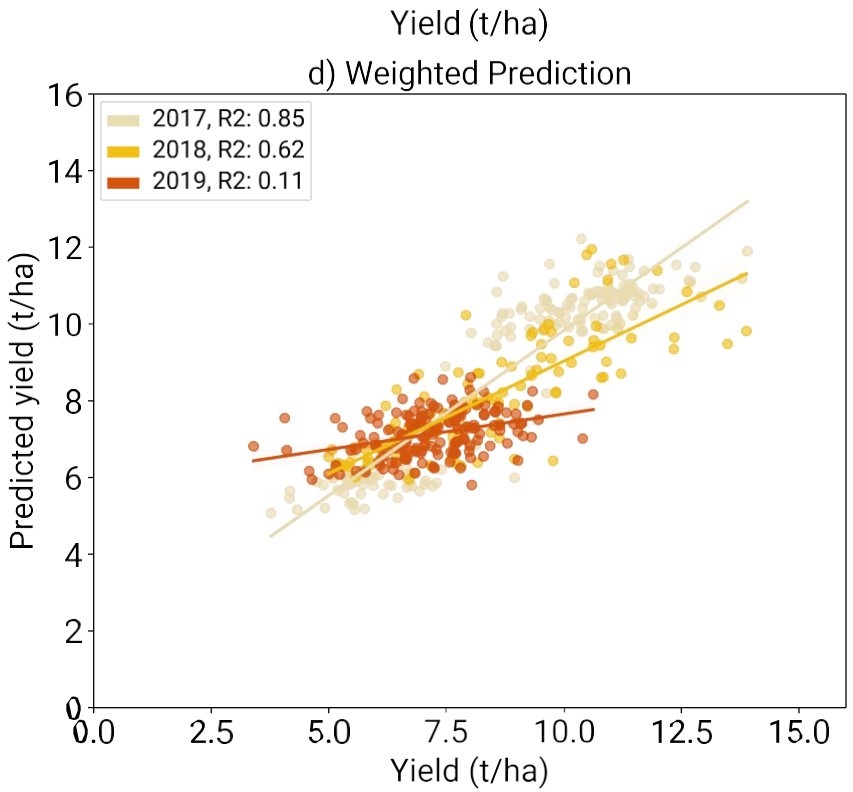

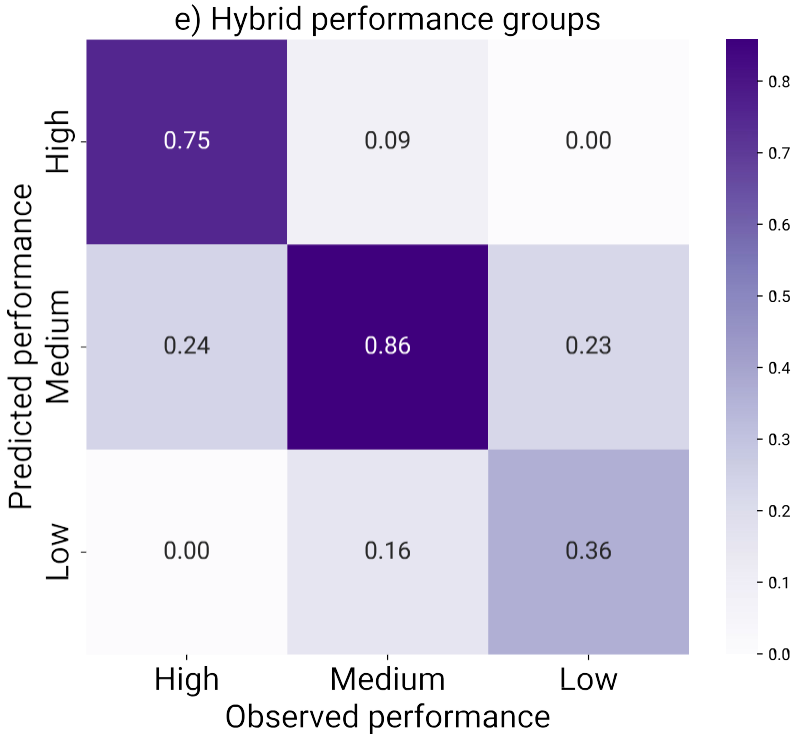

Danilevicz MF, Bayer PE, Boussaid F, Bennamoun M, Edwards D. (2021) Maize yield prediction at an early developmental stage using multispectral images and genotype data for preliminary hybrid selection in the field. Remote Sensing. 13 (19): 3976.

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R gene prediction in canola

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Class	Correct prediction
Rlm1	1951 (89.0%)
Rlm2	2160 (98.5%)
Rlm3	2042 (93.2%)
Rlm4	1977 (90.2%)
Rlm6	2160 (98.5%)
Rlm7	2181 (99.5%)
Rlm9	2031 (92.7%)
RlmS	2179 (99.4%)
LepR1	2151 (98.1%)
LepR2	2178 (99.4%)
LepR3	2178 (99.4%)

Based on phenotypic assays from isolate panel using XGBoost model

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Genotype based quantitative blackleg resistance in canola

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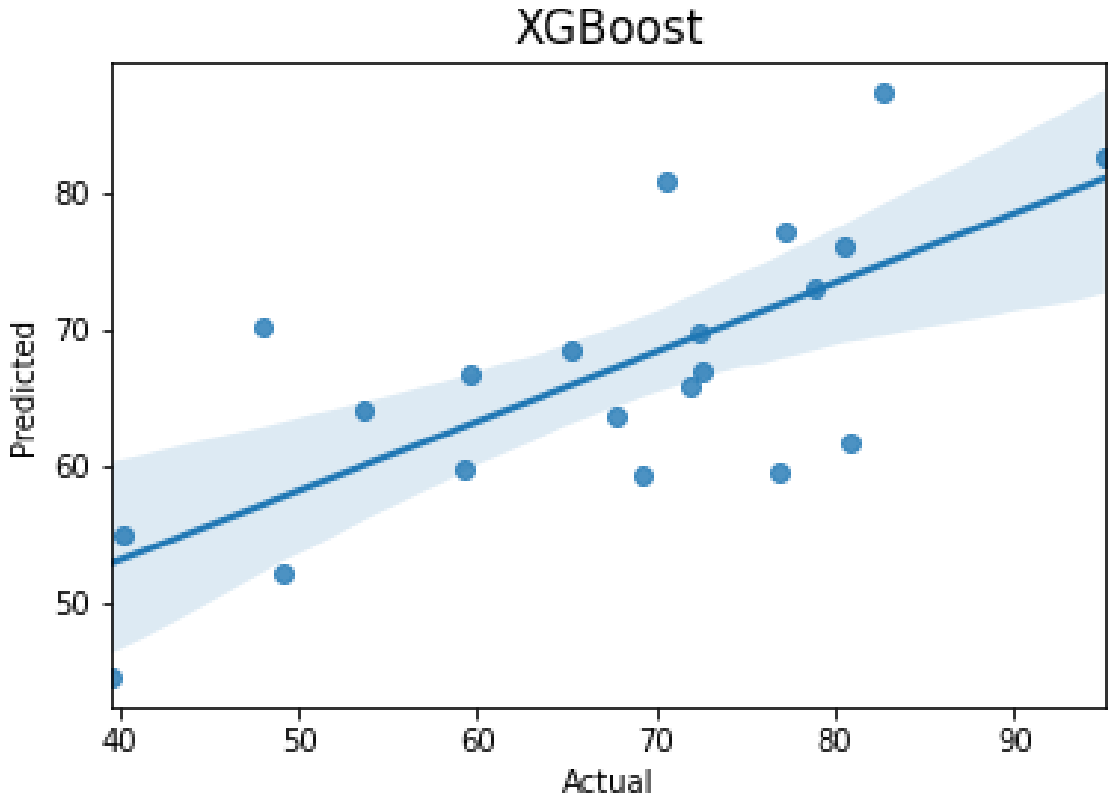

Small dataset, multi location multi year phenotypes
Loci important for prediction overlap with those identified using GWAS

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Next steps

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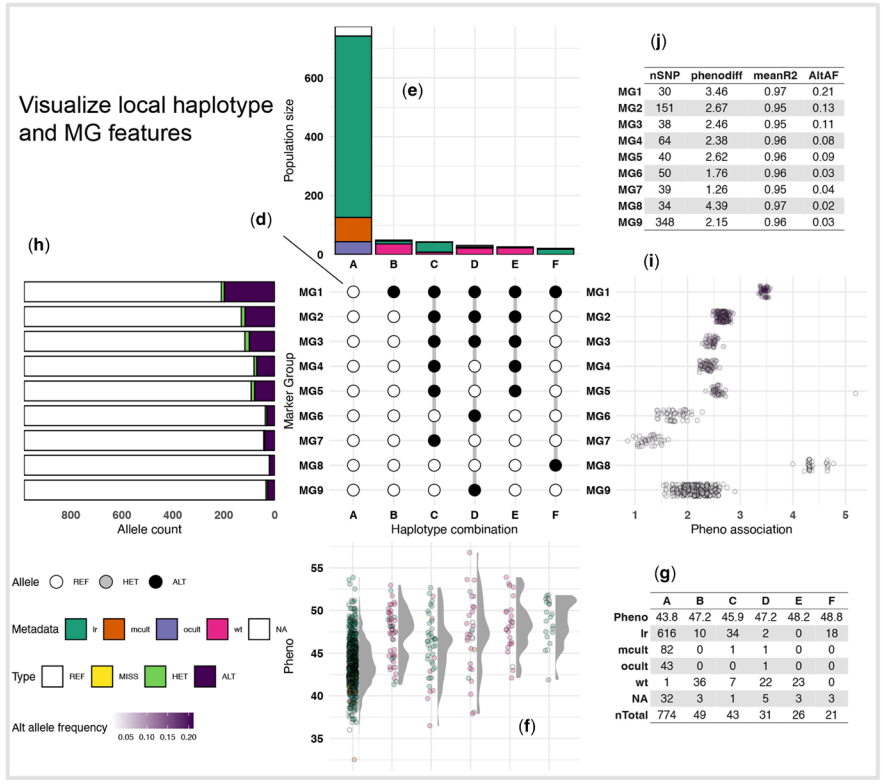
Cluster SNPs into Marker Groups (MGs)

Haplotype individuals by their MG allelles

Optimize haplotyping with clustering tree

Visualize local haplotype and MG features



(j)

	nSNP	phenodiff	meanR2	AltAF
MG1	30	3.46	0.97	0.21
MG2	151	2.67	0.95	0.13
MG3	38	2.46	0.95	0.11
MG4	64	2.38	0.96	0.08
MG5	40	2.62	0.96	0.09
MG6	50	1.76	0.96	0.03
MG7	39	1.26	0.95	0.04
MG8	34	4.39	0.97	0.02
MG9	348	2.15	0.96	0.03

(g)

	A	B	C	D	E	F
Pheno	43.8	47.2	45.9	47.2	48.2	48.8
lr	616	10	34	2	0	18
mcult	82	0	1	1	0	0
ocult	43	0	0	1	0	0
wt	1	36	7	22	23	0
NA	32	3	1	5	3	3
nTotal	774	49	43	31	26	21

Moving from SNPs to haplotypes

Genetics and population analysis

crosshap: R package for local haplotype visualization for trait association analysis

Jacob I. Marsh [1,2], Jakob Petereit [1,2], Brady A. Johnston [3], Philipp E. Bayer [1,2], Cassandria G. Tay Fernandez[1,2], Hawlader A. Al-Mamun[1,2], Jacqueline Batley [1,2], David Edwards [1,2,*]

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Next steps

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Build large language models based on publications (ChatGPT for crops)

Integrate data from knowledge graphs

Develop a comprehensive AI platform for crops and disease

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Thanks

Centre for Applied Bioinformatics

Thanks to my team past and present

Thanks to all my collaborators

Thanks for my funders

Australian Government

Australian Research Council

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