The genetic control of glucosinolate accumulation in the seeds of *Brassica napus*

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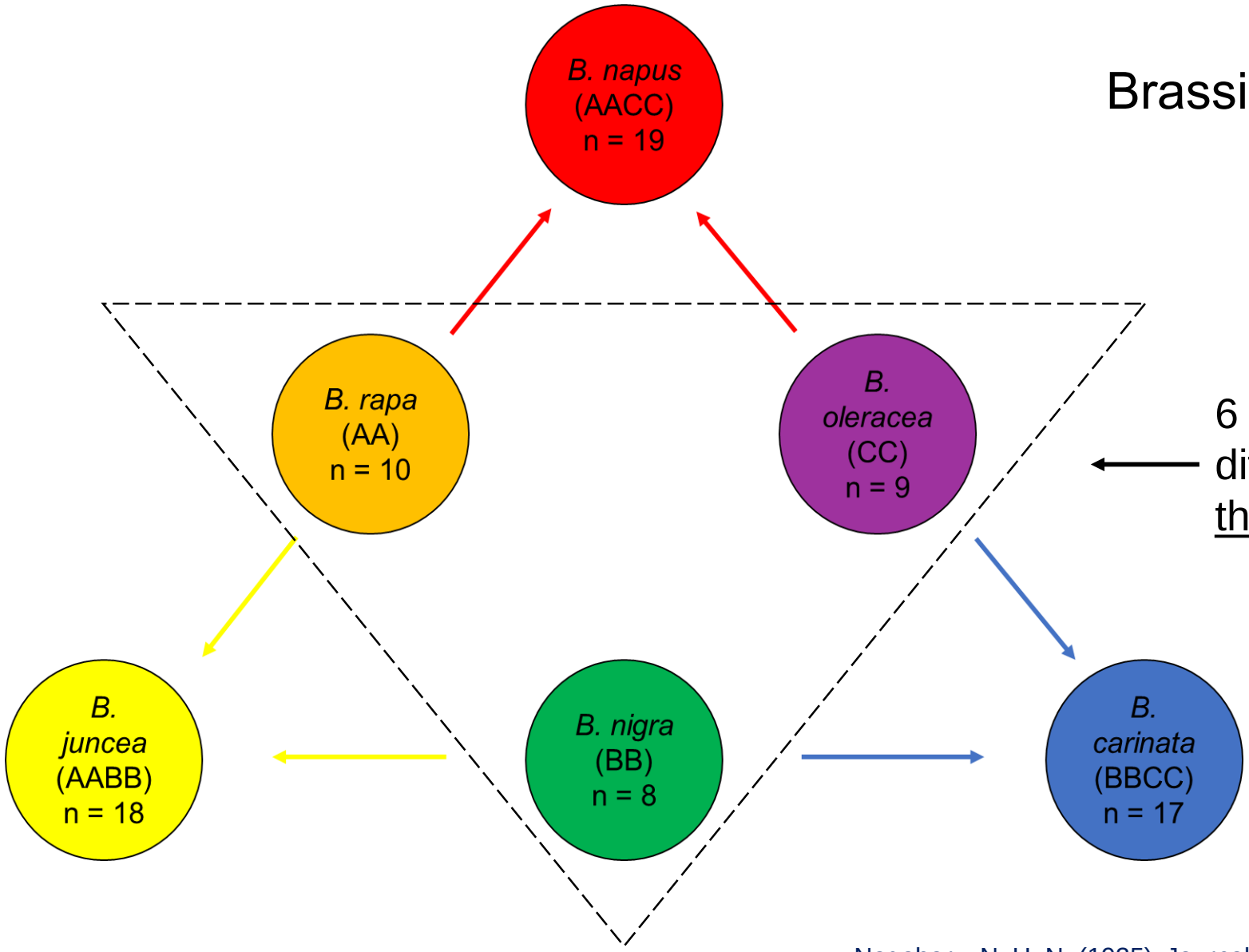
1st year PhD student from Bancroft Lab

Content

- Introduction to *Brassica napus*
- Introduction to Glucosinolates
- Glucosinolates Extraction and Identification

- Project design
  I. Experiment 1
  II. Experiment 2
  III. Experiment 3

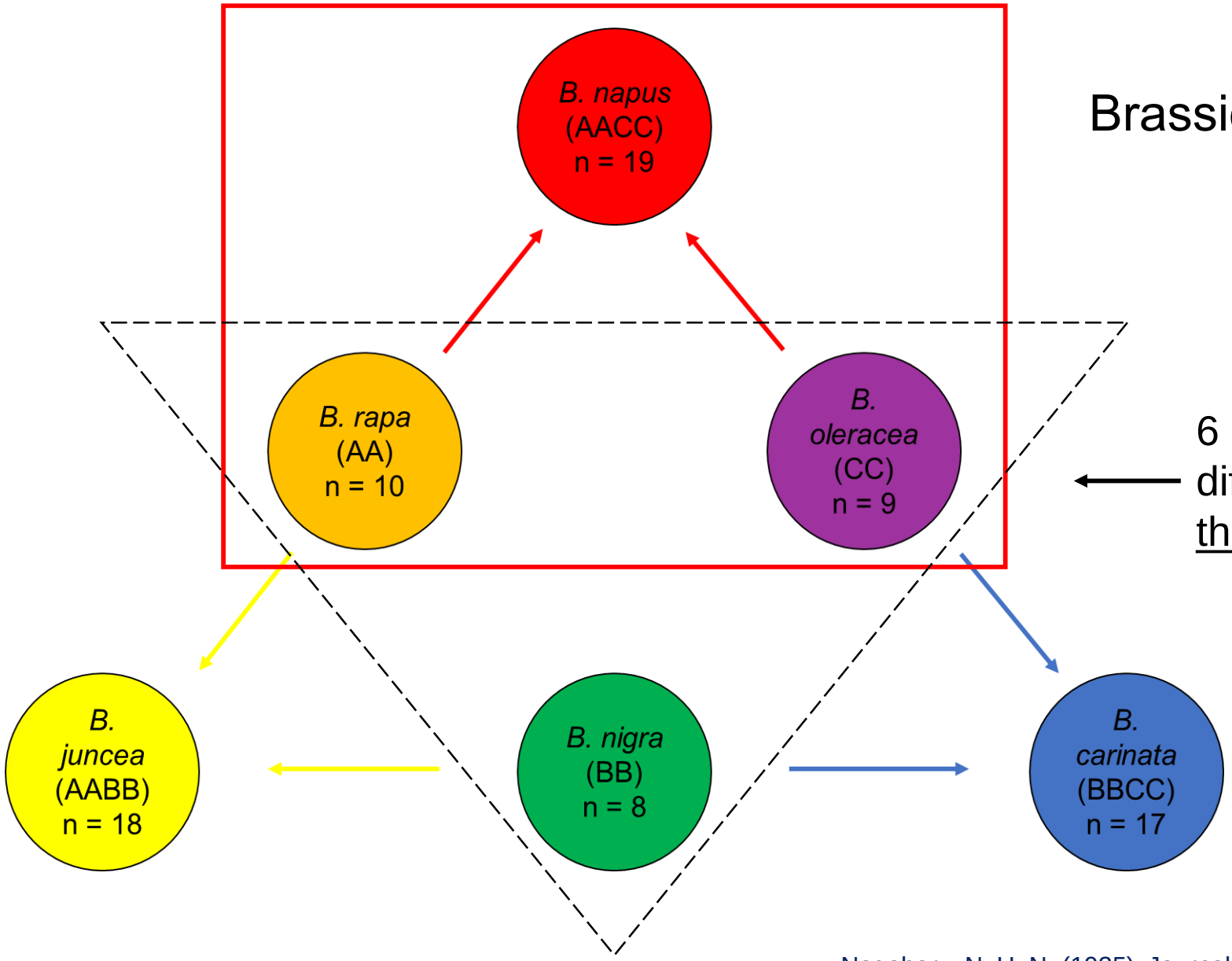
Brassica's triangle of "U"



6 species that share different combinations of <u>three distinct genomes</u>

Nagaharu, N. U. N. (1935), Journal of Japanese Botany, 7(7), pp. 389–452

Brassica's triangle of "U"



6 species that share different combinations of <u>three distinct genomes</u>

Nagaharu, N. U. N. (1935), Journal of Japanese Botany, 7(7), pp. 389–452

B. napus seeds

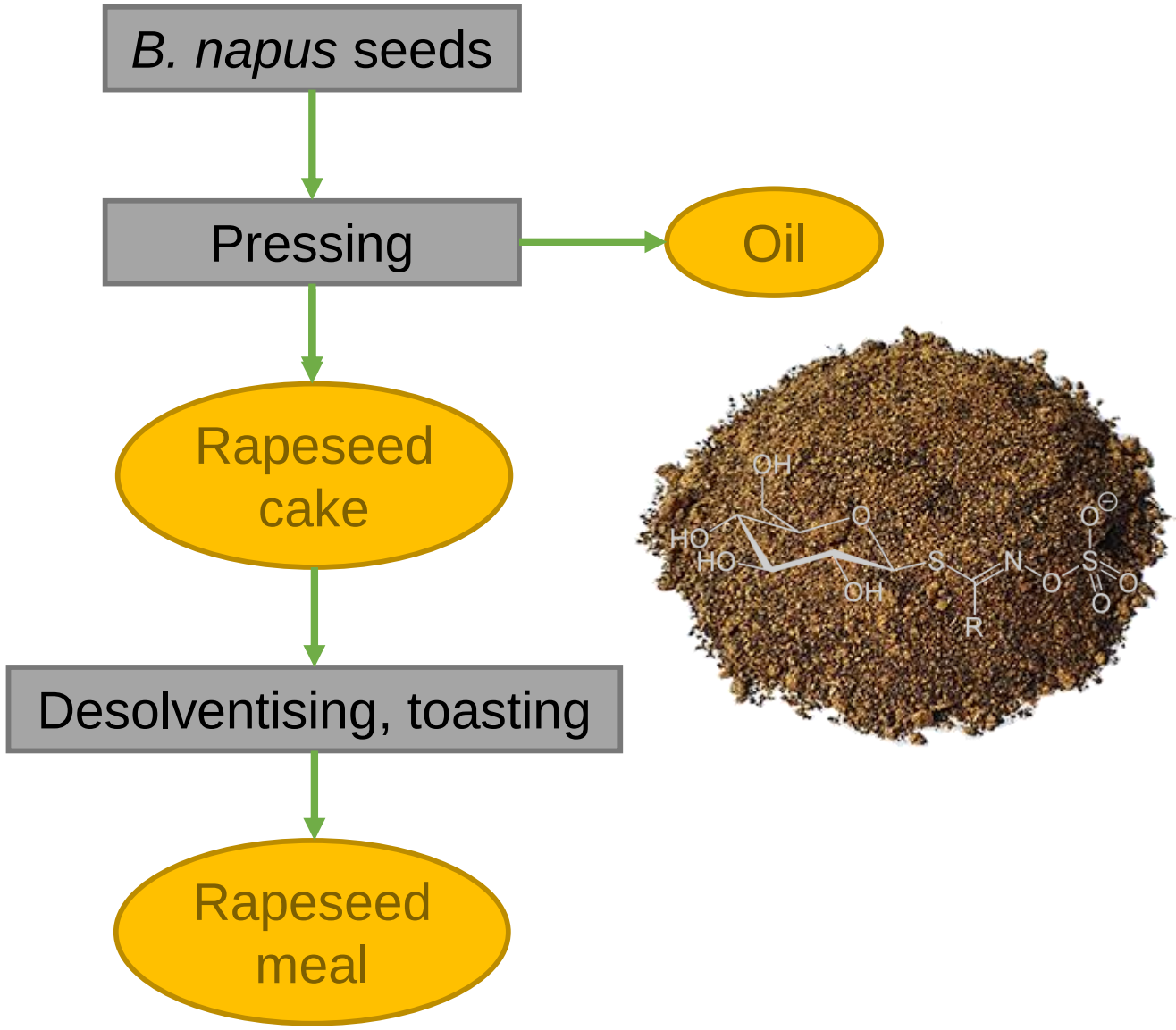
Pressing

Oil

Rapeseed cake

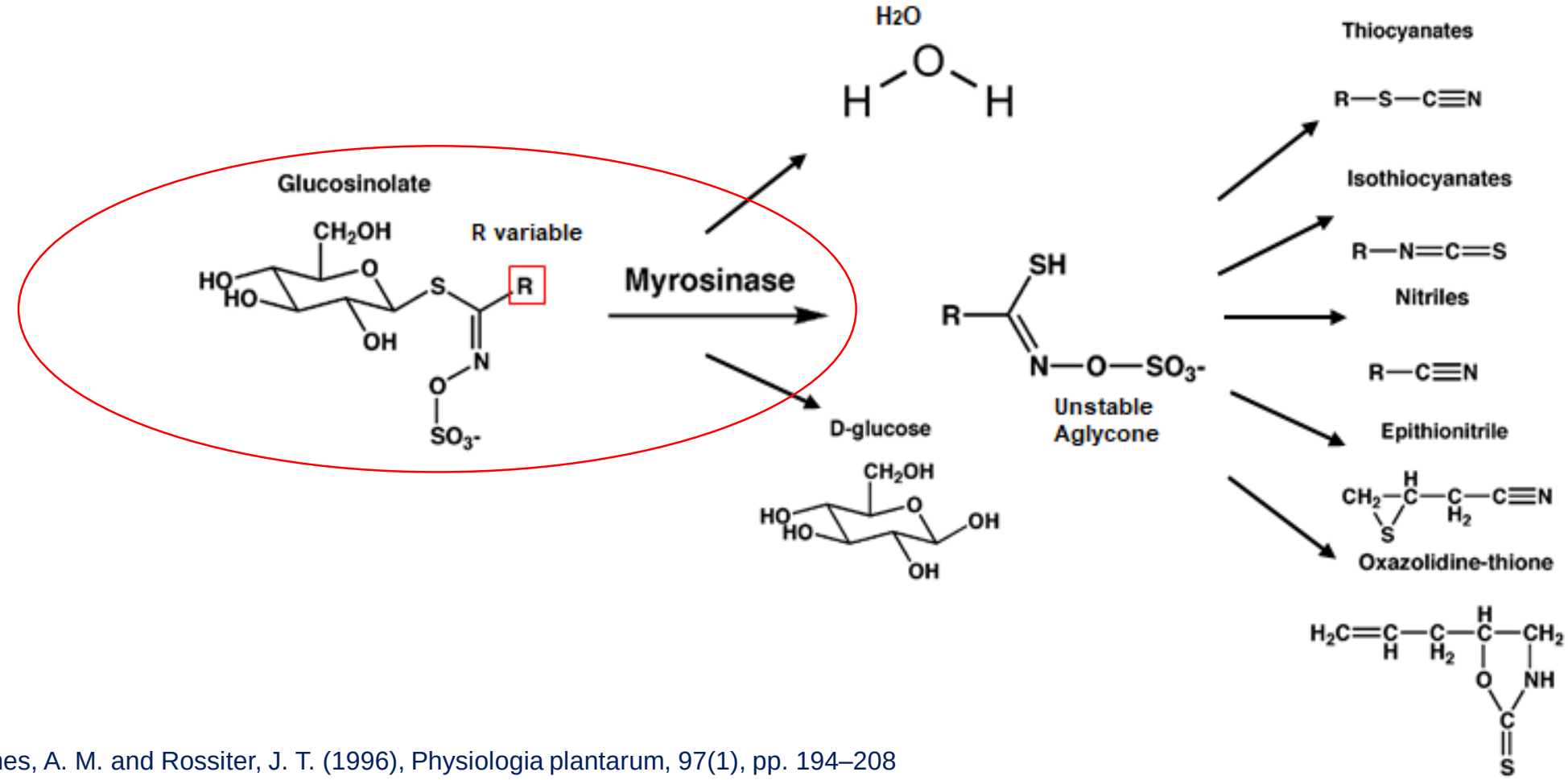
Desolventising, toasting

Rapeseed meal



Diverse biological properties of glucosinolates

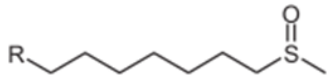
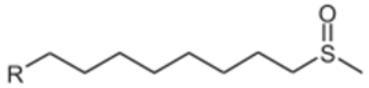
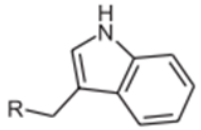
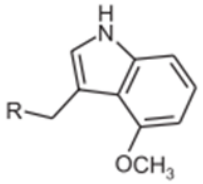
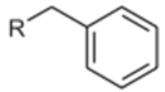
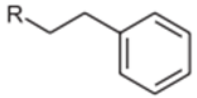
1. Defence Compounds: Antifungal, Antibacterial, Biopesticidal, Bioherbicidal, Antiherbivours
2. Anticarcinogenic
3. Brassica Pungency
4. Antinutritional in feed



Bones, A. M. and Rossiter, J. T. (1996), Physiologia plantarum, 97(1), pp. 194–208

The glucosinolate-myrosinase system is triggered when damage occurs to the plant tissue, myrosinases hydrolyse the GSLs to yield D-glucose and unstable aglycones. Unstable aglycones rearrange to become isothiocyanate or a form of the product.

Side chain structure of some GSL

Trivial name	Side chain structure	
Sinigrin		Aliphatic
Gluconapin		Aliphatic
–		Aliphatic
Glucoibarin		Aliphatic
Glucohirsutin		Aliphatic
Glucoibervirin		Aliphatic
Glucobrassicin		Indole
4-Methoxygluco-brassicin		Indole
Glucotropaeolin		Aromatic
Gluconastrutiin		Aromatic

Kittipol, V. et al. (2019), Journal of plant physiology, 240, p. 152988

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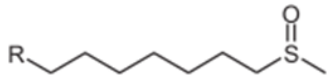
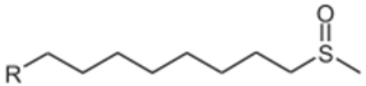
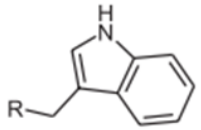
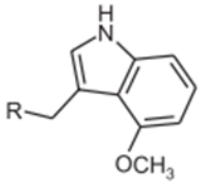
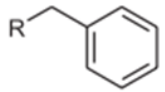
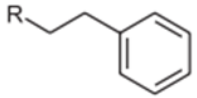
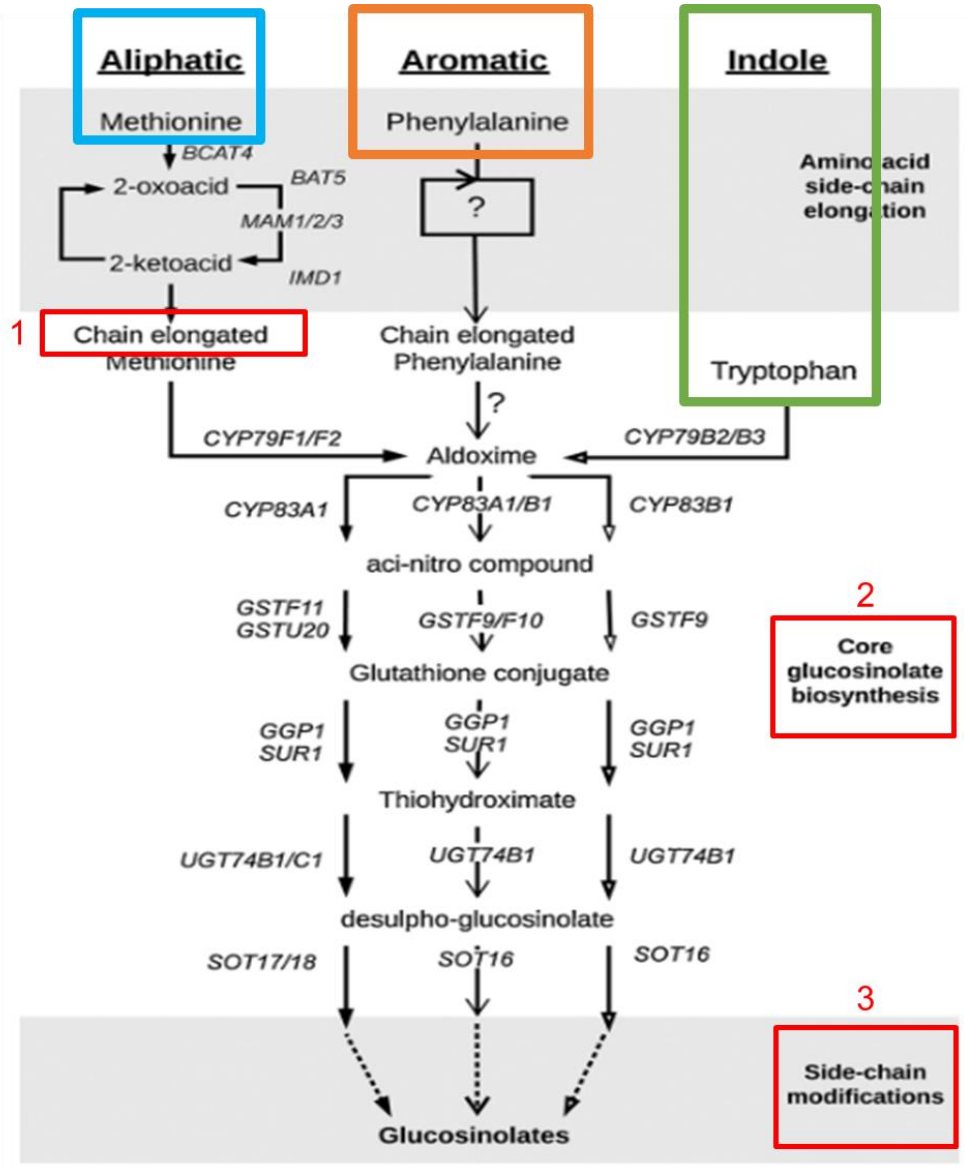
Glucoibarin		
Glucohirsutin		
Glucoibervirin		
Glucobrassicin		Indole
4-Methoxygluco-brassicin		
Glucotropaeolin		Aromatic
Gluconastrutiin		

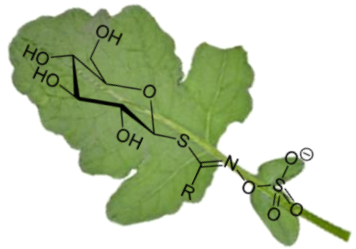
Kittipol, V. et al. (2019), Journal of plant physiology, 240, p. 152988



Doheny-Adams, T. et al. (2017), Plant methods, 13, p. 17

Methods: Glucosinolate extraction, purification and desulfation for HPLC

Sample preparation

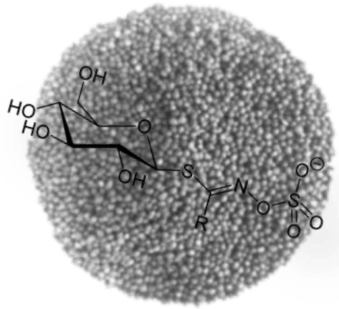


Leaf samples

Treat with liquid Nitrogen
Store at -80ºC
Freeze-dry for 1 day

Measure Weight,

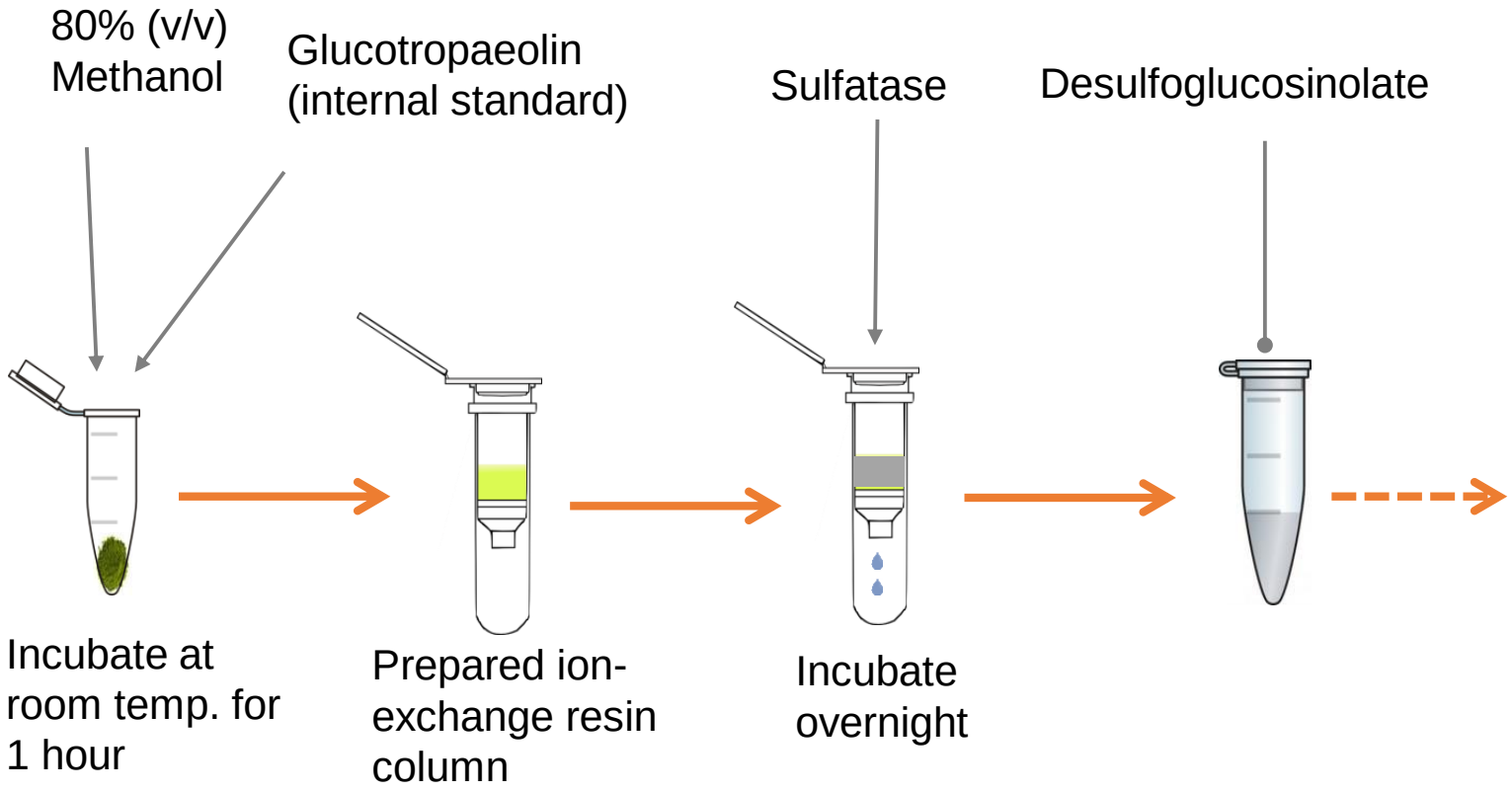
Grind to powder with TissueLyser



Seed samples

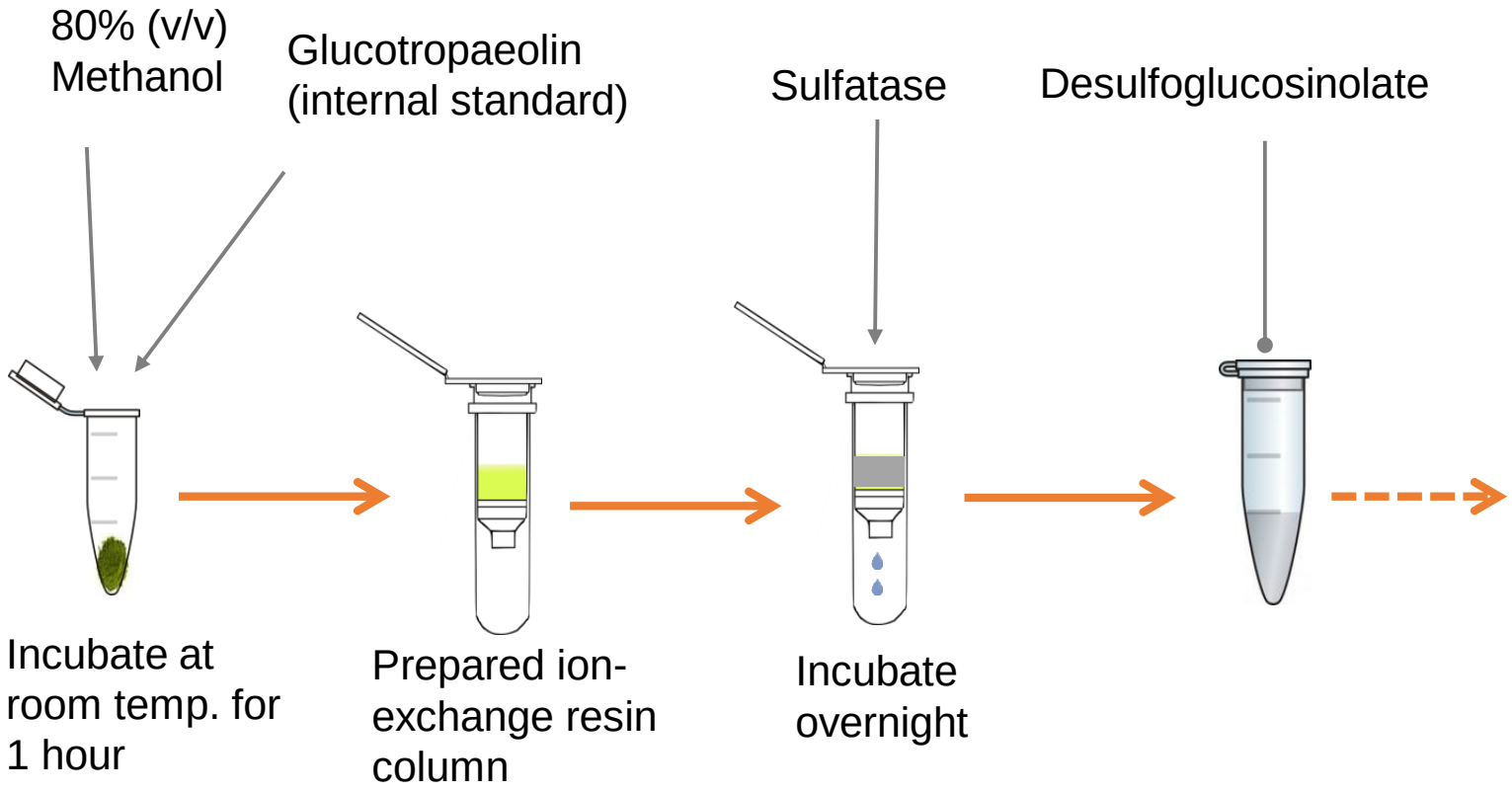
Methods: Glucosinolate extraction, purification and desulfation for HPLC

Extraction & Purification



Methods: Glucosinolate extraction, purification and desulfation for HPLC

Extraction & Purification



HPLC analysis

- C-18 column
- Mobile phase:

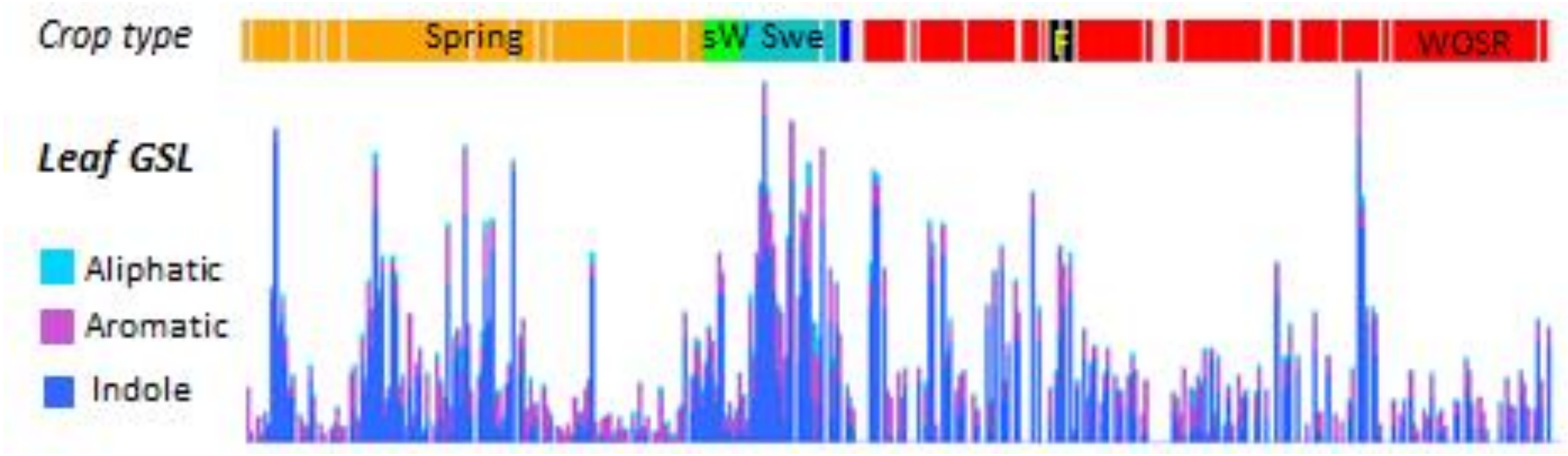
  Water & 20% acetronitile
- Monitor at 229 nm

Experiment 1:

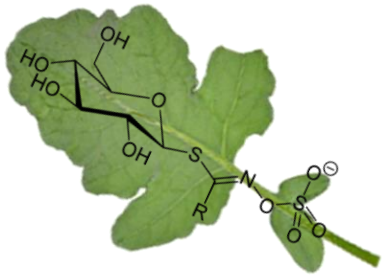
Natural genetic variation for the control of glucosinolate accumulation in seeds.

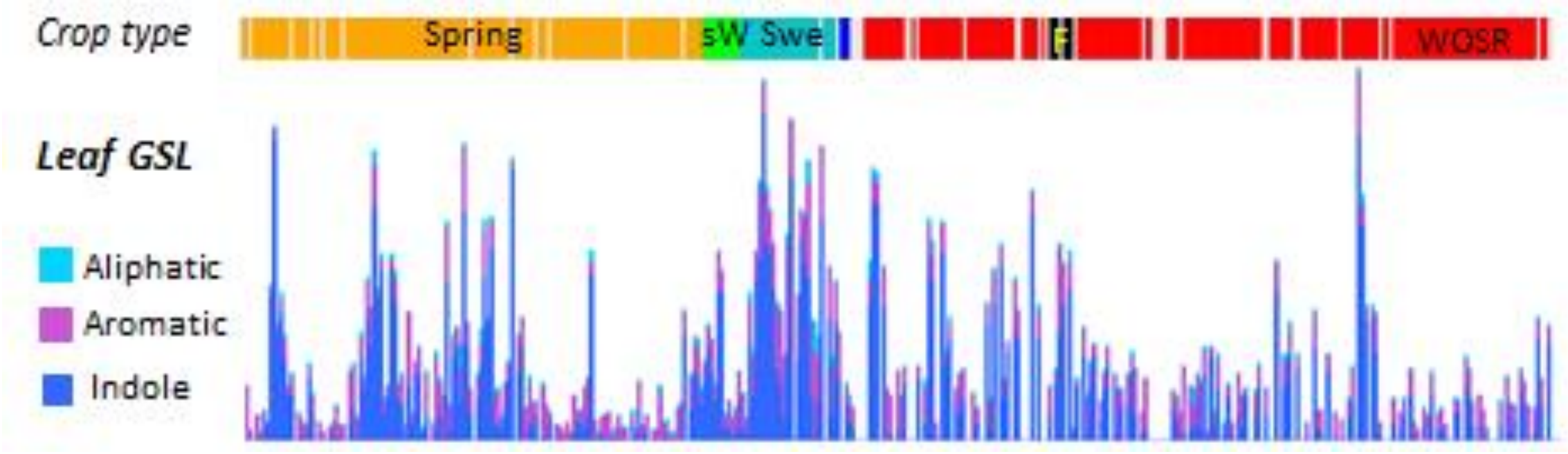
Hypothesis of Experiment 1:

- There are unknown loci in the *B. napus* genome in addition to loci known to control glucosinolate synthesis in leaves (i.e. HAG1 orthologues)
- They quantitatively modulate glucosinolate content and composition in the seeds.

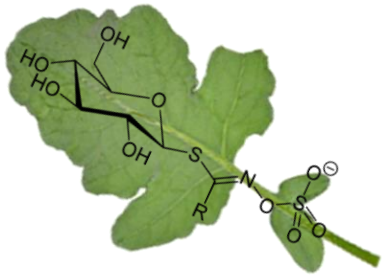


Kittipol, V. et al. (2019), Journal of plant physiology, 240, p. 152988





No.	Croptype count	
1	SpOSR	52
2	WOSR	52
3	Swede	26
4	Semiwinter OSR	11
5	Kale	3
6	Fodder	6



Kittipol, V. et al. (2019), Journal of plant physiology, 240, p. 152988

A subset panel of 150 Brassica napus varieties

Methods: Associative Transcriptomics (AT)

A modified GWAS method using transcribed sequences (mRNA-Seq) to associate trait variation to variation in gene sequence (SNPs) and expression (GEMs).

Reference sequence (AC pan-transcriptome)

Genotypic variation

Markers from mRNA sequences
A) Single nucleotide polymorphism (SNP)
B) Gene expression (GEM)

mRNA Sequencing

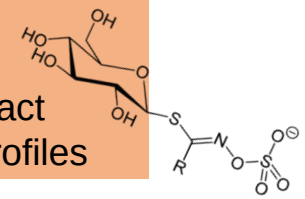
150 varieties of *B. napus* (4 replicates)

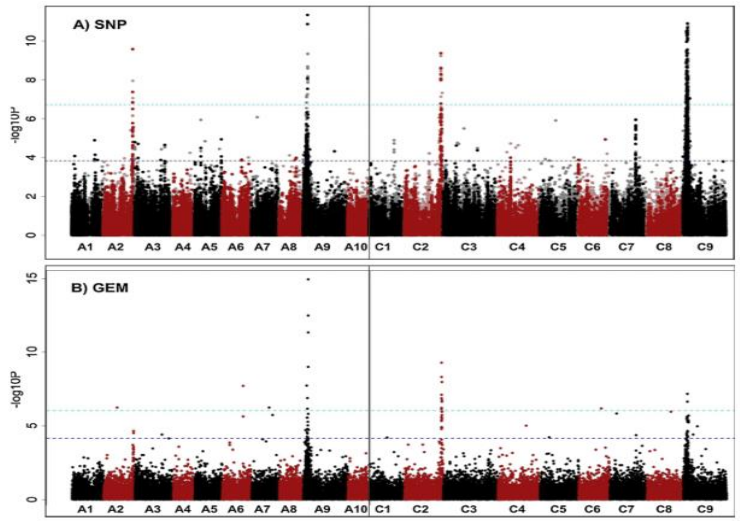
Phenotypic Variation

Seed samples

Glucosinolates extraction

HPLC to extract glucosinolate profiles





An example of visualisation

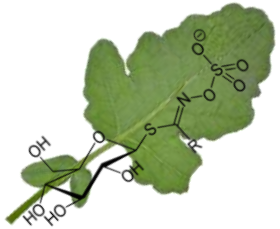
Correlates phenotypic trait variation to Markers

Select candidate genes responsible for seeds' GSL profile

Harper, A. L. et al. (2012), Nature biotechnology, 30, p. 798
Havlickova, L. et al. (2018), The Plant journal, 93(1), pp. 181–192

Methods: Associative Transcriptomics (AT)

Kittipol, V. et al. (2019), Journal of plant physiology, 240, p. 152988

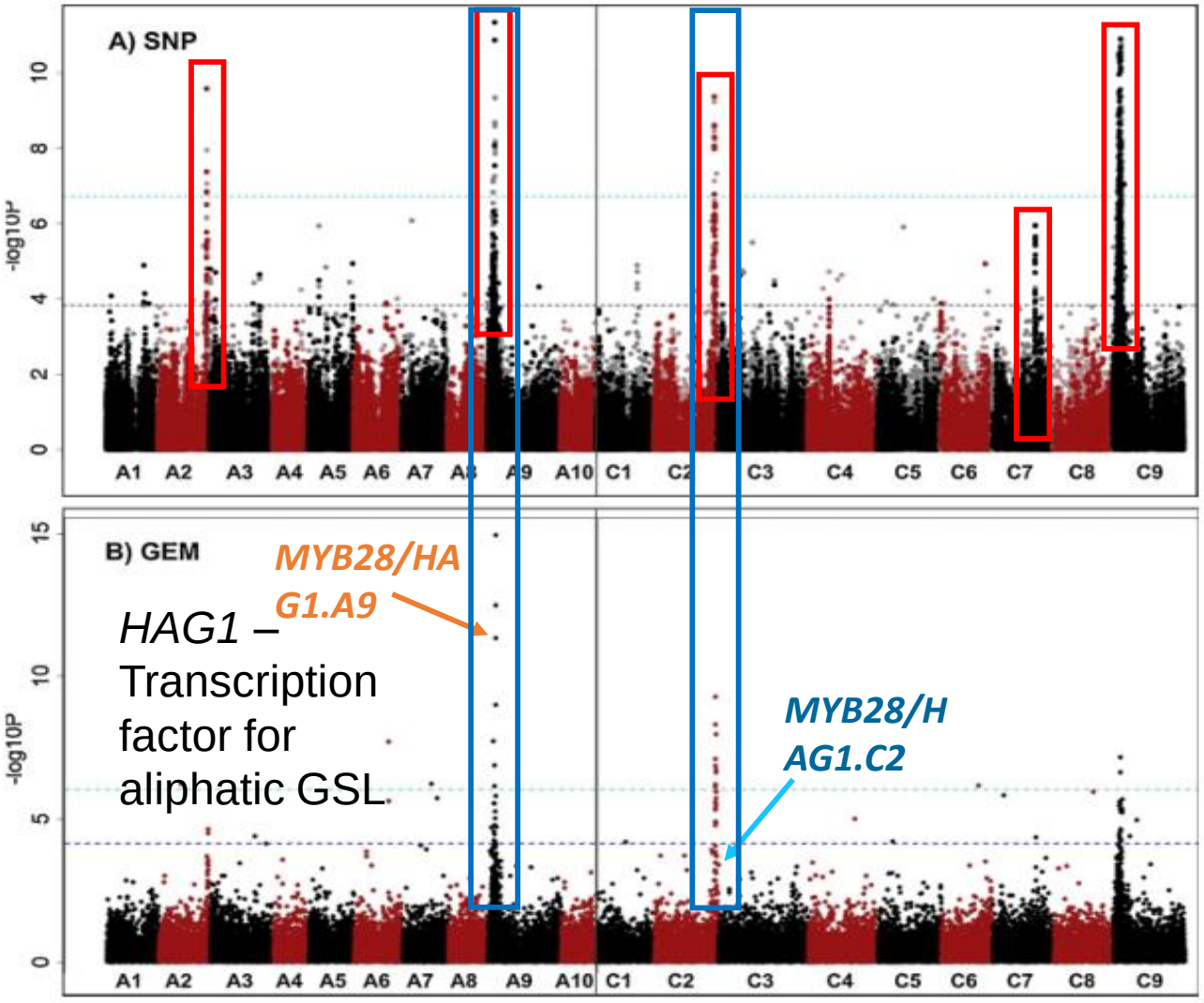


Leaf glucosinolate variation

Significance of Association (P) - Log scale

Genomic order of gene models

Reads per kb per million aligned reads (RPKM) regressed against the trait
R2 and P values were calculated for each gene



Significance of Association (P) - Log scale

SNP Markers position in genome

Bonferroni corrected 5% significance threshold

5% FDR threshold

Experiment 2:

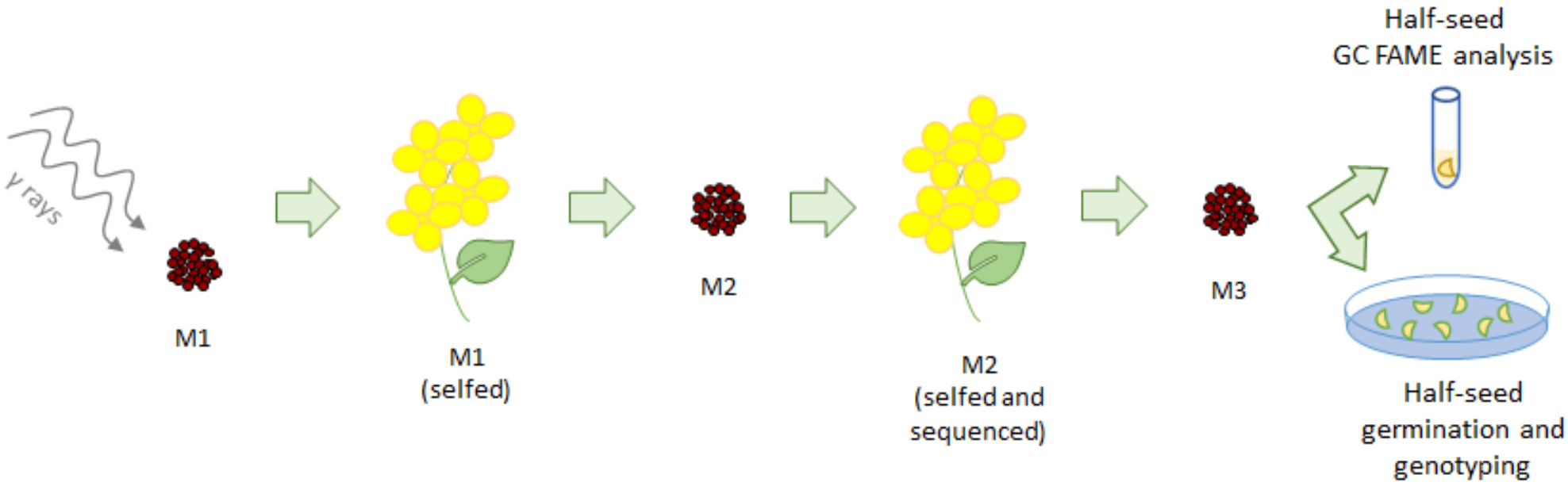
Induced genetic variation for the control of glucosinolate accumulation in seeds by mutation of glucosinolate transporters.

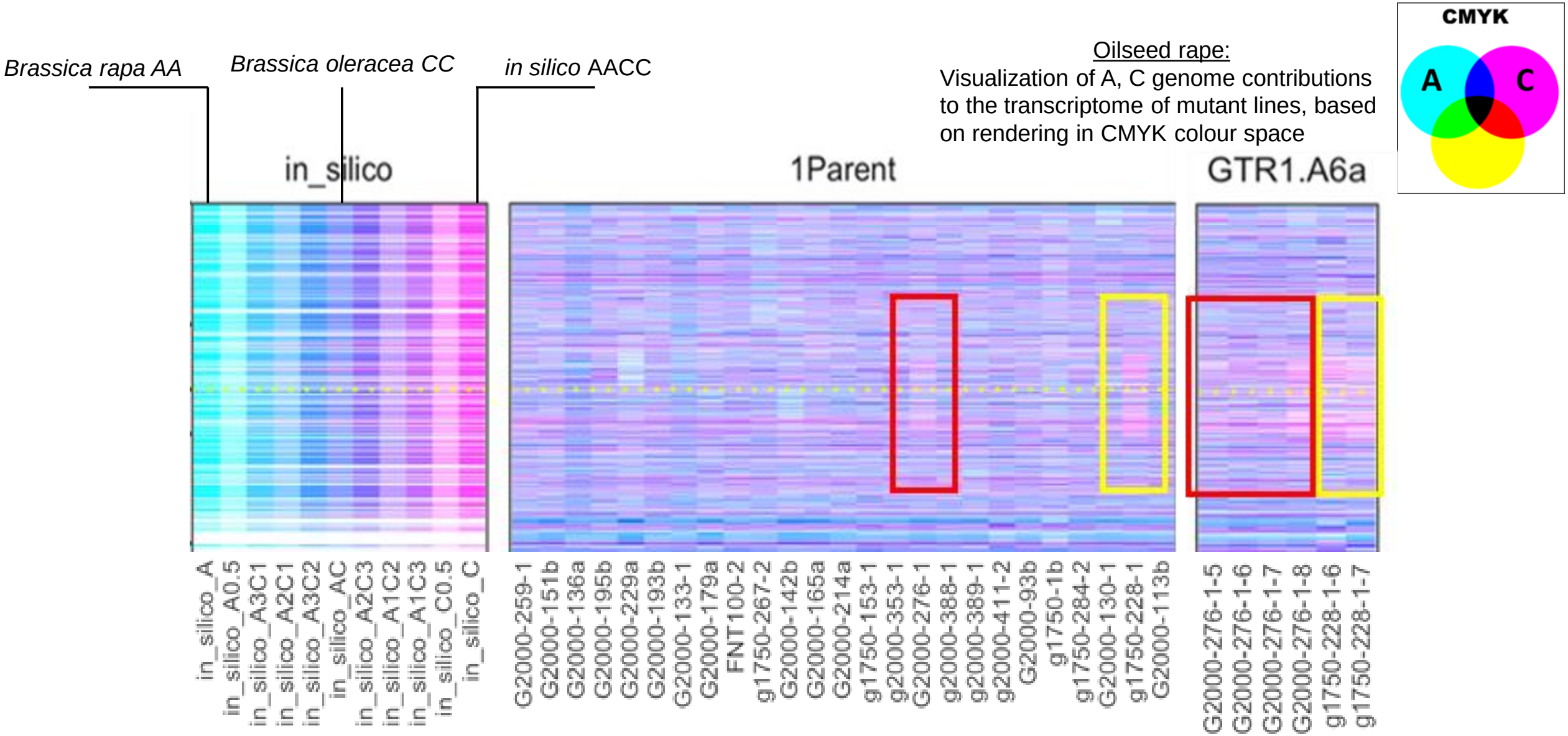
Hypothesis of Experiment 2:

- Inactivation of known glucosinolate transporters (orthologues of GTR1 and GTR 2)
- Reduces glucosinolate content of the seeds by impairing long-distance transport

- Maplus radiation panel generation for the Renewable Industrial Products from Rapeseed (RIPR) genetic diversity pilot study.

- Maplus seeds (M1 generation) were exposed to γ radiation (750-2000 Gy). The seeds were grown, the plants selfed, and the seeds were sown to generate the M2 lines.





Oilseed rape:
Visualization of A, C genome contributions to the transcriptome of mutant lines, based on rendering in CMYK colour space

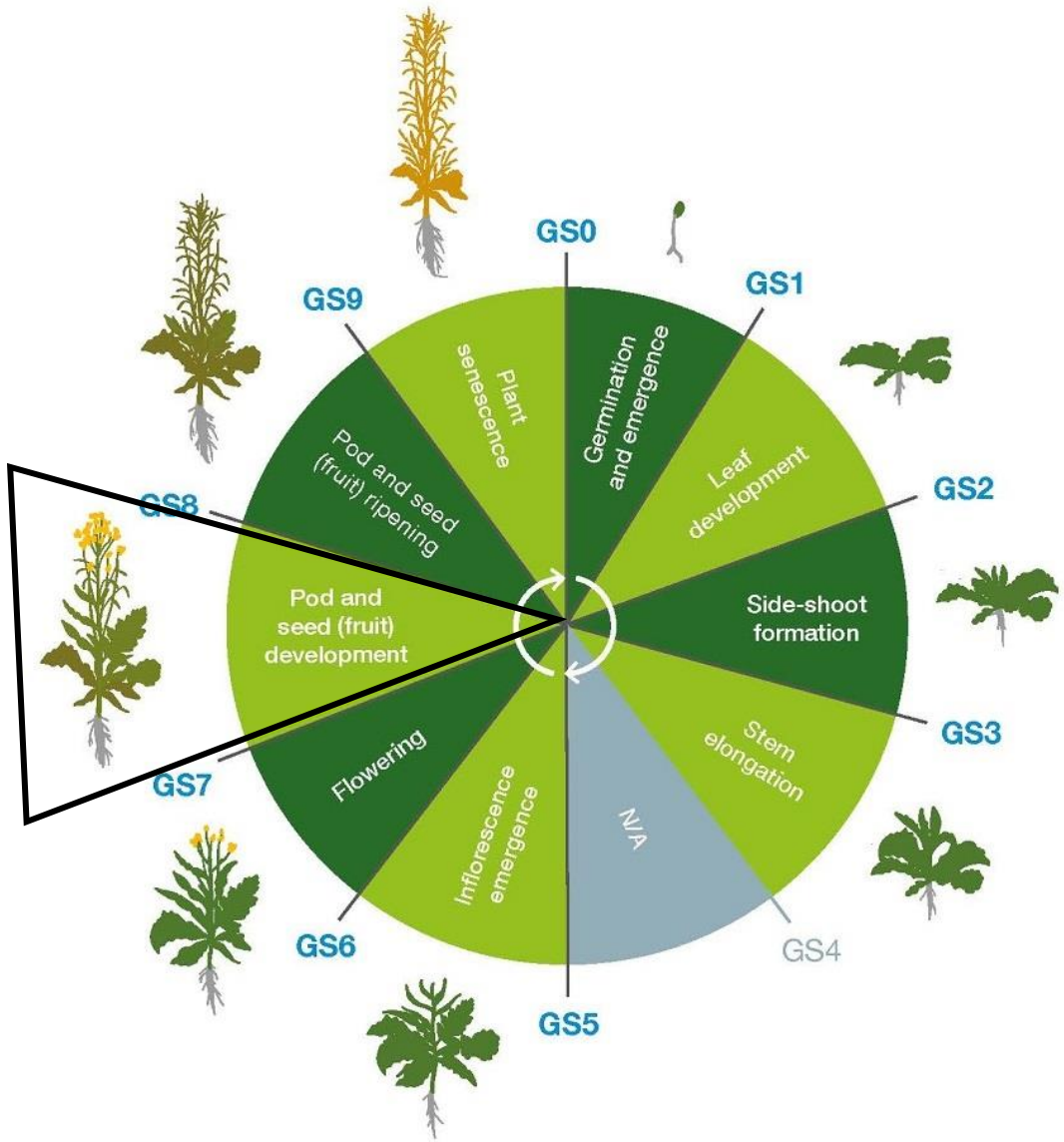
Genome Display Tile Plot of the A6 chromosome of target lines, yellow dashed line is the critical line for gene hit on GTR1.A6a.

Experiment 3:

Induction of glucosinolate synthesis in response to mechanical wounding.

Hypothesis of Experiment 3:

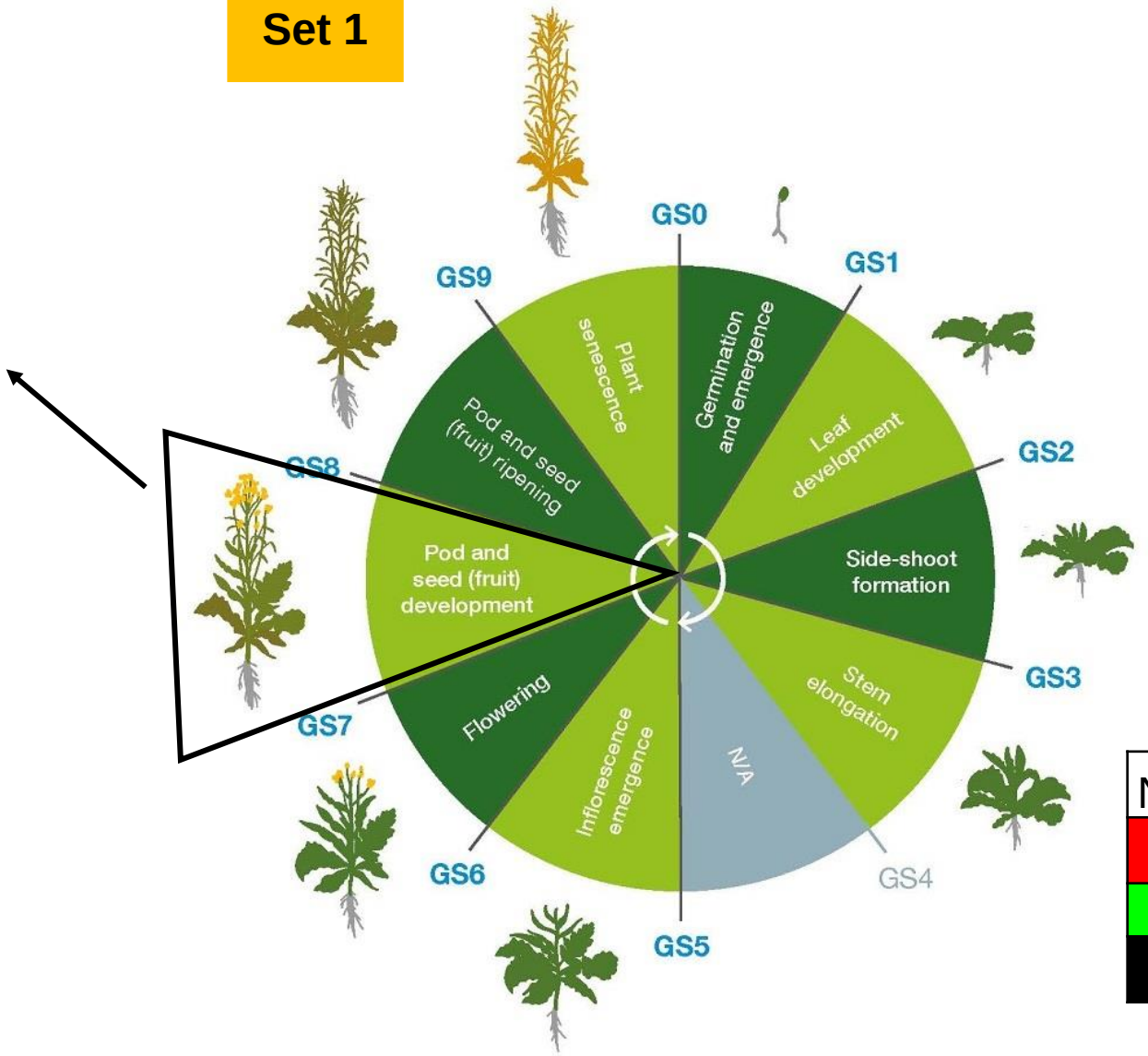
- Mechanical wounding induces gene expression changes in leaves
- Increases the glucosinolate content of seeds



Set 1	Set 2	Set 3

No.	Cultivar count	
1	High GSL	4
2	Low GSL	4
3	Control	4

Set 1



No.	Cultivar count	
1	High GSL	4
2	Low GSL	4
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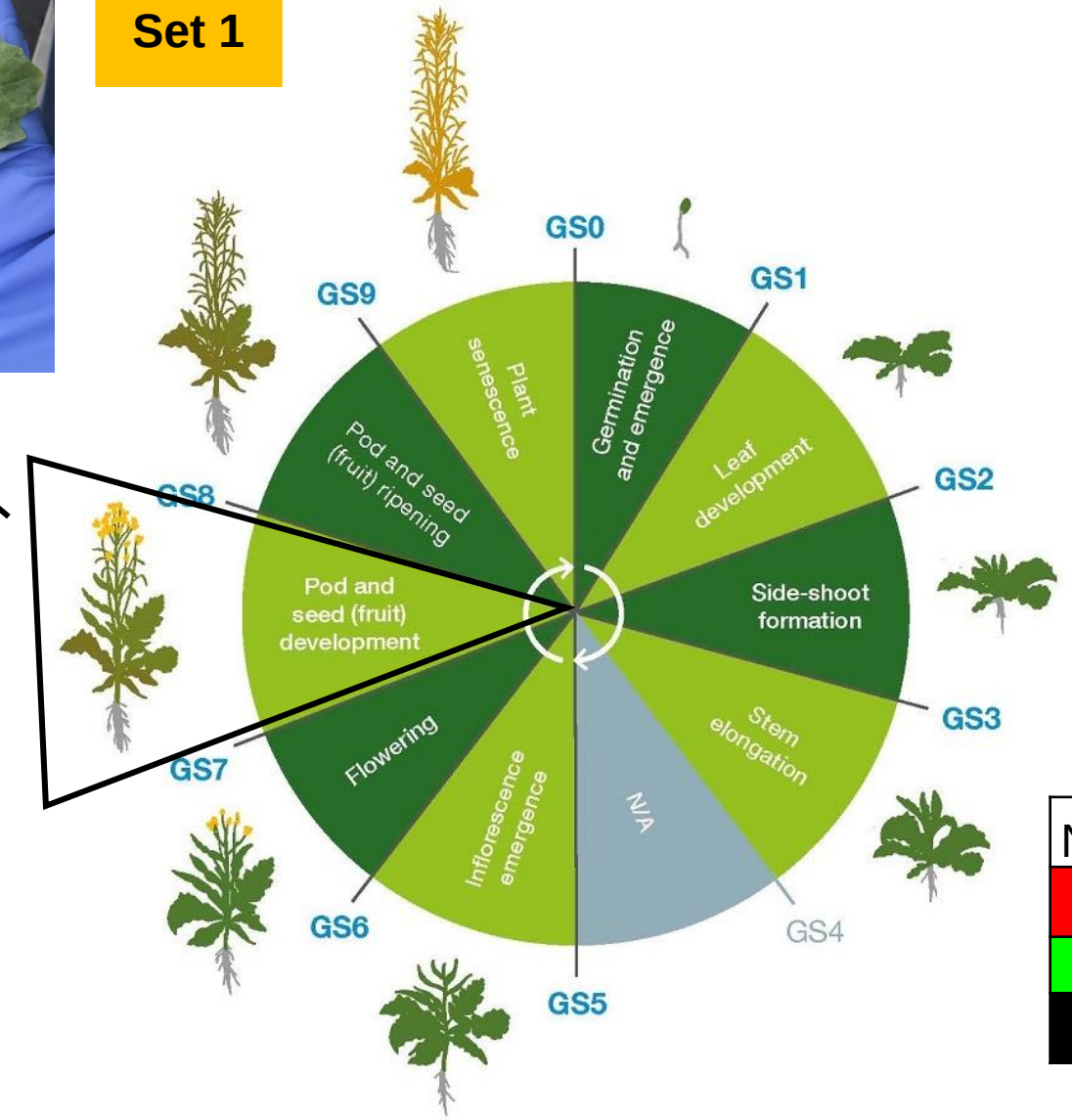
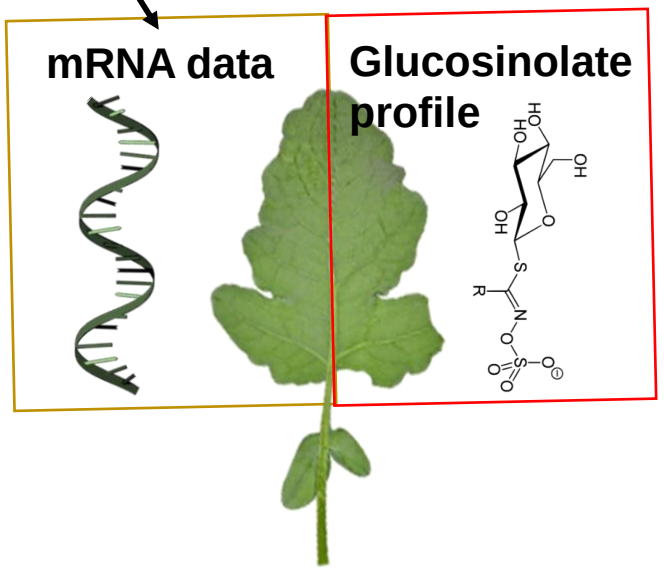
Set 1

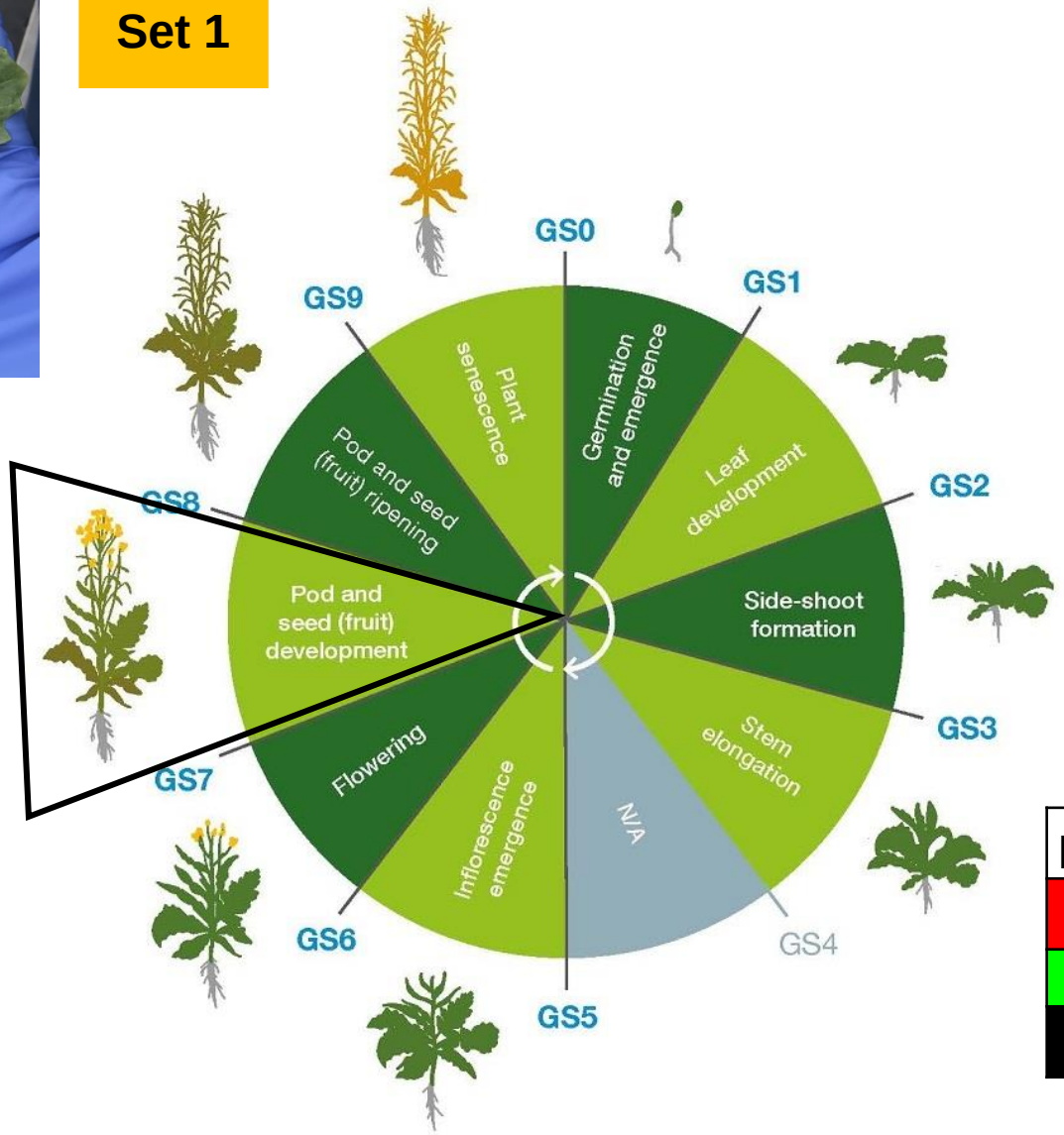


No.	Cultivar count	
1	High GSL	4
2	Low GSL	4
3	Control	4

Set 1

After 24 hours





No.	Cultivar count	
1	High GSL	4
2	Low GSL	4
3	Control	4

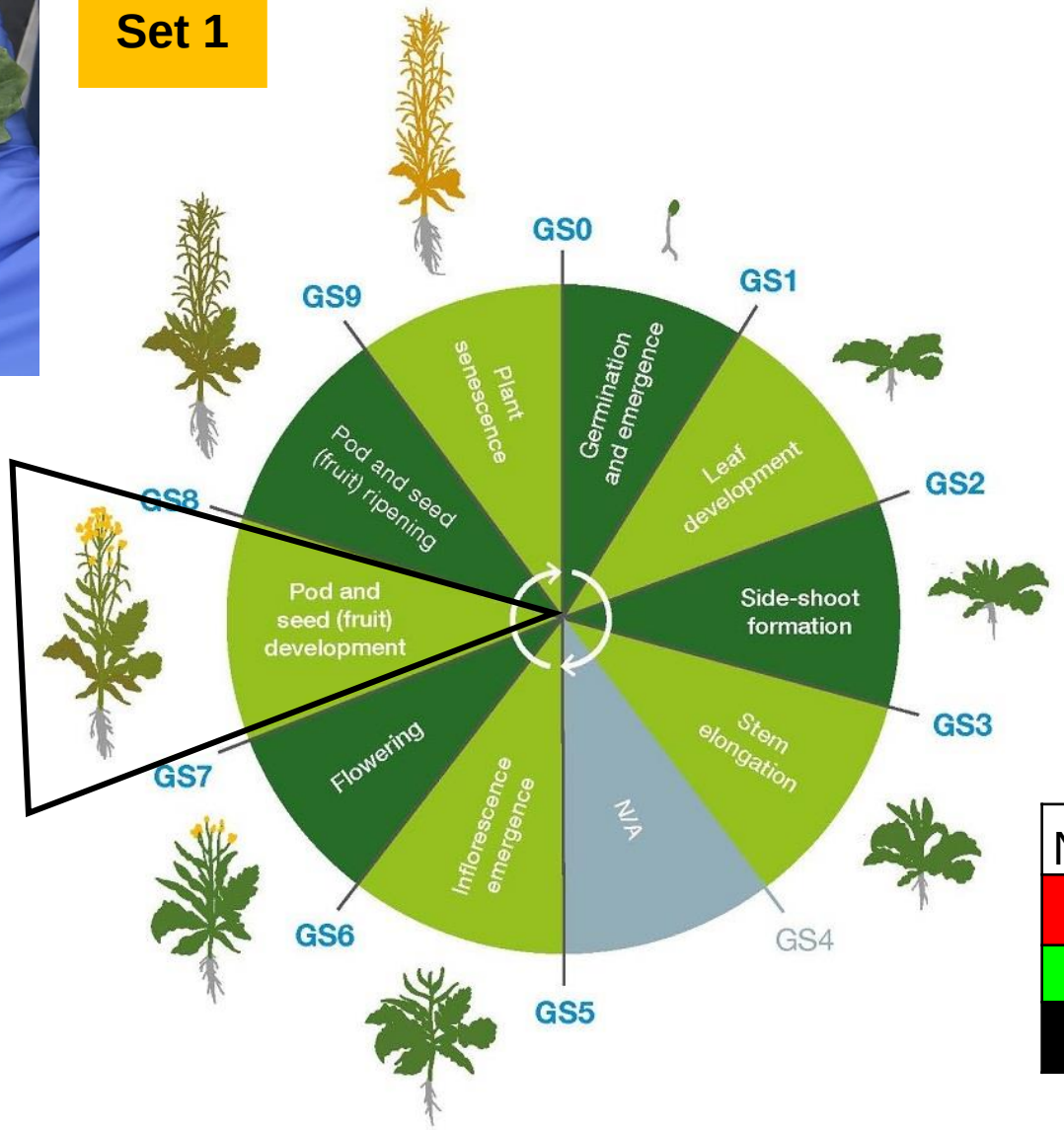
Set 1

After 24 hours

mRNA data

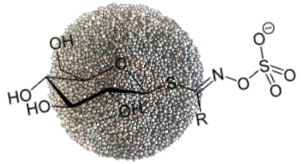
Glucosinolate profile

Set 2



No.	Cultivar count	
1	High GSL	4
2	Low GSL	4
3	Control	4

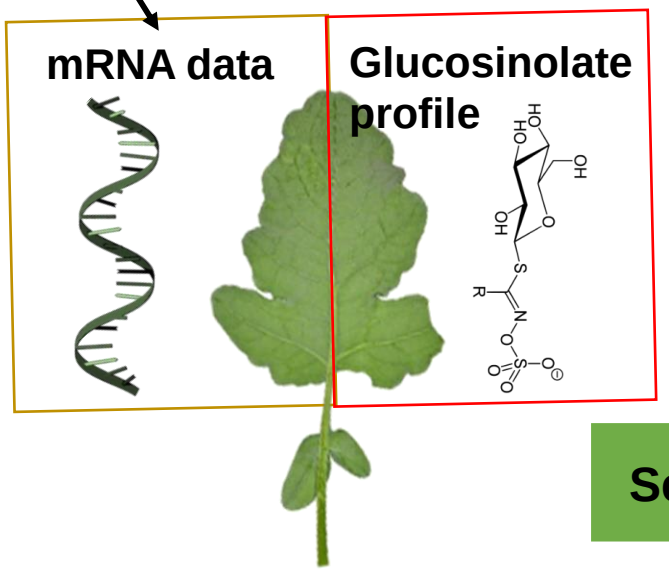
Set 1



Set 3

Grow to seeds to obtain Glucosinolate profile

After 24 hours



Set 2

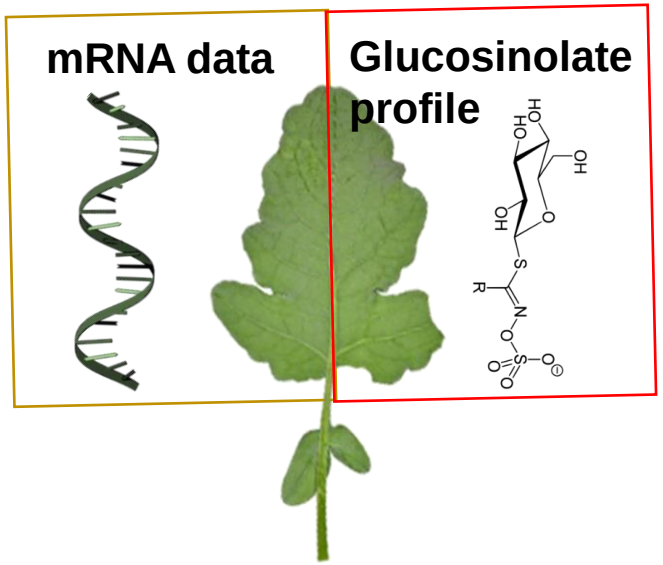
No.	Cultivar count	
1	High GSL	4
2	Low GSL	4
3	Control	4

Set 1

Set 2

Set 3

No.	Cultivar count	
1	High GSL	4
2	Low GSL	4
3	Control	4



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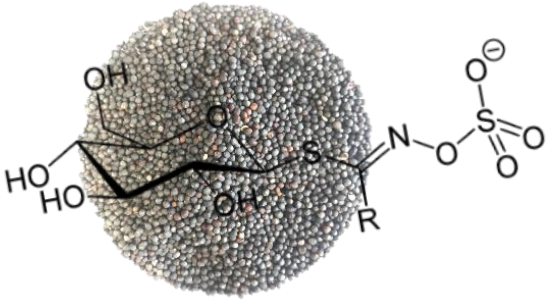
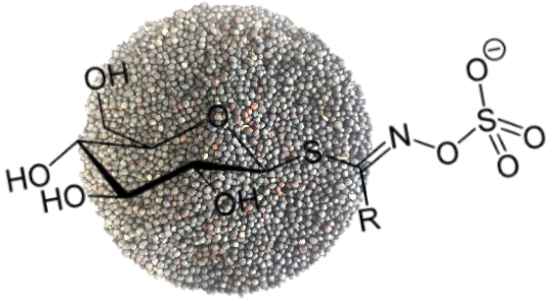


Seed glucosinolate profile



Seed glucosinolate profile

Thank you!