Oral Presentation

Title	Enhancing low seed glucosinolate breeding by editing a transporter gene lacking natural variation in *Brassica napus*
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Presentation Type	Oral
Theme	Genetics, Genomics and Breeding
Session Date, Time and Location	Tuesday, Sep 26, 2023 11:15 - 11:30 Ballroom 1

Enhancing canola breeding by editing a glucosinolate transporter gene lacking natural variation

Regulation mechanism and germplasm creation of high-leaf and low-seed glucosinolate contents in *Brassica napus*

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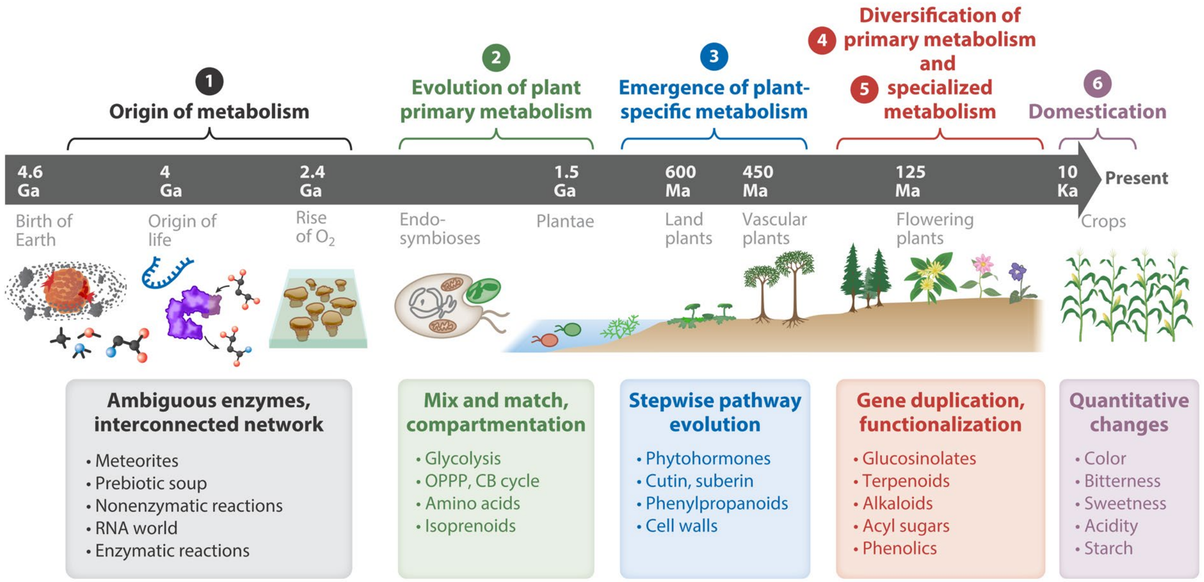
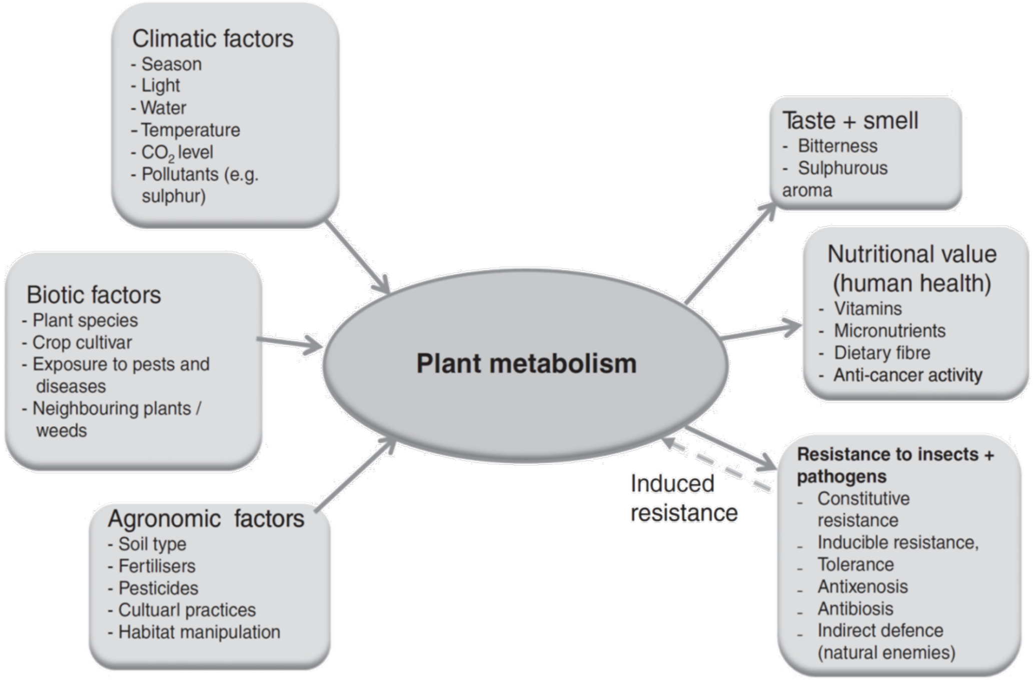
Evolution and function of plant metabolism

Plants produce a huge array of metabolites in environment-dependent evolution.



The overall evolutionary history of plant metabolism.

(Maeda et al., Annu Rev Plant Biol. 2021)



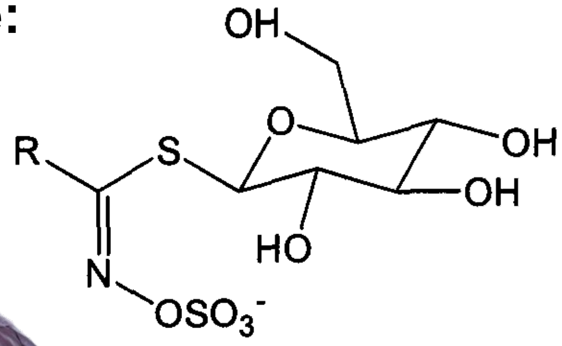
Factors influencing plant metabolism and consequential functional effects.

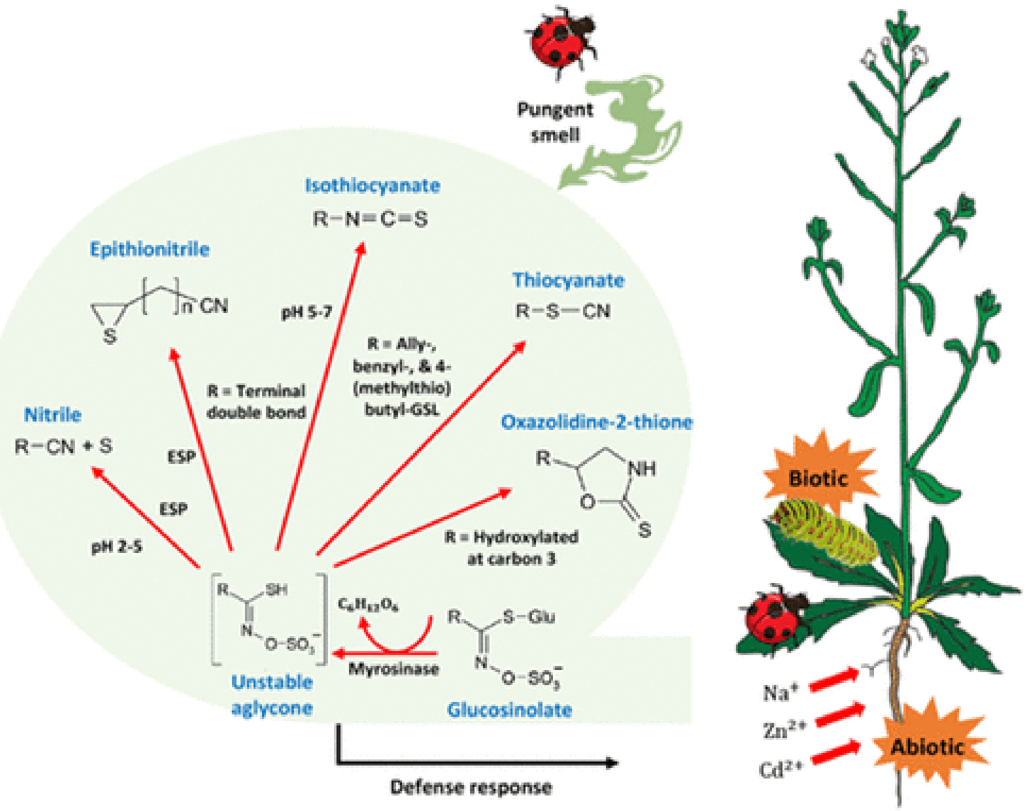
(Björkman et al., Phytochemistry. 2011)

Glucosinolate

Glucosinolates are a well-known group of sulfur-rich secondary metabolites specific to Brassicales and important in plant defense against disease and insects and in human nutrition/health such as anti-cancer effect.

Glucosinolate:

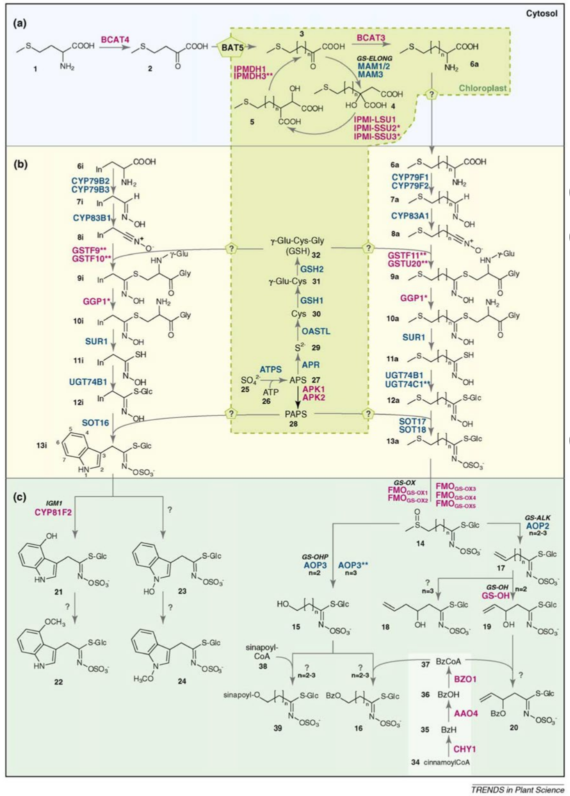




(Harun et al., J. Agric. Food Chem. 2020)

Glucosinolate biosynthesis and transport

Glucosinolates are synthesized in the green organs and subsequent transport to seeds by transporters, related genes have been well characterised in *Arabidopsis*.



Glucosinolate **biosynthetic** pathways in *Arabidopsis*

(Sønderby et al., Trends in Plant Science. 2010)

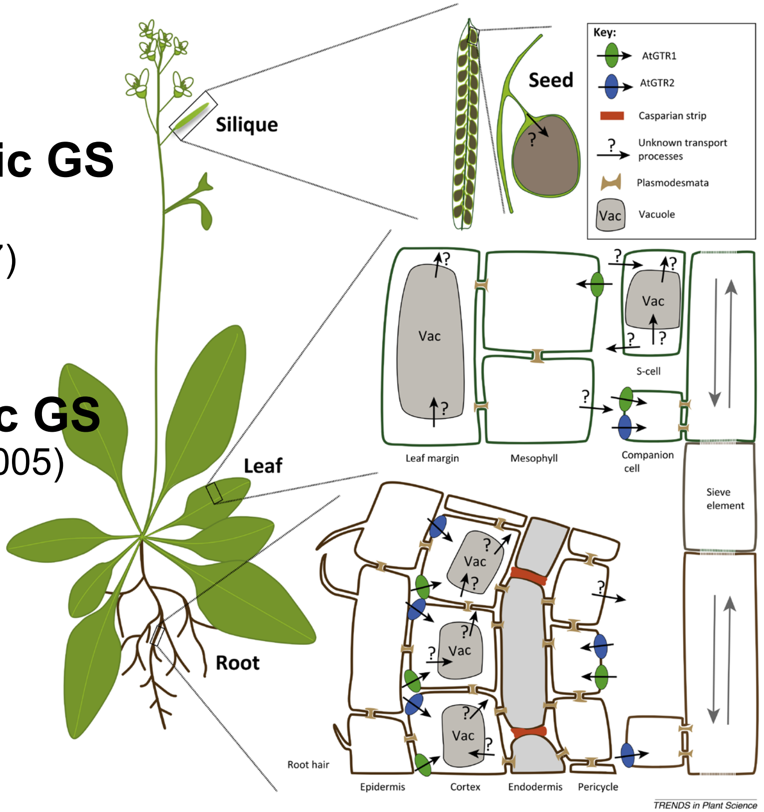
MYB28/29/76 **in aliphatic GS**

(Hirai et al., PNAS. 2007))

(Gigolashvili et al., Plant J. 2007)

MYB34/51/122 **in indolic GS**

(Celenza et al., Plant Physiol. 2005)



Overview of *Arabidopsis* glucosinolate **transport** processes.

(Jørgensen et al., Trends in Plant Science. 2015)

GTR1

GTR2

(Nour-Eldin et al., Nature. 2012)

UMAMIT29

UMAMIT30

UMAMIT31

(Xu et al., Nature. 2023)

Double-low rapeseed breeding

From 1950s, 'Double-low' rapeseed breeding have resulted in the reduction of both leaf- and seed-glucosinolate contents.

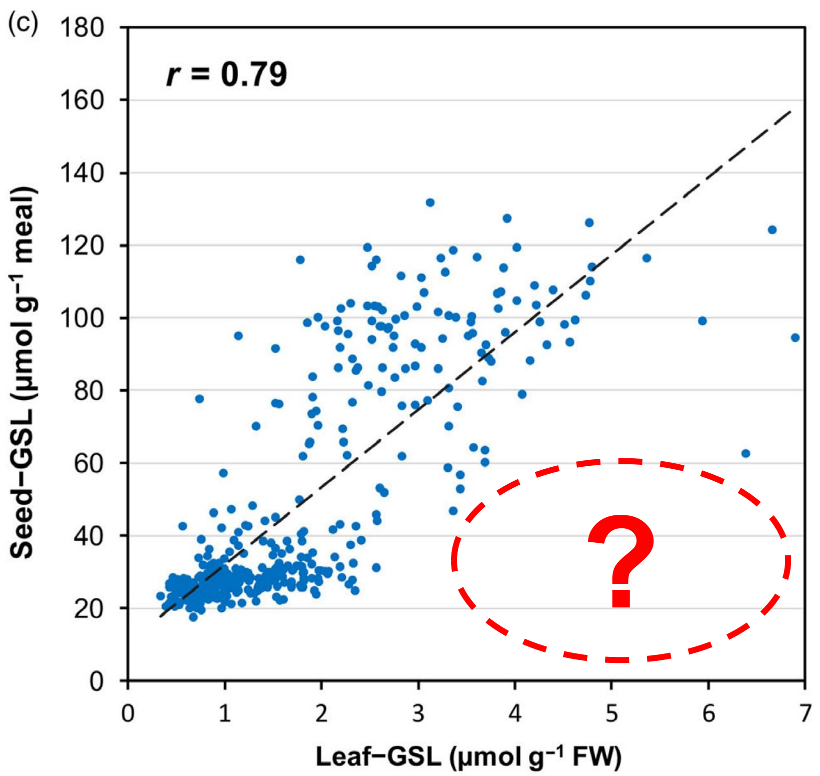
Low-seed glucosinolate content (<30μmol/g)

Low-seed erucic acid content (<2%)

High seed quality, but low leaf resistance.

Low-leaf glucosinolate content

4.0 μmol/g → 0.5 μmol/g



(Liu et al., Plant Biotechnol J . 2019)

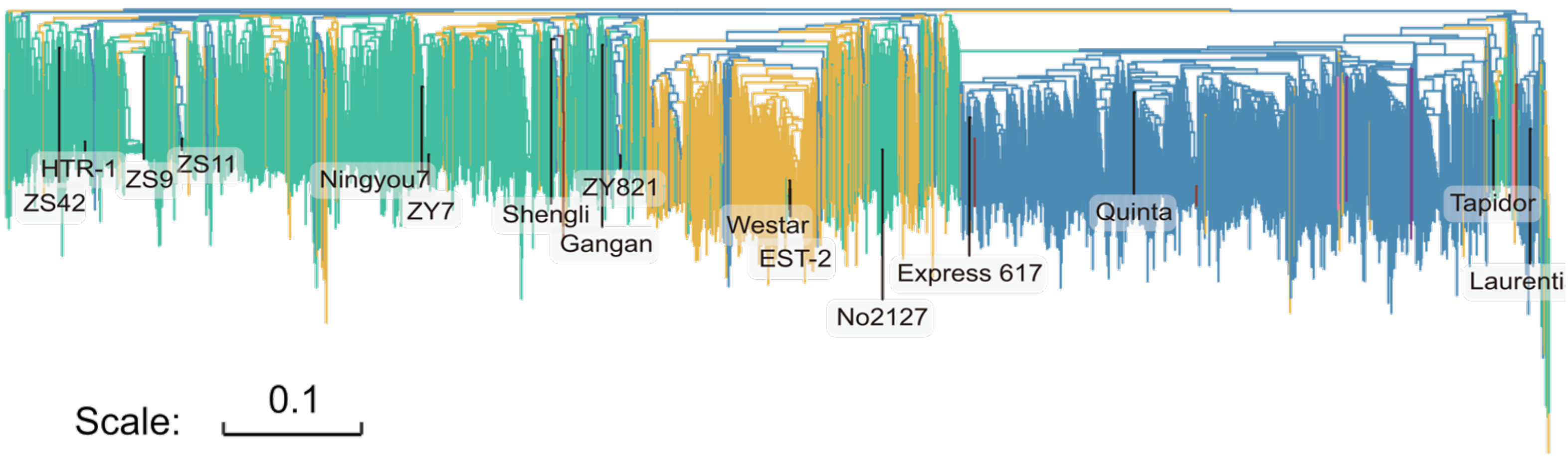
Million dollar questions

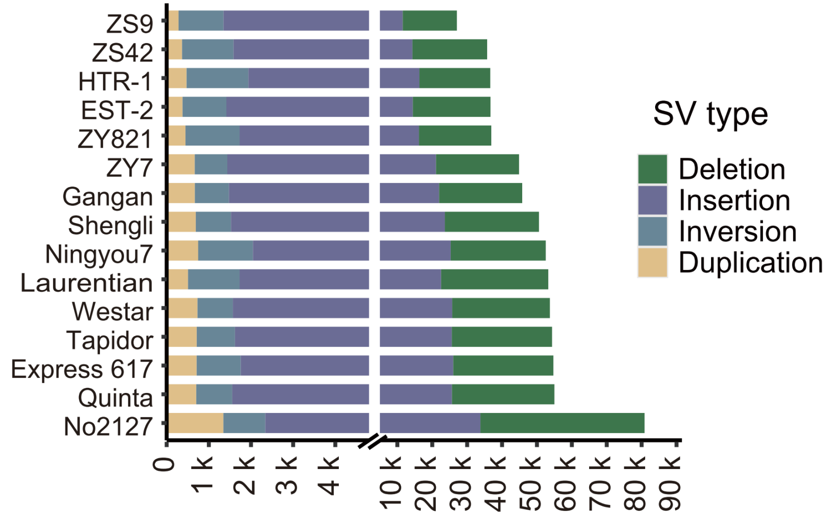
1. Why doesn't it have high-leaf and low-seed glucosinolate contents variety in *B. napus*?

2. How to create the breeding germplasm with high-leaf and low-seed glucosinolate contents in *B. napus*?

Pan-genome and structural variation identification

Construction a high-confidence panSV genome from 16 *de novo B. napus* assemblies.





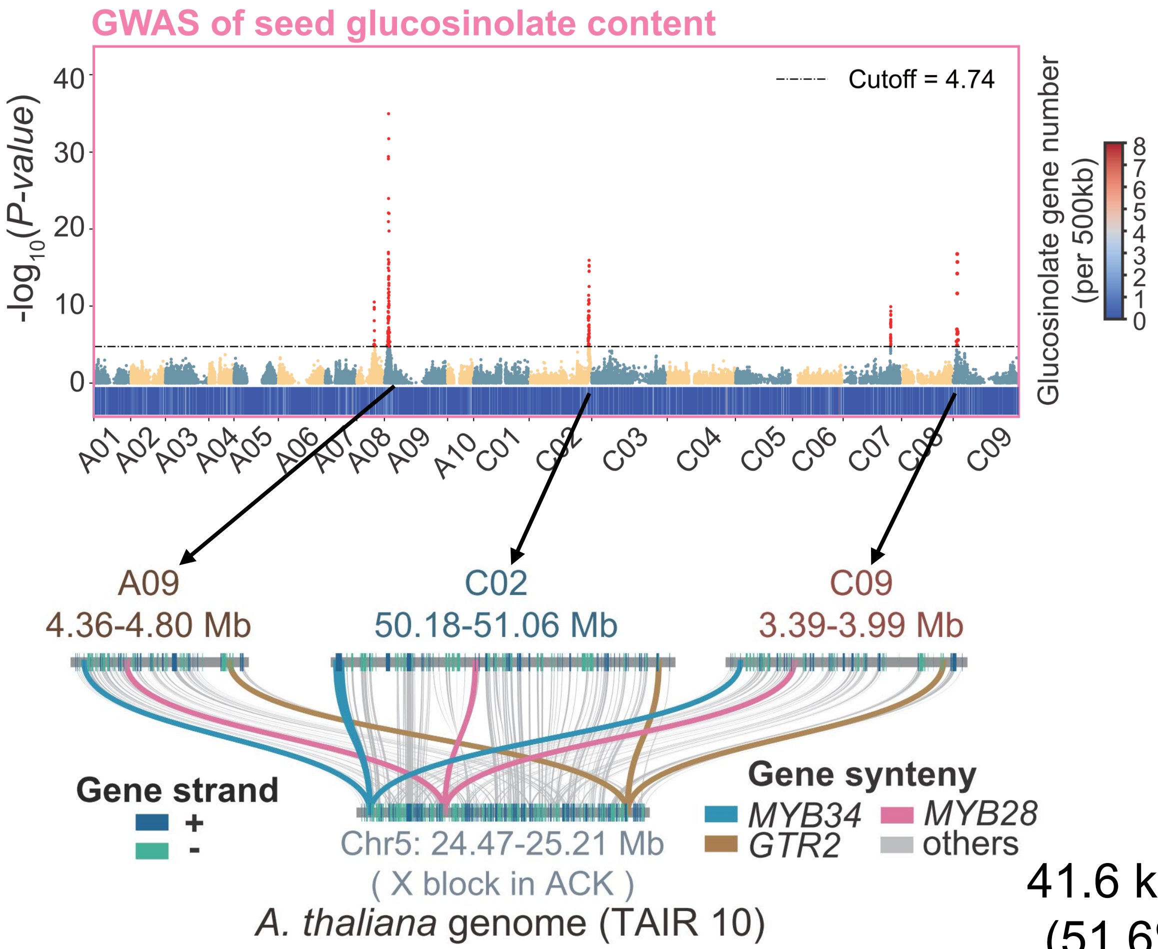
16 *de novo* genome assemblies

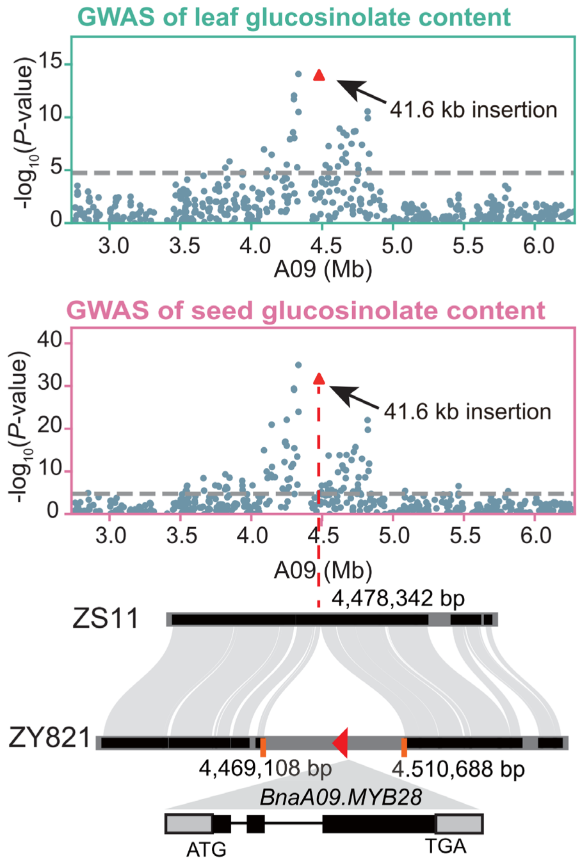
334,461 SVs-based PanSV-genome in 2,105 accessions

(Unpublished data)

Genetic dissection of glucosinolate biosynthesis and transport by PanSV-GWAS

An SV driving the genetic coupling of glucosinolate biosynthesis and transport.

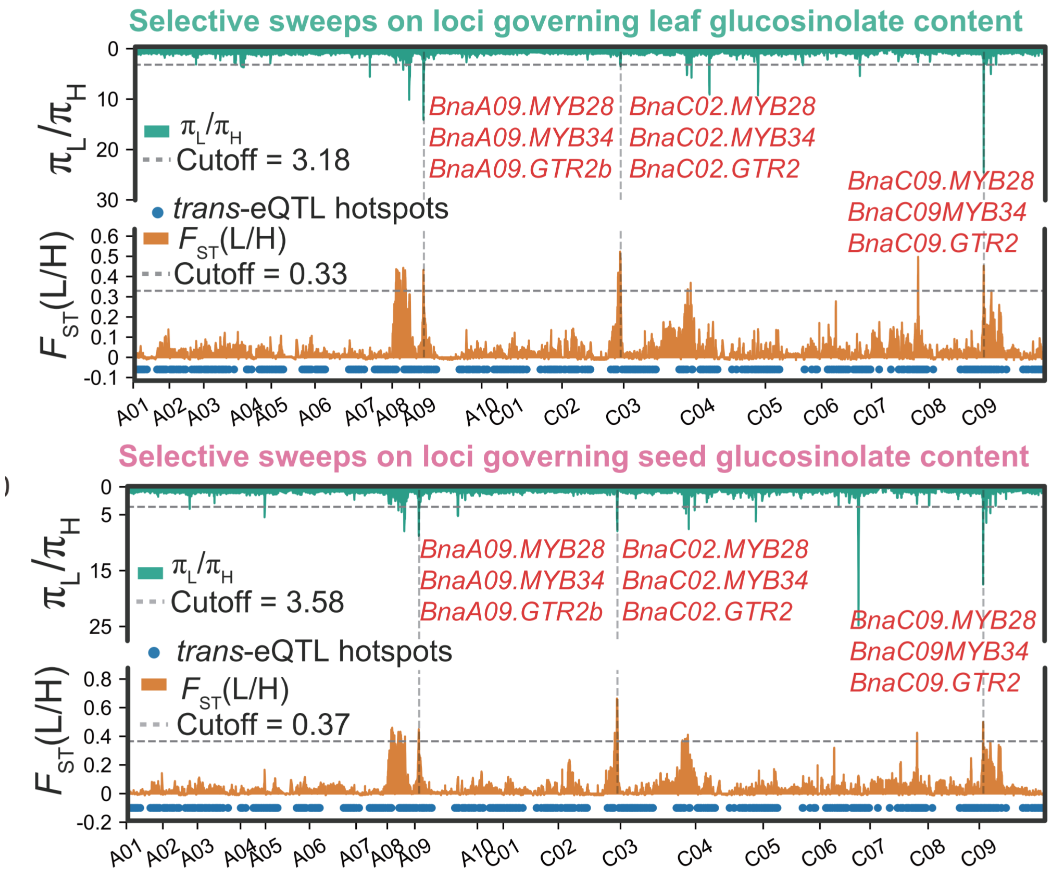




41.6 kb-Deletion harboring *BnaA09.MYB28* explains the highest (51.6%) phenotypic variance of the total glucosinolate content.

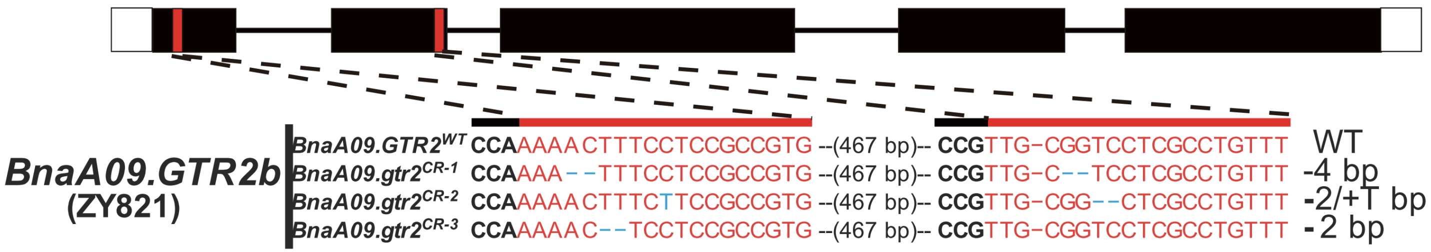
Genetic dissection of glucosinolate biosynthesis and transport

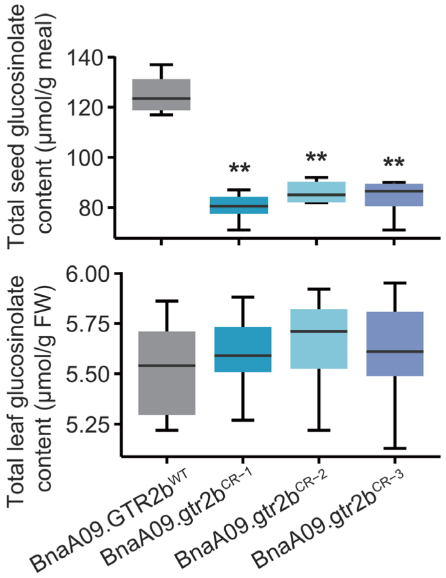
Three loci containing the three genes shows strong selective sweeps.



Germplasm creation of high-leaf and low-seed glucosinolate contents in *Brassica napus*

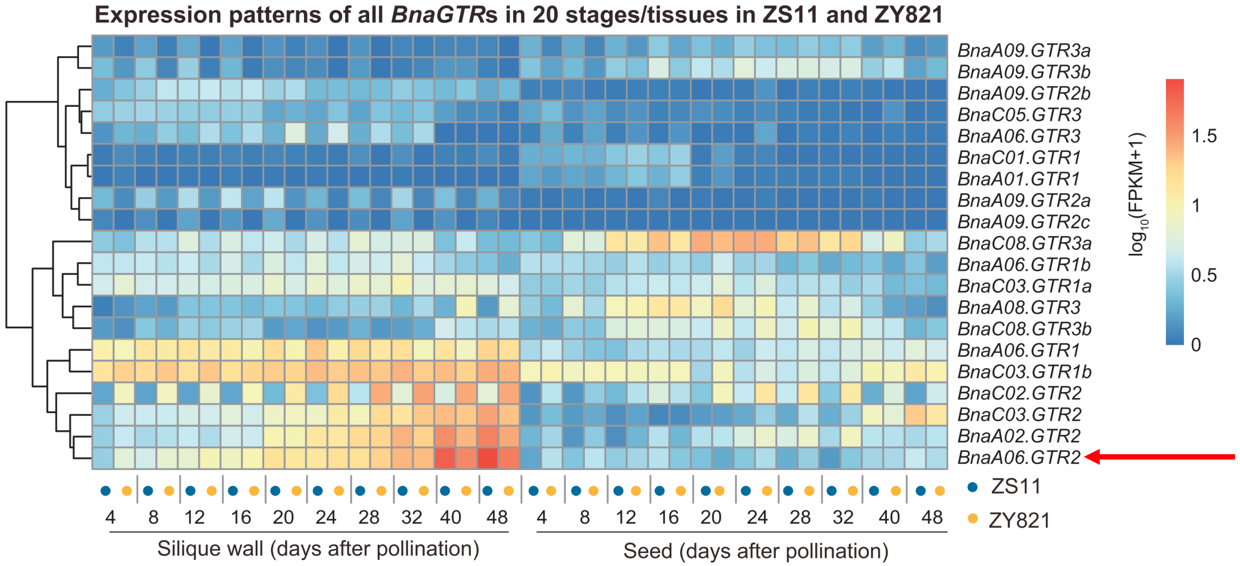
Knockout *BnaA09.GTR2* in high glucosinolate accession ZY821 by CRISPR/Cas9 can reduce seed glucosinolate content, but keep high leaf glucosinolate content.

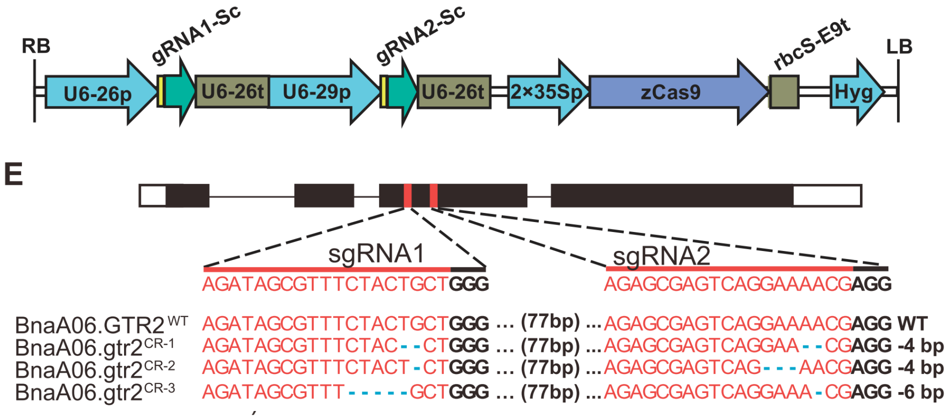




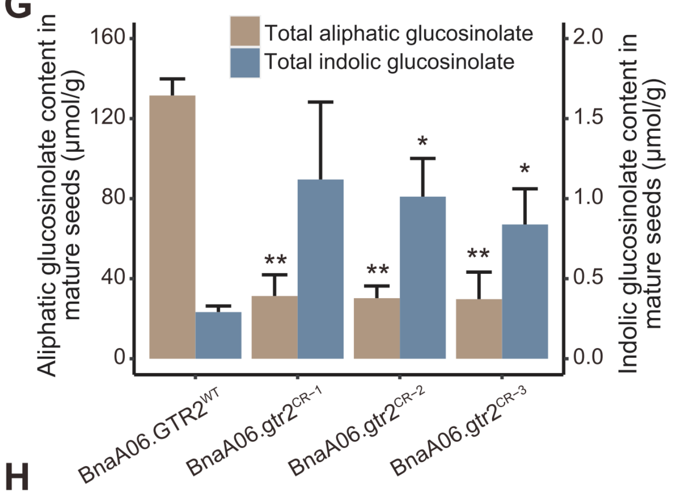
Germplasm creation of high-leaf and low-seed glucosinolate contents in *Brassica napus*

There is no any GWAS/QTL/TWAS signal on *BnaA06.GTR2*, indicate lacking natural variation. Knockout of *BnaA06.GTR2* in high glucosinolate accession ZY821 by CRISPR/Cas9 can significantly reduce seed glucosinolate content.





G

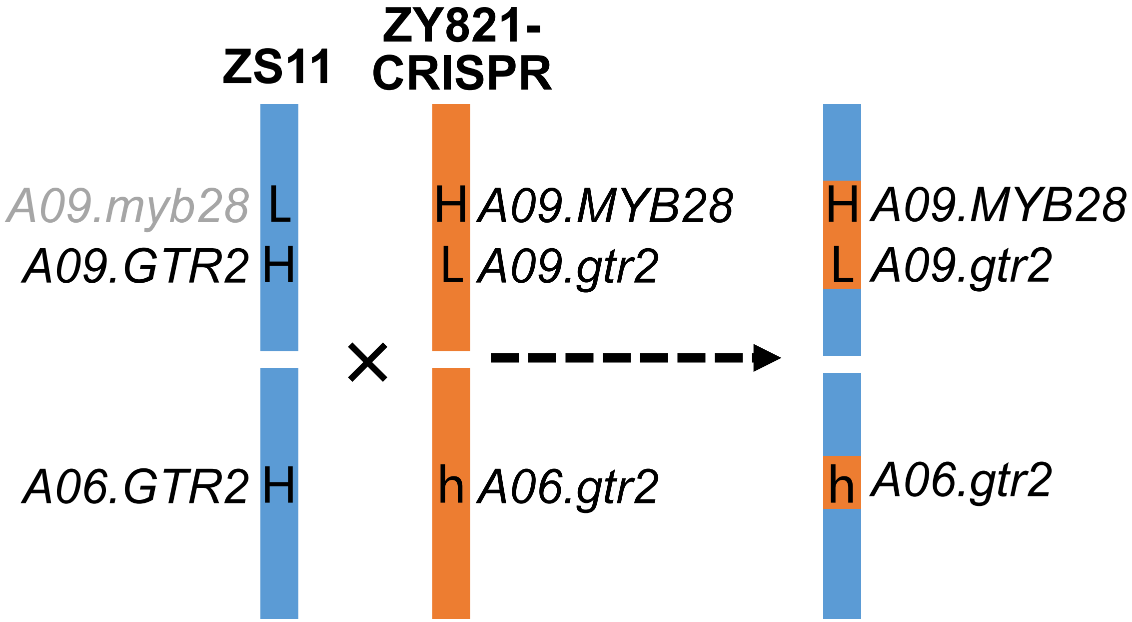


H

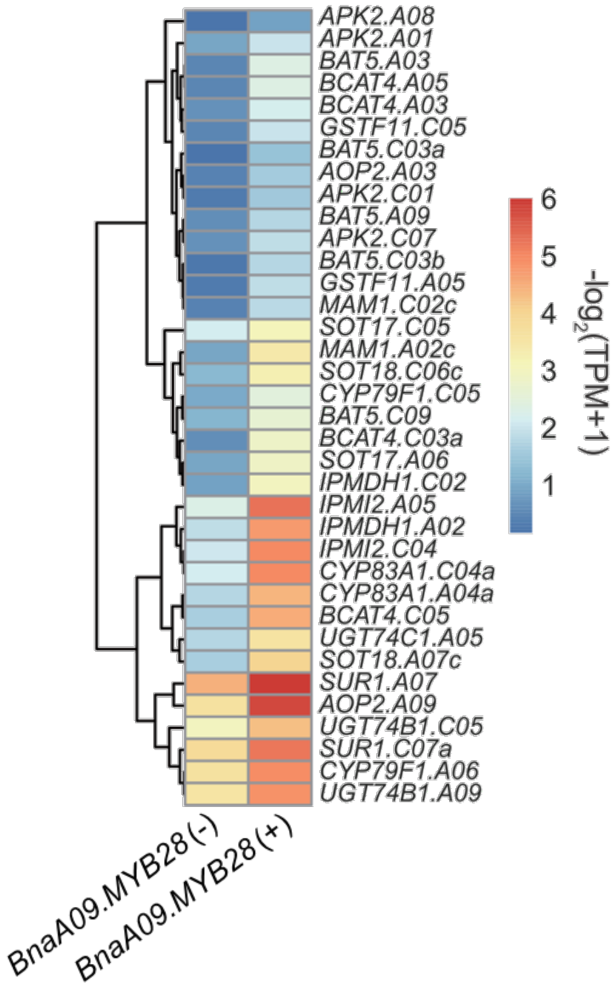
(Enhancing low seed glucosinolate breeding by editing a transporter gene lacking natural variation. He et al., Plant Physiol. 2022)

Germplasm creation of high-leaf and low-seed glucosinolate contents in *Brassica napus*

Introgression of *BnaA09.MYB28/BnaA09.gtr2* and *BnaA06.gtr2* into 'double-low' accession (ZS11) can increase the MYB28-downstream gene expression.



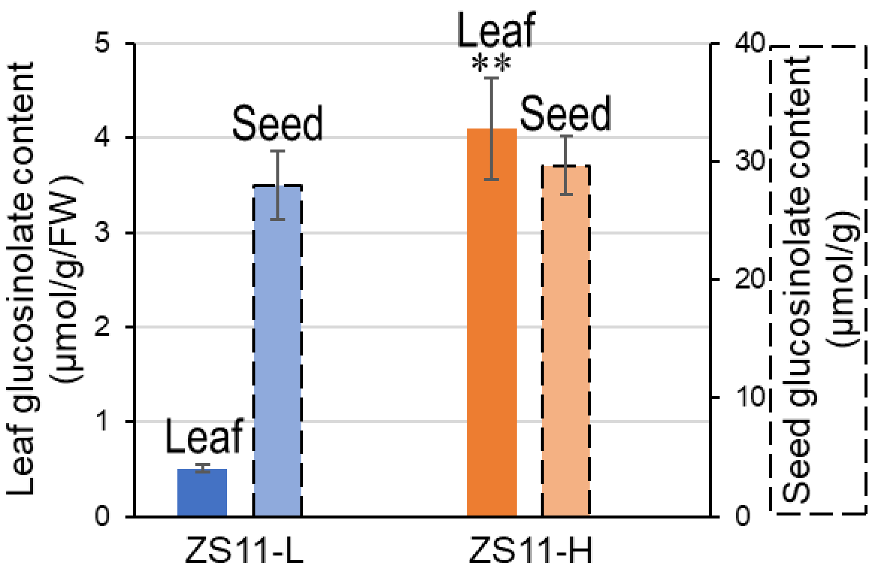
L: Low GS gene
H: High GS gene



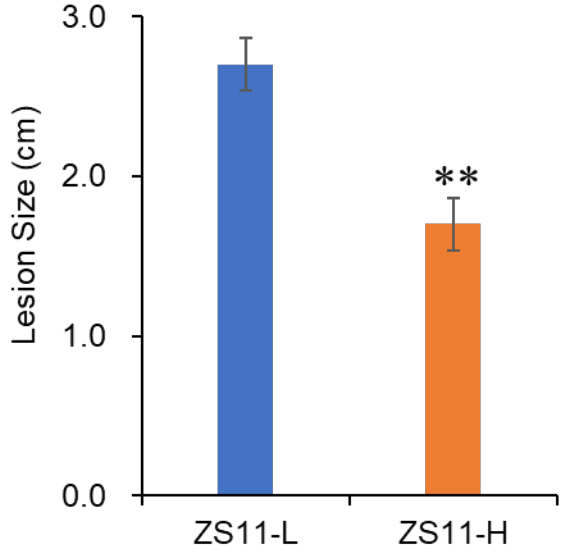
Germplasm creation of high-leaf and low-seed glucosinolate contents in *Brassica napus*

Introgression of *BnaA09.MYB28/BnaA09.gtr2* and *BnaA06.gtr2* can increase leaf-glucosinolate content and keep low seed-glucosinolate content, and increase resistance to disease and pest.

Glucosinolate contents



Inoculation of *S. sclerotiorum*



Summary

- PanSV-genome construction in *B. napus* from 16 *de novo* assemblies.
- We found an SV driving the genetic coupling of glucosinolate biosynthesis and transport, based on linked *MYB28-MYB34-GTR* on chromosomes A09, C02, and C09.
- Absence of *BnaA09.MYB28* have resulted in both low-leaf and low-seed glucosinolate contents in ‘double-low’ rapeseed.
- Introgression of *BnaA09.MYB28/BnaA09.gtr2* and *BnaA06.gtr2* can make high-leaf and low-seed glucosinolate contents in ‘double-low’ rapeseed.

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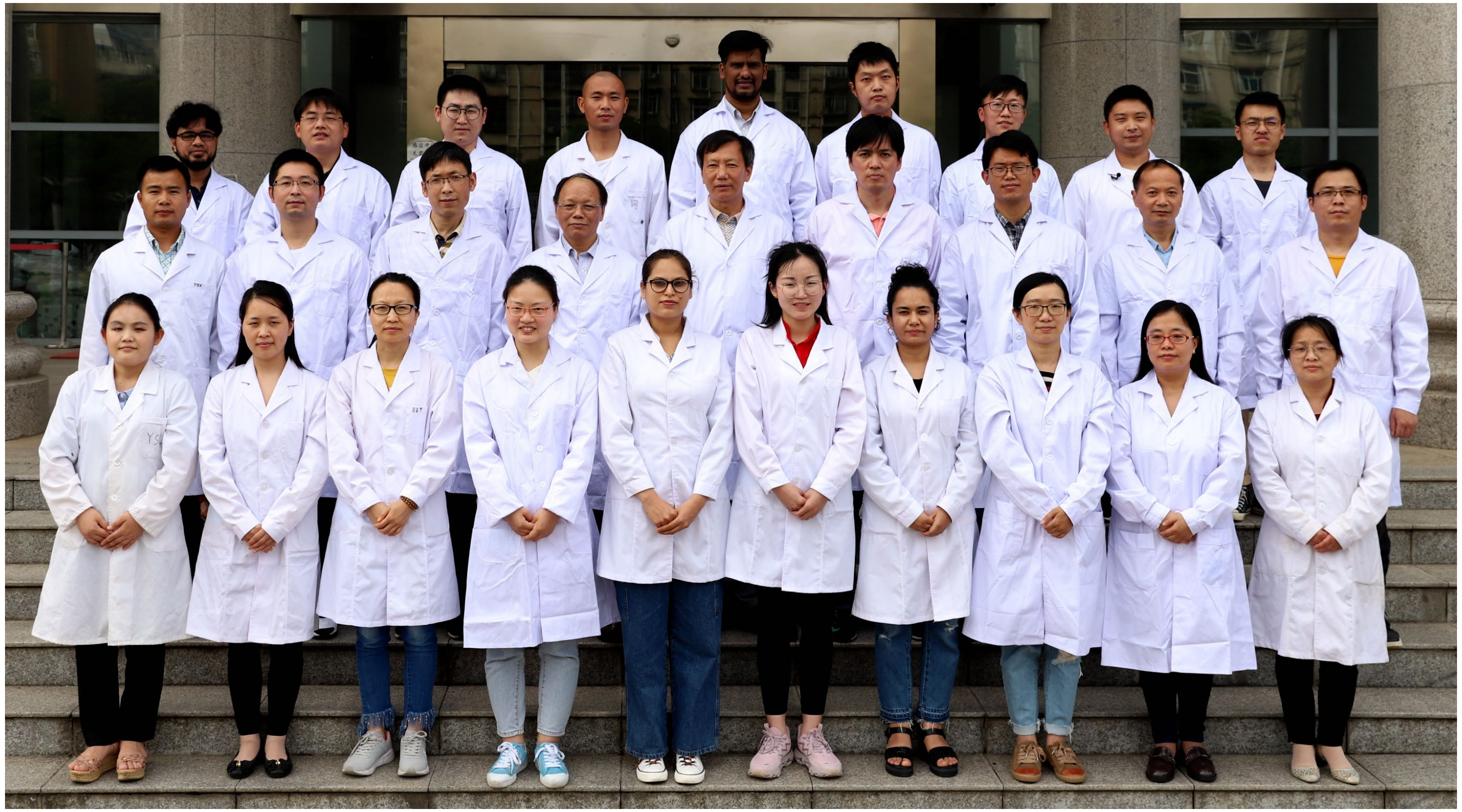
WWAI:

Prof. Harsh Raman



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