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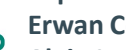
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2S napins are sulfur-rich seed storage proteins. In modern rapeseed varieties '00' without erucic acid (EA) and low in glucosinolates (GSL), the 2S content is generally lower than in older varieties '++' (rich in erucic acid and glucosinolates).

Objective: Identify the genetic and molecular bases of the relationship between the glucosinolate and 2S contents in rapeseed

Material & Methods

Rapeseed

- 174 winter rapeseed accessions : WOSR++ (31), WOSR0+ (15), WOSR +0 (1), WOSR 00 (127) in 9 environments (location x year) and 2 N nutrition regimes
- High dense genotyping assays (270k SNP)
- 12S and 2S contents by size exclusion chromatography
- GSL by NIRS

Arabidopsis

- T-DNA mutants from the SALK collection
- 1 trial (growth chamber, 15 biological repetitions)
- Elementary analysis CNS by Dumas combustion
- 2S content by electrophoresis
- GSL content by UPLC TQD

Genetic and genomic analyses:

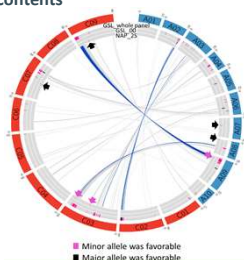
- GWAS was conducted using a mixed linear model including a Kinship matrix (MLM-K)
- Sequence data : WOSR00 DARMOR Bzh [1] and WOSR++ SAREPTA (personal communication).
- Alignment and tree (neighbor joining) with QIAGEN CLC Main Workbench 23.0

2S & GSL QTL



- Identification of 7 loci (QTL) controlling the 2S content located on chromosomes A07, A08, A09, C03 (2), C07 and C09 (Fig.1 – arrows).
- Favorable allele for high 2S content is the minor allele in the population
- 3 co-localizations between QTL for 2S and GSL contents

Fig1. Circos diagram showing the mapping of 2S QTL and GSL QTL onto the rapeseed genome. The GSL QTL were calculated within the whole population ("GSL_whole panel" lane) or the double low subset ("GSL_00" lane). Pairs of homoeologous genes between the A (blue) and C (red) sub-genomes are represented by gray lines (homoeologous genes between QTL and regions carrying no other QTL) or blue lines (homoeologous genes between QTL).



S metabolism genes



Under these QTL, six different genes involved in sulfur-based metabolism pathways were targeted and their orthologs in *Arabidopsis thaliana* were identified.

| Gene | Chromosome | A. thaliana Ortholog |
|----------------|------------|----------------------|
| MYB34 | A09 - 1 | At5g60890 |
| | C09 - 2 | |
| MYB28 | C09 - 2 | At5g61420 |
| ATSD1 | C09 - 2 | At5g48850 |
| GSH2 | C09 - 2 | At5g27380 |
| APK APS kinase | A09 - 3 | At2g14750 |
| IAA28 | C09 - 2 | At5g25890 |

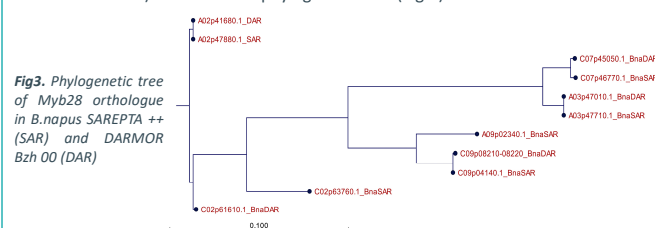
Table. List of targeted genes underlying common QTLs between 2S and GSL with correspondence of Arabidopsis orthologs

Myb28 rapeseed polymorphism



According to the results obtained with the *Arabidopsis* mutant lines and literature, we focused on the *Myb28* gene family in rapeseed.

The resources available led to identify *Myb28* orthologues in oilseed rape (6 orthologues on WOSR++ and 5 orthologues on WOSR00 (loss of the copy on chromosome A09) and to build a phylogenetic tree (Fig.3).



The 4bp insertion on *Myb28* C09 gene of WOSR00 previously identified by [2] was confirmed leading to the production of stop codon and truncation of *Myb28* protein (Fig4).

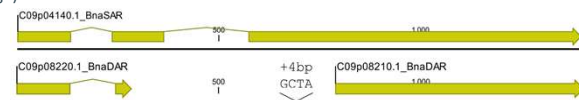


Fig4. Genomic *Myb28* representation of SAREPTA (WOSR++) and DARMOR (WOSR00) on C09 chromosome associated to the translated reading frame

Work in progress : identification of *Myb28* profile in WOSR 00 accessions with high 2S content

Phenotyping of SALK mutants



Homozygous mutant and wild type lines were scored for oil (%C), protein (%N) (data not shown), sulfur (%S), 2S and total GSL contents. Only the *myb28* line (SALK_136312c) has a strong negative impact on all 3 variables observed simultaneously. These results have to be confirmed by a second culture.

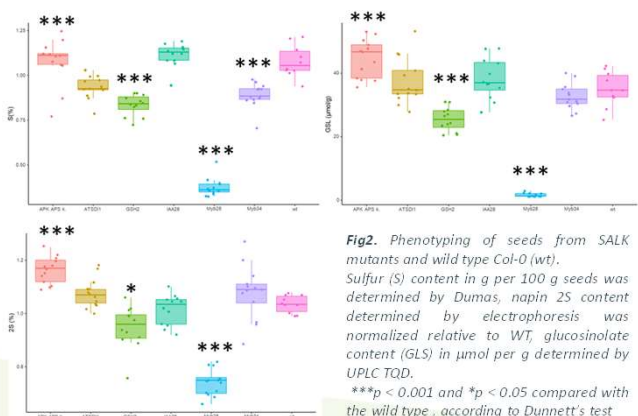


Fig2. Phenotyping of seeds from SALK mutants and wild type Col-0 (wt). Sulfur (S) content in g per 100 g seeds was determined by Dumas, napin 2S content determined by electrophoresis was normalized relative to WT, glucosinolate content (GSL) in µmol per g determined by UPLC TQD. ***p < 0.001 and *p < 0.05 compared with the wild type, according to Dunnett's test

Conclusion & Perspectives

Our results showed that the glucosinolates and 2S napin contents were positively correlated in winter oilseed rape. Crop conversion to low glucosinolate varieties tended to have reduced the 2S content in seeds of the modern-grown varieties. The sulfur metabolic pathway has probably been affected by selection. Our results suggested that *Myb28* genes could be a potential target. These results provided new clues how to improve the seed protein composition in rapeseed.

References

- [1] Rousseau-Gueutin M. et al. *GigaScience* vol. 9,12 (2020): g1aa137.
- [2] Schilbert H.M. et al. *Genes* 13, 1131 (2022).



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