

## QTL-mapping of kaempferols and sinapic acid esters and correlations with other seed quality traits in oilseed rape

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### Background:

Oilseed rape (OSR) meal is a resource-efficient source of protein with a valuable amino acid composition for human nutrition, but its use is limited by antinutritive and off-taste compounds, such as kaempferol glycosides and sinapic acid esters. A thorough understanding of the complex genetic inheritance and trait interactions is crucial for accelerating breeding progress in reducing these undesirable compounds.

### Objective:

Kaempferol 3-O-(2'''-O-Sinapoyl-β-sophoroside) (K3OSS) (Hald *et al.* 2019) has been identified as the main bitter component in OSR protein, but QTL of this and related traits remain largely unknown. This study aims to investigate the genetic variation and inheritance of those compounds in OSR using a doubled haploid (DH) population, to map QTL, identify candidate genes and analyse trait correlations.

### Methods:

To identify QTL, a population consisting of 289 DH lines derived from a cross between the winter line NPZ06 and the yellow-seeded spring line NPZ09, was tested in a two-year field experiment at three environments in Germany. DH lines were genotyped using a 19K SNP Chip. Open-pollinated seeds were analysed using ultra high-performance liquid chromatography and near-infrared spectroscopy. Immature seeds of the parental lines were analysed for differential gene expression.

### Results:

Significant genetic variation for nine individual and total kaempferol glycosides as well as sinapic acid esters were found. Both, K3OSS with a sinapoyl residue and its putative precursor Kaempferol 3-O-(Sophoroside) were significantly positive correlated with sinapine ( $r_s=0.18$ ), and significantly negative correlated with sinapoylglucose ( $r_s=-0.43$ ) and sinapic acid ( $r_s=-0.25$ ). In addition, oil and protein content showed a significant positive and negative correlation with kaempferol glycosides, sinapoylglucose and sinapic acid, respectively.

Over 30 QTL were mapped for above-mentioned traits. Amongst others, a major QTL on chromosome C6 co-localized with individual and total kaempferol glycosides explained 29% of the phenotypic variance for K3OSS. Together with six other QTL, these loci collectively explained 63% of the phenotypic variance. A candidate gene was identified within the confidence interval which is related to an *A. thaliana* gene encoding a flavonol glycosyltransferase (FGT) with activity towards the 3-O and 7-O-position of flavonols. This gene was also differentially expressed between the parents.

Furthermore, another co-localized QTL for K3OSS, individual and total kaempferol glycosides on chromosome A6, was identified and confirmed in a second independent DH population (pers. Comm. Nils Stolte). In both populations, this co-localized QTL was found to be associated with one of several potential flavonol regulator genes recently identified in OSR by Schilbert and Glover (2022).

### Conclusions:

Major and minor QTL for individual and total kaempferol glycosides, including K3OSS, and sinapic acid esters were identified and metabolic interactions between K3OSS and sinapic acid esters were found. A MYB transcription factor gene and a FGT gene were identified as loci controlling the kaempferol biosynthesis.

### References:

Hald *et al.* (2019). Kaempferol 3-O-(2'''-O-Sinapoyl-β-sophoroside) causes the undesired bitter taste of canola/rapeseed protein isolates. *J Agric Food Chem* 67(1):372-378.

Schilbert and Glover (2022). Analysis of flavonol regulator evolution in the Brassicaceae reveals MYB12, MYB111 and MYB21 duplications and MYB11 and MYB24 gene loss. *BMC Genomics* 23:604.