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Unravelling the biosynthetic pathway of bitter-tasting kaempferol derivatives in rapeseed

***Hanna Marie Schilbert*[1]**

Maximilian Schöne[1]
Johanna Pries[1]
Prisca Viehöver[1]
Frank Wolter[2]
Amine Abbadi[2]
Beverley Glover[3]
Daniela Holtgräwe[1]
Bernd Weisshaar[1]

[1] Bielefeld University, Bielefeld, Germany

[2] NPZ Innovation GmbH, Hohenlieth, Germany

[3] University of Cambridge, Cambridge, United Kingdom

Background:

The demand for high quality protein from plants is rising due to concerns about food security, animal welfare, health, and sustainability. Rapeseed (*Brassica napus*) protein has a nutritionally very valuable amino acid composition and high bioavailability. Its use for food is, however, limited by bitter tasting flavonols, namely kaempferol derivatives, present in rapeseed's seeds. Biosynthesis of flavonols is largely uncharacterized in rapeseed.

Objective:

To reduce the bitter tasting kaempferol derivatives in rapeseed seeds, a detailed understanding of their biosynthesis is required. In related species, kaempferol is produced by flavonol synthase (FLS) followed by glycosylation via flavonol glycosyltransferases (FGTs). *FLS* and *FGTs* expression is regulated by MYB transcription factors. We aimed at characterizing these structural and regulatory genes to develop targeted breeding strategies.

Methods:

To identify candidate genes of flavonol biosynthesis we developed the tool "KIPEs", which detects candidates by orthologous relationships and literature based information. The predicted functions of the *FLS* candidates were validated via bioconversion assays and in planta complementation experiments. Phylogenetic analyses were conducted to identify *FGT* and *MYB* candidates based on orthology. To study *MYB* gene duplication and gene loss events, synteny analysis were performed. RNA Seq data was used to analyse organ specific expression patterns of *FLS*, *FGT*, and *MYB* candidates.

Results:

We show that the rapeseed FLS gene family consists of 13 members (Schilbert *et al*. 2021). Five FLSs are expressed in seeds. Among these, two FLS1 homeologs exhibit FLS and flavanone 3 hydroxylase (F3H) activity, while two FLS3 homologs harbour F3H activity and a FLS2 homolog showed neither FLS nor F3H activity.

Among the flavonol regulators, four *MYB12* and three *MYB111* genes were identified in rapeseed, while *MYB11* was lost (Schilbert and Glover 2022). Four *MYB12* and two *MYB111* genes are expressed in seeds, while three MYB12 and two *MYB111* genes are co expressed with homologs of flavonol biosynthesis genes.

Moreover, we identified several *FGT* candidates potentially involved in kaempferol derivatisation that are expressed in seeds.

Conclusions:

We showed that the rapeseed *FLS* gene family consist of 13 paralogs, of which five are expressed in seeds. We demonstrated that FLS from rapeseed is bifunctional by showing that both FLS1 homeologs exhibit F3H and FLS activity. *FLS*, *MYB* transcription factors, and *FGT* candidates can be used to optimize flavonol composition and content e.g., the glycosylation patterns of specific flavonol derivatives.

References:

Schilbert et al. (2021). Characterization of the Brassica napus Flavonol Synthase Gene Family Reveals Bifunctional Flavonol Synthases. *Frontiers in Plant Sciences* 12: 2290.

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.