RaPEQ: Rapeseed protein for human nutrition

JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN

Giessen Plant Breeding

Genetic variation for seed protein quality traits in winter-type accessions of *Brassica napus*

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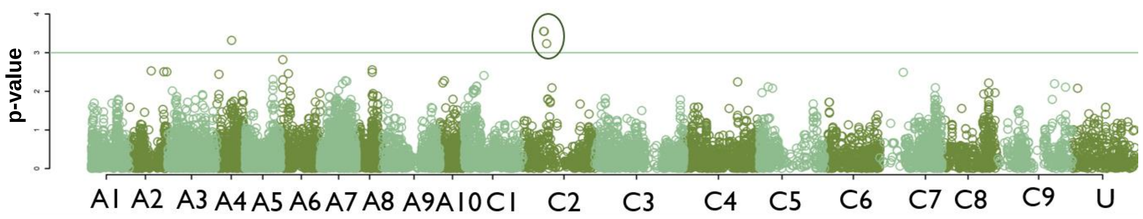
Oilseed rape: Europe‘s major oilseed crop

Brassica napus is Europe‘s leading source for vegetable oil. Besides high quality oil, oilseed rape seeds also contain storage proteins with high nutritional value. In particular, quantities of sulphur-containing amino acids (methionine and cysteine) and lysine are outstanding in comparison to other plant protein sources. Besides the storage protein fractions cruciferin (12S globulin) and napin (2S albumin) a small oleosin fraction with less nutritional relevance is also present.

In this study we screened a broad diversity set (BnASSYST) to identify genotypes with an ideal amino acid composition for human nutrition, and gain first insights into genetic control of protein quality. A total of 158 accessions were genotyped with the Illumina 60K SNP Brassica genotyping array, and self-pollinated seeds from a field trial performed in 2016/2017 were screened for total essential amino acids and the cruciferin:napin ratio.

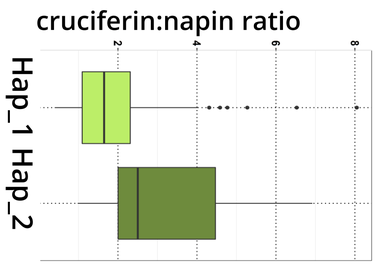
Genetic control of cruciferin:napin ratio

GWAS for cruciferin:napin ratio revealed two variants of a single haplotype block, spanning five SNPs on chromosome C2, which associated with significant differences in the cruciferin:napin ratio. One of the two observed haplotype variants, present in only around 12% of the diversity panel, is associated with an approximately two-fold increase in the cruciferin:napin ratio.



Haploblock on chromosome C2 (LD: $r^2 > 0.8$)

SNP	SNP_1	SNP_2	SNP_3	SNP_4	SNP_5	Frequency
Hap_1	C	G	T	G	A	139
Hap_2	T	A	C	A	T	11



Genome-wide association analysis of protein fractions.

A marker-trait association on chromosome C2 spanning 5 SNP markers in high LD (>0.8). Two haplotype variants show significant differences (0.05) for the cruciferin:napin ratio. The green line indicates a -log10(p-value) of 3.

Ideal protein composition for human nutrition

As shown below, we identified two *B. napus* genotypes whose seed protein fractions perfectly fulfil the current World Health Organisation (WHO) recommendations for nutritional uptake of essential amino acids in adult human beings. With seed-protein of these genotypes, the daily nutritional requirement of a 75kg adult could be achieved with 100g of rapeseed meal.

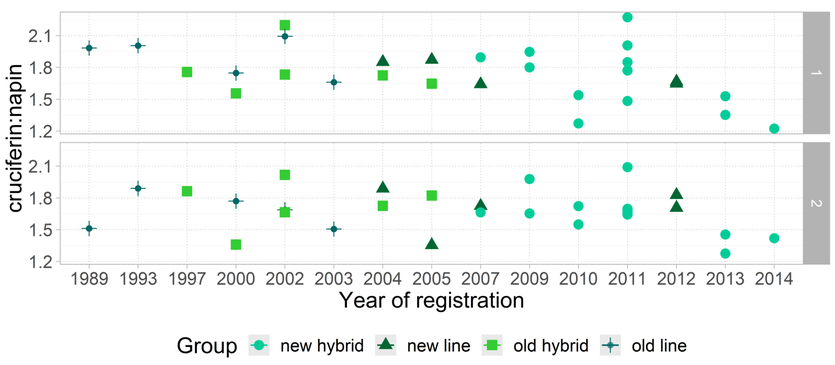
WHO recommended daily intake of essential amino acids (EEA)

For a 75kg human being, compared with amino acid contents in seeds from 158 *B. napus* accessions of the BnASSYST diversity panel.

EEA	Recommended daily intake (mg/75 kg)	EEA content (mg/100g de-fatted meal)				
		Mean	Min	Max	Cultivar 1	Cultivar 2
Histidine	750	931	617	1417	949	1417
Isoleucine	1500	1472	1130	1923	1725	1599
Leucine	2925	2450	1905	3217	3217	2970
Lysine	2250	2041	1628	2527	2305	2527
Methionine	750	659	391	1002	821	875
Cysteine	300	1181	780	1757	1560	1472
Phenylalanine + Tyrosine	1875	2240	1535	3316	3316	2731
Threonine	1125	1331	901	1865	1758	1808
Valine	1950	2041	1430	2680	2669	2121

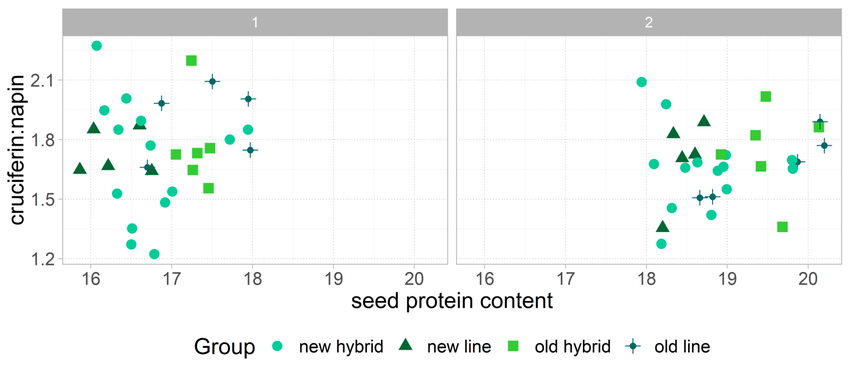
Development of storage protein quality in the past 25 years

A set of 30 divergent WOSR varieties released within the past 25 were used to investigate the development of storage protein quality in the course of breeding progress. Additionally, the effect of contrasting nitrogen fertilisation levels on the seed-protein quality was taken into account.

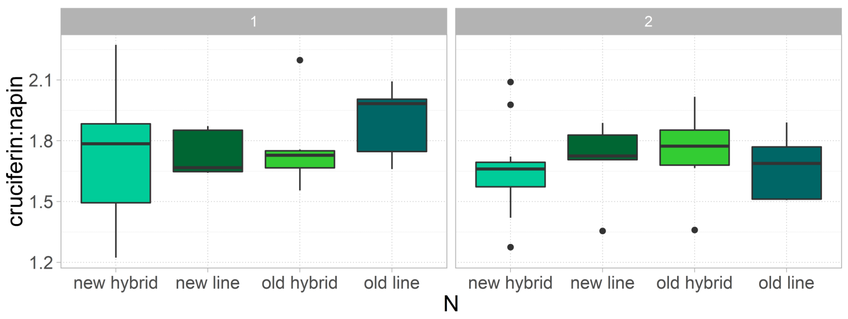


Regression of cruciferin:napin ratio to the year of registration distinguished between the different variety groups (new and old hybrids and old and new lines) and the low (N1) and high (N2) nitrogen fertilisation levels.

Results of SDS-Page analysis for cruciferin:napin ratios of seed samples from a field trial with 30 varieties in 2 N fertilisation levels and 3 locations over two years indicate that the trait is genetical inhertitated, which is supported by calculated the heritabilities of h^2= 0.41, h^2=0.34; and h^2=0.65 for N1, N2 and overall, respectively. Furthermore a shift towards sightly higher napin contents in modern varieties could be observed and presummably lead to an higher nutrional quality of the seed storage proteins in WOSR.



Correlation between cruciferin:napin ratio and the seed protein content



Difference of the cruciferin:napin ratio between the variety groups and N-fertilisation levels.

Conclusions

- Despite the quantitative nature of seed protein traits, we identified an interesting genome region and haplotypes which could help to increase key nutritional traits in oilseed rape protein via haplotype-assisted breeding.
- The results also form the basis for future efforts to identify and utilise relevant genes for mutation breeding towards designed proteins for implementation in human nutrition.
- Declining cruciferin:napin ratios in modern elite varieties suggest an enhanced seed-protein quality despite decreasing seed-protein contents in WOSR

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