Improving rapeseed protein quality for human nutrition

Benjamin Wittkop[1], Isabelle Deppé[1], Andreas Stahl[1], Rod Snowdon[1], Christian Möllers[2], Thomas Hofmann[3], Corinna Dawid[3], Frank Pudel[4], Nino Terjung[5], Jakob Ley[6], Frank Wolter[7], Bernd Weishaar[8]

[1]Justus Liebig University Giessen, [2]Georg August University Göttingen, [3]Technical University Munich, [4]Pilot Pflanzenöltechnologie Magdeburg e.V., [5]German Institute of Food Technologies, [6]Symrise AG, [7]NPZ Innovation GmbH, [8]Bielefeld University

Introduction

Oilseed rape complements high seed oil content and quality with a high-value protein in the meal after oil extraction. To counter dependency on soybean imports in Europe, there is a growing demand for alternative sources of vegetable protein. Rapeseed protein is considered to be of excellent quality for both animal and human nutrition and is competitive with the quality of protein gained from soybean. The quality is mainly determined by the two major storage proteins, the high molecular weight globulins (cruciferin) and the low molecular weight albumins (napin), which respectively account for 60 and 20 % of the total seed protein. Further improvements in quality through reduction of antinutritive properties could open new possibilities to implement rapeseed protein in human consumption.

Project and methods

In order to promote food use of rapeseed protein, the German collaborative project RaPEQ* is combining molecular breeding and genetics, sensomics and technological approaches to increase the quantity and quality of protein fractions obtained from oilseed rape. Ultimately, this integrated approach will help to develop oilseed rape as a sustainable source of nutritious, palatable and techno-functional domestic protein for human consumption. The RaPEQ project incorporates a highly interdisciplinary combination of plant molecular biology, classical and molecular plant breeding, sensory science, processing technology and engineering. The partners from two main areas form integrative subgroups that are interconnected by shared material and intensive information flow. Iterative optimization of plant lines, breeding material, mutants, alleles, molecular markers, functional loci and causative genes on the one hand, and optimisation of processing protocols for optimal extraction of protein fractions as well as their techno-functional and sensory properties on the other. All these well concerted actions, carried out by seven esteemed partners, follow a straightforward but determined goal: optimise seeds from rapeseed lines and seed processing for access to optimal protein for human consumption.

A particularly innovative and synergetic part of RaPEQ is the interdependent interplay between those two areas: input of diverse material preselected for processing, sensory analyses and techno-functional scoring, and finally the combination into GWAS based on material that is genotyped with modern high-throughput methods.

In detail the RaPEQ objectives are:

a) *increase seed protein content and yield while keeping oil content high*

RaPEQ will study and select genotypes that represent correlation breakers for the usually reverse correlation of seed protein and seed oil content, with the goal to localize and identify responsible genes.

b) *establish improved tools for protein analytics to support detection of relevant lines/genotypes*

RaPEQ will develop and optimize rapid, non-destructive NIRS-based analysis methods for protein parameters of rapeseed.

c) *determine the genetic basis of the napin/cruciferin ratio in rapeseed*

RaPEQ will identify and characterize rapeseed lines with contrasting napin/cruciferin ratios adapted to the different purposes and applications of protein fractions.

d) *identify and characterize key molecules contributing to the off-taste of rapeseed protein isolates*

RaPEQ will apply the SENSOMICS approach to identify sensorially adverse compounds in rapeseed protein fractions, addressing both preformed compounds as well as those formed during processing. The pinpointed compounds and potential precursors will subsequently be used as chemotype markers to support line selection in breeding material.

e) *develop processing technology which allows parallel extraction of protein and oil*

RaPEQ will develop methods and technology to increase yield of protein with excellent quality while keeping extraction yield of protein high. Protein fractions will be techno-functionally characterized and initially used for extrusion tests.

f) *to study the genetic basis of accumulation and/or formation of adverse compounds*

RaPEQ will determine heritable components of adverse compound accumulation, and discover genetic variants that control their reduced or eliminated accumulation. Linked molecular marker will be created and relevant loci, genes and/or alleles will be studied.

g) *combine improved genetic variants and improved processing protocols to end up with rapeseed as a popular domestic vegetable protein source for food uses*

RaPEQ will set up technological processes aimed at producing preferentially tasting protein isolates for food applications from optimised source seed, with extruded foods as a first target.

To address the objectives **(a)** and **(c)** the RaPEQ consortium is performing extensive seed quality screening of diverse *B. napus* germplasm and a mutagenised population. Quantitative genetic studies will provide detailed new insight into the genetic determination of seed protein quality, undesirable off-flavors and astringent qualities.

Results and Discussion

Studies have shown that breeding for low glucosinolate content has reduced the relative content of napin in favor of the cruciferin content (Malabat *et al*. 2003). This can be explained by a reduced sulphur availability in seeds of double-low varieties, causing simultaneous downregulation of the respective biosynthetic pathways (Schatzki *et al*. 2014). Furthermore, a positive correlation between nitrogen fertilization level and the absolute quantity of seed protein can be observed (Stahl *et al.* 2016).

To meet the above mentioned environmental modifications on the total seed protein as well as the indirect selection on the protein quality, particular the cru/nap ratio, studies with a small panel of genetic diverse oilseed rape varieties of different seed qualities and from different years of registration were performed and will be continued with a broader germplasm during the project. Besides the influence of nitrogen fertilization levels on the total amount of seed protein the focus of these preliminarily seed quality analyses lie on the change in the cru/nap ratio, in connection with contrasting nitrogen fertilization in old and new oilseed rape varieties as well as to identify any direct breeding, respectively selection effect

on this particular seed quality trait. The results of this first trials indicates that no direct selection on the cru/nap ratio could be observed, although decreasing total seed protein contents in modern cultivars were noticed. However, due to the documented enhanced grain yields in modern oilseed rape varieties the protein yield per area of these cultivars seems to be increased. Finally, the results of the performed field and seed quality trials will provide a starting point towards investigations of the genetic relationships between protein quality, nitrogen use efficiency and agronomic performance.

References

Malabat C, Atterby H, Chaudhry Q, Renard M, Guéguen J (2003) Genetic variability of rapeseed protein composition. In: Proceedings of the 11th international rapeseed conference, Vol I, Kopenhagen, pp 205–208.

Schatzki J, Ecke W, Becker HC, Möllers C (2014) Mapping of QTL for the seed storage proteins cruciferin and napin in a winter oilseed rape doubled haploid population and their inheritance in relation to other seed traits. Theor Appl Genet 127:1213–1222.

Stahl A, Friedt W, Wittkop B, Snowdon RJ (2016) Complementary diversity for nitrogen uptake and utilisation efficiency reveals broad potential for increased sustainability of oilseed rape production. Plant & Soil 400: 245-262, doi: 10.1007/s11104-015-2726-8

Public-private research consortium "RAPEQ: Rapeseed as a domestic protein source of excellent quality for human consumption", funded by the German Federal Ministry of Education and Research (BMBF, FKZ 031B0198A-G)