Improvement of rapeseed meal quality via reduction of seed fibre-fractions

Benjamin Wittkop, Rod Snowdon, Wolfgang Friedt

Department of Plant Breeding, Research Centre for BioSystems, Land Use and Nutrition, Justus Liebig University, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany Email: enjamin.Wittkop@agrar.uni-giessen.de

Abstract

Rapeseed meal is an important product for animal and human nutrition. With drastically rising rapeseed production, an improvement of the quality and economic value of rapeseed meal is an increasingly important breeding aim. The meal possesses about 40% protein with a favorable composition of amino acids. However, due to the high contents of crude fibre and antinutritive components the utilisation of rapeseed meal is limited. To increase the possible use of rapeseed meal a reduction of antinutritive phenolic compounds through breeding of yellow-seeded oilseed rape varieties with thinner seed coats is of very high interest. Yellow seed colour is considered to coincide with low fibre content because the biochemical pathways leading to lignin and seed coat pigments have the same precursors. In this work fibre fractions are being measured in winter oilseed rape populations segregating for seed colour and in genetically diverse black-seeded materials. The major anti-nutritive fibre compounds in the oilseed rape seed coat, namely cellulose, hemicellulose and lignin, are quantified by extraction and measurement of the neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) fractions. Because these measurements are time-consuming and difficult to automate, the aim of this study is to develop near-infrared reflectance spectroscopy (NIRS) calibrations for high-throughput, non-destructive germplasm screening, selection and trait-genotype analysis of these important anti-nutritive rapeseed compounds.

Key words: Rapeseed meal quality - seed colour - fibre fractions - antinutritive compounds - near infrared spectroscopy

Introduction

Oilseed rape (*Brassica napus* L.) is the most important oil crop in Europe. Hence, rapeseed meal as residue after oil extraction is an important product for animal nutrition and a potential source for protein supply in human nutrition. Alongside the oil content and quality, due to the increasing production of oilseed rape the meal quality is becoming an increasingly important factor for the competitive character of oilseed rape. Rapeseed meal possesses about 40% protein with a favorable composition of amino acids; particularly the amounts of the essential sulphuric amino acids methionin and cystein are comparatively increased. Additionally, rapeseed meal is rich in minerals (Mg, P, Ca) and vitamins (tocopherols, cholin). However, the energy value of rapeseed meal as a stock food is limited by high proportions of fibre components, namely lignin, cellulose and hemicellulose, and antinutrive phenolic compounds, which are responsible for the decreased digestibility, the bitter taste, dark colour and the adstringency limited for monogastric animals (poultry and pigs). Major antinutritive and undesirable components in rapeseed meal besides glucosinolates are the phenolic acids, particularly sinapincholin (sinapin), the condensed tannins (proanthocyanidin) and lignin. To increase the quality of the rapeseed meal a reduction of antinutrive and undesirable fibre compounds is of very high interest. The breeding of yellow-seeded oilseed rape varieties is a promising opportunity in this regard, because the thin seed coats of yellow-seeded genotypes are associated with a considerable reduction in the condensed tannins and seed fibre content. Analogous phenotypes in *Arabidopsis thaliana* are collectively known as TT (*transparent testa*) mutants that in some cases regulate the thickness of the endothelium layer and subsequently lead to a thinner seed coat containing lower levels of lignin and proanthocyanidins. In particular, the transcription factors *TT1* and *TT16* are involved in regulation of endothelium cell development, while the endothelium-specific gene *TT10* is presumed to influence both lignin and proanthocyanidin biosynthesis genes in *A. thaliana*. (DEBEAUJON *et al.* 2003). Hence, these genes are interesting candidates for further progress in breeding yellow seeded low lignin and tannin rapeseed genotypes. Dehulling experiments of *B. napus* seeds leads to reduced tannin and fibre contents in different rapeseed varieties, meaning that antinutrtive tannins and fibre compounds are mainly localized in the seed coat (MATTHÄUS 1998). Additionally, different quality investigations of yellow seeded rapeseed varieties show that reductions of seed fibre and tannin contents lead to simultaneously increased oil- and protein contents (MARLES *et al*. 2004, BADANI *et al*. 2006). Since seed colour is very environmentally sensitive the use as a morphological selection marker is difficult. In this regard it is important to establish further analytic methods for screening of germplasm regarding improved seed quality. To determine the total seed fibre content and components the NDF (hemicellulose, cellulose and lignin), ADF (cellulose and lignin) and ADL (lignin) chemical detergent methods are used. However, these techniques are time-consuming and costly. Hence the main aim of this work is to develop low cost, non-destructive, high throughput screening techniques to measure total seed fibre contents. FONT *et al*. (2005) reported about the possibility to determine fibre fractions via near infrared spectroscopy (NIRS) in various *Brassica* species. NIRS is known as a desirable non-destructive high throughput method for the screening of large quantities of

breeding germplasm. With the development of new calibration equations for seed fibre and tannin a useful selection tool could be developed for the breeding of novel yellow-seeded rapeseed genotypes.

Material and Methods

In this study segregating doubled haploid (DH) populations derived from two crosses with different yellow seed sources were used to carry out chemical and molecular analysis. Seed samples were analysed in duplicate for NDF, ADF and ADL following the methods proposed by VAN SOEST (1964). To receive spectral information for the development of new NIRS calibrations with equations for fibre components, the same accessions were scanned in an NIR spectrometer (NIRSystems model 6500, Foss-NIRSystems) in reflectance mode. Spectra were acquired at 2 nm wavelength resolution over a wavelength range from 400 to 2500 nm.

Results and Discussion

First chemical analyses for the seed fibre fractions NDF, ADF and ADL were carried out for selected yellow, brown and black seeded DH-lines. This results showed a broad variation existing for the different seed fibre contents in the investigated rapeseed genotypes: NDF from 10.0 – 23.0, ADF from 5.0 – 13.0 and ADL from 1.0 – 6.0 % dry weight. The yellow-seeded lines showed significantly lower fibre contents than black-seeded lines. The chemical extraction of different raw fibre components showed that the content of ADF in the seed meal is largely influenced by the quantity of lignin (Figure 1), whereas the content of NDF is determined mainly by the amount of hemicellulose (Figure 2).

Lignin and hemicellulose, which are involved in secondary cell wall thickening, are considerably different in yellow and black seeded genotypes. The constituent fibre compounds derive from different biochemical pathways: Lignin is synthesised in the phenylpropanoid pathway and is therefore associated biochemically as a phenolic compound to the seed colour pigments (tannins, flavonoids). On the other hand hemicellulose is a branched polysaccharide. Thus, the synthesis and accumulation of lignin and hemicellulose are presumably influenced by different genes. For further investigations of major genes influencing the various fibre components QTL for the respective traits will be mapped using estimates based on the NIRS calibrations being generated in this study.

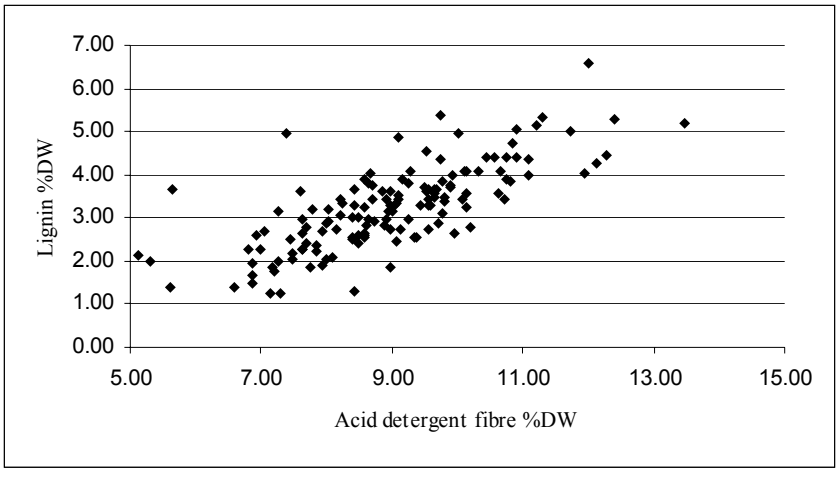

Figure 1: Scatter plot showing the correlation between contents of seed acid detergent fibre (ADF) and lignin (% dry weight). with a coefficient of determination of $R^2 = 0.5621$

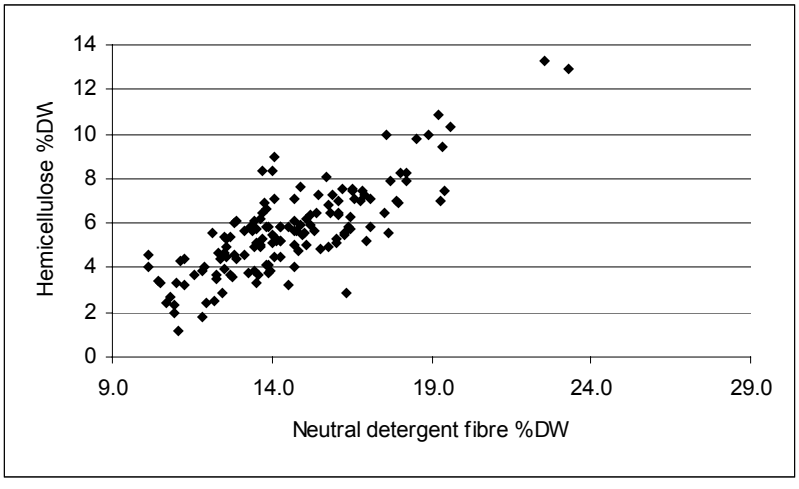

Figure 2: Scatter plot showing the correlation between the contents of seed neutral detergent fibre (NDF) and hemicellulose (% dry weight) with a coefficient of determination of $R^2 = 0.6463$

Dissection of QTL for fibre content into the different components – hemicellulose, cellulose and lignin - may also help to simplify the location of major genes involved in seed fibre content. QTL positions for fibre compounds will be compared with map positions of relevant genes for phenolic compounds, including structural and regulatory *transparent testa* (*TT*) genes from *Arabidopsis thaliana*. In previous work BADANI *et al*. (2006) reported that the seed colour is largely influenced by a major dominant locus on chromosome N18, with additional epistatic loci on N5 and N15. In the present study preliminary QTL analysis for individual fibre compounds and tannins revealed that these three QTL have significant effects on contents of lignin and ADF along with numerous important proanthocyanidins. On the other hand, numerous QTL were detected that do not have a significant effect on seed colour but presumably also contribute to the nutritional quality of the seed. Many of the genes that directly influence specific flavonoid and phenolic compounds are known in *Arabidopsis*, hence this information combined with comparative mapping of candidate genes should help to develop gene-linked selection markers for the breeding of low lignin and low tannin rapeseed genotypes.

Acknowlegements

This work is being carried out as part of the collaborative project "GABI-Kanada: YelLowSin Rapeseed" which is funded by BMBF with support from Deutsche Saatveredelung AG, KWS Saat AG, Norddeutsche Pflanzenzucht H.-G. Lembke KG and SaatenUnion Resistenzlabor GmbH. We thank Nelly Weis and Swetlana Renner for excellent technical assistance.

References

BADANI A. G., R. J. SNOWDON, R. BAETZEL, F. D: LIPSA, B. WITTKOP, R. HORN, A. DE HARO, R. FONT, W. LÜHS, W. FRIEDT, 2006: A model for the inheritance of seed colour in oilseed rape (*Brassica napus L.*) based on analyses of segregation data, QTL and associated quality traits in two genetically distinct crosses. Genome (submitted)

DEBEAUJON I., N. NESI, P. PEREZ, M. DEVIC, O. GRANDJEAN, M. CALBOCHE, L. LEPINIEC, 2003: Proanthocyanidin-Accumulating cells in *Arabidopsis* testa: regulation of differentiation and role in seed development. The Plant Cell 15: 2514-2531

FONT R., B. WITTKOP, A.G. BADANI, M. DEL RIO-CELESTINO, W. FRIEDT, W. LÜHS, A. DE HARO-BAILLON, 2005: Towards a global calibration for acid detergent fibre in rapeseed by visible and near-infrared spectroscopy. Plant Breeding 124: 410-412

MARLES S. & M.Y. GRUBER 2004: Histochemical characterisation of unextractable seed coat pigments and quantification of extractable lignin in the Bassicaceae. J. Sci. Food Agric. 84: 251-262

MATTHÄUS B., 1998: Effect of dehulling on the composition of antinutritive compounds in various cultivars of rapeseed. Fett/Lipid 100, 295-301.

VAN SOEST, P.J., 1964: Symposium on nutrition and forages and pastures: New chemical procedures for evaluating forages. J. Animal Sci. 23, 838-864.