Improvement of oilseed rape meal using near-infrared reflection spectroscopy based selection methods

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Introduction

Oilseed rape (*Brassica napus*) seed meal is an important product for livestock nutrition. With constantly rising seed production in Europe due to higher demands for vegetable oil and biodiesel, an improvement of the seed meal quality is an increasingly important breeding aim in order to increase the overall economic value of the crop. Oilseed rape meal possesses about 40% protein with a favorable composition of amino acids, and high contents of essential amino acids like methionin and cystein. However the utilisation of rapeseed meal in animal nutrition is limited by high content of undigestible and antinutritive fibre components. In particular, the quantity of lignin and other phenolic compounds restricts proportions of oilseed rape meal for the feeding of monogastric livestock (poultry and pigs). Until now most efforts to select for improved meal quality have concentrated on the use of the yellow seed trait as a selection marker for reduced contents of fibre and condensed tannins.

Methods and materials

Reference chemistry measurements from a large panel of winter rapeseed breeding lines segregating for seed colour, fibre and phenolic compounds were used to develop new near-infrared reflection-spectroscopy (NIRS) calibrations for high-throughput screening and selection of important antinutritive fibre components in oilseed rape seed samples. The new NIRS calibrations provide estimates for the contents of neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) in *B. napus* seeds. We screened a large set of genetically diverse oilseed rape germplasm and discovered an unexpectedly broad variation for antinutritive fibre content in black seeded genotypes.

Results and Discussion

Calibration of high-throughput NIRS selection tools for relevant fibre fractions

The new calibration for NDF, ADF and ADL, the major compounds of cell wall encompassing the seed fibre fractions hemicellulose, cellulose and lignin, showed very good correlations and calibration statistics within our panels of calibration genotypes. These new calibrations give acceptable correlations for use in genetic analysis and NIRS-assisted selection and were adapted for application as high-throughput selection tools in the commercial breeding programs of cooperating breeding companies. The calibration for ADL (lignin), the major antinutritive compound in rapeseed meal, showed particularly good prediction with a correlation coefficient of cross validation (R^2_{CV}) of 0.816 and 0.848 for macro- and microcuvettes, respectively, and is therefore of great interest for non destructive analysis in rapeseed breeding. Besides the NIRS-calibrations for standard macrocuvettes (seed volume of 10 ml) additionally calibrations for microcuvettes (seed volume of 1 ml) were developed that show particularly good calibration statistics for ADL, ADF and NDF (Table 1). Accurate calibrations for low seed volumes are a useful breeding tool for selection and screening of nursery germplasm with limited amounts of seeds (early breeding generations).

Table 1. Correlation and cross-validation statistics of new NIRS calibrations for non-destructive high-throughput measurement of antinutritive NDF, ADF and ADL in seed samples from winter oilseed rape. SEC: standard error of calibration; R^2: correlation coefficient; SECV: standard error of cross validation, R^2_{CV}: correlation coefficient of cross validation.

Calibration	SEC	R^2	SECV	R^2_{CV}
NDF macrocuvette (10 ml)	1.551	0.662	1.669	**0.613**
NDF microcuvette (1 ml)	1.601	0.577	1.661	**0.543**
ADF macrocuvette (10 ml)	1.063	0.811	1.128	**0.788**
ADF microcuvette (1 ml)	0.964	0.851	1.072	**0.814**
ADL macrocuvette (10 ml)	0.738	0.844	0.804	**0.816**
ADL microcuvette (1 ml)	0.771	0.869	0.829	**0.848**

Validation of NIRS calibrations in independent seed samples

The cross-validation procedures described above are not always sufficient to describe the accuracy of newly developed NIRS calibrations. For verification of the prediction quality it is necessary to analyse independent and unknown seed samples. Independent validation was carried out using two different validation sets, existing of rapeseed genotypes segregating for seed fibre components from different genetic sources and environments, for standard macrocuvettes (10 ml) (N = 49) and microcuvettes (1 ml) (N = 40). Table 2 shows the statistical results for the independent validations. Again the independent validation for lignin (ADL) is the best and shows a higher accuracy compared to ADF and NDF, with coefficients of determinations (R^2) for e.g. standard macrocuvettes of 0.621, 0.700 and 0.806, respectively. To illustrate the validation results figure 1 shows the graphical regressions of NIRS-predicted and wet chemistry values (reference values) for the contents of the three major fibre fractions using either macro- or microcuvettes. However the results for ADF, representing the sum of lignin and cellulose in the seed meal, are also adequate and robust enough to use these calibrations in breeding programs.

Table 2. Independent validation statistics of new NIRS calibrations for antinutritive NDF, ADF and ADL in seed samples from winter oilseed rape. SEP: standard error of prediction; BIAS: internal error; SEP (C): standard error of prediction (corrected with BIAS), R^2: coefficient of determination.

Validation	SEP	BIAS	SEP (C)	R^2
NDF macrocuvette (10 ml)	1.547	0.208	1.549	**0.621**
NDF microcuvette (1 ml)	1.797	-0.144	1.814	**0.530**
ADF macrocuvette (10 ml)	1.401	-0.043	1.415	**0.700**
ADF microcuvette (1 ml)	1.171	-0.028	1.186	**0.762**
ADL macrocuvette (10 ml)	0.958	0.149	0.956	**0.806**
ADL microcuvette (1 ml)	0.904	0.035	0.915	**0.760**

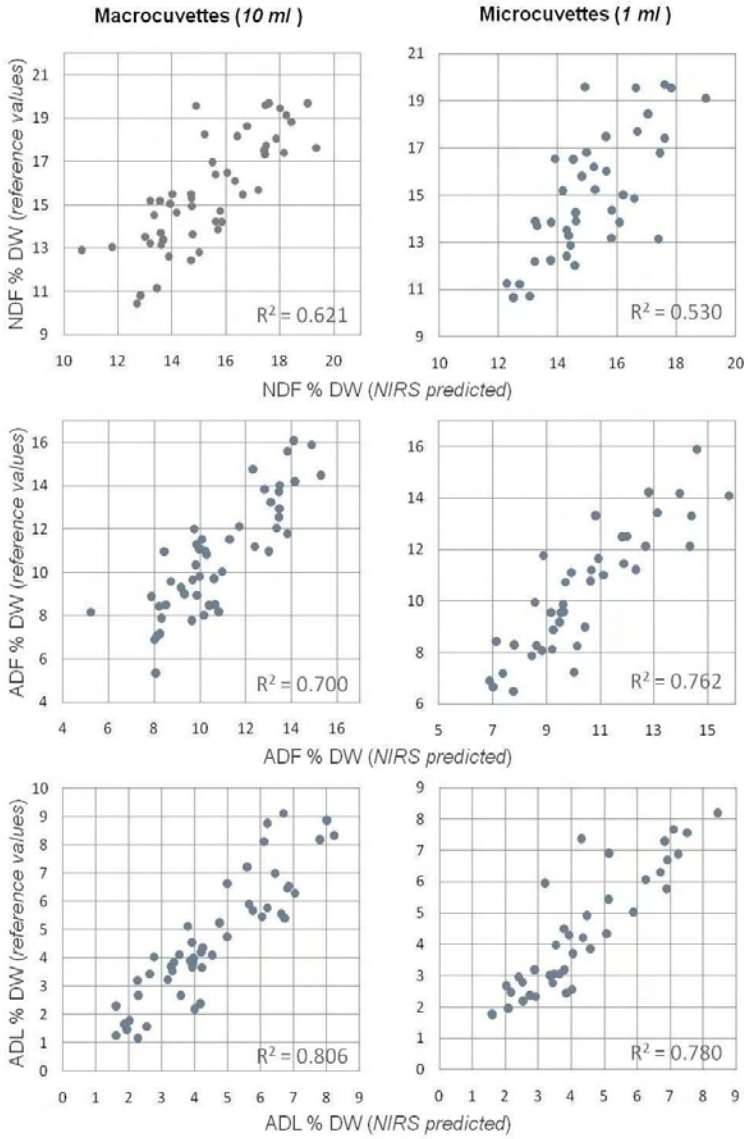

Figure 1. Regression of NIRS-predicted contents and wet chemistry contents (reference values) of neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) in seed samples of *B. napus* for newly developed NIRS calibrations for macrocuvettes (10 ml seed volume) and microcuvettes (1 ml seed volume), R^2 coefficient of determination.

Conclusion

Statistical analysis of new NIRS calibrations for major rapeseed meal fibre components, including cross-validation and independent validation of the calibrations, demonstrated the power of NIRS as a non destructive high-throughput method for the selective breeding of rapeseed varieties with reduced levels of antinutritive fibre fractions, particularly lignin.

Acknowledgements

This work was partly funded by the German Federal Ministry of Education and Research (BMBF) within the GABI-YelLowSin consortium with support from NPZ-Lembke, Deutsche Saatveredelung, KWS Saat and SaatenUnion BioTec.