GENETIC VARIATION FOR SEED FIBRE CONTENT IN A COLLECTION OF EUROPEAN WINTER OILSEED RAPE MATERIAL (*BRASSICA NAPUS* L.)

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ABSTRACT

The oil extracted meal from oilseed rape provides an important protein-rich animal feed. However, compared to soybean meal, crude fibre content of oilseed rape meal is high. Breeding of yellow seeded oilseed rape is one approach to reduce the content of lignin, which is mainly located in the seed coat. Taking advantage of natural genetic variation for neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) among black seeded germplasm may complement this approach. The objective of the present study was to analyse the genetic variation for and the environmental influence on the NDF, ADF and ADL content of 28 current winter oilseed rape cultivars tested in 2008/09 in field experiments at six different locations in Germany. Results showed a large genetic variation for NDF, ADF and ADL contents among current winter oilseed rape cultivars which can be used to develop new improved cultivars with reduced crude fibre content.

INTRODUCTION

The meal of Canola-type oilseed rape (*Brassica napus* L.) is a valuable feedstuff for animals and a potential protein source for human nutrition. However, compared to soybean, fibre content of the oilseed rape meal is high and its energy and protein content is low (Bell 1993). This limits its use in feeding diets. The shortcomings of the oilseed rape meal are mainly attributed to the brown or black fibrous seed hull of oilseed rape, which essentially dilutes the available energy and protein (Clark et al. 2001). Consequently, research has focused on the reduction of the seed hull fraction by breeding yellow seeded oilseed rape and by mechanical separation of the seed hull of black seeded types (Clark et al. 2001). Yellow seeded oilseed rape cultivars are characterized by proportionately less hull and less fibre within the hull than cultivars with black seed coats. For a yellow seeded spring oilseed rape cultivar very much reduced fibre content, consisting of Neutral detergent fibre (NDF), Acid Detergent Fibre (ADF) and Acid Detergent Lignin (ADL) was reported (Relf-Eckstein et al. 2003). NDF comprises hemi-cellulose, cellulose and lignin, ADF comprises cellulose and lignin, and ADL represents the non-digestible lignin fraction (Van Soest et al. 1991), which is mainly located in the seed coat (Wittkop et al. 2009). Despite these achievements, the overall fibre content of yellow seeded types is still high compared to soybean. Apart from studying the fibre content of several yellow seeded types little has been done to investigate the quantitative genetic variation for NDF, ADF and ADL content in black seeded oilseed rape germplasm. The aim of the present study was to analyse the genetic variation and the genotype x environment interactions for NDF, ADF and ADL content of modern winter oilseed rape cultivars tested in field experiments in contrasting environments and to analyse correlations to other seed traits.

MATERIALS AND METHODS

Plant material and field experiments: The seed material consisted of 28 double low quality winter oilseed rape cultivars. In addition to the cultivars listed in Tab.2 the following ones were tested: Katabatic, Elektra, Tassilo, PR44W18, PR44W22, Bellevue, Hybrisurf. The material was tested in 2008/09 at 15 locations in Germany (Bundes- und EU-Sortenversuch 1 Winterraps; Gronow et al. 2009). Field experiments were conducted as a RCB design with 4 replicates for each cultivar at each location. Seed samples were taken after combined harvesting of the yield plots. Samples from the 4 replicates of each cultivar at each location were equally mixed and used for Near-Infrared-Reflectance-Spectroscopy (NIRS) analysis. Based on the mean oil content of the seed samples of the locations, seed samples from each two locations with low, intermediate and high oil content were chosen for the analysis of NDF, ADF and ADL content. For more details about the locations see Gronow et al. (2009).

Analytical methods: Seed samples of about 3 g were scanned with a NIRS monochromator model 6500 (NIRSystems, Inc., Silversprings, MD, USA). Oil, protein and moisture content were determined using the calibration raps2009.eqa provided by VDLUFA Qualitätssicherung NIRS GmbH (Kassel, Germany). Oil and protein content are expressed in % on a seed dry matter basis. Protein content of the oil extracted meal (Prot idM) was calculated by using the seed oil and protein content data obtained from NIRS prediction. NDF, ADF and ADL (% in meal DM) as well as dry matter content (24 hours at 105°C) were determined in 500 mg meal samples according to Van Soest et al. (1991) using the ANKOM™ detergent fibre analysis system with filter bag technology (ANKOM Technology, Macedon, NY, USA).

Statistical analysis: Analysis of variance and calculation of heritabilities were performed by using PLABSTAT software (Utz 2008) considering the locations as random. Mean values of the genotypes across the locations were used to calculate Spearman's rank correlation coefficients between traits.

RESULTS

The analysis of variance showed highly significant effects of the locations and the genotypes on NDF, ADF and ADL content of the defatted seed meal of 28 winter oilseed rape cultivars (Tab. 1). Highly significant effects of the locations and the genotypes on thousand kernel weight (TKW), seed oil and protein content, and protein content of the defatted meal were also found. Comparatively large variance components were detected for the effect of the locations on all traits, except for TKW.

Tab.1: Variance components of NDF, ADF and ADL content and of other seed quality traits in 28 current winter oilseed rape cultivars tested in field experiments at 6 locations

Source of variance	NDF	ADF	ADL	TKW	Oil	Protein	Protein in de-fatted meal
Location	10.7**	7.6**	3.6**	0.05**	6.9**	5.22**	6.21**
Genotype	1.4**	1.4**	0.5*	0.10**	0.9**	0.36**	1.14**
L x G	2.4	2.7	4.5	0.04	0.6	0.33	0.53
h^2	0.78	0.75	0.41	0.94	0.90	0.87	0.93

*; ** Significant at P = 0.05 and P = 0.01, respectively (F test, ANOVA)
h^2 = heritability

Heritabilities were low for ADL, intermediate for NDF and ADF and high for the other traits. Among the cultivars, the NDF content ranged from 26.1 to 32.7% in the dry matter of the defatted seed meal (Tab. 2). Calculated on the basis of dry seeds the NDF content varied from 12.5 to 15.9% (data not shown). ADF content ranged from 20.5 to 26.8% in the dry matter of the defatted meal and from 9.8 to 13.0% in the seeds. In the defatted meal the ADL content varied from 8.5% to 13.2% and calculated on the basis of seeds it varied from 4.1 to 6.4%.

Tab. 2: Seed quality traits of 20 European winter oilseed rape cultivars tested at 6 locations in 2008/2009 (BSV/EUV1)

Cultivar	Type	NDF	ADF	ADL	TKW	Oil	Protein	Protein in defatted meal
ES Alienor	L	26.1	20.5	8.5	5.2	47.8	19.5	37.2
Adriana	L	27.9	22.4	9.1	5.2	49.7	19.2	38.0
Loveli CS	L	28.0	21.1	8.8	4.5	49.8	20.2	40.1
DK Secure	Hzk	28.8	23.8	10.9	4.2	46.8	20.1	37.6
Safran	H	29.3	23.3	9.8	4.4	47.4	19.9	37.7
Limone	H	29.5	23.9	10.3	4.7	48.5	19.2	37.1
Exotic	H	29.5	24.0	10.8	5.1	46.6	20.9	39.0
Lorenz	L	29.7	23.3	9.5	4.4	50.4	18.4	37.0
Cuillin	H	29.8	24.0	11.4	4.5	48.6	19.9	38.6
Monolit	L	29.8	24.2	10.8	5.0	49.5	18.1	35.7
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Visby	H	30.7	23.9	11.5	5.0	47.6	19.3	36.8
Azur	L	30.7	25.7	11.6	4.7	49.1	19.1	37.4
NK Pegaz	L	30.8	25.4	11.0	4.7	48.3	18.5	35.7
PR 45 DR 01	Hzk	30.9	23.9	9.4	4.5	47.8	19.0	36.3
NK Aviator	H	31.1	24.2	10.4	4.0	47.3	19.9	37.7
Zeppelin	H	31.3	25.0	10.7	4.4	49.6	19.3	38.3
Arcadia	L	31.5	26.1	12.0	4.1	48.0	19.4	37.2
NK Caravel	H	31.8	25.1	11.2	4.5	46.8	19.8	37.2
DK Cabernet	L	32.3	26.8	12.3	4.2	48.5	18.7	36.2
NK Morse	L	32.7	26.2	13.2	4.6	48.6	18.1	35.1
Mean		30.1	24.1	10.7	4.6	48.5	19.4	37.5
LSD5%		1.77	1.89	2.42	0.22	0.88	0.66	0.83

H - Hybrid cultivars, L – line cultivars, Hzk – semidwarf hybrid cultivars
LSD5%, least significant difference at the level of probability P = 0.05

Spearman's rank correlations revealed highly significant positive correlations between NDF, ADF and ADL (Tab. 3). All correlations between NDF, ADF, ADL and the other seed traits were negative, although none of them were significant. Closest negative correlations were found for NDF and the other seed quality traits. NDF was more negatively correlated to protein content than to oil content and NDF was also negatively correlated to TKW.

Tab. 3 Spearman's rank correlations for seed quality traits of 28 modern winter oilseed rape cultivars tested in field experiments at 6 locations

Protein	-0.42*						
Oil+Protein	0.80**	0.14					
Prot idM	0.16	0.77**	0.66**				
TKW	0.09	-0.14	0.07	-0.004			
NDF	-0.13	-0.33	-0.38	-0.35	-0.30		
ADF	-0.001	-0.26	-0.22	-0.18	-0.23	0.81**	
ADL	-0.07	-0.14	-0.23	-0.12	-0.05	0.60**	0.84**
	Oil	Protein	Oil+Protein	Prot idM	TKW	NDF	ADF

DISCUSSION

Within the present study a significant and large quantitative variation was found for NDF, ADF and ADL content among 28 current winter oilseed rape cultivars evaluated in field experiments at 6 different locations in Germany. The variance components showed a very dominant effect of the locations on the NDF and ADF content of the defatted meal (Tab. 1). This was not surprising since the 6 test locations were selected among 15 locations based on large differences in mean seed oil

content. Remarkably, the heritability for ADL content was much lower than for ADF and NDF content. Earlier results indicated that temperature affects seed colour, with higher temperatures leading to brighter seed colour (Burbulis and Kott 2005 and references therein)

The Spearman rank correlation coefficients showed as expected a close correlation between NDF, ADF and ADL content. Most of the other correlation coefficients were not significant, because of the limited number of genotypes tested. However, the correlations indicate some trends. NDF, ADF and ADL contents were in descending order negatively correlated with the seed protein content but they were not at all correlated with seed oil content. These results are in contrast with the results of Wittkop et al. (2009) who observed in a set of 54 dark-seeded winter oilseed rape varieties a negative correlation between NDF and oil content (r=-0.51**). In conclusion, the results of the present study revealed a large genetic variation for NDF, ADF and ADL content among modern European winter oilseed rape cultivars. With medium to high heritabilities and the availability of NIRS calibrations for NDF, ADF and ADL content, the prospects for reducing fibre content in winter oilseed rape can be considered as good.

ACKNOWLEDGEMENTS

The authors express their gratitude to Rolf Jeromin and Uwe Ammermann for their technical assistance and to Sortenförderungsgesellschaft (SFG) in Bonn for providing seed sample material from the Bundes- und EU-Sortenversuch Winterraps 2008/2009 field experiments. Zoran Dimov gratefully acknowledges a fellowship from the DAAD.

REFERENCES

Bell JM (1993) Factors affecting the nutritional value of canola meal: a review. Can J Anim Sci 73:679–697

Burbulis N, LS Kott (2005) A new yellow-seeded canola genotype originating from double low black-seeded *Brassica napus* cultivars. Can. J. Plant Sci. 85: 109–114

Clark WD, HL Classen, RW Newkirk (2001) Assessment of tail-end dehulled canola meal for use in broiler diets. Canadian Journal of Animal Science 81:379-386

Gronow J, W Sauermann, G Barthelmes (2009) Sortenversuche 2009 – Mit Winterraps, Futtererbsen und Sonnenblumen. UFOP Schriften, Heft 37/Agrar (E-Book). http://www.ufop.de/publikationen_schriften.php

Relf-Eckstein, J., Rakow, G. and Raney, J. P. 2003. Yellow seeded *Brassica napus* – a new generation of high quality canola for Canada. *In* Proc. 11th Int. Rapeseed Cong., Copenhagen, Denmark. July 2003. pp. 458–460

Utz HF (2008) PLABSTAT (Version 2N), a computer program for the computation of variances and covariances. Institute of Plant Breeding, Seed Science, and Population Genetics, University of Hohenheim, Stuttgart, Germany (http://www.uni-hohenheim.de/ipspwww/soft.html)

Van Soest PJ, JB Robertson, BA Lewis (1991) Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 74:3583–3597

Wittkop B, RJ Snowdon, W Friedt (2009) Status and perspectives of breeding for enhanced yield and quality of oilseed crops for Europe. Euphytica 170:131–140