

## Genetic Variation of Traits Affecting Meal Quality in Black × Yellow Seeded Doubled Haploid Population of Winter Oilseed Rape

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**Abstract.** The by-product of oil production from oilseed rape (*Brassica napus* L.) is protein rich rapeseed meal. It is of great interest to improve the quality of rapeseed meal for poultry feed by reducing the level of anti-nutritional factors, mainly fibre and glucosinolates. The aim of the study was to assess genetic variation of traits affecting rapeseed meal quality in seeds from the M305 (black-seeded) × Z114 (yellow seeded) population of winter oilseed rape doubled haploid (DH) plants. The influence of weather conditions on these traits was tested under two-year field growing conditions in Poland. Significant effect of genotypes and the year of experiment was found for all of the studied traits, apart from neutral detergent fibre (NDF). The significant phenotypic variation in all of these traits makes future selection to improve quality of rapeseed meal possible. It was also found that all of the traits, apart from neutral detergent fibre, are regulated in a complex genetic manner involving additive and epistatic gene action. NDF is regulated by the additive gene action indicating that this trait might be easier for selection. Low heritability found for seed colour, fibre and glucobrassicin indicates great environmental effect on these traits. Large phenotypic variation for protein, oil, acid detergent fibre, neutral detergent fibre and glucosinolates found in this study will allow future genetic mapping analysis to identify key genes regulating these traits. The application of such genetic markers could enhance breeding programs aiming to improve oilseed rape which could be successfully introduced as poultry feed.

**Key words:** rapeseed meal quality, yellow-seeded oilseed rape, protein, oil, fibre.

### INTRODUCTION

Oilseed rape (OSR) also known as rapeseed, canola (*Brassica napus*), is the second largest oil producing crop in the world (FAO). The by-product of oil production from this crop is protein rich rapeseed meal (RSM) or cake. When compared to protein meals from soybean or other legumes, RSM contains an excellent balance of essential amino acids, including high levels of desirable sulphur containing amino acids (cystine, methionine) and a slightly limited amount of lysine (Gacek et al., 2018). However, improvement of RSM is desired in order to make it a useful source of protein, especially for poultry feed, by reducing level of anti-nutritional components such as glucosinolates

(GLS), fibre, non-starch polysaccharides (NSP), and phytic acid present in rapeseed meal (Nesi et al., 2008; Swiech et al., 2016). Phytic acid is a primary storage form of *P* in the seed, while also being a low bioavailability source of *P* and also reduces the bioavailability of other essential nutrients especially cations like Fe or Zn. Glucosinolates induce enlargement of thyroid, therefore have negative effect on growth and health of animals (Quinsac et al., 1994; Swiech et al., 2016). The content of GLS has been significantly reduced in modern double-zero cultivars of OSR ( $< 25 \mu\text{mol g}^{-1}$  of seeds) but it is still desired to reduce its content especially for poultry feed purposes. Fibre, mainly present in the seed coat, reduces metabolizable energy content, protein digestibility, and bioavailability of minerals, therefore lowering its level in RSM meal is of great interest (Simbaya et al., 1995; Rahman et al., 2001; Mailer et al., 2008). The Van Soest system (Vansoest et al., 1991) classifies fibre as neutral detergent fibre, NDF (hemicellulose, cellulose, and lignin) and acid detergent fibre, ADF (cellulose and lignin).

Yellow-seeded oilseed rape genotypes of *B. napus* have a thinner and translucent seed coat resulting in lower fibre proportion and consequently higher protein and oil content in seeds (Rahman & McVetty, 2011). The level of proanthocyanidins and tannins, the major compounds involved in seed pigmentation known to reduce digestibility of seed meal, is also lowered in yellow-seeded OSR (Rahman & McVetty, 2011). The study on nutritive value of seed meal derived from yellow-seeded *B. napus* indicated that it could be successfully used in poultry industry for broilers (Slominski et al., 1994; Slominski et al., 1999). In contrast, the nutritional value and digestibility of yellow-seeded cakes was not improved when fed to young pigs, most likely due to presence of non-starch polysaccharides (NSP) and pectins in RSM (Simbaya et al., 1995; Rahman et al., 2001; Swiech et al., 2016). Although yellow-seeded OSR genotypes seem to be valuable for feeding industry, these varieties also have a number of negative qualities, many associated with yield, that limit their current utility for the wider market. Thus efforts have been dedicated to create new 'triple low' varieties with ultra low fibre meal (less than 2% of acid detergent lignin) and enhanced yield qualities from yellow by black seeded crosses. The yellow-seeded genotypes and intermediate genotypes derived from a cross between yellow-seeded and black-seeded OSR are valuable material in genetic and genomic studies aiming to understand genetic regulation of traits affecting RSM quality (Liu et al., 2013; Stein et al., 2013; Wang et al., 2015). Seed coat colour is a complex quantitative trait, known to be controlled by the interaction of many genes which are greatly affected by maternal and environmental effects (Penfield & MacGregor, 2017). The genetic analysis of yellow-seeded lines indicated that three to four genes, all in homozygous recessive state, were involved in regulation of seed coat colour (Rahman et al., 2001). Numerous genetic mapping studies have been performed in *Brassica* to understand the genetic basis of seed colour (Snowdon et al., 2010; Liu et al., 2013; Qu et al., 2015; Stein et al., 2017; Wang et al., 2017; Behnke et al., 2018) but the improvement of RSM as poultry feed is still a challenge. Further investigations of genetic regulation of traits affecting RSM quality in oilseed rape using different genetic backgrounds and environments, as well as development of targeted mapping populations would be advantageous for future marker assisted breeding programmes in this crop species.

Here a winter oilseed rape black-seeded  $\times$  yellow-seeded mapping population of DH plants has been analysed under field conditions to assess genetic variability of traits affecting RSM quality including oil, protein, fibre and GLS content in seeds. The results

illustrate large genetic variation for these traits and interrelationship among them. The analysis of heritability and gene effect on the studied traits allowed estimation of genetic basis controlling these traits. Moreover, the data obtained could be used in our subsequent mapping studies to identify molecular markers associated with RSM quality in *B. napus*.

## MATERIALS AND METHODS

### Plant material

The experimental population consisted of 78 doubled haploid (DH) lines, developed from F<sub>1</sub> plants of a cross between M305, a black-seeded *B. napus* DH line and Z114, a yellow-seeded *B. napus* DH line. The two parental lines were selected for contrasting seed coat colour and seed fibre content. The population was developed in the Laboratory of Plant Tissue Culture, Plant Breeding and Acclimatization Institute-National Research Institute (PBAI-NRI), Oilseed Crops Division in Poznan, Poland through microspore culture according to the procedure described by Cegielska-Taras (Cegielska-Taras et al., 2002).

### Field experiments

The M305×Z114 mapping population lines and parental lines were cultivated in PBAI-NRI experimental field in Poznan, Poland during two growing seasons: 2015/2016 and 2017/2018. The field trials were carried out in randomized block design with three replicates of double 2 m-long rows with 30 cm spacing seeded at a rate of 100 seeds per each row. The field was managed with standard methods. Seeds were harvested at maturity from five self-pollinated plants per genotype and threshed for further analysis.

### Phenotypic evaluation

The contents of oil, protein, fibre (ADF and NDF), glucosinolates (GLS): 4-OH glucobrassicin, glucobrassicin, glucobrassicinapin, gluconapin, napoleiferin, progoitrin, and total GLS were determined in the Laboratory of Biochemistry PBAI-NRI in Poznan from each seed sample using near-infrared reflectance spectroscopy (NIRS) - Infracore 1255 analyser (Michalski & Czernik-Kolodziej, 2000). The quantification of seed coat colour was determined with The Hunter Labs spectrophotometer (Colorflex) and classified using 0 (black) to 5 (yellow) scale (Michalski, 2009).

### Statistical analysis

The normality of the distributions of the traits value were tested using Shapiro-Wilk's normality test (Shapiro & Wilk, 1965). The two-way analysis of variance (ANOVA) were performed in order to verify the zero hypothesis of a lack of effects of years, DH lines and year × DH line interaction in terms of the values of the observed traits. The minimal and maximal values of the traits, as well as the arithmetic means and coefficients of variation (CV in %), were calculated. Fisher's least significant differences (LSDs) were estimated at a significance level of  $\alpha = 0.001$ . The relationships between the observed traits were assessed based on Pearson's correlation coefficients.

Estimation of the additive gene effect and additive-by-additive interaction of homozygous loci (epistasis) effect on the basis of phenotypic observations of DH lines and on the basis of parental observations as well as the test statistics to verify hypotheses about genetic parameters different than zero were described by following Bocianowski et al. (2017).

The heritability values were estimated using the standard units method of Frey and Horner (1957) in each year of study. Quantitative estimates of the number of genes segregating for observed traits were made using the Wright's formula (Frey and Horner 1957; Bjarko and Line 1988).

The statistical analysis was performed using GenStat 18 software package (<https://genstat.kb.vsnl.co.uk/>).

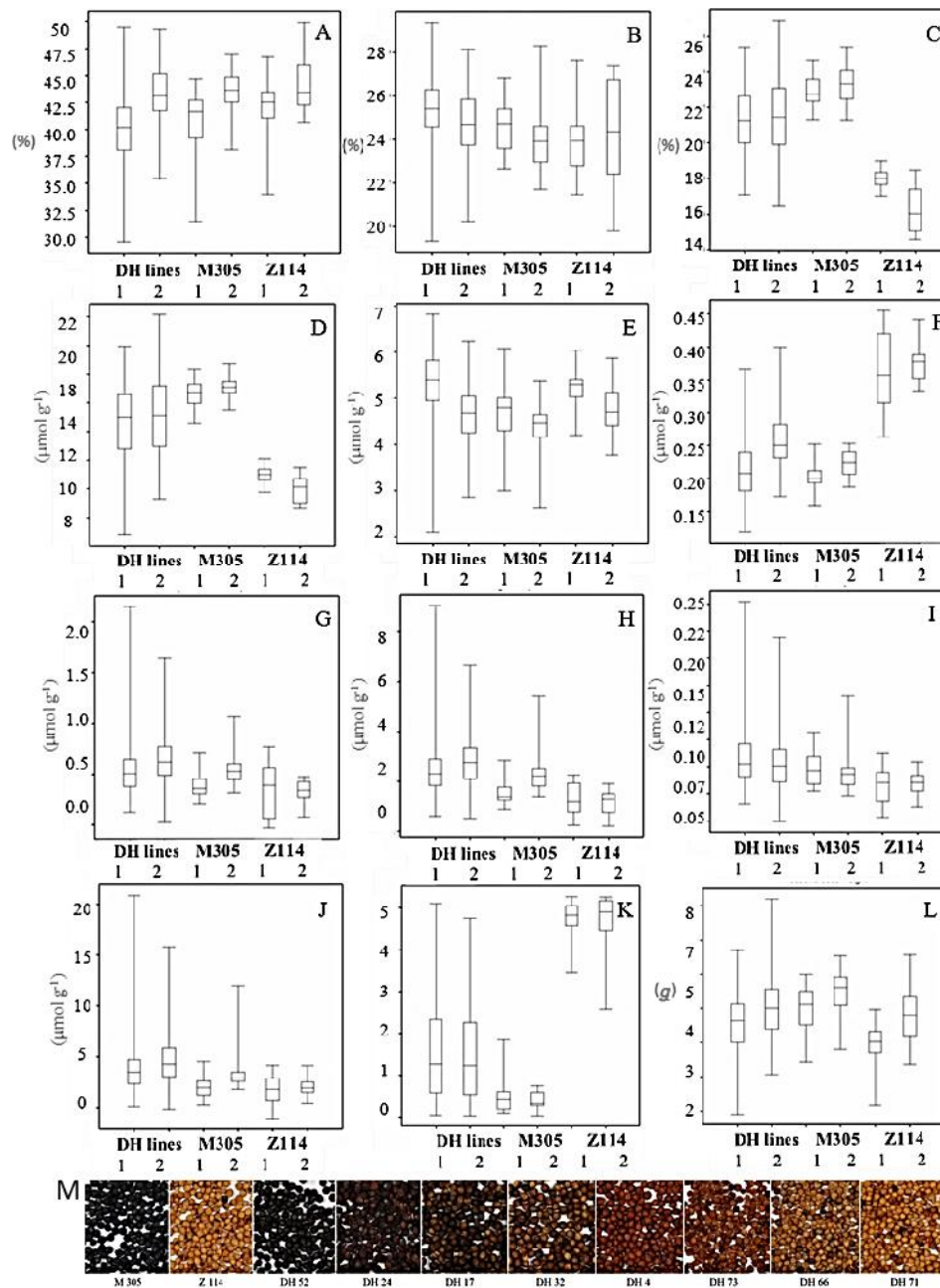
## RESULTS

### Phenotypic variation of seed coat colour, oil, protein, fibre, and glucosinolates in seeds

Traits affecting RSM quality, including content of oil, protein, fibre (ADF and NDF), two indole glucosinolates: 4OH-glucobrassicin (4OH-GBS), glucobrassicin (GBS), four alkene glucosinolates: glucobrassicinapin (GBN), gluconapin (GNA), napoleiferin (NAP), progoitrin (PRO), and seed coat colour (SCC) were measured in the parental lines and each of the 78 DH lines of the *B. napus* population. The parental lines contrasted in SCC: line M305 displayed black seeds and Z114 yellow seeds. The SCC in the DH lines showed great variation in seed coat colour representing black, dark brown, dark brown with single light colour seeds, light brown, red, yellow with single brown seeds, yellow seeds (Fig. 1, M).

The results of ANOVA revealed that the main effects of years were significant for all tested traits, except NDF. The main effects of DH lines and year  $\times$  DH lines interactions were statistically significant for all traits (data not shown). Higher or lower values for all of the tested traits in DH lines when compared to the parental lines indicate transgressive segregation in this population (Fig. 1, A–J and Table 1).

The mean oil content in Z114 seeds was 42%, 44% in 2016 and 2018, respectively whereas in M305 was 40%, 43% in 2016 and 2018, respectively. DH lines showed greater variation in oil content ranging from 30% -49% in both years of the study. The mean content of seed protein in the parental lines was 24% (Z114) and 25% (M305) in 2016, with similar values in 2018. The protein content in the DH lines showed bigger variation, ranging from 19% to 29% in 2016 and 20% to 28% in 2018. The mean content of NDF contrasted in seeds of the parental lines, with 18% (2016) and 16% (2018) for Z114 and 23% in 2016, 2018 for M305 line. The NDF content in the DH lines ranged from 17% to 25% in 2016, and 16% to 27% in 2018. The mean content of ADF in the parental lines was 11% (2016) and 10% (2018) for Z114 and about 17% (2016, 2018) for M305. The content of ADF fibre ranged in DH lines from 7% to 20% in 2016, and 9% to 22% in 2018. The mean content of 4OH-GBS glucosinolate was slightly higher for Z114 (5.2 and 4.7  $\mu\text{mol g}^{-1}$  in 2016 and 2018, respectively) than for M305 (4.6 and 4.4  $\mu\text{mol g}^{-1}$  in 2016 and 2018, respectively). In DH lines the variation for this glucosinolate ranged between 2.1–6.8  $\mu\text{mol g}^{-1}$  in 2016 and 2.86–6.24  $\mu\text{mol g}^{-1}$  in 2018.



**Figure 1.** Boxplots showing content of (A) oil, (B) protein, (C) NDF fibre, (D) ADF fibre, (E) 4OH glucobrassicin, (F) glucobrassicin, (G) glucobrassicinapin, (H) gluconapin, (I) napoleiferin, (J) progoitrin, (K) seed coat colour, and (L) thousand seed weight in seeds of the M305×Z114 mapping population (DH lines) and the parental lines M305 and Z114 in two growing seasons (1) 2015/2016 (2) 2017/2018. (M) The images panel shows variation in seed coat colour in the parental lines: M305, Z114 and selection of DH lines representing colours black (DH52), dark brown (DH24), dark brown with single light colour seeds (DH17), light brown (DH32), red (DH4, DH73), yellow with single brown seeds (DH66), and yellow seeds (DH71).

The variation of GBS content ranged from 0.1 to 0.4  $\mu\text{mol g}^{-1}$  in seeds collected in both years. Mean GBN content was lower in Z114 (0.33  $\mu\text{mol g}^{-1}$  in both years) than in M305 (0.38 and 0.56  $\mu\text{mol g}^{-1}$  in 2016 and 2018), whereas in the DH lines it ranged from 0.03 to 2.2  $\mu\text{mol g}^{-1}$ . Mean GNA seed content was slightly lower in Z114 (1.3  $\mu\text{mol g}^{-1}$  in 2016 and 1.1  $\mu\text{mol g}^{-1}$  in 2018) when compared to M305 parent (1.5 in 2016 and 2.3  $\mu\text{mol g}^{-1}$  in 2018). The mean NAP content was similar in both years of the study and was 0.08  $\mu\text{mol g}^{-1}$  for Z114 and 0.09  $\mu\text{mol g}^{-1}$  for M305, whereas in the DH lines NAP content ranged between 0.05–0.2  $\mu\text{mol g}^{-1}$ . The mean PRO content was slightly higher in the black-seeded parent M305 (1.9 and 3.5  $\mu\text{mol g}^{-1}$  in 2016 and 2018, respectively) when compared to yellow seeded parent Z114 with 1.8 and 2.01  $\mu\text{mol g}^{-1}$  in 2016 and 2018, respectively. The content of PRO showed great variation in the DH lines which ranged between 0.1–21  $\mu\text{mol g}^{-1}$  in both years of the study. Together this data shows that the largest variation among the traits affecting RSM quality was found in the DH lines from the M305×Z114 mapping population, whereas parental lines contrasted the most for SCC, ADF, and NDF. All of the traits were affected by the weather conditions (temperature and rainfall) which differed extremely in both years of the study (data not shown).

**Table 1.** Performance of traits affecting RSM quality for 2016 and 2018 in M305×Z114 mapping population

Trait	Year	Z114 mean	M305 mean	DH mean	DH range
Oil	2016	41.56 ± 3.1	40.35 ± 3.4	39.8 ± 3.5	29.55–49.46
	2018	44.04 ± 2.6	43.4 ± 2.06	43.29 ± 2.5	35.45–49.29
Protein	2016	24.02 ± 1.5	24.58 ± 1.1	25.42 ± 1.3	19.33–29.36
	2018	24.4 ± 2.4	24.02 ± 1.5	24.68 ± 1.5	20.25–28.17
NDF	2016	18.01 ± 0.5	22.92 ± 0.9	21.32 ± 1.7	17.06–25.4
	2018	16.25 ± 1.2	23.25 ± 0.9	21.41 ± 2.1	16.47–26.86
ADF	2016	11.01 ± 0.5	16.64 ± 0.9	14.62 ± 2.3	6.79–19.95
	2018	10.01 ± 0.9	17.17 ± 0.7	15.14 ± 2.6	9.27–22.15
4OH-GBS	2016	5.21 ± 0.4	4.61 ± 0.7	5.3 ± 0.7	2.1–6.82
	2018	4.72±0.5	4.44 ± 0.5	4.67 ± 0.7	2.86–6.24
GBS	2016	0.37±0.05	0.2 ± 0.01	0.21 ± 0.04	0.12–0.37
	2018	0.38 ± 0.02	0.22 ± 0.01	0.26 ± 0.04	0.17–0.4
GBN	2016	0.33 ± 0.2	0.38 ± 0.1	0.52 ± 0.2	0.13–2.15
	2018	0.34 ± 0.1	0.56 ± 0.1	0.63 ± 0.2	0.03–1.64
GNA	2016	1.32 ± 0.6	1.54 ± 0.4	2.44 ± 0.9	0.62–9.04
	2018	1.14 ± 0.4	2.34 ± 0.8	2.75 ± 0.9	0.54–6.65
NAP	2016	0.083 ± 0.01	0.099 ± 0.01	0.108±0.02	0.066–0.252
	2018	0.085 ± 0.01	0.095 ± 0.01	0.102 ± 0.02	0.051–0.22
PRO	2016	1.82 ± 1.3	1.96 ± 1.02	3.77 ± 2.3	0.1–20.9
	2018	2.01 ± 1.09	3.5 ± 2.09	4.59 ± 2.2	0.17–15.8
SCC	2016	4.72 ± 0.4	0.55 ± 0.5	1.58 ± 0.4	0.04–5.1
	2018	4.69 ± 0.8	0.4 ± 1.2	1.51 ± 1.1	0.03–4.74
TSW	2016	3.97 ± 0.6	4.97 ± 0.6	4.53 ± 0.8	1.9–6.7
	2018	4.81 ± 0.9	5.52 ± 0.6	5.01 ± 0.8	3.07–8.2

NDF: neutral detergent fibre; ADF: acid detergent fibre; 4OH-GBS: 4OH-glucobrassicin; GBS: glucobrassicin; GBN: glucobrassicinapin; GNA: gluconapin; NAP: napoleiferin; PRO: progoitrin; SCC: seed coat colour; TSW: thousand seed weight.

**Table 2.** Correlations between traits affecting RSM quality for 2016 (above diagonal) and 2018 (below diagonal) in M305×Z114 mapping population

	Oil	Protein	NDF	ADF	4OH-GBS	GBS	GBN	GNA	NAP	PRO	SCC	TSW
2016												
2018												
Oil	1											
Protein	-0.41***	1										
NDF	0.08	-0.44***	1									
ADF	0.12	-0.33**	0.96***	1								
4OH-GBS	0.43***	0.13	-0.44***	-0.41***	1							
GBS	0.08	-0.16	-0.91***	-0.71***	0.21	1						
GBN	-0.13	0.34**	0.21	-0.19	-0.1	-0.2	1					
GNA	0.04	0.22	0.04	-0.28*	0.12	0	0.85***	1				
NAP	-0.16	0.22	0.41***	0.13	-0.41***	-0.26*	0.62***	0.43**	1			
PRO	-0.14	0.31**	-0.32**	-0.25*	-0.11	-0.07	0.82***	0.82***	0.59**	1		
SCC	-0.11	0.12	-0.90***	-0.91***	0.35**	0.9***	-0.26*	-0.14	-0.41***	-0.21	1	
TSW	0.16	0.04	0.03	0.03	0.01	0	-0.17	-0.02	-0.1	0	-0.32**	1

NDF: neutral detergent fibre; ADF: acid detergent fibre; 4OH-GBS: 4OH-glucobrassicin; GBS: glucobrassicin; GBN: glucobrassicin; GNA: glucobrassicin; NAP: napoleiferin; PRO: progoitrin; SCC: seed coat colour; TSW: thousand seed weight.

### Correlations between traits affecting RSM quality

The correlations between Oil-Protein, Oil-4OH-GBS, Protein-NDF, Protein-ADF, NDF-ADF, NDF-GBS, NDF-SCC, ADF-4OH-GBS, ADF-GBS, ADF-SCC, GBS-NAP, GBS-SCC, GBN-GNA, GBN-NAP, GBN-PRO, GNA-NAP, GNA-PRO, NAP-PRO were statistically significant across both years (Table 2). Correlation coefficients statistically significant in 2016 and not-significant in 2018 were observed for: Oil-NDF, OIL-ADF, OIL-GBN, OIL-NAP, OIL-PRO, OIL-SCC, OIL-TSW, NDF-GBN, NDF-GNA, NDF-TSW, ADF-GBN, ADF-NAP, 4OH-GBS-GBS, 4OH-GBS-GNA, 4OH-GBS-TSW, GBN-TSW, GNA-SCC, NAP-TSW (Table 2). Correlation coefficients statistically significant in 2018 and not-significant in 2016 were observed for: Protein-GBN, Protein-PRO, NDF-4OH-GBS, NDF-NAP, NDF-PRO, ADF-GNA, 4OH-GBS-NAP, 4OH-GBS-SCC, GBN-SCC, NAP-SCC, and SCC-TSW (Table 2). For ADF-PRO the observed correlation coefficients was significant in both years but negative in one year and positive in the other (Table 2). The correlations between the above traits are important factors to be considered in breeding programmes of improved RSM quality in oilseed rape.

### Genetic regulation of traits affecting seed meal quality

To elucidate the mechanism of inheritance and genetic regulation of the traits affecting RSM quality, the estimation of heritability and the effect of additive and epistatic gene action were performed in the M305×Z114 mapping population (Table 3). The analysis of inheritance in the mapping population showed low heritability estimates for SCC (0.1), ADF (0.2), NDF (0.1), and GBS (0.1) in both years. High heritability estimates were observed for oil (0.9), protein (0.9), GBN (0.9 in 2016), GNA (0.9 in 2016), PRO (0.9), and NAP (0.8). The number of genes segregating for the analysed traits ranged from 0.0 (for protein content in 2018 and progoitrin in 2016) to 18.9 (for glucobrassicin in 2016) (Table 3). The number of genes segregating were significant negative correlated with heritability in 2018 ( $r = -0.889$ ,  $p < 0.001$ ) and not statistically significant in 2016 ( $r = -0.527$ ,  $p = 0.078$ ) (Table 3). Both additive and epistatic gene effects were found to be significant for SCC and GBN, GBS, GNA, NAP, PRO content in seeds whereas significant additive effect and non-significant epistasis was found for TSW, 4OH-GBS, ADF, and NDF fibre. The seed oil and protein content showed additive gene effect only in the first year of study.

**Table 3.** Estimation heritability of additive and epistatic effects as well as the number of genes segregating for RSM quality traits M305×Z114 mapping population

Trait	Year	Additive effect based on parental lines	Additive effect based on DH lines	Epistatic effect based on DH lines	Mean value	Heritability	Number of genes segregating
Oil	2016	-0.605	6.442*	-0.484	39.749	0.916	0.2
	2018	-0.320	3.980	-0.479	43.285	0.946	0.1
Protein content	2016	0.280	2.648*	-0.364	25.459	0.898	3.7
	2018	-0.190	2.235	-0.215	24.680	0.947	0.0
PRO	2016	0.070	3.957***	1.290***	3.737	0.997	0.0
	2018	0.746*	4.176***	1.121**	4.592	0.769	0.2
NDF	2016	2.455*	2.813*	-0.160	21.276	0.157	2.3
	2018	3.500*	3.955*	-0.179	21.410	0.134	3.4



Table 3(continued)

ADF	2016	2.815*	3.827*	-0.182	14.598	0.210	1.6
	2018	3.580*	4.787*	0.110	15.136	0.193	1.9
40H-GBS	2016	-0.300	1.266*	-0.171	5.294	0.624	0.4
	2018	-0.138	0.907*	0.048	4.673	0.848	0.5
GBS	2016	-0.081**	0.097**	0.019*	0.216	0.124	18.9
	2018	-0.077*	0.084*	0.020*	0.259	0.108	4.8
GNA	2016	0.109	1.665***	0.445**	2.408	0.958	0.0
	2018	0.599*	1.641***	0.197*	2.752	0.435	0.9
NAP	2016	0.008	0.042**	0.011**	0.107	0.751	0.1
	2018	0.005	0.045**	0.007*	0.102	0.851	0.1
GBN	2016	0.025	0.354***	0.089**	0.518	0.960	0.1
	2018	0.109*	0.412***	0.035*	0.629	0.582	0.3
SCC	2016	-2.085***	2.086***	0.638***	1.618	0.123	3.3
	2018	-2.145***	2.055***	0.711***	1.508	0.115	4.4
TSW	2016	0.503*	1.607**	-0.379*	4.511	0.489	0.9
	2018	0.356	1.245*	0.165	5.009	0.551	2.0

\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ ; NDF: neutral detergent fibre; ADF: acid detergent fibre; 40H-GBS: 4OH-glucobrassicin; GBS: glucobrassicin; GBN: glucobrassicinapin; GNA: gluconapin; NAP: napoleiferin; PRO: progoitrin; SCC: seed coat colour; TSW: thousand seed weight.

## DISCUSSION

The significant effects of years on all tested traits, apart from NDF, were most likely caused by the differences in weather conditions in both experimental seasons. The rainfall and temperature recorded in meteorological station at PBAI-NRI for the growing seasons 2016 and 2018 (between April and September) showed extreme differences, with very high temperatures and very low rainfall level in 2018 (data not shown). The significant effect of weather on the content of fatty acid, glucosinolates and oil in seeds of winter oilseed rape have already been observed in previous studies (Liersch et al., 2013; Lääniste et al., 2016; Nowosad et al., 2017).

The ideal range of seed fibre content in OSR cultivars which could be used as poultry feed should be on a similar level to that found in the yellow-seeded genotypes. Unfortunately, the yellow-seeded OSR displays many unfavourable traits of agronomic importance (pre-harvest sprouting, seed deterioration during storage and problems with oil extraction) which makes them difficult to incorporate into the market. One of the strategies of RSM improvement would be introducing intermediate SCC genotypes of OSR with enhanced nutritional value. It is expected that reduction of seed coat thickness in yellow seeded genotypes results in increased contribution of the embryo to the seed weight and for this reason the content of oil and protein should rise too (Slominski et al., 1999). In contrast to this view the protein and oil content in the yellow-seeded line Z114 was not much higher than in the black-seeded parent M305. The selection of the parental lines for the development of our mapping population was based on great contrast in seed coat colour and seed fibre content, not protein and oil. In other studies no significant effect on oil content was observed in the yellow seeded genotypes too, which indicates a variation in protein and oil content in yellow-seeded genotypes (Wittkop et al., 2009). The observed variation for seed protein content (19–29%) in M305×Z114 mapping population was slightly smaller when compared to values in other studied population

KNDH (19–34%) (Chao et al., 2017). The protein content showed negative correlation with oil and fibre content which was also observed in other studies (Hannoufa et al., 2014). Seeds containing less fibre have thinner seed coat with decreased amount of cell wall polysaccharides which can cause increased carbon availability for protein deposition (Stombaugh et al., 2000). The analysis of the 78 DH lines in the mapping population allowed identification of line DH53 which contained lower amount of fibre and slightly higher protein content. This finding indicates that selection for lighter colour seeds could lead to higher protein content in seeds. The NDF content in the mapping population varied in both seasons between 16% and 27%, whereas in other studies the content of NDF ranged between 11–21% (Snowdon et al., 2010; Wittkop et al., 2012). The ADF fibre ranged in DH lines from 7% to 20% in 2016, and 9% to 22% in 2018, which shows wider variation in comparison to previously published studies (Mailer et al., 2008; Dimov et al., 2012). Strong negative correlation found between SCC and fibre is expected since yellow-seeded genotypes contain lower levels of fibre (Rahman & McVetty, 2011). Interestingly, negative correlation in both years was also observed between fibre and GBS content. In Supranto study (PhD thesis <https://d-nb.info/1052337953/34>) the QTL for GLS colocalises with QTL for cellulose content which indicates that such correlation could be due to common QTLs for these two traits. Although no correlation was found between progoitrin and seed coat colour, the yellow-seeded parental line (Z114) contained lower level of progoitrin when compared to M305 line. It would also be very desired to eliminate progoitrin from the intermediate genotypes of OSR, which could be challenging since this glucosinolate showed negative correlation with fibre content in 2018. In some of the DH lines, the mean value of this major compound responsible for anti-nutritional effect in RSM was greatly reduced (e.g.  $0.9 \mu\text{mol g}^{-1}$  in line DH78) which makes these lines valuable genetic material for future breeding improvement work.

Both additive and epistasis gene effects were found to be significant for SCC, GBN, GBS, GNA, NAP and PRO content in seeds. It means that these traits are regulated in a complex manner involving many genes with small effect and also by gene-by-gene interactions. Significant additive effect and non-significant epistasis for TSW, 4OH-GBS, ADF, and NDF fibre means that these traits are regulated by many genes with small individual effect. Selection for genotypes with low fibre content in the future should be less challenging since this anti-nutritional component was found to be regulated by the additive action of genes.

## CONCLUSION

In conclusion, this data shows that seed quality traits affecting RSM quality are complex and are regulated by interaction of genes which are influenced by the environment. Although some interesting DH lines were found in this study which are already being introduced to breeding programmes, future work includes application of the latest bioinformatic tools in genetic mapping studies using this population to identify key genomic regions affecting RSM quality traits. Recent advances in sequencing technologies and bioinformatics facilitate high density marker discovery with sufficient resolution to give closely linked markers with traits of interest using the DH mapping population developed in this study (Gacek et al., 2017). Identification of such regions

would enhance selection process of valuable genetic material for improvement of RSM quality regardless of environmental impact.

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