Genetic variation of seed phosphorus concentration in winter oilseed rape and development of a NIRS calibration

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Received: 10 September 2020 / Accepted: 5 February 2021 / Published online: 5 March 2021
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Abstract Phytic acid is the major organic phosphorus storage compound in rapeseed. Following oil extraction, the defatted meal is used in feed mixtures for livestock. However, monogastric pigs and chickens can only poorly metabolize phytate. Hence, their excrements are rich in phosphorus (P), which when applied as manure may lead to eutrophication of surface waters. The aim of the present study was to analyze the genetic variation for total and organic P concentration (i.e. mainly phytate) in rapeseed and to compare the results with soybean. Two sets of rapeseed material were tested in field experiments in different environments with varying soil P levels and harvested seeds were used for seed quality analysis. Results revealed significant genotypic differences in total seed P concentration, which ranged from 0.47 to 0.94%. Depending on the experiment, the heritability for total P concentration ranged from 52 to 93%. The organic P portion of total P concentration was above 90% for current rapeseed hybrids. In both sets, there was a significant positive correlation between seed protein and P concentration. A NIRS calibration for total P concentration in intact seeds showed in cross validation a standard error of 0.05% and a coefficient of determination of $R^2 = 0.83$. Total P concentration of soybean seeds and meal was between 0.55 and

0.65%, and around 1.1% for rapeseed meal. Rapeseed meal had a twofold higher ratio of total P to nitrogen concentration as compared to soybean which could be considered adverse when the meal is used for feeding livestock.

Keywords Phytate · Phytic acid · Rapeseed · Soybean · Phosphorus · Seed quality

Introduction

Phytic acid (*myo*-inositol-1,2,3,4,5,6-hexakisphosphate) is the major organic phosphorus (P) storage compound in seeds (Sparvoli and Cominelli 2015). In rapeseed, phytic acid levels ranging from 2 to 4% in seeds, 2 to 5% in the defatted meal and 5 to 7% in protein concentrates have been reported (Thompson 1990; Matthäus et al. 1995; Lickfett et al. 1999). Depending on the soil P concentration, up to 90% of the seed P is stored as phytate and only about 10% is given in other binding forms like phospholipids in membranes, nucleic acids or inorganic (Lickfett et al. 1999; Lott et al. 2000). Following oil extraction, the defatted meal is commonly applied in mixtures for feeding cows, pigs and chickens. Attempts are underway to use rapeseed protein as replacement for meat in food products (Campbell et al. 2016; Hald et al. 2018). Due to its strong chelating characteristic, phytate

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reduces the bioavailability of essential minerals (e.g., Ca^{2+} , Zn^{2+} , Mg^{2+} , Mn^{2+} , and $\text{Fe}^{2+/3+}$) that may cause nutritional deficiencies in populations of developing countries. Humans and non-ruminant pigs and chicken can only poorly metabolize phytate. Consequently, their excrements are rich in phytate. When applied as manure, P is released from phytate by microbial enzymes and may lead to eutrophication of surface waters, which is an environmental concern (Dodds and Smith 2016). To improve the P availability to monogastric livestock and to reduce the P concentration in the manure, external phytases are applied to feed (Adem et al. 2014; Darambazar et al. 2019; Hanna et al. 2020). Genetically increasing the non-phytate P concentration and decreasing the phytic acid concentration in the seeds could be an efficient way to improve the P availability and thereby the meal and protein quality of oilseed rape. The phytic acid percentage of total P concentration depends on the soil P concentration. Lickfett et al. (1999) found in a greenhouse pot experiment, that with decreasing P supply not only the total P concentration in the seeds decreased but also the phytate portion of it. However, since most European soils have a high P supply level (Tóth et al. 2014; Schoumans et al. 2015), the phytate percentage of seeds will in general be high. Beyond, P-deficient oilseed rape plants seem to be able to increase phosphate availability in the rhizosphere by root exudation of e.g. organic acids such as malic and citric acid (Hoffland 1992). There are almost no studies on genetic diversity of phytate and total P concentration in seeds of rapeseed (Yang et al. 2011). The objective of this work was to analyze the total P concentration of 20 different winter oilseed genotypes in field experiments performed on a soil that has received nil and high P fertilizer levels for more than 20 years. Furthermore, current rapeseed hybrids were tested in 2016/2017 in field experiments at six different environments in Germany. Harvested seeds were analyzed for organic, inorganic and total P concentration. Since the majority of the seed P is stored in the organic form, it was also tempted to develop a NIRS calibration for total P concentration. In order to compare the results of rapeseed with those of soybean, a limited number of soybean seed and meal samples were analyzed for their P and nitrogen concentration.

Material and methods

Plant material

Twenty different winter oilseed rape genotypes were used in experiment I (Table 2). This material consisted of old line and hybrid cultivars, inbred and resynthesized lines. The plant material varied in their seed quality from canola (00-quality) to high concentrations of erucic acid in the oil and of glucosinolate in the seed (++-quality). In field experiment II 31 current winter oilseed rape hybrid cultivars (00-quality) as listed in Algermissen et al. (2017) were used. In addition, Volker Hahn (University of Hohenheim) provided soybean seeds of the three cultivars Regina, ES Mentor und Lissabon. Seed samples provided in triplicate were derived from field seed increase generations at Eckartsweier (University of Hohenheim) and from two environments in Austria. Furthermore, soybean meal samples of five different shipments of Brazilian origin and rapeseed meal samples of six different shipments of German origin were provided by the Raiffeisen Warengenossenschaft Niedersachsen Mitte e.G.

Field experiments

Plant material used in field experiment I was tested in 2005/2006 at the experimental station Reinshof of the Georg-August-Universität, Göttingen in North-Western Germany. The field experiment was performed as a split plot design with four replicates and two different soil phosphate concentrations (P0 and P1; together 160 plots). Plot size was 2.0×1.5 m. The soil of the P0-variant did not receive any P fertilizer since 1983 and the complete above ground organic matter of different crops was removed every year at harvest. The calcium-acetate-lactate (CAL) extractable P was 1.8 mg P/100 g soil, i.e. supply level 'class B' according to the German classification system which means a poorly P supplied soil (www.vdlufa.de 2020). The high P1-variant was fertilized until 1995 with the ninefold P amount of what was removed with the harvest. From 1995 on no P fertilizer was applied. However, leaves and straw always remained on the plots. In 2005, the soil of the P1-variant contained 11.7 mg CAL-P/100 g soil which is equivalent to soil P supply level 'class D', i.e. high. Nitrogen (180 kg N/ha) and potassium fertilizer as

well as micronutrients were applied according to local custom. Sowing was done end of August 2005 and harvest in the second half of July 2006. At maturity seed yield per plot was determined by combined harvesting. Additional field experiments with 76 genotypes (lines, hybrids and resynthesized genotypes) were performed in 2006/2007 at Çanakkale (Turkey), Düşhorn, Krelingen and Reinshof (all located in north-western Germany) with two P fertilizer levels and 2 replicates. Beside 17 genotypes from field experiment I, this set comprised a much larger genetic variation. Seed samples were harvested from open pollinated plants and were analyzed for seed quality traits as described above for field experiment I. Results from this experiment are not reported, because they were very similar to the results from field experiment I. However, the recorded spectra were used along with the results from the total P analysis for the NIRS calibration development.

Field experiment II was performed in 2016/2017 at 6 different environments with three to four replicates in Germany. Mixed seed samples from the replicates per genotype and the six environments Boldebeck, Tützpatz, Borwede, Sonnenwalde, Seligenstadt und Boxberg were provided (Algermissen et al. 2007). While the soil of the environment Seligenstadt and Boldebeck had very high supply levels ('class E', 19 and 16 mg CAL-P/100 g soil), Boxberg and Tützpatz had a high supply level ('class D', 12 and 11 mg CAL-P/100 g soil; Algermissen et al. 2017, www.vdlufa.de 2020). P concentrations of the soils at Sonnenwalde and Borwede were not available.

Analytical methods

Soil P concentrations was analysed using the CAL extraction method (Schüller 1969) and the phosphate concentration in the extract was determined spectrophotometrically (Murphy and Riley 1962). Total P concentration in the seeds was determined according to the Molybdän-Vanadin-Method of Scheffer and Pajenkamp (1952). Dry seeds of rapeseed and soybean (5–7% moisture concentration) as well as the meal samples were ground using a coffee mill (Bosch KM13). The meal was dried at 100 °C to constant weight. 250 mg dry meal was transferred to digestion vessels and 5 ml of concentrated nitric acid (65%) and 3 ml of hydrogen peroxide (30%) were added. The vessels were closed and incubated in a microwave

(Model Ethos.lab, MLS GmbH) at 200 °C and 15 bar for 90 min. At the end the solution was transferred to a graduated flask and diluted with Seralpur® water to 25 ml. 2 ml of the extraction volume were mixed with 7.5 ml of the P yellow solution and again diluted to 25 ml with Seralpur water. After 60 min, the extinction was measured spectrophotometrically at 436 nm (Helios Gamma, UNICAM) by using a 1 cm flow-through cell. After development of a calibration curve and determination of the extinction coefficient, the phosphate concentration was calculated.

Inorganic P was extracted according to Bollons and Barraclough (1997). 250 mg dry meal were transferred to plastic bottles and 25 ml of acetic acid (2%, 0.33 M) were added. The bottles were incubated for 30 min on a horizontal shaker (130 rpm; Edmund Bühler GmbH, Swip) and the suspension was filtrated (Filter MN 615 ¼ 15 cm). P concentration was determined by P blue staining according to Murphy and Riley (1962). In brief, 2 ml of the extract were mixed with 4 ml of the staining solution. After an incubation of 60 min, the inorganic P concentration was determined spectrophotometrically at 882 nm. Organic P concentration was calculated as the difference between total and inorganic P concentration.

Seed quality traits oil, protein and glucosinolate concentration were determined by NIRS using the calibration raps2007.eqa and raps2016.eqa provided by VDLUFA Qualitätssicherung NIRS GmbH (www.vdlufa-nirs.de). The sum of oil and protein was calculated for each seed sample. Protein concentration of the rapeseed seed samples were converted to nitrogen concentration by Factor 6.25^{-1} . The nitrogen concentration of the soybean seeds, the soybean meal and rapeseed meal samples were determined by applying the DUMAS method with two technical replicates per sample.

NIRS calibration development

In total NIRS spectra of 1202 seed samples were recorded from experiment I and from field experiments in 2006/2007. Reference analysis for seed P concentration was performed on all samples. The frequency distribution of the seed P concentration of all samples was almost normal distributed. Redundant spectra with intermediate concentrations were removed. Representative samples were kept in the calibration set (n = 629; Table 5). The NIRSystems

6500 (Foss A/S, Hillerød, Denmark) and small ring cups with approx. 3 g whole seed filling were used to record the spectra in a wavelength range of 400–2500 nm with a resolution of 2 nm. WinISI II Software Version 1.5 (Infrasoft International) and a MPLS algorithm with a combination of math treatments (1,4,4,1), SNV and Detrend correction was applied. Two outlier elimination passes were allowed and spectral outliers with $t > 2.0$ and $GH > 3$ were not considered for calibration development (Williams and Sobering 1996). Calibration performance was assessed by standard error of calibration (SEC), coefficient of determination of calibration (RSQ), standard error of cross-validation (SECV) and coefficient of determination in cross validation (1-VR). The standard deviations (SD) and the means for the calibration sets as well as range of the calibration were taken from the WINISI software.

Statistical analysis

Analysis of variance, heritabilities and least significance differences (LSD5%) were performed by using PLABSTAT software (Utz 2011). A mixed model was used with P level as fixed factor and genotype as random factor. Field experiment II was designed as a random complete block with environments and genotype considered as random factors. Mean values of the genotypes across the environments were used to calculate Spearman's rank correlation coefficients between traits. Kruskal–Wallis-test (TIBCO Statistica ver. 13.3) was applied to test for significant difference between samples of soybean seeds, soybean and rapeseed meal. Significant differences at $p = 0.05$ were marked with small letters a, b, c, d (Fig. 2).

Results

The soil P level of experiment I had a significant effect on the total seed P concentration of the rapeseed genotypes (Table 1). The seed P concentration at the low P soil level ranged from 0.47% (cv. Lirajet) to 0.85% (RS6). At the high P soil level, the seed P concentration ranged from 0.69% (cv. Bristol) to 0.94% for RS6. Mean total seed P concentration at low and high soil P level was 0.61% and 0.80%, respectively (Table 2). Significant differences between the 20 genotypes were found and the mean total seed P

concentration over both soil P levels ranged from 0.59% for cv. Lirajet to 0.89% for resynthesized genotype RS6 (Tables 1, 2). No significant effect of the soil P level was found on seed yield and on the oil, protein and glucosinolate concentration. There was no significant genotype $\times$ soil P level interaction for all traits and heritabilities were high. Correlations between seed P concentration as a mean over both soil P levels were positive with protein ($r_s = 0.82$, $p = 0.01$) and negative with oil concentration ($r_s = -0.66$, $p = 0.01$). The correlation between the mean seed P concentration of the genotypes tested at P0 and P1 was $r_s = 0.85$ ($p = 0.01$).

For total P, inorganic and organic P significant differences between the 31 hybrid cultivars of experiment II were found (Table 3). Heritabilities were 86% for seed inorganic P concentration, 52% for total and 48% for organic P concentration. No significant difference for the percentage of organic P of the total P concentration ($\%P_{org}$ of P_{tot}) was detected among the genotypes. Hence, the heritability was estimated as 7%. The heritability for the ratio inorganic P to nitrogen (P_{inorg}/N) was 84%, whereas it was only 45% for the ratio total P to nitrogen concentration (P_{total}/N). Significant genotypic differences were found for the concentrations of oil, protein, glucosinolate and for the sum of oil and protein (Table 3). Total P concentration in the hybrid varieties ranged from 0.61 to 0.82% (Table 4). The organic part of the total P was estimated 92.4% without much variation. While total and organic phosphorous concentration were closely correlated, there was only little correlation to inorganic P (Table 5). Moreover, correlations were positive to protein concentration, to the ratio of P_{tot}/N , and negative to oil concentration.

The NIRS calibration set consisted of the maximum range of total P concentration (0.48 to 1.07%, Table 6). The average Global H-value was 0.97 and the slope was 0.94. The standard error of calibration was 0.04 and the coefficient of determination 0.89. In cross-validation the standard error was slightly higher and the coefficient of determination was lower. Figure 1 shows the correlation between the reference and the NIRS predicted values. As found in the first and the second experiment, in the NIRS calibration set there was a significant positive correlation between total seed P and the protein concentration ($r_s = 0.57$; $p = 0.01$) and a negative correlation to the oil concentration ($r_s = -0.51$; $p = 0.01$).

Table 1 Variance components for total Seed P concentration (P) of 20 rapeseed genotypes (G) tested in 2005/2006 in field experiment I at two different soil P levels

Source DF	P 1	G 19	G × P 19	Heritability h^2 (%)
<i>Trait</i>				
P (%)	0.0183**	0.0057**	0.0002	93
Oil (%)	− 0.07	6.34**	0.26	95
Protein (%)	0.01	4.90**	0.12	96
Glucosinolate (μmol/g)	5.25	711.18**	3.05	99
Seed yield (g)	49.7	8336.2**	− 1534.6	83

G × P = Genotype × P level interactions, DF = degrees of freedom

*, **Significant at $p = 0.05$ and $p = 0.01$, respect**Table 2** Total seed phosphorus concentration of 20 rapeseed genotypes tested in field experiment I with two contrasting soil P levels (P0 = low; P1 = high) and their respective means

Genotyp	Mean P0 + P1	P0	P1
RS6	0.89	0.85	0.94
R53	0.82	0.73	0.90
DP HB 1	0.80	0.65	0.94
Emerald	0.77	0.68	0.86
G65	0.76	0.63	0.88
S27	0.76	0.67	0.85
RS3	0.73	0.62	0.84
DH Samourai	0.73	0.64	0.81
Express	0.68	0.59	0.76
Oase	0.67	0.56	0.77
Trabant	0.67	0.60	0.75
Apex	0.66	0.57	0.74
DH Mansholts	0.66	0.56	0.77
Talent	0.66	0.55	0.78
Aurum	0.65	0.58	0.72
Mohican	0.64	0.53	0.75
Sollux	0.63	0.53	0.74
Elektra	0.63	0.55	0.70
Bristol	0.62	0.54	0.69
Lirajet	0.59	0.47	0.71
Mean	0.70	0.61	0.80

The analysis of the soybean seed samples revealed similar P concentrations and P distributions as found for rapeseed seeds (c.f. Table 4 and Fig. 2). Rapeseed meal showed the highest total-P concentration from all samples. Soybean seeds had twofold higher N

concentrations than rapeseed seeds. And, the soybean meal samples had 35% higher N-concentration as rapeseed meal. Accordingly, the P to nitrogen ratios were twofold lower. Interestingly, the organic P portion (P_{org}/P_{tot}) of the soybean meal was higher compared to the soybean seeds.

Discussion

Results from the first field experiment showed that there are significant differences for the seed P concentration between the genotypes. Despite large differences in soil P level, the first experiment did not reveal significant genotype × soil P interaction for total seed P concentration and the other seed quality traits. The low soil P level of 1.8 mg CAL-P (100 g soil)^{−1} led as a mean over all genotypes to a reduction of seed P concentration by 25%. According to the results of Lickfett et al. (1999), a reduced plant supply with P leads to a reduced phytate portion of total seed P concentration. Thereby, the P availability for monogastric livestock could be enhanced and the phytate concentration in the manure reduced. The non-significant effect of the soil P level on seed yield, oil and protein concentration in the first experiment was confirmed in a more recent study performed on the same field plot by Römer et al. (2017). They reported that in 2015 the yield of the hybrid cultivar Visby was not affected by the low soil P level of 1.5 mg (100 g soil)^{−1} compared to the other plots with higher soil P levels. This did also not have an effect on seed oil and protein concentration. Obviously, winter oilseed rape

Table 3 Variance components of 31 rapeseed hybrid genotypes (G) tested in 2016/2017 in field experiment II at six different environments (E)

Source DF	G 30	E 5	G × E 149	Heritability h^2 (%)
Trait				
P _{total} (%)	0.1490**	1.908**	0.8426	52
P _{inorganic} (%)	0.1140**	0.1834**	0.1148	86
P _{organic} (%)	0.1298**	0.1537**	0.8489	48
%P _{organic} of P _{total}	0.0413	− 0.0213	3.1766	7
Oil (%)	0.1504**	0.4555**	0.0977	90
Protein (%)	0.0340**	0.4409**	0.0342	86
Sum oil + protein (%)	0.1373**	0.0460**	0.0483	95
Glucosinolate (μmol/g)	0.5632**	0.7425**	0.3253	91
Nitrogen (N %)	0.0009**	0.0113**	0.0009	86
P _{total} /N	0.0135*	0.0183**	0.0996	45
P _{inorganic} /N	0.0111**	0.0189**	0.0132	84

G × E = Genotype × environment interaction,
DF = degrees of freedom

'Original values × 10;

"Original values × 100; *,

**significant at $p = 0.05$

and $p = 0.01$, respect

Table 4 Descriptive statistics for the variation of seed quality traits in the 31 rapeseed hybrid genotypes tested in 2016/2017 in field experiment II at six different environments

Trait	Min	Max	Mean	SD	LSD5%
P _{total} (%)	0.61	0.82	0.72	0.054	0.10
P _{inorganic} (%)	0.047	0.063	0.053	0.004	0.01
P _{organic} (%)	0.56	0.76	0.67	0.052	0.11
%P _{organic} of P _{total}	90.6	93.6	92.4	0.755	2.03
Oil (%)	41.8	43.5	42.7	0.408	0.36
Protein (%)	17.9	18.8	18.3	0.199	0.21
Sum oil + protein (%)	60.0	61.7	61.0	0.382	0.25
Glucosinolate (μmol/g)	10.7	13.9	12.6	0.786	0.65
Nitrogen (N%)	2.86	3.02	2.93	0.033	0.03
P _{total} /N	0.21	0.28	0.25	0.017	0.04
P _{inorganic} /N	0.016	0.021	0.018	0.011	0.0013

Table 5 Spearman rank (r_s) correlations among seed quality traits among the 31 hybrid varieties of experiment II

	P _{total}	P _{inorganic}	P _{organic}	Oil	Protein	Oil + prot	Glucosinolate
P _{inorganic}	0.46**						
P _{organic}	0.99**	0.42*					
Oil	− 0.28	− 0.22	− 0.23				
Protein	0.54**	0.30	0.56**	− 0.23			
Oil + protein	− 0.04	− 0.16	− 0.03	0.90**	0.14		
Glucosinolate	0.36*	0.04	0.40*	0.40*	0.57**	− 0.23	
P _{total} /N	0.96**	0.40*	0.97**	− 0.24	0.47**	− 0.04	0.32

*, **significant at $p = 0.05$
and $p = 0.01$, respectively

is P uptake and utilization efficient, which may be explained by good subsoil rooting of this winter annual crop (Barraclough 1989). The situation may be different with spring crops like rapeseed and others which develop a shallower root system.

The second field experiment also revealed large and significant effects for the genotype and the environment for all seed P fractions. However, genotype × environment interaction effects could not be tested, since only one bulk sample of the four replicates of the field experiment of the different environments was

Table 6 NIRS calibration for total seed P concentration (% of dry matter) of a genetic diverse set of seed samples of rapeseed

Trait	N	Population			Calibration		Cross-validation	
		Mean	SD	Range	SEC	RSQ	SECV	1-VR
P	629	0.75	0.13	0.48–1.07	0.04	0.89	0.05	0.83

SD standard deviation, SEC standard error of calibration, RSQ coefficient of determination, SECV standard error in cross-validation, 1-VR coefficient of determination in cross-validation

Fig. 1 Near-Infrared Reflection Spectroscopic (NIRS) calibration plot of total seed phosphorus concentration (%P)

available. Notably, heritability was high for inorganic seed P concentration but only moderate for organic and total seed P concentration. This suggests that the genotype $\times$ environment interaction was considerable for phytate portion which in turn affected the heritability of total P concentration. Non-significant differences for the portion of organic of total P among the genotypes can be explained by the low heritability and the narrow genetic variation of the adapted high yielding rapeseed hybrid material. The relatively high heritability for inorganic P concentration indicates that the applied extraction method was reproducible. In contrast to the present study, McKie and McCleary (2016) extracted the meal with hydrochloric acid in a much higher concentration and a longer incubation period (0.66 M, 3 h). Such a treatment may result in higher inorganic P concentrations. However, the 90% portion of organic P concentration found for all genotypes corresponds well to the above 90% reported by Lott et al. (2000). As discussed above, the high

portion of organic P at high soil P levels is in accordance with the results of Lickfett et al. (1999). A reduced phytate portion of total seed P concentration can only be expected at lower P soil levels. Since there was no effect of low soil P levels on seed yield and other seed quality traits in the first experiment and by Römer et al. (2017), the P fertilizer level could be reduced and thereby, the P availability for monogastric livestock could be enhanced and the phytate concentration in the manure reduced. Even though the soil P level of the six environments were comparatively high, a similar variation in total seed P concentration was observed among the 31 hybrid cultivars as for the 20 genotypes tested in the first experiment. This underlines the dependence of the seed P concentration on the genotype and opens the possibility to manipulate it through breeding.

Preliminary attempts to develop a fast and non-destructive method to determine phytate concentration in rapeseed seeds and meal were successful. De Boever et al. (1994) developed a NIRS calibration for total and phytate P concentration based on 81 oilseed meal samples. Also, Möllers et al. (1999) generated a NIRS calibration for phytate concentration based on spectra of 38 seed samples that were derived from a greenhouse experiment with low and high P fertilizer levels (Lickfett et al. 1999). This set of 38 samples was extended by including 122 preselected seed samples to give a calibration file of 160 rapeseed samples. The coefficient of determination between the reference method and the NIRS predicted phytate values was $R^2 = 0.97$ and the standard error in cross validation was 0.17 (Möllers et al. 1999). The NIRS calibration for total seed P concentration developed in the present study comprised a much larger germplasm set. The calibration resulted in a coefficient of determination and a standard error in cross validation of 0.83 and 0.05, respectively. This indicates that NIRS calibrations for total seed P concentration in intact seeds of

Fig. 2 Phosphorus and nitrogen concentration and ratio of soybean seeds, soybean and rapeseed meal (mean and standard deviations of $n = 3$ to 6 independent samples)

different seed qualities can be developed. Although the NIRS calibration is based on total seed P concentration, the close correlation between total and organic P concentration found in experiment II indicates that selection for reduced organic P concentration should be effective. Tahir et al. (2012) reported that NIRS was able to predict phytate P much better than total P in canola meal and other feed ingredients. This is to be expected because only organic molecules absorb light in the near-infrared region. The results of this study show positive correlations between seed total P concentration and protein concentration in all three

data sets. This suggests that NIRS calibrations for phytate concentration may be based to some extent on cross correlations. However, as shown above for experiment I, there was no significant effect of the soil P level on neither seed protein nor oil concentration. Beside oil and protein there is a relatively high fiber concentration in rapeseed and it would be interesting to learn about the effect on seed P concentration by increasing the seed protein concentration through genetically reducing seed fiber concentration (Dimov et al. 2012; Behnke et al. 2018).

The higher total P concentration of the rapeseed meal samples compared to the mean seed concentration of rapeseed hybrids can be explained by the P enrichment in the meal following oil extraction. Rapeseed has about 45% oil and 20% protein in the seeds (Dimov et al. 2012). Since soybean seeds have higher protein (37 to 41% for the three genotypes) and lower oil concentration (about 20%) than rapeseed, there is a much-reduced P enrichment in the soybean meal following oil extraction. Therefore, the total P concentration of rapeseed meal is almost two times that of soybean meal calculated per unit nitrogen (0.2 vs 0.09; Fig. 2). Similar ratios are obtained on seed basis. The lower organic P portion of the soybean meal compared to the soybean seeds and the rapeseed seed and meal samples awaits further confirmation and clarification. Feeding monogastric livestock with a larger portion of rapeseed meal hence may lead to undesirable higher phytate concentrations in their excrements.

In conclusion, the results of this study show that there is genetic variation for total seed P concentration among adapted line and hybrid rapeseed cultivars. The NIRS calibration for total seed P concentration can be used along with other routinely applied NIRS calibrations for other seed quality traits (e.g. oil, protein, glucosinolate, moisture, etc.) to select for lower seed P concentration in rapeseed. A moderate reduction of the phytate portion appears possible by reducing soil P level which is in line with the current policy. In addition, further genetic variation can be found when screening genetic resources, i.e. additional resynthesized rapeseed lines and diploid ancestor species genotypes. Recently, considerable progress has been achieved in reducing seed phytate concentration in rapeseed by analyzing seed derived from a TILLING population (Sashidhar et al. 2019) and from a CRISPR/Cas9 experiment (Sashidhar et al. 2020). Breeding of low phytate rapeseed is absolutely necessary to reduce the environmental impact and to make local rapeseed meal a prime source for feeding and the protein for food production. As stated in Schoumans et al. (2015), the management of P in Europe has to change because global reserves of P are running out. There are many options to reduce the P fertilizer to an acceptable level and to recycle P fertilizer from human and animal waste streams. Thereby, the European P cycle could be completely

closed and European water quality could be improved (Schoumans et al. 2015).

Acknowledgements We thank breeding companies for providing seed samples from field experiments, the Raiffeisen Warengenossenschaft Niedersachsen Mitte e.G. for soybean and rapeseed meal samples, Volker Hahn for soybean seed samples, and Susanne Koch and Dietrich Kaufmann for technical assistance.

Author contributions JE, JEW and CM carried out the research and analyzed the data. CM, JE and BS interpreted the data and wrote the manuscript.

Funding Open Access funding enabled and organized by Projekt DEAL.

Declarations

Conflict of interest The authors declare that there are no conflict of interest.

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