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INFLUENCE OF P-FERTILIZER ON PHYTIC ACID CONTENT IN SEEDS OF *BRASSICA NAPUS* L. AND DEVELOPMENT OF A NIRS CALIBRATION

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ABSTRACT

The main storage form of phosphorous in the seeds of Brassica napus is phytic acid. The reduction of phytic acid content in the meal is of interest, because of its role in binding phosphorous as well as other essential minerals which then are not available for monogastric animals. Although suitable analytical tests are available, little is known about the genetic variation of the phytic acid and total P content in the seeds of *Brassica napus* and the influence of P-fertilization. Therefore, a greenhouse experiment with the two winter rapeseed cultivars 'Lirajet' and 'Bristol' was performed. These two cultivars were grown under 4 different soil P levels, ranging from P-starvation to luxurious supply (0.6, 2.1, 3.5 and 11 mg P/100g soil). Seeds were harvested from the plants and the phytic acid content as well as the total P content were determined. The phytate-phosphorus content ranged from 0.05 to 0.69% in the seeds. The results from the phytic acid analysis obtained by a photometric assay and by HPLC were highly correlated (r=0.96) and were used independently to develop near-infrared reflectance spectroscopy (NIRS) calibrations. The developed NIRS calibrations were highly correlated (R^2=0.97) with the corresponding reference methods and are now used to screen *Brassica* germplasm for genotypes with a low phytic acid content in the seeds, which can then be used in breeding programmes.

KEYWORDS

near-infrared reflectance spectroscopy, phosphorous

INTRODUCTION

The high nutritional value of oilseed rape meal (*Brassica napus* L. var. oleifera) resulting from a high energy and protein content and a favourable amino acid composition is restricted by its content of glucosinolates, tannins, phenolic acids and phytic acid, which are referred to as anti-nutritive compounds (Matthäus et al. 1995a). Phytates, the salts of phytic acid, myoinositol-hexa*kis*phosphate (IP6), are the main storage of phosphorus in grains and seeds (Marschner 1995). Phytates are low digestible for monogasters, reduce the resorption af calcium, iron, magnesium, zinc and other trace elements and form complexes with basic amino acids (Shah et al. 1979, Atwal et al.1980, Marquard 1993). As a result, large amounts of phytate-P are transferred with the manure to the environment, where it may contribute to the eutrophication of soils and waters (Feil and Fossati 1997). Supplementing diets with phytase - as it is already practised in intensive livestock areas - and growing cultivars with low levels of phytic acid in their seeds might help to reduce this

problem (Feil and Fossati 1997). The content of phytic acid in rapeseed and its products is generally higher than in other oilseeds (Matthäus et al. 1995a). However, until now very little is known about the genotypical variation for and the influence of P level in the soil on the phytic acid content in seeds of oilseed rape. The present experiment was performed to analyse the effect of increasing P fertilizer levels on seed phytate content and to develop a NIRS calibration suitable for screening large sample numbers.

METHODS AND MATERIALS

Two winter oilseed rape cultivars (cv. Lirajet and cv. Bristol) which differ in phenological properties (seed yield, date and duration of flowering, maturity) were grown in a growth chamber at 4°C for 8 weeks. After vernalization, adhesive soil was rinsed off from the roots with demineralized water and the plants (2 foliage-leaves, EC 17) were transferred to the greenhouse and planted into PVC-tubes (40 cm length, 10 cm i.d., 1 plant per pot, 5 replicates), containing 4 kg of a 1:1-mixture of silty loam from the subsoil of a luvisol and quartz sand. The Calcium lactate acetate soluble P content (P_{CAL};Schüller 1969) of the soil was adjusted to 0.6 (P1), 1.9 (P2), 3.1 (P3) and 10.6 (P4) mg P 100 g^{-1} dry soil, resp., by addition of KH_2PO_4. The P-supply per pot ranged from 24, 74, 124 to 424 mg, representing a low to adequate level of available phosphorus for the plants (in Germany 4.5 -9 mg P 100 g^{-1} soil). All other nutrients (NKS) were added in suitable amounts and plants were watered regulary. At maturity, seeds were harvested. Total phosphorus was determined by the Molybdat-Vanadat-method (Scheffer and Pajenkamp 1952). Phytic acid was analyzed by HPLC as described by Matthäus et al. (1995b) and by a photometric test (Thies 1991).

RESULTS AND DISCUSSION

With increasing P supply mean seed yield for the 2 genotypes increased from 3.5 g over 9.9 and 10.2 to 11.9 g per plant (data not shown), indicating severe P starvation only at the lowest P level. The P content in the seeds increased from 0.24 to 0.72% and the means were significant different for the 2 genotypes (Tab. 1). The seed phytate-P (IP6-P) content increased tenfold from 0.06% to 0.6% as determined by HPLC analysis.

Tab. 1: P and phytate-P content of two oilseed rape cultivars at different P supply.

P supply [mg pot^{-1}]	P content of the seeds [%]			IP6-P content of the seeds [%]		
	cv. Bristol	cv. Lirajet	Mean	cv. Bristol	cv. Lirajet	Mean
24 (P1)	0.23	0.26	0.24a	0.05	0.07	0.06a
74 (P2)	0.30	0.39	0.35b	0.16	0.22	0.19b
124 (P3)	0.41	0.48	0.45c	0.29	0.38	0.34c
424 (P4)	0.63	0.81	0.72d	0.50	0.69	0.60d
Mean	0.39a	0.48b	0.44	0.25a	0.34b	0.28

Numbers in a column followed by different letters indicate significant differences at $P < 0.05$.

From the P fertilization experiment 38 seed samples were anlysed also by the photometric test. The photometric values were highly correlated with the HPLC results (r=0.96; Fig. 1), indicating that the photometric tests is specific for phytate and can be used in larger *Brassica* germplasm screenings. The regression formula developed from the data shown in Fig. 1 is y=-120,044+136,086x.

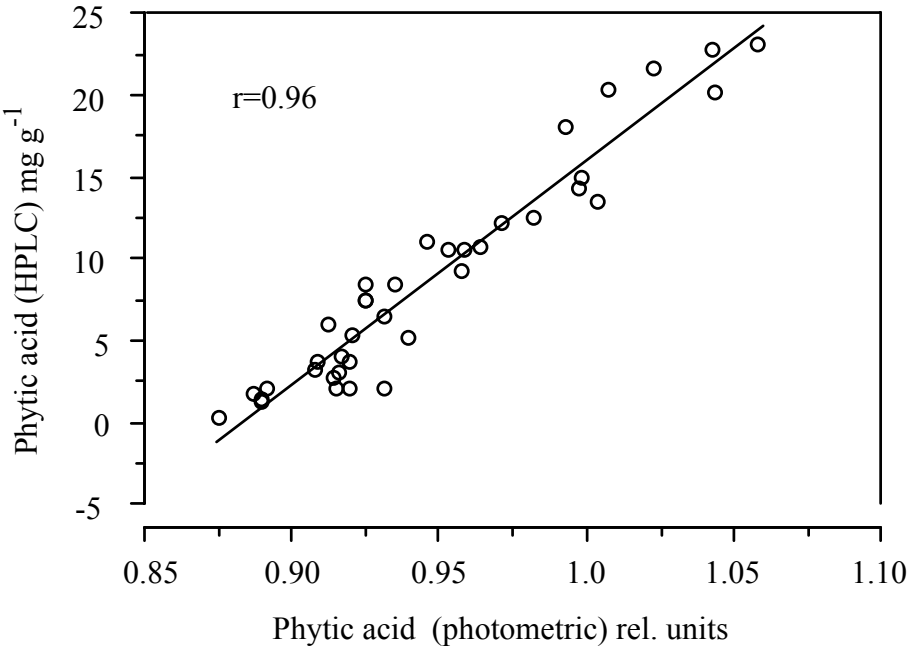

Fig. 1: Scatter plot of phytic acid content as determined by a photometric test and by HPLC in a set of 38 seed samples of *Brassica napus* plants grown at different phosphorous supply.

With the results obtained from the photometric test and the HPLC analysis two independent NIRS calibrations were developed (details not shown). The NIRS calibrations were highly correlated (R^2=0.97) with the corresponding reference methods. The results of the calibration using the photometric values are shown in Fig. 2.

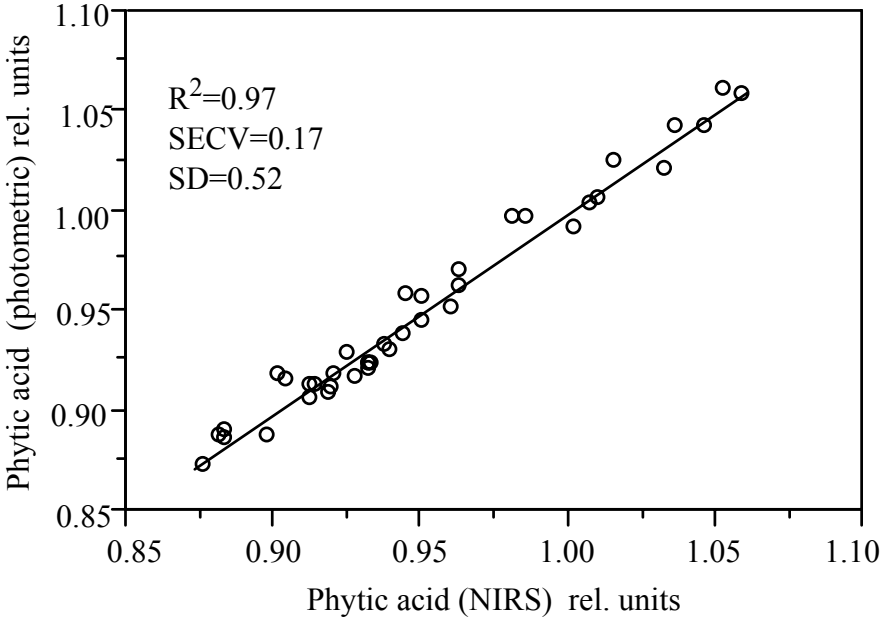

Fig. 2: Calibration plot for phytic acid, determined by a photometric test, in a set of 38 rapeseed samples. R^2=coefficient of multiple determination in calibration, SECV=standard error of cross validation, SD=standard deviation of the population.

The NIRS calibrations developed from the 38 seed samples of the P fertilization experiment were used to screen additional 544 represenative seed samples from the Göttinger *Brassica napus*

germplasm collection for additional variability for phytic acid content. 122 seed samples were selected and analysed by the photometric test and an expanded NIRS calibration was developed based on the total of 160 seed samples (Fig. 3). The possibility of using near infrared reflection spectroscopy to predict phytate-P in vegetable feedstuffs has also been described by De Boever et al. (1994).

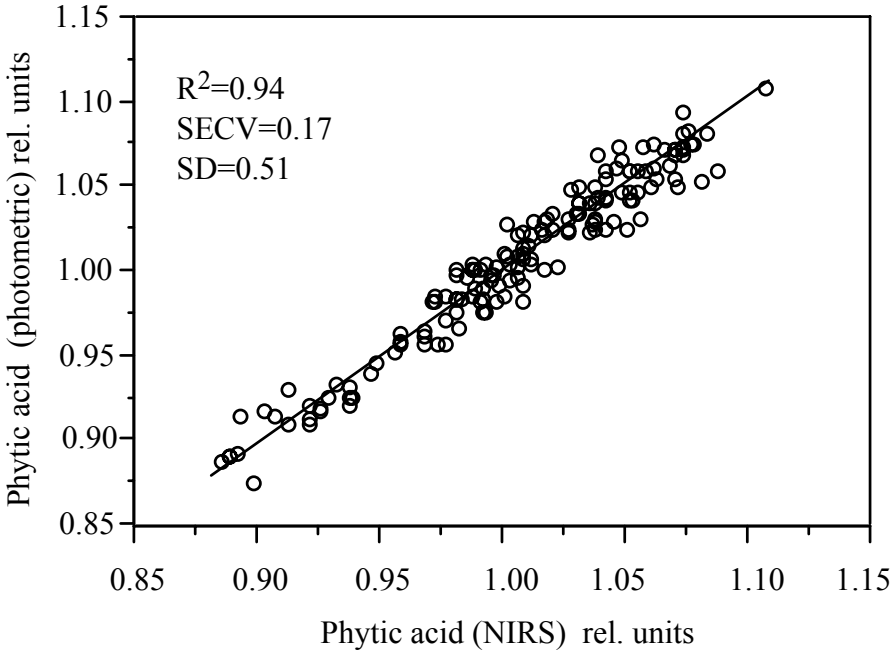

Fig. 3: Calibration plot for phytic acid, determined by a photometric method, in a set of 160 rapeseed seed samples. R^2=coefficient of multiple determination in calibration, SECV= standard error of cross validation, SD=standard deviation of the population.

The phytic acid content of the 122 selected seed samples ranged between 10-30 mg g^{-1}, thus showing a large variability for this trait. A variability from 20-40 mg g^{-1} was also reported by Matthäus et al. (1995a, and refs. therein). Among the seed samples screened no entry with phytate contents below 10 mg g-1 was found. However, the chances of finding genotypes with lower contents are reasonable, since phytic acid is a secondary compound, which is probably not required in large quantities in the seed to maintain vital functions. At the current P supply levels of most of our soils phytic acid as a P reservoir is not anymore necessary during seed germination. The results shown in Figs. 1-3 demonstrate that both the photometric test and the NIRS can be used in rapeseed breeding programmes for the determination of the seed phytic acid content. However, NIRS has clear advantages over the photometric test, as it is non-destructive and it can be simultaneously performed with NIRS analyses of other quality traits, e.g oil, protein, and glucosinolate content, as already routinely done in rapeseed breeding programmes.

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