Genetic variation, inheritance and development of NIRS-calibrations for sinapic acid esters in oilseed rape (*Brassica napus* L.)

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ABSTRACT

Sinapic acid esters (SAE) are important antinutritional compounds in oilseed rape (*Brassica napus* L.). Their high content of 1% in seeds is believed to limit use of oilseed rape meal as a source of high-quality protein for food production. The developed near infrared reflectance spectroscopy (NIRS) calibration equations for sinapin, sinapoylglucose and the total SAE content showed high R^2 in cross validation from 0.79-0.81, indicating that the calibrations are useful to estimate the contents of the SAE components in breeding material. In field experiments high heritabilities were found for sinapine (0.78), the total content of SAE (0.86) and for sinapoylglucose (0.95).

Key words: NIRS, sinapine, sinapic acid ester, GxE-Interactions

INTRODUCTION

SAE are important antinutritional compounds in oilseed rape. The concentration of these phenolic compounds is much higher than in other oilseeds, accounting for about 30 times the amount found in soybean. Because of their antinutritive nature, the high content of these compounds limits the use of oilseed rape meal as a source of high-quality protein for food production (Marquard 1993). The main objectives of this work were to study the potential of NIRS to determine the SAE composition and content in a large number of seed samples, the identification of material with reduced levels of SAE compounds, and the investigation of genotype x environment interactions.

MATERIALS AND METHODS

A collection of 184 rapeseed samples were scanned by a NIRS 6500 equipment using small ring cups. The samples included genotypically divergent winter rapeseed material (breeding lines, resynthezised rapeseed, genebank material and lines derived from a mutation experiment) cultivated at the experimental station Reinshof near Göttingen in 1997-2000. The scanned samples were analysed by HPLC for sinapoylglucose, sinapoylcholine (sinapine), and 8 additional not fully characterized compounds containing sinapic acid at IPB Halle (Milkowski *et al*. 2000). Preliminary NIRS-calibrations were developed and used to analyse 24.224 NIRS-spectra of seed samples harvested in the year 2001 from different locations. Most of the samples were provided by the companies NPZ, Hohenlieth, and DSV, Thüle. The spectra provided by the breeders were standardized to the NIRS equipment in Göttingen. Based on the variation in SAE content and composition, geographical origin and spectral divergence (global H-value of the WinISI 1.04 software) additional seed samples were selected and analysed by HPLC. A total of 549 samples were used to develop the final calibration equations for sinapine, sinapoylglucose, for the sum of the not fully characterized SAE and for the total content of SAE.

In a field experiment carried out at 2 locations over 2 years 70 lines were tested in double rows in two replications in a completely randomised block design. The tested lines consisted of 16 DH-lines derived from the cross DH Mansholts' x Express, 46 DH-lines of the cross DH Samourai x Sv0565, the cultivars Apex, Express, Mohican and the line DH Mansholts' and two times each the lines Sv0565 and DH Samourai. Plants were bagged during flowering to achieve self pollination.

RESULTS

The 549 selected seed samples for the development of NIRS calibration equations showed a large variation for SAE composition and content (Tab.1). There was a more than threefold variation for total SAE content. Some seed samples were either completely devoid of sinapoylglucose or of the sum of not fully characterized SAE (Tab. 1, Fig. 1). Sinapine is reported to be with 70% the predominant SAE component in rapeseed (Kolodziejczyk *et al*. 1999). This is in accordance with the 66% found in the present study. However, Fig. 1 shows a considerable variation in the SAE composition among the samples and the sinapine content ranges from about 25%-100% of total SAE content.

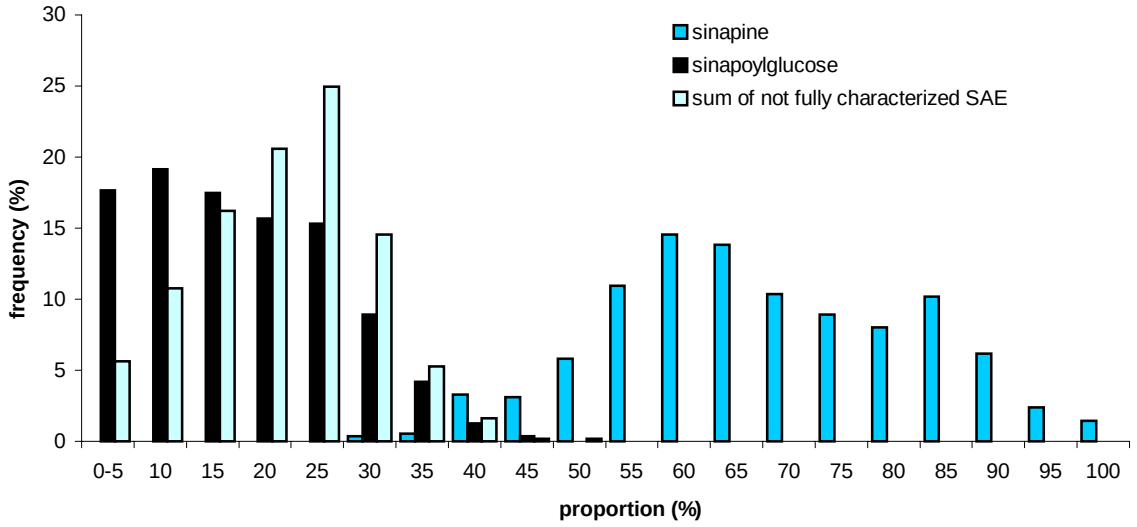

Fig. 1. Frequency distribution of the proportions of the SAE components (n=549)

The NIRS calibration equations developed on the basis of the 549 selected rapeseed samples showed medium to high R^2 in cross validation (R^2_{cv}, Tab. 1), indicating that the calibrations are useful to estimate the contents of the SAE components. The fractions of explained variance of cross-validation, R^2_{cv}, agreed well with R^2_c of calibration for all variables. The standard errors SEC and SECV are also low and prove the good precision of this calibration equations.

Tab. 1. Calibration and cross validation statistics for analysis of SAE content by NIRS in a set containing n= 549 samples of *Brassica napus*

				calibration		cross validation	
	mean	SD	range	R^2_c	SEC [a]	R^2_{cv}	SECV [b]
	---------- (mg g^{-1}) ----------				(mg g^{-1})		(mg g^{-1})
sinapine	7.46	1.42	3.2 – 11.7	0.86	0.54	0.81	0.62
sinapoylglucose	2.10	1.47	0.0 – 6.5	0.87	0.53	0.79	0.67
sum of not fully characterized SAE [1]	1.51	0.81	0.0 – 3.9	0.76	0.40	0.69	0.45
total SAE content [1]	7.89	1.73	2.7 – 13.1	0.85	0.64	0.80	0.77

[1] calculated as sinapic acid [a] SEC = standard error of calibration [b] SECV = standard error of cross validation

With the developed calibration equations additional 24.224 NIRS of seed samples were analysed. Tab. 2 shows the big range in total SAE content and composition in these samples.

Tab. 2. Variation of SAE content analysed by NIRS from 3 locations in 2001 (in mg g^{-1})

	NPZ (n=10399)		DSV (n=11276)		Göttingen (n=2549)		Express (Gö.) (cultivar, n=42)	
	range	mean	range	mean	range	mean	range	mean
sinapine	3.1 – 10.5	6.7	2.5 – 13.5	6.7	3.8 – 13.3	8.2	7.3 – 8.8	7.8
sinapoylglucose	1.2 – 5.4	3.1	0.0 – 5.6	3.0	0.0 – 5.8	2.4	1.8– 3.1	2.6
sum of not fully characterized SAE [1]	0.4 – 3.9	1.4	0.3 – 4.0	1.4	0.4 – 4.2	1.5	1.5 – 2.0	1.8
total SAE content [1]	4.0 – 12.6	8.1	4.3 – 14.3	7.9	4.1 – 13.8	8.9	7.6 – 8.9	8.4

[1] calculated as sinapic acid n= number of seed samples

The field experiments showed highly significant differences for the lines (L) as well as highly significant interactions with the environments (LxE). The relatively large variance components for L resulted in medium to high heritabilities for sinapine (h^2= 0.78), the total content of SAE (h^2= 0.86) and for sinapoylglucose (h^2= 0.95) (Tab. 3).

Tab. 3. Variance components of total SAE content and composition

	range	mean	variance components			h^2
	-------(mg g^{-1})-------		L	L x E	error	
sinapine	2.3 – 9.5	6.69	0.2378**	0.1111**	0.3021	0.78
sinapoylglucose	0.0 – 4.6	2.41	0.5558**	0.0579**	0.1098	0.95
sum of not fully characterized SAE [1]	0.0 – 3.0	1.49	0.2473**	0.0151**	0.0522	0.94
total SAE content [1]	4.4 – 11.7	7.83	1.0120**	0.0611**	0.2254	0.86

*p = 0,05 **p = 0,01 [1] calculated as sinapic acid
L = genotype E = environment L x E = genotype x environment h^2 = heritability

DISCUSSION AND CONCLUSION

The results show a large variation for all SAE compounds in rapeseed germplasm, which can sufficiently well be estimated by NIRS calibrations. The developed NIRS calibrations, together with the large variation and the high heritabilities are a good basis for breeding for a low SAE content.

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REFERENCES

Kolodziejczyk, P., W. Xiaoyan, M. Marianchuk, L. Wanli, R. Amarowicz, 1999: Phenolics In Rapeseed: Capillary Electrophoresis as a novel analytical method for detection of sinapine, sinapic acid esters and ferulates. 10th International Rapeseed Congress, Canberra

Marquard, R., 1993: Zuchtziele bei Raps im Hinblick auf die Qualität von Rapsschrot. Fat. Sci. Technol., 95. Jahrgang, 557-560

Milkowski, C., A. Baumert, D. Strack, 2000: Cloning and heterologous expression of a rape cDNA encoding UDP-glucose:sinapate glucosyltransferase. Planta 211, 883-886

Velasco, L., B. Matthäus, C. Möllers, 1998: Nondestructive Assessment of Sinapic Acid Esters in *Brassica* Species: 1. Analysis by Near Infrared Reflectance Spectroskopie. Crop. Science, Vol.38, No.6