Influence of pollen genotype on rapeseed quality – Analyses of single seeds by near-infrared reflectance spectroscopy (NIRS)

Nang Hseng Hom, Andreas Girke, Christian Möllers and Heiko C. Becker
Institute of Agronomy and Plant Breeding, Von-Siebold-Str. 8, 37075 Göttingen, Germany
E-mail: nhom@gwdg.de; cmoelle2@gwdg.de; hbecker1@gwdg.de

ABSTRACT

It is often assumed, that major rapeseed quality traits like oil, protein and glucosinolate content are only determined by the genotype of the mother plant and not at all influenced by the genotype of the pollinator. However, knowledge on the pollinator influence is very limited so far. If the pollinator genotype is of influence on the individual seed quality, it would be meaningful to select already among individual F_2seeds, as traditionally done for fatty acids by the half-seed method. A non-destructive method for the analysis of glucosinolate, oil, and protein content in single seeds by Near infrared reflectance spectroscopy (NIRS) was developed.

Key words: Single seed selection - Near infrared reflectance spectroscopy (NIRS) - protein content - glucosinolate content – seed quality

INTRODUCTION

In recent years, rapeseed quality is of increasing interest for various food and non-food uses. Different quality characteristics are required for the use of the seed for human consumption, feeding livestock, or as sources for the many non-edible products, such as detergents, lubricants, cosmetics, hydraulic oils, or biodiesel (Shahidi, 1990; Kimber and McGregor, 1995). To fulfil all future requirements, improvement of the seed quality is one of the most important objectives in rapeseed breeding (Becker et al., 1999).

It is often assumed, that most rapeseed quality traits like oil, protein and glucosinolate content are only determined by the genotype of the mother plant and not influenced by the genotype of the pollinator, whereas the fatty acid composition is well-known to be determined by the genotype of the embryo. However, experimental knowledge on the pollen influence on seed quality traits is very limited. Preliminary observations (Möllers, unpublished) indicate, that at least in cases where the mother genotype is very low in glucosinolate content, seeds from pollination with a high glucosinolate parent show an increased content when compared with the mother plant after self-pollination (Table 1).

Table 1. Influence of pollen genotype on glucosinolate content (Möllers, unpublished)

Genotype	Glucosinolate (µmol/g)		
	Total	Progoitrin	Gluconapin
Ha 699 (Mother)	6.8	2.6	1.1
Ha 699 x Mansholts (F_1 seed)	26.2	15.9	3.2
Manholts (Father)	111.4	66.7	27.7

If seed quality is determined by the embryo, the F_2 seeds grown on an F_1 plant already segregate and selection between individual seeds is possible like traditionally done for fatty acids by the half-seed method. For a non-destructive analysis of oil content and fatty acid composition of individual seeds, the near-infrared reflectance spectroscopy (NIRS) has successfully been applied (Velasco et al., 1999). The objectives of this project is to develop calibrations for the analysis of oil, protein and glucosinolate content of single seeds by NIRS and to select among F_2 single seeds by NIRS.

MATERIALS AND METHODS

1. Development of the calibration equations

Three calibration equations for oil, protein and glucosinolate content were developed. The samples for the calibration were selected from different years, locations and genotypes including large ranges for specific traits. For NIRS analyses, a special adapter was used for intact single seeds. This adapter was made of 4mm thick teflon with 38 mm diameter and a 3 mm central hole. This adapter was inserted into the standard ring cup and the single seeds were put into the central hole of it and covered with card board. Then they were scanned by NIRS monochromator model 6500, and their spectra collected between 400-2500nm., registering the absorbance values (log1/R) at 2nm intervals for each sample. After NIRS scanning, the same seeds were analysed by reference methods: repeated extraction with a solution Iso-octan: Iso-propanol 9:1 for oil content (% of seed weight), Dumas combustion for protein (% of seed weight) and High-performance liquid chromatography (HPLC) for GSL (µmol/g), respectively. Calibration equations were developed under WinISI II Project Manager v 1.02a, with spectral information from 1100-2500 nm and using modified partial least squares (MPLS).

2. Selection among F_2 seeds

After the calibration equations were developed, they were used to predict the oil, protein and glucosinolate content of five segregating F_2 populations derived from three crosses between parents with high and low oil content and two crosses between parents with high and low glucosinolate content. Five F_1 plants per cross were analysed with 200 F_2 seeds each.

RESULTS AND DISCUSSION

A close relationship between NIRS and reference values were found in all three traits. The NIRS statistics of the developed calibrations for oil (% of seed weight), protein (% of seed weight) and glucosinolate (µmol/g) content of single seeds are presented in Table 2. It can be demonstrated that NIRS is a suitable method for a non-destructive analysis of important quality traits in single seeds.

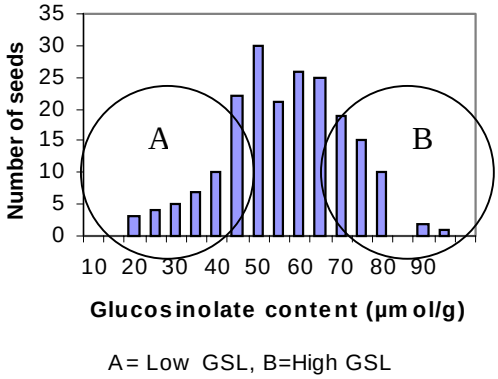
Table 2. NIRS statistics of the calibrations for oil, protein and GSL in single seeds

	Calibration						Cross-validation	
	n	Mean	Range	SD	SEC	RSQ	SECV	1-VR
Oil content	206	45.7	26.2- 61.1	6.5	0.98	0.98	1.14	0.97
Protein content	157	20.9	14.7- 32.1	3.6	0.38	0.99	0.74	0.96
Glucosinolate	113	32.6	0.6-118.9	27.2	5.15	0.96	9.58	0.88

SD = standard deviation, SEC = standard error of calibration, RSQ =coefficient of determination, SECV =standard error of cross-validation, 1-VR = percentage of variation in reference values explained by NIRS.

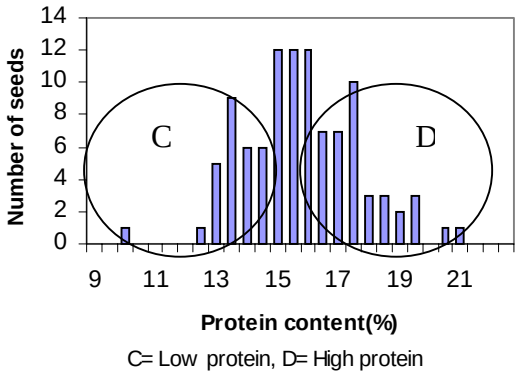
These NIRS calibrations were used to analyse single F_2 seeds harvested on individual F_1 plants. Based on these results a selection experiment was initiated. The principle is illustrated in Fig. 1 and Fig. 2 for the seeds of one F_1 plant. In total 200 F_2 seeds of this plant were analysed for glucosinolate content, and the 40 seeds with lowest and highest glucosinolate content, respectively, were selected (Fig. 1). The remaining 120 seeds were then analysed for protein content, and again the 40 seeds with lowest and highest protein content, respectively, were selected (Fig. 2). The original aim was to select for oil content, but at the time the experiment was started, no reliable NIRS calibration for oil content was available. Therefore selection was performed for protein content instead, because in segregating populations, oil and protein content generally show a close negative correlation.

However, this selection among individual F_2 seeds is based on three hypotheses: (1) the seed quality is determined by the embryo, that means by the genotype of the F_2 seed, not by the genotype of the F_1 mother plant, (2) the variation in seed quality among F_2 seeds has a sufficiently high heritability, that means environmental factors influencing differences among individual seeds on the same mother plant do not mask all genetic differences, and (3) the seed quality can be reliably measured by NIRS.



A= Low GSL, B=High GSL

Fig. 1. The distribution of glucosinolate content among 200 single seeds/plant and selected area for low and high glucosinolate content (µmol/g)



C= Low protein, D= High protein

Fig. 2. The distribution of protein content among 120 single seeds and number of selected seeds for low and high protein content (%)

To test these hypotheses, the selected material was sown in replicated field trials at three locations in Germany in autumn 2002. Response to selection measured after harvest 2003 will show in how far a selection by NIRS among single seeds is an efficient way to improve rapeseed quality.

ACKNOWLEDGEMENTS

The authors thank Uwe Ammermann, Hans-Otto Heise, Gabi Kolle, Nicole Ritgen-Homayounfar, and Sonja Yaman for their excellent technical assistance in performing reference analyses, developing the plant material and skilful manufacturing the NIRS teflon adapters. This project is financially supported by the “Gesellschaft zur Förderung der privaten deutschen Pflanzenzüchtung“ (GFP) and the German Academic Exchange Service (DAAD). The financial support of the UFOP (Union for the Promotion of Oil and Protein Plants) for attendance at the congress is gratefully acknowledged.

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