Genetic variation of glucosinolates in young leaves of winter rapeseed (*Brassica napus* L.)

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Abstract

Increased levels of glucosinolates (GSL) in greenmatter of rapeseed (*Brassica napus* L.) and other *Brassica* species are believed to confer resistance to certain pests and diseases. To study the genetic variation of leaf GSL and their genetic correlation with undesired seed GSL, 140 winter rapeseed accessions with a wide range of seed GSL concentrations were grown in the field. Leaf samples were taken in late fall, dried and analyzed by High Performance Liquid Chromatography (HPLC). Seed GSL ranged from 2.1 to 139.5 µmol/g drymatter (DM) and leaf GSL ranged from 2.4 to 53.7 µmol/g DM with a correlation of r = 0.58 ($p \leqslant 0.01$). This indicates that there is sufficient genetic variation to select for higher levels of leaf GSL and that to a limited extent this can be done without increasing the undesired seed GSL. Leaf samples were also scanned with Near Infrared Reflectance Spectroscopy (NIRS). A NIRS calibration was developed with the recorded spectra that is suitable to conduct efficient leaf GSL analysis by NIRS for total GSL concentration (1-VR = 0.86) as well as for some individual GSL. For confirmation, a subset of 40 most informative accessions are currently evaluated in field and greenhouse experiments.

Introduction

In rapeseed, much research has been conducted on the genetics of seed GSL. Eventually, this research led to the development of rapeseed cultivars with low seed GSL concentration that drastically increased the economic value of this crop. Little focus, however, has been put on the genetics of GSL concentration in vegetative tissue of rapeseed. Since increased levels of greenmatter GSL are discussed to confer pest and disease resistance (Mithen, 1992), it should be desirable to breed for increased GSL concentration of greenmatter in rapeseed. However, some studies indicate that greenmatter GSL and seed GSL are correlated (Jürges, 1982; Schilling and Friedt, 1991). Therefore, it is not clear how feasible it is to select for increased GSL levels in the greenmatter of the plant while maintaining low GSL quality in the seed. This mainly depends on how strong the correlation is and if it can be overcome.

To study the genetic variation of leaf GSL in *B. napus*, 140 winter rapeseed accessions with a wide range of seed GSL concentration were grown in the field and leaf samples were analyzed with HPLC and NIRS. Our objectives were (i) to estimate the variation for leaf GSL in rapeseed, (ii) to estimate the correlation between leaf and seed GSL, and (iii) to develop a NIRS calibration for leaf GSL.

Material and Methods

One hundred and forty winter rapeseed accessions with a wide range in seed GSL (based on NIRS) were sown on 26 August 2005 at the University of Göttingen field station as non replicated observation plots. Seeds were planted in 2.5 m long and 0.6 m wide plots with a row spacing of 0.3 m and a planting density of 27 seeds m^{-2}. Leaf samples were taken on 21 November 2005. In each plot, young leaves of about 10 cm by 15 cm without yellowing or signs of damage were sampled from ten plants, put on ice for transport and dried for one day at 60° according to Zobelt and Marquard (1988). Dried samples were ground and the spectra of the leaf meal were recorded with NIRS (Model 6500, Foss NIR Systems, Inc., Silver Springs, MD) at a wavelength of 400-2500 nm. Samples were then analyzed with HPLC (Gynkotek, Munich) modified after Thies (1979) with glucotropaeolin as internal standard. NIRS calibrations were developed with Modified Partial Least Squares followed by cross validation using WinISI II Project Manager (v. 1.04).

Results

Seed GSL for the 140 rapeseed accessions determined by NIRS ranged from 2.1 to 139.5 µmol/g drymatter (DM) while leaf GSL determined by HPLC ranged from 2.4 to 53.7 µmol/g DM. The correlation between seed and leaf GSL was r = 0.58 and significant at $\alpha = 0.01$ (see Fig. 1). The most prevalent individual GSL was gluco*brassica*napin (GBN) ranging from 0.2 to 29.5 µmol/g DM followed by progoitrin (PRO) ranging from 0.1 to 26.0 µmol/g DM. The sum of alkenyl GSL ranged from 0.7 to 46.0 µmol/g DM and the sum of indole GSL ranged from 0.2 to 6.2 µmol/g DM. The concentration for the phenyl GSL nasturtiin (NAS) ranged from 0.1 to 5.5 µmol/g DM.

The coefficient of determination for the NIRS calibration of leaf GSL was relatively high with R2 = 0.90 for total GSL concentration, R2 = 0.93 for the group of alkenyl GSL, R2 = 0.82 for the group of indole GSL, and a considerable variation of R2 for each individual GSL (see Tab. 1). This could be validated by also high coefficients of determination for cross validation (1-VR) except for indole GSL.

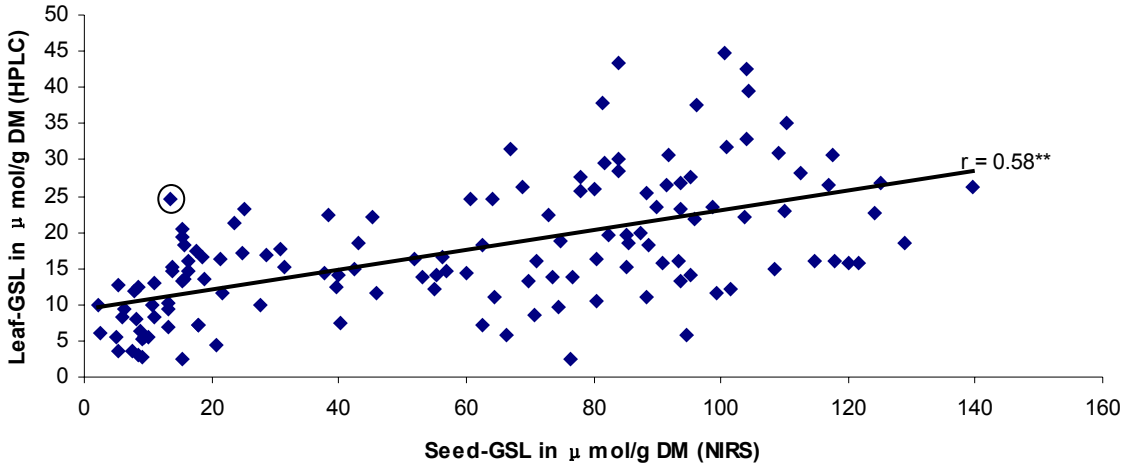

Figure 1: Correlation between leaf and seed glucosinolates (GSL) of 140 winter rapeseed accessions grown in non replicated observation plots. Leafs were sampled 13 weeks after sowing and analyzed by High Performance Liquid Chromatography (HPLC). Seeds were analyzed with Near Infrared Spectroscopy (NIRS). The observation for the cultivar 'Bristol' discussed in the text is marked with a circle.

Table 1: Near Infrared Spectroscopy (NIRS) calibration results for GSL dried leaf samples of 134 winter rapeseed accessions grown in the field. Leafs were sampled 13 weeks after sowing and analyzed by High Performance Liquid Chromatography (HPLC). Listed are for each GSL the sample size (N), mean in µmol/g drymatter (DM), standard deviation (SD) in µmol/g DM, minimum (Min) and maximum (Max) in µmol/g DM, standard error of calibration (SEC), R2 of calibration (RSQ), standard error of cross validation (SECV), and R2 of cross validation (1-VR).

Constituents[a]	N	Mean[b]	SD[b]	Min[b]	Max[b]	SEC	RSQ	SECV	1-VR
PRO	133	4.6	3.9	0.1	26.0	1.16	0.82	1.41	0.74
SIN	128	0.1	0.2	0.0	1.0	0.07	0.22	0.08	0.09
GNL	127	0.3	0.3	0.0	2.5	0.12	0.12	0.12	0.06
GNA	132	2.3	1.7	0.0	10.8	0.88	0.47	1.02	0.30
GBN	133	7.5	5.0	0.2	29.5	1.17	0.93	1.66	0.86
Alkenyl GSL	133	14.7	9.1	0.7	46.0	2.15	0.93	2.86	0.88
GBC	137	1.2	0.8	0.1	4.8	0.36	0.71	0.60	0.22
4ME	129	0.4	0.3	0.0	2.3	0.22	0.08	0.23	0.02
4OH	130	0.7	0.5	0.0	3.4	0.19	0.70	0.24	0.50
Indole GSL	138	2.4	1.0	0.2	6.2	0.41	0.82	0.71	0.48
NAS	130	1.1	0.7	0.1	5.5	0.41	0.08	0.42	0.04
Total GSL	134	18.1	9.5	1.9	49.7	2.78	0.90	3.28	0.86

[a] PRO = Progoitrin, SIN = Sinigrin, GNL = Gluconapoleiferin, GNA = Gluconapin, GBN = Gluco*brassica*napin, GBC = Glucobrassicin, 4ME = 4-Methoxyglucobrassicin, 4OH = 4-Hydroxyglucobrassicin, NAS = Nasturtiin

Discussion

We observed a considerable variation for total leaf GSL among the 140 winter rapeseed accessions ranging from as low as 2.4 µmol/g DM to beyond 50 µmol/g DM. Looking just at the accessions with lower seed GSL, our observed variation is similar to that of Schilling and Friedt (1991), who reported leaf GSL concentration from about 5 to 24 µmol/g DM in 32 winter rapeseed F_1-hybrids not exceeding total seed GSL concentration of 32 µmol/g DM. Jürges (1982) reported less variation for leaf GSL ranging from 1.0 to 15.5 µmol/g DM for 18 winter rapeseed cultivars ranging from 0.7 to 84.4 µmol/g DM in seed GSL concentration. However, leaves in this experiment were harvested just before flowering, while we as well as Schilling and Friedt (1991) sampled during late fall / early winter. Although our experiment was not replicated and results need to be confirmed, it suggests that there is sufficient variation in winter rapeseed to select for increased levels of leaf GSL.

A clear correlation was observed between leaf and seed GSL. This correlation was medium with r = 0.58 ($p \leqslant 0.01$) indicating that to some extent it should be possible to select for increased levels of leaf GSL without increasing undesired seed GSL. An interesting accession is the cultivar 'Bristol' who sticks out among accessions with less than 20 µmol/g DM seed GSL; with a leaf GSL concentration of 24.6 µmol/g DM, it exceeds even some of the accessions with more than 120 µmol/g DM seed GSL concentration (Fig. 1). Still, this correlation is low compared to r = 0.76 reported by Jürges (1982) and r = 0.85 reported by Schilling and Friedt (1991) and needs to be confirmed in replicated experiments.

We were able to predict total GSL concentration in leaves by NIRS as indicated by a high 1-VR from the calibration and by a low standard error of cross validation (SECV) compared to the standard deviation (SD) (Table 1). The major individual

GSL PRO and GBN could also be predicted as well as the sum of alkenyl GSL, while 4-methoxyglucobrassicin (4OH) and the sum of indole GSL could be predicted only with medium precision. The remaining GSL that were detected by HPLC seemed to be in a too low concentration in order to be predicted by NIRS. Although the NIRS calibration seems suitable to predict the major GSL in leaf meal, it needs to be extended for plant material grown in different environments.

Prospects

Forty three most informative accessions were selected from our experiment and are currently being investigated in replicated field and greenhouse experiments to confirm the above reported results. In addition a quantitative trait locus (QTL) study is being conducted in a segregating doubled haploid population of winter rapeseed in order to map QTL for GSL concentration in various vegetative plant parts. Progress on this will be presented and discussed in the context of cultivar development.

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