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Glucosinolate profiles of disomic rapeseed-radish chromosome addition lines

Glucosinolatprofile von disomen Raps-Rettich Additionslinien

Abstract

The glucosinolate (GSL) content and -profile of a complete series of nine disomic rapeseed-radish chromosome addition lines ($2n = 38 + 2$, *a* to *i*) was estimated and compared to that of radish as donor and rapeseed as recipient of the alien chromosomes. Modification of identical genetic rapeseed background by single radish chromosomes allows the assignment of GSL biosynthesis steps to individual radish chromosomes. Addition line *a* was found to have a five times higher aliphatic GSL content than the rapeseed parent. Radish-specific GSLs, glucoraphenin and glucoerysolin, were synthesized exclusively in addition line *g*. Addition line *i* produced sinigrin, not detectable in both parents. The variations in GSL content are not correlated with resistance against beet cyst nematode (*Heterodera schachtii* Schmidt).

Key words: Chromosome addition, rapeseed, oil radish, glucosinolate profile, *Heterodera schachtii*, resistance

Zusammenfassung

Glucosinolatgehalte und Glucosinolatmuster einer kompletten Serie von neun disomen Raps-Rettich Chromosomenadditionen ($2n = 38 + 2$, *a* bis *i*) wurden bestimmt und mit denen des Chromosomenspenders Rettich und des Chromosomenempfängers Raps verglichen. Der Effekt einzelner Rettichchromosomen im identischen Raps-Hintergrund erlaubte die chromosomale Zuordnung von Schritten der Glucosinolat-Biosynthese. Die Addi-

tionslinie *a* wies den fünffachen Glucosinolatgehalt auf wie der Rapselter. Die rettichspezifischen Glucosinolate Glucoraphenin und Glucoerysolin wurden ausschließlich in der Additionslinie *g* nachgewiesen. In der Additionslinie *i* wurde außerdem Sinigrin nachgewiesen, welches in keinem der Eltern gebildet wird. Der Glucosinolatgehalt ist nicht mit der Resistenz gegenüber dem Rübenzystennematoden (*Heterodera schachtii* Schmidt) korreliert.

Stichwörter: Additionslinien, Raps, Rettich, Glucosinolatgehalt, *Heterodera schachtii*, Resistenz

Introduction

Glucosinolates (GSLs) are secondary plant metabolites found in sixteen families of dicotyledonous plants, especially in the family Brassicaceae. The myrosinase-glucosinolate system is known to be a very effective defense system in higher plants. In uninjured plant cells myrosinase and GSLs are kept separated from one another. Under conditions of tissue damage, myrosinase and its substrates, the GSLs, come together, producing biologically highly active thiocyanates, isothiocyanates, nitriles and epinitriles, which have antimicrobial effects and influence insect behaviour (CHEW, 1988; GIAMOUSTARIS and MITHEN, 1995). The effect of degradation products of GSLs on reduction of soil borne pests is used also for the biofumigation technique.

Furthermore, GSLs have an important impact on the usability of plant products as food and feed. Rapeseed (*Brassica napus* L.; genome AACC, $2n = 38$) is the third

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leading source of both vegetable oil and oil extraction meal worldwide. The dramatic increase of cultivation area started some 30 years ago with the release of the first double-zero or canola quality varieties (< 2% erucic acid and < 18 μmol total GSL/g dry matter). By introgression of recessive genes from the Polish cultivar 'Bronowski' into high-yielding erucic acid-free material, the seed aliphatic GSL content was reduced from above 100 $\mu\text{mol/g}$ to less than 18 $\mu\text{mol/g}$. The decreased seed GSL content was entirely due to a reduction of aliphatic GSLs, derived from methionine, whereas the levels of indolic GSLs, derived from tryptophan, remained approximately constant (TOROSER et al., 1995).

Because radish (*Raphanus sativus* L.: genome RR, $2n = 18$) is a potential donor of several interesting traits for *Brassica* breeding (resistance to clubroot, *Turnip Mosaic Virus*, nematodes) there is a long history of experiments to hybridize radish and *Brassica* species starting with KARPECHENKO (1924). Hybrids between rapeseed and radish were realized by protoplast fusion (LELIVELT and KRENS, 1992) and sexual hybridization (THIERFELDER, 1994) to transfer resistance against the beet cyst nematode (BCN), *Heterodera schachtii* Schmidt, into rapeseed. The BCN is a worldwide important pathogen in sugar beet cropping areas. In rapeseed no resistance to BCN has been found (HARREWIJN, 1987; LELIVELT, 1995; FATEMY and ABOOTORABI, 2002). So rapeseed as a tolerant good host for BCN could not be included in rotations of dense sugar beet cropping. For biological control BCN-resistant varieties of white mustard (*Sinapis alba* L.) or oil radish (*Raphanus sativus* L. ssp. *oleiferus* DC.) are used as intercrops. These crops stimulate the hatching of BCN L₂ larvae from eggs and after penetration of the larvae into the root they inhibit further nematode development. According to WYSS et al. (1984) the BCN-resistance in oil radish results from degradation of the nematode feeding sites, so that the juveniles suffer from a lack of nutrients and the male/female nematode ratio increases (MÜLLER, 1985; LELIVELT and HOOGENDOORN, 1993). In this way cultivation of trap crops on severely infected soils can reduce the nematode population density significantly (NICOLAY and SIKORA, 1989).

For the transfer of resistance against BCN a *Raphano brassica* interspecific hybridization program was started by CLAUSS (1978) and continued by PETERKA et al. (2004). Multiple monosomic rapeseed-radish chromosome additions were developed and described using molecular and cytogenetic markers. BCN-resistance tests in this material identified radish chromosome *d* as the carrier of nematode resistance. A resistance gene *Hs1^{Rph}* was mapped on chromosome *d* by QTL analysis (BUDAHN et al., 2009). Investigations in progenies of monosomic rapeseed-radish additions segregating for this chromosome showed no correlation of BCN-resistance with the GSL content in the roots (PETERKA et al., 2004), whereas other authors suspected aliphatic GSLs to have a nematocidal activity (LAZZERI et al., 1993; SUMBAYAK, 1997).

In contrast to modern rapeseed varieties, oil radish has a high content of erucic acid and aliphatic GSLs. Genetic

studies of glucosinolate synthesis have been done in *Arabidopsis thaliana*, *Brassica oleracea* and *Brassica napus*, but never in *Raphanus*. BUDAHN et al. (2008) described the development of a complete set (*a* to *i*) of nine disomic rapeseed-radish addition lines ($2n = 38 + 2$). Using this material the analysis of the effects of individual radish chromosomes on identical genetic background of rapeseed should allow chromosomal localization of genes for GSL biosynthesis. Common action of genes controlling specific steps of biosynthesis from high-GSL radish with those of the double-zero rapeseed in the addition lines should give insight into their interaction. This allows the prediction of effects on GSLs after successful transfer of genetic material from radish to rapeseed. A specific goal of this investigation was to verify the hypothesis that BCN-resistance could be transferred without affecting GSL profile of double-zero rapeseed.

Material and methods

Plant material

Seeds were used from the complete set of nine disomic rapeseed-radish addition lines, *a* to *i*, the double-zero winter rapeseed variety 'Madora', as recipient, the tetraploid oil seed radish A24 (strain 2544) and the tetraploid fodder radish A107 (strain 101/77), both parts of the pedigree of the addition lines as chromosome donors (PETERKA et al., 2004).

Detection of glucosinolates

GSLs in seeds were determined using the methods described by SCHÜTZE et al. (2004). Ten seeds of each line were analyzed by single grain analysis. The seeds were not defatted (Tab. 1).

Results

Total glucosinolate content

The chromosome-recipient rapeseed 'Madora' as a double-zero variety had a total GSL content in the seeds of 21.2 $\mu\text{mol/g}$. The chromosome-donor radish lines A24 and A107 exhibited high levels of 178.5 and 130.2 $\mu\text{mol/g}$, respectively. The average of total GSLs for the complete set of addition lines was 28.5 $\mu\text{mol/g}$. The mean of individual lines varied between 13.1 and 70.7 $\mu\text{mol/g}$ (Fig. 1). This variation was solely associated with the differences in the concentration of aliphatic GSLs, whereas indolic GSL content showed nearly the same absolute level throughout all parental and addition lines. In consequence the ratio of indolic to aliphatic GSLs differed considerably in the material. Rapeseed had an indolic GSL percentage of 44.4%, the two radish lines 5.2 and 6.7%, respectively, and the addition lines varied between 10.3% and 49.7%. Because of these pronounced differences of variation, the two GSL classes will be considered separately.

Tab. 1. Seed glucosinolates analyzed

Trivial name	Side chain	Class
sinigrin	2-propenyl	aliphatic
progoitrin	2-hydroxy-3-butenyl	aliphatic
gluconapin	3-butenyl	aliphatic
glucoallyssin	5-methylsufinylpentyl	aliphatic
glucobrassicinapin	4-pentenyl	aliphatic
glucoraphanin	4-methylsufinylbutyl	aliphatic
glucoraphasatin	4-methylthio-3-butenyl	aliphatic
glucoraphenin	4-methylsufinyl-3-butenyl	aliphatic
glucoerysolin	4-methylsulfonylbutyl	aliphatic
glucobrassicin	3-indolylmethyl	indolic
neoglucobrassicin	1-methoxy-3-indolylmethyl	indolic
4-methoxyglucobrassicin	4-methoxy-3-indolylmethyl	indolic
4-hydroxyglucobrassicin	4-OH-3-indolylmethyl	indolic

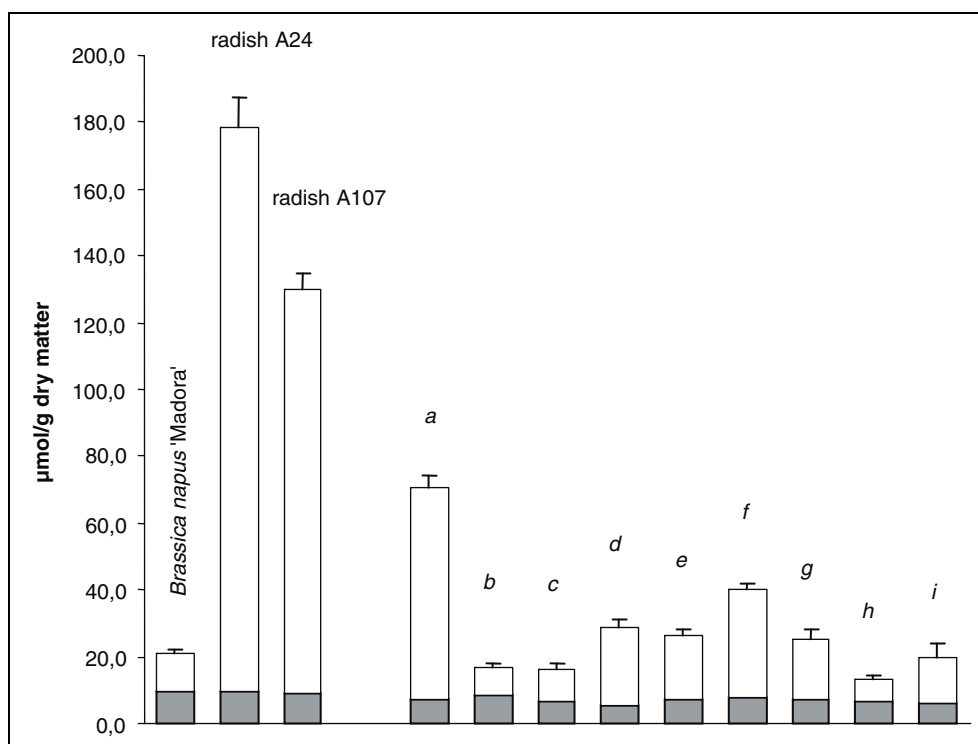


Fig. 1. Content of total seed glucosinolates (GSLs) of chromosome donors radish A24 and A107, chromosome recipient *Brassica napus* 'Madora' and the disomic addition lines a to i (indicated by corresponding letter). Error bars indicate standard error of the mean of total GSL-levels. $n=10$ for all genotypes. Grey bar: indolic GSLs; open bar: aliphatic GSLs.

Aliphatic glucosinolates

Most rapeseed-radish addition lines showed significant differences in the aliphatic GSL content compared to rapeseed 'Madora' (Tab. 2). Five chromosomes, a, d, e, f, g, increased the aliphatic GSL content significantly by 6.4 $\mu\text{mol/g}$ up to 51.6 $\mu\text{mol/g}$. Two lines, additions b and h, had significantly lower contents of aliphatic GSLs than 'Madora', which is already a double-zero variety. The highest content of 63.4 $\mu\text{mol/g}$ aliphatic GSL was observed in addition line a. Its total GSL content of 70.7 $\mu\text{mol/g}$ is in the range of a traditional rapeseed variety. Addition line d, which is resistant to the beet cyst

nematode, has a doubled aliphatic GSL content compared to 'Madora', but by far not as high as for addition lines a and f.

Rapeseed-specific glucosinolates

The four aliphatic GSLs, glucoallyssin, glucobrassicinapin, gluconapin and progoitrin were detected in rapeseed 'Madora' but were not present in the radish lines (Fig. 2). All addition lines expressed the rapeseed-specific GSLs. In two lines with low total GSL content, b and h, some minor components could not be detected. Similar to the rapeseed parent, the butenyl GSLs gluconapin and pro-

Tab. 2. Content of aliphatic glucosinolates in rapeseed and radish parents and disomic rapeseed-radish chromosome additions

	BCN resistance	$\mu\text{mol/g}$ dry matter	Effect of radish chromosome
<i>Brassica napus</i> 'Madora'	susceptible	11.8	
Radish A24	resistant	169.2	
Radish A107	resistant	121.5	
addition <i>a</i>	susceptible	63.4	51.6**
<i>b</i>	susceptible	8.3	-3.5*
<i>c</i>	susceptible	9.8	-2.0
<i>d</i>	resistant	23.3	11.5**
<i>e</i>	susceptible	19.2	7.4**
<i>f</i>	susceptible	32.1	20.3**
<i>g</i>	susceptible	18.2	6.4**
<i>h</i>	susceptible	6.4	-5.4**
<i>i</i>	susceptible	14.2	2.4

*/** different from *B. napus* 'Madora' at 0.01/0.001 significance level with Student's t-test

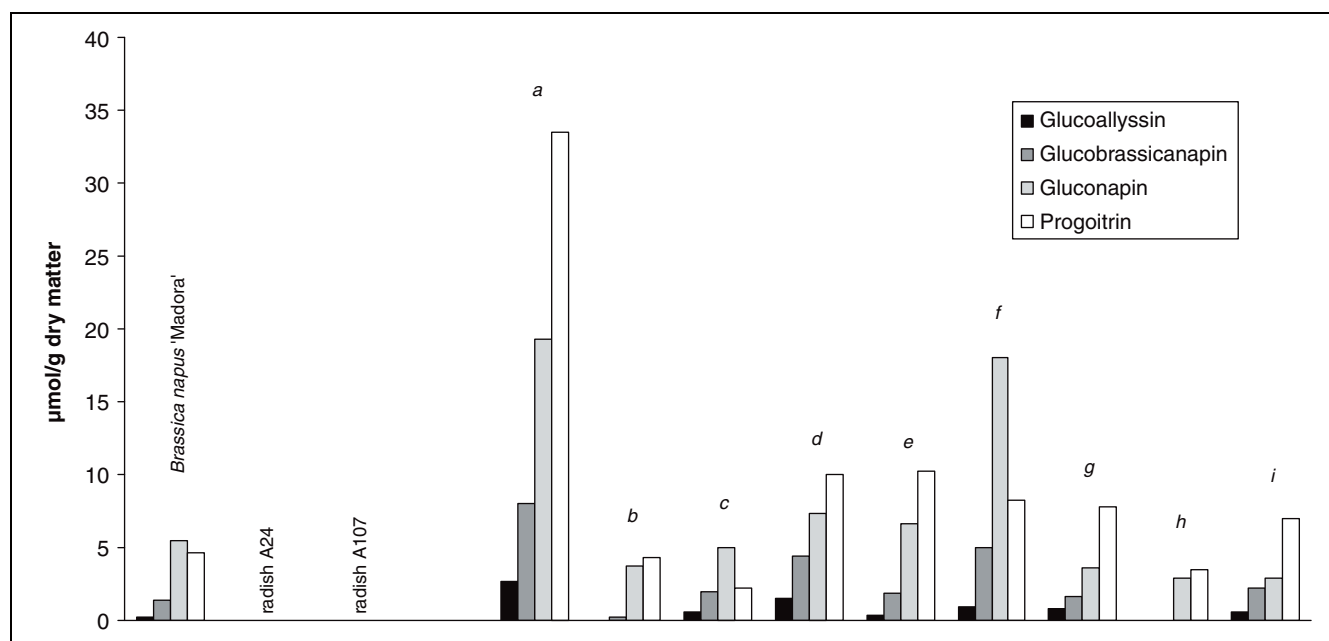


Fig. 2. Content of the rapeseed-specific aliphatic GSLs glucoallyssin, glucobrassicinapin, gluconapin and progoitrin in seeds of disomic rapeseed-radish chromosome addition lines.

goitrin had higher concentrations than the pentenyl GSLs glucoallyssin and glucobrassicinapin. Glucobrassicinapin content was consistently higher than glucoallyssin. In contrast to rapeseed, most addition lines had more progoitrin than gluconapin in their seeds, with exception of lines *c* and *f*.

Radish-specific glucosinolates

Four aliphatic GSLs, glucoraphenin, glucoerysolin, glucoraphanin and glucoraphasatin, were produced by oil radish but not by rapeseed (Tab. 3). GSLs with side-chains longer than four C-atoms were not produced.

Radish-specific GSLs were found only in seeds of addition line *g* but by far at lower concentration than in oil radish parents. The two major radish specific GSLs were detected in all investigated seeds of line *g*, glucoraphenin from 1.13 to 2.35 $\mu\text{mol/g}$ (mean 2.1 $\mu\text{mol/g}$) and glucoerysolin from 1.43 to 4.38 $\mu\text{mol/g}$ (mean 2.3 $\mu\text{mol/g}$).

Non-parental glucosinolates

Sinigrin was found neither in rapeseed nor in radish nor in the addition lines with exception of addition *i*. All ten grains of addition *i* contained sinigrin in concentrations between 0.46–7.44 $\mu\text{mol/g}$ (data not shown).

Tab. 3. Seed content ($\mu\text{mol/g}$) of the radish-specific aliphatic GSLs glucoraphenin, glucoerysolin, glucoraphanin and glucoraphasatin in the rapeseed-radish chromosome additions

	Glucoraphenin	Glucoerysolin	Glucoraphanin	Glucoraphasatin
<i>Brassica napus</i> 'Madora'	0	0	0	0
radish A24	89.7	73.7	4.6	1.3
radish A107	67.2	51.4	2.8	0.2
addition <i>a</i>	0	0	0	0
<i>b</i>	0	0	0	0
<i>c</i>	0	0	0	0
<i>d</i>	0	0	0	0
<i>e</i>	0	0	0	0
<i>f</i>	0	0	0	0
<i>g</i>	2.1	2.3	0	0
<i>h</i>	0	0	0	0
<i>i</i>	0	0	0	0

Indolic glucosinolates

4-Hydroxyglucobrassicin was the main indolic GSL (93.7%) in parental lines and the additions. Glucobrassicin, 4-methoxyglucobrassicin and neoglucobrassicin occurred in traces.

Discussion

Independence of aliphatic and indolic GSL content

Most of the added oil radish chromosomes (seven of nine) significantly influenced the total GSL content of rapeseed by changing the aliphatic GSLs without exhibiting any effect on the indolic GSLs. Five chromosomes had promoting effects but two chromosomes lowered the GSL content even beyond that of the double-zero rapeseed. The higher aliphatic GSL content could be expected from the differences between the chromosome donor radish and the recipient rapeseed with higher aliphatic GSL of the former but similar indolic GSL levels. The introgression of genes from the low-GSL cultivar Bronowski in the modern rape cultivars had only reduced methionine-derived GSLs, with no effect on the level of GSLs derived from tryptophan or phenylalanine (MITHEN, 1992).

Radish chromosome effects on aliphatic glucosinolate content

Most chromosomes (*a*, *d*, *e*, *f*, *g* and *i*) enhanced the aliphatic GSL content. The introgression of radish chromosomes in rapeseed background results in increased content for the **rapeseed-specific** aliphatic glucosinolates. All of them were increased nearly in the same ratio. It seems that the introgression of individual radish chromosomes provides precursors for rapeseed aliphatic GSL synthesis.

No individual radish chromosome was able to establish the high GSL content of radish in rapeseed background. This must be the result of the concerted action of several

loci, distributed over different radish chromosomes. Adding the effects of all nine radish chromosomes to mean GSL content of 'Madora' the radish range of more than 100 $\mu\text{mol/g}$ aliphatic GSL in seeds is reached. In the allopolyploid *B. napus*, TOROSER et al. (1995) and UZUNOVA et al. (1995) mapped four QTLs with similar additive effects on total seed aliphatic GSLs.

Surprisingly the chromosomes *b*, *c* and *h* had decreasing effects in the background of double-zero rapeseed. Such a transgression was also found by TOROSER et al. (1995) but in that case a high content transgression for genes from low content parent.

Radish chromosome *a* has the greatest effect on total GSL content

Addition of radish chromosome *a* enhanced the aliphatic GSL content of 'Madora' fivefold. DELOURME et al. (1998) found that the introgression of a radish fragment carrying the restorer gene *Rfo* for the *Ogu*-INRA cytoplasmic male sterility in rapeseed increases the content of all aliphatic GSLs. We were able to map this radish fragment using molecular markers on chromosome *a* of our radish map (under publication). DELOURME et al. (1998) had two hypotheses for high seed GSL content of restorer lines: 1) a gene controlling GSL content in the rapeseed genome very close to radish introgression or 2) a radish gene that is not responsible for radish specific GSLs but for enhancement of all aliphatic GSLs. With our data we can clearly show that the second hypothesis is true. The dense map of radish chromosome *a* presented by BUDAHN et al. (2009) can help to select for shorter *a* chromosome fragment in rapeseed restorer line. LI and QUIROS (2002) have shown that in *Brassica* the *GS-Elong* locus encoding methyl alkyl malate synthases controls the first steps in aliphatic GSL-pathway. It must be proved if a highly active allele of this gene is located on chromosome *a* of radish.

Additionally, in field experiments growing all rapeseed-radish addition lines in double-rows side by side, it

was observed that the plants of addition line *a* were selectively and hardly attacked by the larvae of cabbage root fly (*Delia radicum* L.) (data not shown). The cabbage root fly is the main root herbivore described for crucifers and can be a severe pest in natural and in agricultural systems (FINCH, 1993). It is a chewing specialist root herbivore that feeds on the roots of several crucifer species. Cabbage root fly was intensively studied for its contact chemoreception playing a decisive role for oviposition (COAKER and FINCH, 1973; STÄDLER and SCHÖNI, 1990). ROESSINGH et al. (1992) showed that tarsal chemoreceptors of *Delia radicum* L. were stimulated by GSLs, with the highest effect of glucobrassicin. In our study we determined the seed GSL content and not that for the leaf material, but GLEN et al. (1990) showed a good correlation between GSL profiles of seeds and leaves of seedlings. The same was shown for *Arabidopsis* by KLIEBENSTEIN et al. (2001). Glucobrassicin is an indolic GSL, present in nearly the same concentration in all addition lines. So it should be proved if aliphatic GSLs have here the distinguishing effect. ROESSINGH et al. (1997) have shown, that also non-GSL substances such as the 'cabbage identification factor' (CIF) play a role in chemoreception of the cabbage root fly. Content of this factor was not studied in this set of rapeseed-radish addition lines, but could give interesting insights into insect-plant interactions.

Synthesis of radish-specific GSLs in rapeseed background

Disomic rapeseed-radish addition line *g* is the only one in which radish specific GSLs have been synthesized. The concentration of glucoraphenin and glucoerysolin is only 2.7% and 3.7% of the mean of both radish parental lines, respectively. Glucoraphenin, known to have anticarcinogenic effect (inducer of phase II detoxification enzymes) has not been detected in addition line *g*, presumably because it is already present in radish with by far lower concentration than glucoraphenin and glucoerysolin. HALL et al. (2001) and KLIEBENSTEIN et al. (2001) showed that in *Arabidopsis* the production of GSLs with different classes of side chains is controlled by one genetic locus *GS-AOP* with three alleles: *GS-ALK* is responsible for the reaction from methylsulfinylalkyl- to alkenyl- and *GS-OHP* further to hydroxyalkyl- side chains. Allele *GS-null* (both absent) accumulates GSLs with methylsulfinylalkyl side chains as it is also the case for radish. In the background of rapeseed these precursors are processed to alkenyl and hydroxyalkenyl GSLs.

Glucosinolate content of disomic rapeseed-radish addition line *d*

GSL content of addition line *d*, resistant to beet cyst nematode *Heterodera schachtii*, was enhanced in comparison to rapeseed 'Madora'. The amount of indolic GSLs was nearly unchanged, but the content of aliphatic GSLs has been doubled. In this study GSL content of the roots has not been determined. Generally, the GSL content in the roots and also the aliphatic/indolic GSL-ratio is lower as it was shown in *Arabidopsis* (BROWN et al.,

2003). All other addition lines though they have equal, lower or even higher GSL contents, are BCN-susceptible. Therefore the variation in the content of aliphatic GSLs is not connected with nematode resistance. The analysis of individual GSLs gave also no evidence for correlation with resistance. This is in accordance with results of PETERKA et al. (2004) obtained with monosomic additions. In breeding experiments using addition line *d*, the transfer of BCN-resistance to rapeseed should be possible by spontaneous (meiotic) or artificial (irradiation) intergenomic recombination without increasing the GSL content in the seed over the limit decisive for food quality.

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