BREEDING FOR A REDUCED GLUCOSINOLATE CONTENT IN THE GREEN MASS OF RAPESEED TO IMPROVE ITS SUITABILITY FOR BIOGAS PRODUCTION

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COVER: DETAIL OF RAPESEED SELFING BAGS
PHOTOS: KWS SAAT AG

ZÜCHTUNG AUF EINEN NIEDRIGEN GLUCOSINOLATGEHALT IN RAPSBLÄTTERN ZUR VERBESSERUNG DER EIGNUNG ZUR BIOGASGEWINNUNG

BREEDING FOR A REDUCED GLUCOSINOLATE CONTENT IN THE GREEN MASS OF RAPESEED TO IMPROVE ITS SUITABILITY FOR BIOGAS PRODUCTION

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PREFACE

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

SYMBOLS

4ME:	4-Methoxy-glucobrassicin
bf:	Begin of flowering
ef:	End of flowering
GCA:	General Combining ability
GSL:	Glucosinolate
GBC:	Glucobrassicin
GBN:	Glucobrassicanapin
GNA:	Gluconapin
GNL:	Gluconapoleiferin
h^2:	Heritability
HPLC:	High Pressure Liquid Chromatography
NAS:	Gluconasturtiin
NL ODM:	Norm Liter Organic Dry Matter
NEO:	Neo-glucobrassicin
PRO:	Progoitrin
SCA:	Specific Combining Ability
SIN:	Sinigrin
r^2:	Correlation coefficient

1 General Introduction

Rapeseed (*Brassica napus* L.) is an economically important crop belonging to the *Brassicaceae*. After the release of canola quality cultivars, which contain low erucic acid and low glucosinolate content, rapeseed became an important oil seed crop in temperate areas. The worldwide production area of winter rapeseed is the second largest for an oilseed crop after soybean (FAO 2007). The seeds are pressed to gain rapeseed oil. Rapeseed oil is, after corn oil, the second richest natural source of phytosterols contributing up to 1% of the crude rapeseed oil (Gordon and Miller 1997).

Unfavorable traits within seeds of *Brassica napus* L. are the anti-nutritive components such as phytic acid, glucosinolates, sinapine, and tannins. These are not desired in the rapeseed meal. Favorable traits are unsaturated fatty acid, high oleic acid and protein content. The anti-quality chemical components (glucosinolates, tannins, erucic acid, sinapine, phytic acid) are studied for their anti-nutritional and toxic effects, which might have an inhibiting effect on the fermentation processes. *Brassica napus* L. is favorable for its higher polyunsaturated fatty acid composition and oleic acid content (Aslam et al. 2009).

Phytosterols are predominantly present in oilseed plants and cereal lipids and their content in vegetables, fruits, nuts and berries is considerably lower (Piironen et al. 2003). The application for renewable energy is in conflict with the requirements for local food production and the land use policy. In the last decades, the area coverage and amount of production continuously increased in Europe and China.

Seeds of winter rapeseed are pressed to extract oil and used as a renewable resource for bio-products and bio fuel (Qian et al. 2009). The feeding quality of winter rapeseed has a large potential but is restricted because the meal is not well digested by monogastric animals, such as swines and poultry. Some glucosinolate types provoke a health promoting effect (Verhoeven et al. 1997; Fahey et al. 2001). In the early seventies winter rapeseed breeders developed ‘double zero’ rapeseed (Holst and Williamson 2004) with a low glucosinolate and a low erucic acid content. Further the focus on nitrogen and sulfur fertilization is the main breeding standard. Insect resistance and biogas applications, compromises the reducement of the glucosinolate molecule in the leaves, stems, seeds and even the roots. The glucosinolate-myrosinase system is a meaningful plant defense system, which acts against herbivores and pests. The variation of the aliphatic glucosinolates has been mainly attributed to genetic variation (Giamoustaris and Mithen 1996), while the concentration of the indole forms has been proposed to be regulated primarily by environmental and/or physiological factors (Brown et al. 2002). In *Brassica* genus, alkenyle glucosinolates are mostly present (Giamoustaris and Mithen 1996), whereas indole glucosinolates are present in a minority.

Winter rapeseed (*Brassica napus* L.) is a biannual herbaceous species, which reaches a height of 30 till 150 centimeter. The roots can form a pale or fine root system, depending on the cultivars the leaves are widely differing. Some Resynthesized rapeseed lines are similar to European winter rapeseed cultivars, whereas others have a quite unique pattern. Resynthesized rapeseed is a valuable source for broadening the genetic variation in present breeding material of *Brassica napus* L. However, different lines differ widely in their suitability for this purpose (Becker et al. 1995).

With a decreasing fossil fuel supply, the interest for biogas production increased over the past decades, resulting in local renewable energy production initiatives. In the past ten years winter rapeseed has received much attention as an alternative source of energy. When grown in a sustainable land-use management system, winter rapeseed has a huge potential for the production of renewable energy, also by the production of biogas from whole plant biomass. An agricultural system used for the cultivation of winter rapeseed with a double-cropping-system, where winter rapeseed is used before maize in winter and early spring is a good option. Within this crop system, two energy crops are harvested in one rotation and hence a higher biomass harvest per hectare is reached.

In the past ten years winter rapeseed has received much attention as an alternative source of energy. In Germany, winter rapeseed can become a potential crop for the production of biogas. The use of crop variation for the production of biogas has several advantages. Firstly due to the double-cropping a higher biomass per hectare is accumulated. Secondly a diversified intercropping system leads to more ecological diversity.

GLUCOSINOLATES

Glucosinolates occur as secondary metabolites of almost all plants of the order *Brassicales* (including the family *Brassicaceae*, *Capparidaceae* and *Caricaceae*), but also in the genus Drypetes (family *Euphorbiaceae*). These secondary components are sulfur containing bindings with an anti-bacterial and anti-fungal role (Mikkelsen et al. 2002). The biogas production process with winter rapeseed as a substrate is possibly inhibited by glucosinolates. Aside from a high biomass value for a sufficient biogas conversion (Ofori and Becker 2007) also a favorable composition of the biomass for components such as low leaf glucosinolate content is valorized.

Glucosinolates are composed of a ß-thioglucose moiety, a sulphonated oxime moiety and a variable side chain, derived from an amino acid (Figure 1) (Halkier and Du 1997, Giamoustaris and Mithen 1996, Mithen 2001).

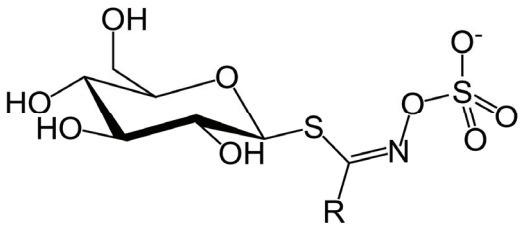

Figure 1. Structural formula of the glucosinolate molecule (Halkier and Du 1997)

The 9 glucosinolates identified in rapeseed are grouped into alkenyle glucosinolates, indole glucosinolates and phenyl glucosinolates based on whether they are derived from methionine, phenylalanine, or tryptophane, respectively (Halkier and Du 1997).

The content and composition of the glucosinolates are influenced by the (1) genotype (Stephani 1985) (2) development state and (3) environment and age of the plants, (1) the genetic constitutions of the different rapeseed lines, cultivar, influence the content of glucosinolates. The earliest visible characterization of the genetic constitution can be seen by the pattern diversification in the third and fourth leaf stadium, whereas concentration increases is assumed to occur later (Stephani 1985). A precondition for the development of a selection technique using leaves is the phenotypical characterization of the glucosinolate contents in different development stages, different environmental conditions and diverse material (Stephani 1985). (2) The leaves of younger plants have a lower glucosinolate contents as those from ripe plants (Stephani 1985). Glucosinolates occur in several parts of the plant such as the root, the stems and the leaves, but their highest concentration is usually in the seeds (Xue et al. 1992). In former studies (Stephani, 1985; Jürges 1982) the green material was separated into generative and vegetative material, or the material was distinguished based upon the position of the leaves on the main stem. This was necessary in order to prove the influence of the state of development (ontogenetic phase) on the leaf glucosinolate content and composition. Stages of accumulation during vegetative growth and seed maturation are followed by intense degradation during flowering, germination and early growth stages of seedlings. The two main groups of glucosinolates, namely alkenyles and the indoles, vary according to the ontogenetic stage of the plant. The alkenyles and the indoles increase initially, being at their maximum level in an early vegetative phase. After this the concentrations are lowering slowly, to remain in a further development on a constant level.

This diversity in the amount and structure of the glucosinolates suggests a storage and/or hormonal function of the glucosinolates in rapeseed. (3) Environmental effects, such as nitrogen fertilization, water and sulfur supply influence the glucosinolate content (Wielebski et al. 1999). The nitrogen supply has a reducing

effect on the glucosinolate content in the leaves, whereas the sulfur supply influences the increase of the glucosinolate content in the leaves.

The biosynthesis of glucosinolates consists of three phases. The example for the alkenyle glucosinolates is given. The first common step for the precursor amino acids is the formation of the aldoxime structure. An amino acid is elongated with methylene; this is accomplished after deamination of the amino acid to the 2-oxo acid (Kliebenstein et al. 2001). The 2-oxo acid can then be reanimated to the elongated amino acid or undergoes further elongation. By conversion to an aldoxime via one of a series of cytochrome P450 oxygenases, the amino acid starts the pathway of glucosinolate biosynthesis. The resulting amino acid is then converted to a glucosinolate by three additional steps. Firstly the synthesis of the chain-elongating amino is accomplished, thereafter glucones are added and finally the synthesis is completed with the elongation of the side-chain (Kliebenstein et al. 2001b).
The anti-bacterial functioning of glucosinolates is caused by the presence of the myrosinase enzyme. The myrosinases are localized in `myrosin´ cells, which are scattered throughout most plant tissues (Matile 1980). When the plant tissue is damaged, glucosinolates come into contact with the myrosinase enzyme (Wittstock et al. 2004). Seeds differ morphologically from leaf and stem glucosinolates. The glucosinolates are localized inside the sub-cellular compartments of the same plant cell. Glucosinolates are situated inside the vacuole (Bones and Rossiter 1996). In contrast, with seeds, plant parts are expected to be more fragile.

The glucosinolates are then hydrolyzed in a number of substances, which can have toxic effects against bacteria and fungi for example the isothiocyanate, oxazolidinthione or rhodanid, thiocyanate, nitrile, and or episulphide (Wittstock et al. 2004, Buchner 1988). The isothiocyanate, thiocyanate, nitrile and epithionitrile belong towards the antibodies, which are responsible for the biological activities of the glucosinolates, such as their participation in plant defense and response to environmental changes (Grubb and Abel 2006), which include pathogen attack, UV radiation and drought. Isothiocyanates serve as a gustatory stimulus for insect pests of cruceriferous plants, while their isothiocyanate cleavage products act as feeding and oviposition attractants for many insect species (Beck and Reese 1976). These components are also goitrogenic and result in depressed growth of animals fed on meals containing these components. Goitrin has a well documented anti-nutritional property and thiocyanates are the main anti-nutritional factors. The goitrin component is derived from the intra-molecular cyclisation of 1-isothiocyanatobut-3-en-2-ol to goitrin, which is an inhibitor of thyroid peroxidase and prevents oxidation of iodide for subsequent iodination of tyrosine residues in the biosynthesis of thyroxines. Thiocyanate anions act as a competitive antagonistic inhibitor of iodide

and thus prevent iodide uptake by the thyroid, but this effect can be largely overcome if enough iodide is supplied in the diet (Bones and Rossiter 2006).

The central hypothesis is that winter rapeseed genotypes with an increased amount of secondary glucosinolate components inhibit the bacterial flora in the reaction process. This, while a possible negative effect, might be caused by the decomposition products of glucosinolates. Their negative or inhibiting effects on fermentation bacteria might be caused by the transformation of glucosinolates into isothiocyanates, thiocyanate and other inhibiting secondary components.

This hydrolisation towards nitrile or isothiocyanate is pH dependent.

A pH of 5-7 leads towards isothiocyanate, and a more acid pH improve the development of nitrile. Other variables such as temperature and presence of other cofactors such as iron are also conditioning the situation towards a specific end product (Hom 2004). To reduce their inhibiting effects on bacterial growth or increase their positive effects such as insect attacks, the glucosinolates can be diminished in the leaves, stems and seeds of winter rapeseed.

Reducing glucosinolates within the vegetative substances of the plants might open up possibilities for the further development of winter rapeseed as an energy plant. Precondition for this is the selection of winter rapeseed genotypes, with lower glucosinolate content in the leaves and the stems and the compliance of the previous statement with the main hypothesis of this dissertation. This genetic variation can be sought in forage rapeseed and interspecific crosses between turnip rape and cabbage (Krähling 1987).

Sinigrin is dominant in the seeds, glucotropaeolin occurs only in the leaves of winter rapeseed (*Brassica napus* L.). With the exception of some resynthesized lines, expressing low SIN content within their leaves (Mithen, written communication). Leaves and stems contain different types of glucosinolates, such as PRO, GNA, GBN and GNL. These types differ for their relative and total distribution. Seeds of winter rapeseed contain a larger total amount of glucosinolates as the leaves.

Table 1 present the trivial, semi-systematic names and abbreviations of the glucosinolates inside leaves, stems and seeds (Bennert 1992).

High biomass cultivars are poorly investigated for their secondary components, such as glucosinolates. Knowledge concerning such components could be used too for cultivar development. The dominant glucosinolate types in *Brassica napus* L. are given (Table 1).

Table 1. The glucosinolates, semi-systematic names and abbreviations (Bennert 1992)

TRIVIAL NAME	SEMI SYSTEMATIC	REST	ABB.
Alkenyle glucosinolates			
Sinigrin	2-propenyl	CH2=CH-CH2-	SIN
Gluconapin	3-butenyl	CH2=CH-CH2-CH2-	GNA
Glucobrassicanapin	4-pentenyl	CH2=CH-CH2-CH2-	GBN
Progoitrin	2-Hydroxy-3- butenyl	CH2=CH-CHOH-CH2-	PRO
Gluconapoleiferin	2-Hydroxy-4-pentenyl	CH2=CH-CH2-CHOH-CH2	GNL
Glucoraphanin	4-Methylsulphinylbutyl	CH3-CS(O)-CH2-CH2-CH2-CH2-	GRA
Glucoalyssin	5-Methylsulphinylpentyl	CH3-SO-CH2-CH2-CH2-CH2-CH2-	GAL
Indole glucosinolates			
Glucobrassicin	3-indolemethyl-	R1:-H,R2:-H	GBC
4-hydroxyglucobrassicin	4-hydroxy-3-indolemethyl	R1:-OH,R2:-H	4OH
4-methoxyglucobrassicin	4-Methoxy-3-indolemethyl-	R1:-OCH3,R2:-H	4ME
Neo glucobrassicin	1-Methoxy-3-indolemethyl-	R1:-H,R2:-OCH3	NEO
Phenyl glucosinolates			
Glucotropaeolin	Benzyl-	-CH2-CH2	GTL
Nasturtiin	Phenylethyl--		NAS

Genetics of the leaf glucosinolates within *Brassica napus* L. have been poorly investigated (Stephani 1985). The approach, a set of field experiments, one repetition in Göttingen and one field experiment in Einbeck, combined with a harvest at the beginning of flowering and different leaf stages over different years, is unique and has never been implemented so far. Winter rapeseed is well suited for biogas energy purposes, because of their high biomass production in early spring time. Glucosinolates are molecules, which role are questioned, by their very low content, their relative amount vary between 2% in the beginning of the vegetation and 0.1% in the end (Zukalova and Rostlinná 2002).

Biochemical laboratory analyses of the vegetative and generative material (stems, leaves and seeds) identifies the individual glucosinolate types.

A precondition for the development of a selection system using leaves is the phenotypical characterization of the glucosinolate content in different development stages, different environmental conditions and diverse material (Stephani 1985) (2) The leaves of younger plants have higher glucosinolate content as those from ripe plants (Stephani 1985).

The two main groups of glucosinolates, namely alkenyles and the indoles, vary according to the ontogenetic stage of the plant. The alkenyles and the indoles increase initially, being at their maximum level in an early vegetative phase. After

this the concentrations are lowering slowly, to remain in a further development on a constant level. Koritsas et al. (1991) observed an increase of the indole glucosinolates, glucobrassicin and neo-glucobrassicin, after mechanical wounding or infestation of intact rapeseed plants.

The glucosinolate content in the seeds is determined by the mother plant, and is independent from the genetic constitution of the seeds. Through stable or segregating interaction crossing makes it possible to regulate the glucosinolate content in the seed (Röbbelen 1973). The characteristic glucosinolate poverty is ascertained by more (two till three) recessive either partial recessive genes (Lein 1972). The direct selection of green mass values highly in forage rape, its applicability however is under discussion for the indirect selection of the seeds, which has a higher technical simplicity (Stephani 1985). Some research has sought to study the correlation between leaf and seed glucosinolate concentrations (Schilling and Friedt, 1992). In some cases pollen influences were observed for aliphatic glucosinolate, total glucosinolate contents but maternal additive and dominant effects were more important (Hom 2004). In any case the pollen genotype has to be considered as disturbing factor when harvesting open pollinated plants and selecting single seeds in the segregating F2 generation (Hom 2004). In comparison with other directly expressed traits, the concentrations of most glucosinolates are rather lately expressed; besides this a relative high number of genes are at its base.

The correlation between leaf and seed glucosinolate concentrations (Schilling and Friedt 1992) has been analyzed before. It is apparent that within the range of material currently available, leaf and seed glucosinolate concentrations may be under separate genetic control (Mithen 1992). According to Toroser et al. (1995) glucosinolates are synthesized in the pod walls of oilseed rape and most of them are transferred as intact glucosinolates to the seeds. The glucosinolate pattern in leaf tissue originates by the novo glucosinolate biosynthesis (Magrath and Mithen 1993).

EXPERIMENTAL VARIATION OF LEAF AND STEM GLUCOSINOLATES

Variation for glucosinolate content or variability of glucosinolate content within leaves, stems has been poorly investigated. Leaf stage development might influence the glucosinolate content in the leaves. One of the main criteria, relevant for field experiments is the stage of development and experimental variation (Figure 2). In 2007, 2008 and 2009, a standard HPLC methodology for peak detection was further developed. In 2009 freeze-drying of the leaves was applied.

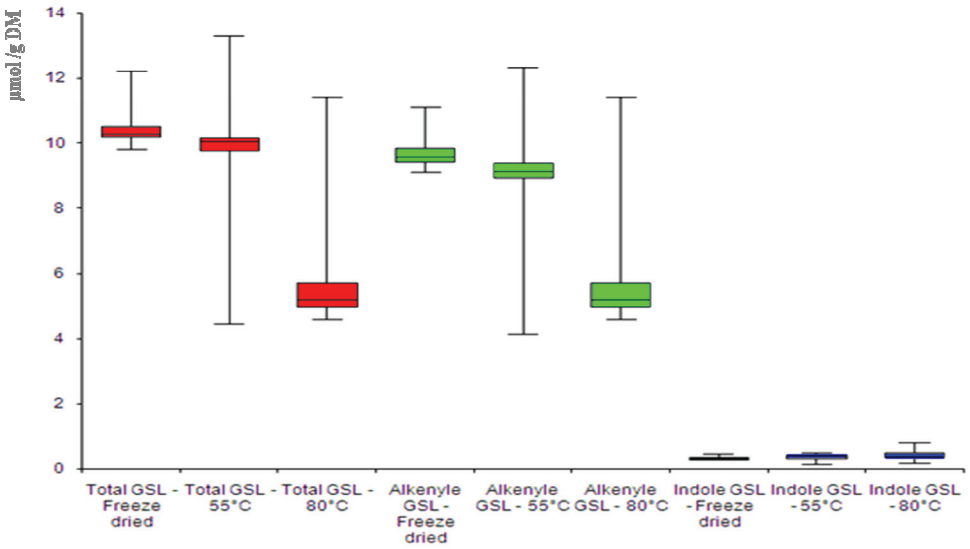

Figure 2. Experimental variation, oven drying (55°C, 80°C) versus freeze drying of leaf glucosinolates

A too high drying temperature or damaging during the sampling can be a cause for relative low measured glucosinolate values. The drying temperature in this study was 60°C, under these conditions a total maximal recovery efficiency of 61% can be expected (Stephani 1985). An optimal temperature between of 50°C and 60°C is chosen, for this temperature a maximal recovery efficiency of 75% is possible. In all cases an optimal air circulation in the oven is guaranteed, therefore a not too large sampling quantity is investigated.

GLUCOSINOLATE ANALYSIS

Glucosinolate identification and quantification was based on the internal standard method (peak area and retention time) with reference samples. The quantification of the leaf and stem material can be done by using glucotropaeolin and sinigrin as an internal standard. The accuracy of the method was verified using a seed sample of the cultivar Linetta analyzed at the beginning of each sampling.

Preparation of the samples and HPLC analysis

1. About 200mg of the milling material is given in 70/17 polypropylene-tubes and the weight is notated

2. The tubes are heated for 10 minutes in a water bath at 75°C, to inactivate the myrosinase.
3. 2 ml of 70% methanol is added in the sampling tubes, this resolves the glucosinolates out of the sample
4. 200µl of intern standard (5mmol gluco-tropaeolin-SIN/l water) is added
5. The content of the tubes is mixed with the mixomat
6. Extraction by 75°C for 10 minutes
7. Mixing after 5 minutes and after the extraction
8. Centrifugation for 4 minutes at 4000-5000 rpm
9. Decantation of the rests in 70/17 polypropylene-tube
10. 2ml 10% methanol is given in the sediment in the sampling tubes (75°C)
11. Repetition of the steps 5-8
12. Decantation and aggregation of the rests
13. Mixing of the rests with the mixomat
14. Insertion of 500µl extract on a 20 mg sephadex DEAE-A25 ion exchange column
15. Flushing the column 2 times with 1 ml water
16. Addition of 100µl purified depleted (1:2, 5) sulphohydrolase on the columns
17. Desulphatising over night at a temperature of 39°C
18. Flushing of the column 3 times with 500 HPLC water and the solution in 3 ml polystyrole tubes
19. Hoisting 0.7 ml of the extract in a nozzle and spraying in a HPLC sampling tube computation of the glucosinolate content

To obtain the area values from the glucosinolate contents, following magnitudes were used:
- Area-value of the internal standard (GTL)
- Response factor of the internal standard (0, 95)
- Sample weighing in gramm
- Area-value of the glucosinolate value
- Response factor of the investigated glucosinolate

Following formula calculates the content for the glucosinolate PRO with the response factor 1.09: (Area PRO x 1.22 x 1.2) (Area GTL x 0.95) x weight in g) = quantity of PRO in µmol /g D.M. The product from the area surface value PRO and response factor is multiplied with 1.2, this coincides with the amount of added internal standard 1.2µmol (200µl 16mmol/l gluco-tropaeolin). As an alternative analysis method spectroscopic analysis can be used, this summarizes all methods for which electro magnetique radiation is used within the plant material. The difference between the basic radiation level and the radiated light, results for the energy difference between the ground radiation level and the reached wave length of the radiation emitted by the components (Wüst and Rudzik 1994).

SEASONAL VARIATION

The evaluation trials were conducted at one location in 2007 and two locations in 2008 and 2009, with one replication at each location. Every field experiment consisted of one replication per double row. In the first field experiment, resynthesized lines, which are grown under temperate regimes, were randomly selected. A focus on biomass and plant stages was set. 44 resynthesized winter rapeseed lines are tested within a field trial on 2 locations, these parental lines are analyzed for their total leaf, stem and seed glucosinolate patterns. Sowing dates were chosen in August, which are optimal for *Brassica napus* L. in climatic zones with a cold winter and warm summer, expressing its relevance to fulfill the vernalisation requirements of *Brassica napus* L. The plants of all accessions were grown under temperatures, normal for a western European country. A three year experiment with the first year having one location was undertaken in the field conditions during August-April. The biomass of winter rapeseed reaches a peak value in spring time at the end of April or beginning of May. The seeds which are kept in paper bags are used for line maintenance. Seed storage was performed over annual selfing of at least 3 plants.

Over several years, at least 100 seeds were maintained per location. Seed storage is kept free from moths and mice using insecticides and rotenicides. Besides this, flowering is an important morphological trait, the influence on the leaf and stem glucosinolate content is tested experimentally by scoring the beginning and ending of flowering. In 2007, 44 genotypes were randomly selected for their naturally variation in leaf glucosinolate content. Two locations with similar soil conditions were chosen, this to find out the environmental effect on the leaf and stem glucosinolates. Two plants of *Brassica napus L.* were sampled for their leaves and stems in April, seeds were harvested in July.

In the autumn of 2007 and 2008, 11 cultivars were harvested. This was done to follow the glucosinolate profile along the developmental state of plants (seasonal variation). A modern cultivar, a resynthesized rapeseed (Express and S3) and a forage rapeseed cultivar (Nikos) were included Figure 3.

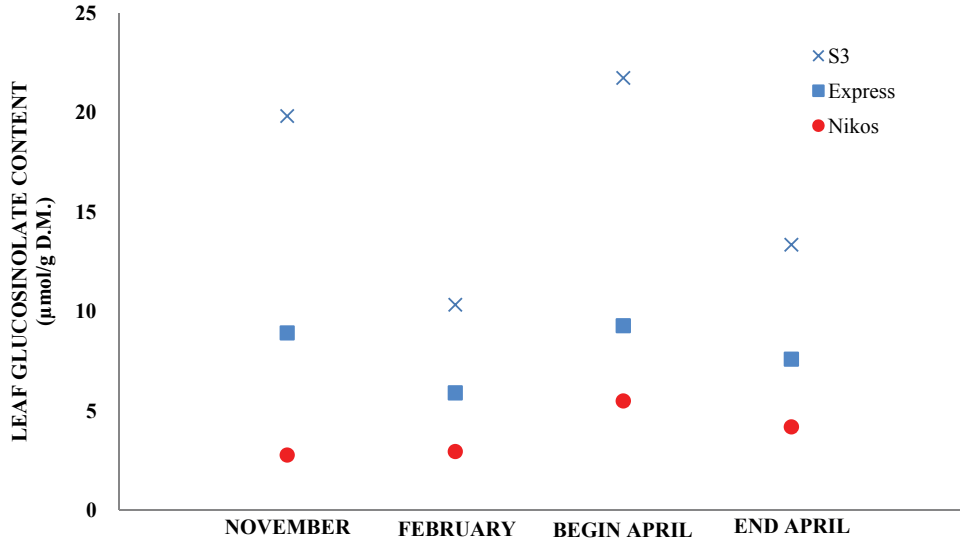

Figure 3. Seasonal variation of leaf glucosinolates (in µmol/g D.M.)

The levels of all major glucosinolates increased from September to November in plants from three resynthesized lines.

The vigorous plants, originating from high biomass yielding cultivars were grown and harvested for their stems and leaves in the beginning of May. Begin of flowering was scored to find specific genetic relations between morphological visible traits and leaf and stem glucosinolates. Further 44 resynthesized genotypes were grown at two locations, in double rows with about 100 seeds per row and harvested for their leaf and stem glucosinolate content in May.

PLANT MATERIAL AND EXPERIMENTAL SETUP

One of the aims of the study was to analyse the genetic variation in glucosinolate content and composition in genetically diverse winter rapeseed material, including modern cultivars (n=15), double haploid lines (n=8) and resynthesized lines (n=32). The first rapeseed population (n =44) was established in a field experiment in a single environment, with several replications per genotype made in different plots. Of the 44 accessions, one forage rapeseed cultivar namely Nikos was included. Some of the material consisted of double haploid lines of the F1 generation of the cross between Gaoyou and Sollux. The parent line Sollux was released in 1973 by ZG Winterraps (German Democratic Republic). Gaoyou is an inbreed line from a

cross between the Chinese breeding line `695` and the Japanese cultivar Nongling 18 developed by pedigree selection and registered in 1990 by Zhejiang Agricultural University. It behaves more like spring rapeseed because it has poor winter hardiness and no vernalisation requirement (Zhao et al. 2005). Except a Turkish cultivar (Eskisehir), one semi-synthesized (H111-2) and a list of elder and modern cultivars, all the other accessions are resynthesized rapeseed lines. The choice for resynthesized lines instead of hybrids is based on the easiness of crossing and presence of partial self fertility which makes plant reproduction possible. The resynthesized plant material is originating from the Brassica assortment of the Göttingen institute. This assortment was largely established from interspecific crossing between cabbage and turnip rapeseed and proved to be useful to investigate the variability of the trait glucosinolate concentration and composition within rapeseed. Some interspecific crosses were sown in Reinshof in 2006, whereas in 2007 they were sown at two locations namely Reinshof and Einbeck.

In the spring of 2007 the green material of 44 accessions and 24 crosses was harvested from the field plots at the beginning of flowering. The beginning of the flowering was characterized as the time, where the first flowering buds became visible. For each sample, two plants were cut, whereof a part of the stem, few leaves and later the seeds (of other plants) were harvested.

In 2007 and 2008 Hybrid crosses were grown in the field in a randomized block design. Further glucosinolate and oil content and other relevant parameters such as protein content were tested for the double haploid lines.

SOWING AND GROWING CONDITIONS

Brassica napus L., which is cultivated mostly within temperate climatic zones is sown in August. The environmental conditions are tabulated in table 2. The seeds are harvested in July. The biomass of winter rapeseed reaches a peak value in spring time at the end of April or beginning of May. Except for anti-bacterial effects, originating from cyanogenic glucosides, also complex biological interactions e.g. with insects have been scored. Glucosinolates and their breakdown products have been known for their fungicidal, bactericidal, nematocidal and allelopathic properties and have recently attracted intense research interest because of their cancer and chemo protective attributes (Fahey et al. 2001). The breeding of winter rapeseed with a focus on leaf, stem and seed glucosinolates is for this matter quite diverse, winter rapeseed plays a role as a renewable energy crop as well as in animal feeding and biofumigation applications.

Table 2. Precipitation and temperature for the two environments

	Average temperature (°C)		Rainfall (mm)	
Göttingen	5.8	13.8	303.5	561.3
Einbeck	6.0	14.8	394.2	718.4
Site location	Average min. t (°C)	Average max. t(°C)	Growing season	Total year
Year 2007				
Göttingen	6.1	14.2	299.1	740.3
Year 2008				
Göttingen	5.8	13.8	303.5	561.3
Einbeck	6.0	14.8	394.2	718.4
Year 2009				
Göttingen	5.0	13.6	299.0	670.2
Einbeck	6.2	15.7	384.5	700.0

QUANTITATIVE TRAIT LOCI MAPPING

The phenotypic variation of many complex traits of agricultural or evolutionary importance is influenced by multiple quantitative trait loci (QTLs), their interaction, the environment, and the interaction between QTL and the environment. With a population of double haploid lines it is possible to create a map and it is possible to observe such segregation. Based on a population of double haploid lines and an available subset of 133 markers it is possible to create a map, which situates the leaf glucosinolate trait within the Brassica genome. The estimation of the gene number requires the environmental variance, the variance caused by the double haploid lines and the variance caused by the parental lines.

Linkage analysis in plants typically localizes QTLs to 10 to 20 cM intervals because of the limited number of recombination events that occur during the construction of mapping populations and the cost for propagating and evaluating a large number of lines (Doerge 2002).

Analysis of molecular markers and identification of quantitative trait loci (QTL) can help to explore the genetic basis of glucosinolate traits within leaves and stems of winter rapeseed. Molecular plant breeding offers a jumping leap for the further improvement of winter rapeseed lines. A large widely adapted mapping population is necessary to identify the available functional loci. The two lines, which are used, namely Sollux and Gaoyou are cultivars, adapted to western European and Chinese conditions (Zhao et al. 2005).

Seed glucosinolate mapping has received great attention in QTL mapping in the past decade. Efforts to explore genetic traits such as leaf glucosinolate content are poorly made, while leaf and stem glucosinolates as a quantitative trait in *Brassica napus* L. are not investigated properly.

The trait expression for itself is assumed to be less influenced by their distribution across the genome. Whether a QTL is exploited in a winter rapeseed breeding programme is rather a question of finding the favorable alleles, towards QTL and candidate gene identification with the help of DNA sequencing.

INTER-CROPPING AND AGRICULTURAL SYSTEM

Except for winter rapeseed breeding initiatives for biogas purposes, alternative intercropping systems with C4 crops, such as maize and sorghum are known to be successful. Higher biomass levels and better substrate composition (proteins, dry matter, lignin, etc.) are reached.

Intercropping systems with winter rapeseed as a crop diversify the agro-ecological setting. This is to improve the biological decomposition processes during fermentation. It is known that the biological processes required for methane gas production need a sufficient amount of dry matter. Besides the dry matter content, biogas substrate requires a high nutrient, protein content and well-adapted organic substrate composition. Advantageous for an early harvest and a continuous flow to the reactor is that biomass production of winter rapeseed reaches an early peak value in April. Winter rapeseed as an intercrop with maize offers a possible high agronomical potential. Until now winter rapeseed was never closely investigated for its methane production potential before. By the cultivation of winter rapeseed as a renewable energy crop the emission of greenhouse gasses through the burning of fossil fuels could be reduced. High yielding biomass crops offer an alternative way for renewable energy production. Sufficient energy is stored through the process of photosynthesis and this green energy is reused during fermentation processes and methane production. Except from transport costs, renewable energy crops are believed to be ecologically less demanding.

These types of crops need a lower application of pesticides and herbicides. A long term plant breeding approach in compliance with a sustainable agricultural system offers perspectives for renewable energy production. A closer look is made to see relations between glucosinolates within the leaves and the stems and the biogas production. The latter two biosynthetic steps mainly depend on temperature and cell moisture content, which is different in the leaves, stems as in the seeds. Physiological differences between sink and source in relation with their respective

plant parts are interesting for exemplifying the differences in genetic variation. *Cruciferous* species are all able to synthesize glucosinolates. Sink and source relations for these secondary components are however complex and less understood.

The favorable agricultural system used for the cultivation of winter rapeseed is in a double-cropping-system with maize, where two energy crops are harvested in one rotation. The use of crop variation for the production of biogas has several advantages. Firstly due to the double-cropping a higher biomass per ha is accumulated and secondly a diversified intercropping system leads towards more ecological diversity. Until now, plant breeding regularly improved the suitability of rapeseed for biogas extraction by increasing its dry matter, nutrient and protein content. A secondary plant component which role is questioned by its very low content between 2% in the beginning of the vegetation and 0.1% in its end (Zukalova and Vasák 2002) are glucosinolates. The genetic reducement of the glucosinolate contents in the vegetative substances of the plants opens up possibilities for the further development of rapeseed as an energy plant. Precondition for this is the selection of rapeseed genotypes with reduced glucosinolate content in the leaves and the stems. This genetic variation can be sought in forage rapeseed and interspecific crosses between turnip rape and cabbage (Krähling 1987). These crops are well suited for biogas energy purposes, because of their high biomass production and occasional low glucosinolate level in the leaves.

Demanding energy production and plant phytopathological effects, which are driven by global effects such as climate change, or local effects such as an increased pest situation are a challenging setting for winter rapeseed breeding. Local breeding of resynthesized winter rapeseed lines, considering the effect of a long term breeding approach can help to breed lines with for example a higher biomass or a higher dry matter content for local renewable energy purposes.

The oil of winter rapeseed can be used for biodiesel, or human nutrition purposes. Modern cultivars produce seeds with a low glucosinolate and a high protein and oil content, considering these traits, lines that are used in modern applications such as the production of biodiesel or animal nutrition (rapeseed meal) are further introgressed with lines that have genes responsible for glucosinolate content within the leaves and stems. Whole breeding programs are designed to further optimize the selection processes for these specific traits of interest. As selection within a modern population of winter rapeseed is coping with modern and alternative human and animal nutrition related requirements, such as plant diseases, the urge for renewable energy production or the definition of new high yielding crops focusing on a higher biomass production, which are necessary to cope with increasing populations, is needed. From a plant perspective other requirements such as herbivore defense strategies or herbivore induced metabolites that are emitted might play an important

role in attracting or repelling insects. Glucosinolates are sulfur containing secondary components that fulfill such complex biological roles. With this wide field of application and gap in knowledge, the study of the leaf glucosinolate trait remains a challenge in plant breeding.

OBJECTIVES AND RESEARCH QUESTIONS

The objectives of this dissertation are to create an overview of the genetic variation of leaf glucosinolates, their distribution and inheritance. Therefore the total glucosinolate content in samples of intact seeds of *Brassica napus* L. and the potential of individual glucosinolates in leaves stems and seeds is analyzed. With this it is important to investigate also the biological relationship with seed glucosinolates, with a high correlation between seeds and leaves, the seed glucosinolate content could be a useful mean to preselect the material for reduced leaf glucosinolate content in a fast and reliable way.

- To explore and define the genetic basis of the synthesis of glucosinolates in the green matter of oilseed rape and their interactions with pathogens during biogas production, three experiments are simultaneously performed.
- To investigate the genetic variation of the glucosinolate contents and pattern in the vegetative tissue in classical breeding material and resynthesized rapeseed lines. To verify this, several crosses between resynthesized cultivars were tested over several years in several places.
- To acquire information that determines the influence of the glucosinolate content and pattern in the green matter on the biogas production.
- To develop and characterize a quantitative trait loci map based on a mapping population from a cross. To analyse total glucosinolate content in samples of intact seeds of *Brassica napus* L.

Glucosinolates within leaves and stems of winter rapeseed inhibit the bacterial processes within a biogas fermentation unit. In this way the fermentation biology and the methane digesting process is influenced.

The specific objective is to identify the influence of genetics behind the variation expressed in leaf, stem and seed glucosinolates. Results compromise the effect of heterosis. The genotypic value of leaf and stem glucosinolates for their additive and dominant effects is evaluated after hybridization by the means of the general and specific combining ability tests.

What is the genetic variation and relation for leaf stem and seed glucosinolate content within winter rapeseed? Therefore the genetic variation of the leaf, stem and seed glucosinolate content in resynthesized winter rapeseed lines is investigated.
To explore the genetic pattern of crosses between resynthesized lines and modern breeding material, to identify the genetic effects from the environmental effects, testcrosses are approved for their leaf and stem glucosinolates. Last but not least the glucosinolate content of some testcrosses are evaluated and correlated with the methane production in the fermentation unit, this to decide on the relative importance of the glucosinolate trait in relation with bacterial fermenting processes.

How many genes are involved in the glucosinolate regulation process? This study wants to describe the genetic variability for glucosinolate content in a double haploid population derived from the cross 'Sollux x Gaoyou'. The main objective in this part is to investigate the segregation pattern and polymorphisms of the glucosinolate traits in the green mass of winter rapeseed. Does the leaf and stems glucosinolate content influence the methane production during fermentation process?

To acquire information that determines the influence of the glucosinolate content and distribution in the different plant parts on the methane production. In this way a closer look on the suitability of winter rapeseed as an energy crop is made.

Genotype times environmental interactions are evaluated. Lack of correlation of genotype performance across environments would have substantial impact on selection. Resources about the genetics of the glucosinolate content within the leaves and stems are scarcely found.

Focusing on parent-offspring and heterosis effects, breeding values of glucosinolates can be obtained e.g. by application of suitable schemes, such as a factorial design or by crossing parental forms with a varying glucosinolate content. The number of inbred lines tested is higher than that using diallel crosses.

Testcross evaluation of winter rapeseed after one growing cycle helps to estimate the genetic variance for leaf glucosinolates. Normally several cycles of testcrosses are helpful to find the best genotypes, this for low or high leaf glucosinolate content. The number of testcrosses cycles depends on the selection gain and the probability of identifying the best genotypes. The optimum number of selection cycles is related with the genetic variance within and between the crosses or the heritability of the trait.

Crossbreeding is principally applied to exploit breeding complementarities. Usually factorial schemes that are too complicated have been proposed to maximize the

genetic gain, considering both additive and non-additive genetic effects. In this situation a quite simple approach has been applied. The breeding objectives are meant to maximize the heterosis and segregation inside the single breeding populations. The high potential breeding material can then be used for recurrent selection to further function as donor in breeding programs and introgression of desired genes. This is particularly useful when further enhancing genetic progress inside the resulting breeding lines.

2 Genetic variation in leaf and stem glucosinolates in resynthesized lines of winter rapeseed (Brassica napus L.)

Accepted by Genetic Resources and Crop Evolution

ABSTRACT

Glucosinolates are secondary components characteristic for the *Brassicaceae* with complex biological functions. Glucosinolates in the seeds are anti-nutritive when feeding animals and their inheritance have been extensively investigated. Much less is known about the genetics of glucosinolates in leaves and stems, which may attract some insects, while repelling others. They may also inhibit bacterial processes of importance when using green biomass for the production of biogas. The objective of this study was to analyse the genetic variation of total and individual glucosinolates in the green material of rapeseed. For this 28 resynthesized winter rapeseed lines were tested at two locations. There was a large variation in leaf glucosinolate content between 0.10 and 4.75 µmol/g dry matter. The predominant leaf glucosinolates are the alkenyle glucosinolates progoitrin, gluconapin and glucobrassicanapin. The line R53 is exceptional, while combining a relative high content of the indole glucosinolate glucobrassicin with low alkenyle glucosinolates in the leaves. The total glucosinolate concentration in the stems and leaves is not correlated with the seed glucosinolate concentrations. Heritabilities are above $h^2 = 0.60$ for progoitrin, $h^2 = 0.65$ for gluconapin, $h^2 = 0.30$ for glucobrassicanapin and $h^2 = 0.52$ for total glucosinolate content in the leaves. In conclusion, resynthesized rapeseed is an important genetic resource to modify the leaf glucosinolate content and composition of rapeseed.

INTRODUCTION

Glucosinolates with more than one hundred different side chain structures have been described (Mithen 2001). In the *Brassicaceae*, the main groups are the aliphatic or alkenyle glucosinolates (derived from methionine), the phenyl or aromatic glucosinolates (from phenylalanine or tyrosine) and indole glucosinolates (from tryptophane). Depending on structural differences, alkenyle, aromatic and indole glucosinolates produce different toxic end-products after cleavage by the myrosinase enzyme (Fenwick et al. 1983). The alkenyle glucosinolates are dominant in *Brassica* napus L. and are systematically classified as 2-propenyl (sinigrin=SIN), 3-butenyl (gluconapin=GNA), 2-hydroxy-3-butenyl (progoitrin=PRO), 4-pentenyl (glucobrassicanapin=GBN) and 2-hydroxy-4-pentenyl (gluconapoleiferin=GNL) glucosinolates (Mithen 2001), also the aromatic glucosinolate NAS (gluconasturtiin), and GBC, NEO, 4OH is classified; see systematic names in Table 1.

Table 1 Glucosinolates detected in leaves and seeds (adapted from Velasco and Becker 2000)

SYSTEMATIC NAME	TRIVIAL NAME	ABBREVIATION
2-Propenyl	Sinigrin	SIN
2(R) 2-hydroxy-3-butenyl Progoitrin	Progoitrin	PRO
4-pentenyl Glucobrassicanapin	Glucobrassicanapin	GBN
2-hydroxy-4-pentenyl Napoleiferin	Gluconapoleiferin	GNL
3-indolylmethyl Glucobrassicin	Glucobrassicin	GBC
N-methoxy-3indolylmethyl	Neoglucobrassicin	NEO
2-Phenylethyl Gluconasturtiin	Gluconasturtiin	NAS
4-Hydroxy-Glucobrassicin	4OHglucobrassicin	4OH

The genetic variation and inheritance of seed glucosinolates is well known. Compared to this, the knowledge on glucosinolates in leaves and stems is still rather limited. Therefore the objective of this study is to investigate the genetic variation of glucosinolate content and composition in green material of rapeseed. As material, resynthesized rapeseed lines from interspecific hybridization between cabbage (*B. oleracea* L.) and turnip rape (*B. rapa* L.) (Gland et al. 1981) are used, because in such material the maximum amount of genetic variation available in *Brassica napus* L. can be expected.

MATERIAL AND METHODS

Materials

The material consisted of 28 resynthesized lines with very broad genetic background both for the *B*. *oleracea* L. and the *B*. *rapa* L. parent (Table 2). For comparison, the common German winter rapeseed cultivar 'Express' was included as check.

Table 2. Origin of resynthesized winter rapeseed lines used in the field experiments

LINE	MOTHER	FATHER
S3	*B. rapa* L. ssp. *rapa*	*B. oleracea* convar. *acephala* var. *sabellica*
H231	*B. oleracea* L. convar. *capitata* (L.) Alef. var. *capitata* L.	*B. rapa* ssp. p*ekinensis* (Lour.) Hanelt
H327	*B. oleracea* convar. *capitata* var. *capitata*	*B. rapa* ssp. *nipposinica* (Bailey) Hanelt var. *perviridis* Bailey
R75	*B. oleracea* convar. *acephala* (DC.) Alef.	*B. rapa* ssp. *oleifera*
H19	*B. oleracea* convar. *capitata* var. *sabauda* L.	*B. rapa* ssp. *pekinensis*
G35	*B. oleracea* convar. *capitata* var. *sabauda*	*B. rapa* ssp. *oleifera*
H344	*B. oleracea* convar. *capitata* var. *sabauda*	*B. rapa* ssp. *pekinensis*
L122	*B. oleracea.* convar. *capitata* var. *sabauda*	*B. rapa* ssp. *pekinensis*
G2	*B. oleracea* convar. *capitata* var. *sabauda*	*B. rapa* ssp. *oleifera*
R28	*B. oleracea* convar. *capitata* var. *capitata*	*B. rapa* ssp. *oleifera*
H357	*B. oleracea* convar. *capitata* var. *capitata*	*B. rapa* ssp. *pekinensis*
R59	*B. oleracea* convar. *capitata* var. *capitata*	*B. rapa* ssp. *oleifera*
R1	*B. oleracea* convar. *capitata* var. *capitata*	*B. rapa* ssp. *rapa*
R53	*B. oleracea* convar. *capitata* var. *capitata*	*B. rapa* ssp. *pekinensis*
G56	*B. oleracea* convar. *capitata* var. *capitata*	*B. rapa* ssp. *nipposinica* var. *perviridis*
R64	*B. oleracea* convar. *capitata* var. *capitata*	*B. rapa* ssp. *rapa*
R12	*B. oleracea* convar. *capitata* var. *capitata*	*B. rapa* ssp. *pekinensis*
R73	*B. oleracea* convar. *capitata* var. *capitata*	*B. rapa* ssp. *oleifera*
H4	*B. oleracea* convar. *acephala* var. *sabellica* L.	*B. rapa* ssp. *pekinensis* var. *laxa* (Tsen et Lee) Hanelt
L239	*B. oleracea* convar. *gemmifera* (DC.) Gladis	*B. rapa* ssp. × *chinensis* (L.) Hanelt
R19	*B. oleracea* convar. *gemmifera*	*B. rapa* ssp. *oleifera*
H196	*B. oleracea.* convar. *acephala* var. *gongylodes* L.	*B. rapa* ssp. *chinensis*
L341nc	*B. napus* L. ssp. *napus*	*B. rapa.* ssp. *pekinensis*
S14	*B. napus* var. *pabularia* (DC.) Reichb. *x B. oleracea.* convar. *acephala* var. *sabellica*	*B. rapa.* ssp. *oleifera*
S15	*B. napus* × *B. rapa* ssp. *oleifera* (DC.) Metzg.	*B. oleracea* var. *gemmifera* DC.
S16	*B. napus* × *B. rapa* ssp. *oleifera*	*B. oleracea* convar. *acephala* var. *sabellica*
S30	*B. napus* × *B. rapa* ssp. *oleifera*	*B. rapa* ssp. *pekinensis*
S31	*B. oleracea* L. convar. *botrytis* var. *italica* Plenck	*B. rapa* ssp. p*ekinensis*
R19	*B. oleracea* convar. *gemmifera*	*B. rapa* ssp. *oleifera*
H196	*B. oleracea.* convar. *acephala* var. *gongylodes* L.	*B. rapa* ssp. *chinensis*
L341nc	*B. napus* L. ssp. *napus*	*B. rapa.* ssp. *pekinensis*
S14	*B. napus* var. *pabularia* (DC.) Reichb. *x B. oleracea.* convar. *acephala* var. *sabellica*	*B. rapa.* ssp. *oleifera*

Field experiments

The resynthesized lines were sown in two row plots of 2.5 m length with 10 cm plant distance, at two locations, Einbeck and Göttingen in the 2007/2008 season. At beginning of May, the leaves and stems were harvested as random sample of 10 green fresh leaves and stems from each plot, cooled during transport, and dried in an oven at 55 °C (McGregor and Love 1978). At maturity, the pods of 3 open pollinated plants were harvested; more than 100 seeds were stored for further analysis.

Glucosinolate analysis

Glucosinolate profiles of stems, leaves and seeds were analyzed by HPLC (High Pressure Liquid Chromatography). After heating 200 mg of milled material twice for 10 minutes at 75 °C; glucosinolates were extracted and hydroxylated using concentrates of both 70 % and 10 % methanol. After decantation the extract was passed through sephadex micro-columns. After rinsing the columns with 1 ml of water and addition of a sulphatase, these were incubated over night at 40 °C. The desulfo-glucosinolates were eluated by 500 µl of water. An ultraviolet detector (190 - 400 nm) was used for peak detection. Glucosinolates are expressed in µmol/g dry matter (D.M.). For seed meal containing SIN, glucotropaeolin (200µl 6 mM) was used as an internal standard. For leaf and stem material, SIN (200 µl, 6 mM) was used as an internal standard (Spinks et al. 1984). The HPLC analyses were performed at least three times for each sample, and the results were averaged.

Statistical analysis

An analysis of variance was performed with location and genotype as factors. For comparison of glucosinolate content between lines least significant differences (P=0.05) were calculated. The software Plabstat (Utz 1996) was used for all statistical analyses

RESULTS

To explore the genetic resources of the glucosinolates in *Brassica napus* L. a set of plant parts (seeds, leaves and stems) originating from resynthesized lines was analyzed for their glucosinolate content.

Genotype times environmental interaction was investigated for the leaves, stems and seeds of the 28 resynthesized lines (Figure 1).

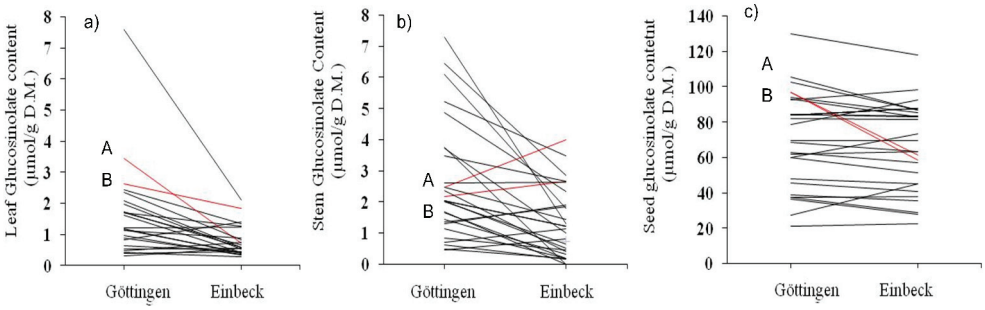

Figure 1 Genotype times environmental interaction for the leaves, stems and seeds (A, B are genotypes marked in red, to exemplify different types of interactions between glucosinolate content and the environment, a) A is performing better in Göttingen as in Einbeck, relation is opposite in Einbeck as in Göttingen b) A has the highest glucosinolate content in Göttingen and Einbeck, c) Glucosinolate content is similar in Göttingen but differs in Einbeck).

The maximum glucosinolate content for the leaves was reached for the cultivar "Sollux" with a glucosinolate value of 9.91 µmol/g D.M., the resynthesized line "S3" also has a high glucosinolate concentration in its leaves with a value of 4.75 µmol/g D.M.

The seed glucosinolates of the resynthesized lines are given in Table 3.

Table 3. Seed glucosinolates for the resynthesized lines

	SEED GLUCOSINOLATES								
	PRO	**SIN**	**GNL**	**GNA**	**GBN**	**GBC**	**4OH**	**NAS**	**SUM**
S3	48.83	1.29	0.57	25.80	1.31	0.23	4.55	0.53	83.22
H231	33.50	1.37	3.93	22.13	4.34	0.10	3.13	0.75	69.33
H327	73.04	2.37	0.51	30.57	4.74	0.21	5.91	0.38	117.89
R75	44.90	0.94	0.15	28.86	3.27	0.35	2.52	0.14	81.32
H19	6.48	0.33	1.76	6.70	0.57	0.19	6.01	0.29	22.40
G35	55.74	0.93	0.38	22.71	2.36	0.08	2.71	0.13	85.15
H344	50.89	2.45	0.52	35.63	2.84	0.17	5.27	0.44	98.35
L122	38.49	0.20	0.26	12.06	7.75	0.06	2.94	1.35	63.50
G2	14.80	3.46	3.61	22.30	2.70	0.12	3.67	0.60	51.42
R28	39.50	2.91	0.12	8.47	3.24	0.16	3.15	0.71	58.46
H357	60.88	0.76	0.46	13.98	4.54	0.03	2.29	0.10	83.06
R59	30.32	0.90	5.79	17.29	1.37	0.27	5.32	0.19	61.59
R1	14.27	0.50	2.74	13.56	3.91	0.33	7.29	2.38	45.30
R53	21.31	0.20	0.98	8.18	2.57	0.79	5.82	0.58	40.75
G56	44.02	1.32	12.45	15.14	1.04	0.33	8.40	0.21	83.08
R64	45.75	1.29	4.41	28.95	3.07	0.22	7.80	0.69	92.30
R12	18.65	0.67	3.23	8.42	0.61	0.15	5.60	0.24	37.74
R73	43.91	2.18	1.42	8.69	2.00	0.08	4.53	0.22	63.10
H4	13.60	2.71	0.27	5.17	1.62	0.45	3.51	0.36	27.88
L239	16.93	0.43	1.34	3.00	0.79	0.31	4.86	0.34	28.55
R19	27.86	0.75	2.80	13.23	3.10	0.20	6.70	1.10	56.01
H196	15.97	1.69	3.33	9.15	0.32	0.04	4.65	0.31	35.50
L341nc	26.00	0.51	0.40	6.76	5.55	0.68	4.52	0.16	45.23
S14	50.96	2.07	1.03	22.23	4.50	0.05	4.77	1.21	86.92
S15	53.66	1.61	0.37	25.50	1.65	0.12	4.05	0.58	87.74
S16	53.00	1.14	0.56	24.80	2.79	0.26	3.97	0.25	86.90
S30	33.91	1.20	0.40	8.96	6.39	0.16	5.26	0.57	56.96
S31	43.76	1.79	0.41	20.53	1.34	0.52	4.48	0.52	73.46
MEAN	35.61	1.35	2.11	16.58	2.79	0.23	4.80	0.56	64.23
VALUES									
EXPRESS	8.61	0.32	0.07	2.71	5.13	1.68	0.40	0.46	19.57
LSD 0.05	14.89	1.90	1.85	8.97	3.51	0.46	3.29	0.87	23.12
MINIMUM	6.48	0.20	0.12	3.00	0.32	0.03	2.29	0.10	22.40
MAXIMUM	73.04	3.46	12.45	35.63	7.75	0.79	8.40	2.38	117.89

The results for leaves and stem are given in Table 4.

Table 4. Leaf and stem glucosinolate content (in µmol/g D.M.) in *Brassica napus*

	LEAF							STEM						
LINE	**PRO**	**GNL**	**GNA**	**GBN**	**GBC**	**NAS**	**SUM**	**PRO**	**GNL**	**GNA**	**GBN**	**GBC**	**NAS**	**SUM**
S3	2.00	0.00	1.65	0.75	0.11	0.25	4.75	3.28	0.03	0.57	0.19	0.05	0.17	4.28
H231	0.18	0.38	0.01	0.04	0.11	0.11	0.83	0.13	0.45	0.11	0.04	0.11	0.11	0.95
H327	0.30	0.12	0.48	0.42	0.28	0.42	2.02	0.98	0.35	0.93	0.42	0.21	0.21	3.10
R75	0.14	0.32	0.25	0.22	0.09	0.10	1.12	0.63	0.21	0.16	0.22	0.17	0.17	1.56
G2	0.13	0.14	0.00	0.03	0.04	0.00	0.34	0.79	0.27	0.09	0.35	0.16	0.30	1.94
G35	0.06	0.31	0.00	0.02	0.01	0.00	0.41	0.45	0.27	0.12	0.27	0.04	0.10	1.24
H344	0.24	0.04	0.37	0.39	0.04	0.37	1.44	1.43	0.00	0.51	0.70	0.10	0.23	2.97
L122	0.46	0.01	0.15	0.42	0.03	0.15	1.22	1.93	0.03	0.24	1.01	0.18	0.23	3.61
R28	0.43	0.05	0.22	0.24	0.31	0.17	1.42	1.05	0.02	0.32	0.56	0.08	0.51	2.53
H357	0.10	0.03	0.05	0.59	0.30	0.05	1.12	0.23	0.25	0.03	0.09	0.12	0.16	0.87
R59	0.24	0.00	0.81	0.71	0.05	0.81	2.62	0.55	0.00	0.38	0.36	0.06	0.29	1.63
R1	0.20	0.37	0.27	0.23	0.06	0.27	1.40	0.82	0.33	0.51	0.73	0.36	0.42	3.16
R53	0.09	0.00	0.02	0.05	0.31	0.02	0.50	0.15	0.00	0.00	0.03	0.29	0.45	0.92
G56	0.43	0.00	0.19	0.14	0.09	0.19	1.05	3.50	0.01	0.21	0.57	0.16	0.20	4.64
R64	0.11	0.28	0.11	0.04	0.02	0.11	0.67	0.47	0.00	0.08	0.13	0.06	0.18	0.91
R73	0.19	0.29	0.09	0.00	0.06	0.09	0.72	0.64	0.33	0.08	0.08	0.13	0.09	1.34
S30	0.17	0.03	0.06	0.02	0.03	0.06	0.38	0.88	0.00	0.29	0.15	0.14	0.17	1.61
R12	0.13	0.00	0.00	0.05	0.03	0.00	0.21	0.11	0.00	0.03	0.06	0.04	0.07	0.31
H4	0.07	0.00	0.01	0.00	0.01	0.01	0.10	0.19	0.00	0.07	0.05	0.04	0.05	0.39
H19	0.08	0.00	0.01	0.16	0.03	0.01	0.30	0.08	0.26	0.01	0.04	0.10	0.18	0.65
L239	0.15	0.00	0.05	0.08	0.08	0.05	0.40	1.09	0.02	0.08	0.19	0.08	0.15	1.60
R19	0.38	0.00	0.21	0.18	0.04	0.21	1.04	0.61	0.00	0.05	0.25	0.11	0.31	1.32
H196	0.13	0.02	0.04	0.00	0.10	0.04	0.32	0.28	0.06	0.06	0.04	0.10	0.09	0.61
L341nc	0.13	0.00	0.08	0.01	0.03	0.08	0.34	0.38	0.00	0.02	0.11	0.08	0.09	0.66
S31	0.16	0.03	0.17	0.09	0.09	0.17	0.70	1.14	0.00	0.56	0.28	0.16	0.28	2.41
S16	0.20	0.45	0.02	0.10	0.07	0.02	0.87	1.93	0.34	0.38	1.03	0.13	0.54	4.33
S14	0.63	0.34	0.23	0.81	0.07	0.23	2.31	2.11	0.36	0.27	0.89	0.11	0.20	3.93
S15	0.27	0.39	0.19	0.14	0.04	0.11	1.14	1.31	0.33	0.30	0.16	0.06	0.20	2.35
MEAN	0.28	0.13	0.21	0.21	0.09	0.15	1.06	0.97	0.14	0.23	0.32	0.12	0.22	1.99
EXPRESS	0.27	0.06	0.15	0.40	0.07	0.08	1.13	0.30	0.00	0.17	0.19	0.05	0.16	0.92
LSD 0.05	0.67	0.42	0.57	0.59	0.29	0.46	1.84	1.77	0.41	0.40	0.44	0.11	0.26	2.47
MINIMUM	0.06	0.00	0.00	0.00	0.01	0.00	0.10	0.08	0.00	0.00	0.03	0.04	0.05	0.31
MAXIMUM	2.00	0.45	1.65	0.81	0.31	0.81	4.75	3.50	0.45	0.93	1.03	0.36	0.54	4.64

The mean level of total glucosinolates in the seeds is 64.23 µmol/g D.M. and for the leaves 1.06 µmol/g D.M., and stems 1.99 µmol/g D.M. The dominant glucosinolates belong to the alkenyles (PRO, GBN, GNL and GNA) in seeds as well as in stems and leaves, SIN and 4OH are only present in the seeds. Total leaf glucosinolate values range from 0.10-4.75 µmol/g D.M. Alkenyles are the most dominant glucosinolate group in the seeds (70-80%) followed by the indole glucosinolate GBC (10%) and the phenyl type NAS (10%). Leaves and stems have dominant concentrations of PRO and GNA. In the leaves the most prevalent individual

glucosinolate was PRO (0.06 - 2.00 µmol/g D.M.) followed by GBN (0.00 - 0.81 µmol/g D.M). NAS was the major glucosinolate type in the phenyl group (0.00 – 0.81 µmol/g D.M.). The indole group was dominated by GBC (0.01-0.31µmol/g D.M.).

The genotype S3 has the highest content of leaf glucosinolates associated with high seed glucosinolate content. Least significant differences showed in the leaves of S3 significantly higher total glucosinolate content and levels of PRO and GNA compared with the rest of the resynthesized lines. H4 has the lowest leaf glucosinolate content; in this line alkenyle glucosinolates are almost absent. H327 has the highest seed glucosinolate content, whereas H19 had the lowest seed glucosinolate content. This corresponds both with a high and low leaf glucosinolate content respectively 2.02 µmol/g D.M. and 0.30 µmol/ D.M. The line R53 combines a very low leaf alkenyle content (PRO, GNA, GNL, GBN) and high leaf indole (GBC) glucosinolate content Express is the standard cultivar chosen for comparison with the resynthesized rapeseed lines. Express has the lowest seed glucosinolate content, but average leaf glucosinolate content.

An analysis of variance for leaves, stems and seeds shows highly significant differences for total glucosinolates among locations and genotypes (Table 5).

Table 5. Mean squares of the analysis of variance for glucosinolate content (µmol/g D.M.)

MATERIAL	DF	PRO	SIN	GNL	GNA	4-OH	GBN	GBC	NAS	SUM
LEAVES (n=28)										
GENOTYPE (G)	27	0.27*	/	0.05	0.22**	/	0.12	0.02	0.06	10.77**
LOCATION (L)	1	0.89**	/	0.71**	0.67**	/	0.99**	0.04	0.05	1.67*
G X L	27	0.11	/	0.04	0.08	/	0.08	0.02	0.05	
h^2		0.60	/	0.19	0.65	/	0.30	0.00	0.21	0.52
STEMS (n=25)										
GENOTYPE	24	1.58*	/	0.05	0.1**	/	0.19**	0.01**	0.03*	3.43*
LOCATION	1	8.17**	/	0.66**	0.05	/	0.04	0.018*	0.01	19.06**
G X L	24	0.74	/	0.04	0.04	/	75.91	74.21	51.57	1.44
h^2		0.53	/	0.21	0.62		0.76	0.74	0.52	0.58
SEEDS (n=28)										
GENOTYPE	27	575.05**	2.3*	18.76**	159.83**	5.5*	7.67*	0.08	0.52**	1204**
LOCATION	1	178.43*	0.15	0.16	21.98	13.77*	0.67	0.08	1.04*	571*
G X L	27	51.80	0.84	0.80	18.81	2.52	2.88	0.05	0.18	125
h^2		0.91	0.63	0.96	0.88	0.54	0.62	0.35	0.65	0.90

Depending on the genotype the level of PRO and GNA varies significantly in the leaves and the stems. In the stem, also GBN, GBC, and NAS show significant genotypic differences. In the seeds, for all glucosinolates except GBC significant genotypic variance was observed. The heritability estimates are high for total and major glucosinolate types of the alkenyles group (PRO, GNA, GBN) within the leaves and stems (Table 5). For total glucosinolate content heritability is very high for seeds (h^2= 0.90), and lower for leaves (h^2 = 52) and stems (h^2 = 0.58).

The correlation between the different leaf glucosinolate types are significant for GNA, PRO (0.87**) and GNA, GBN (0.69**), which are alkenyle glucosinolates related with each other (table 6). The minor glucosinolate types NAS and GBC, belong to the aromatic and indole glucosinolate groups are also significantly correlated [0.76**]. However significant correlations between the main indole glucosinolate type (GBC) and alkenyle types are absent figure 2.

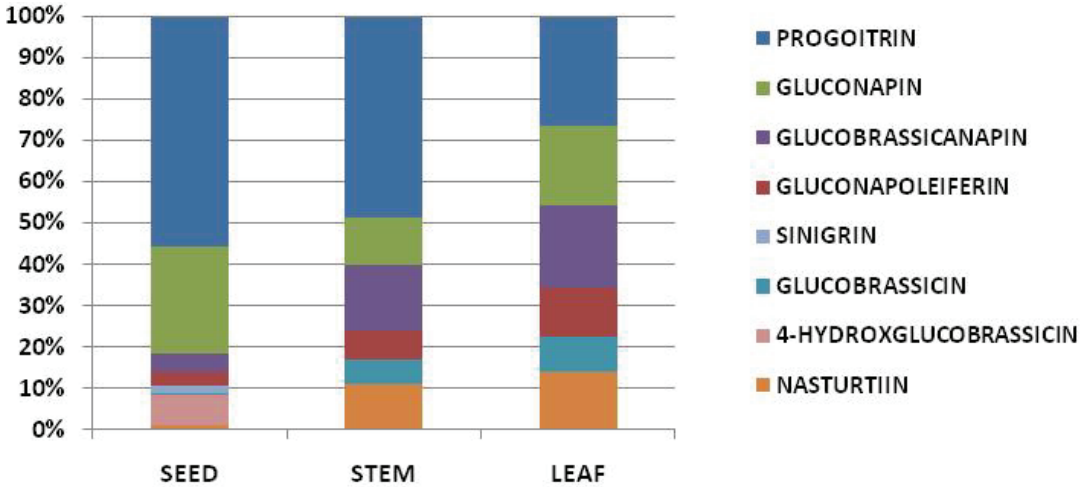

Figure 2. Relative distribution of different glucosinolate types within the leaves, stems and seeds

Table 6. Phenotypical correlation coefficients for leaf glucosinolate types

r^2	PRO	GNL	GNA	GBN	GBC	NAS
GNL	-0.13					
GNA	0.87**	-0.17				
GBN	0.60	-0.09	0.69**			
GBC	0.08	-0.16	0.09	0.24		
NAS	0.00	-0.08	0.04	0.25	0.76**	
SUM	0.86**	0.00	0.88**	0.82**	0.36	0.36

*,** significant at p = 0.05, p = 0.01

A clear difference between glucosinolate types within the seeds stems as well as in the leaves is observed. A relative increasing amount of PRO for the leaves, stems as in the seeds are observed (24% in the leaves, 48% in the stems and 56% in the seeds). GNL contributes in a smaller amount for the total glucosinolate content, with values from 11% in the leaves, 7% in the stems and 3% in the seeds. The same for GBN (leaves=18%, stems=16% and seeds 4%) and the indole glucosinolate GBC (8% in the leaves, 6% in the stems and almost absent in the seeds).

Highly significant (p = 0.01) correlations are found between the content of glucosinolates in the stems and leaves (r^2=0.65), Figure 3.

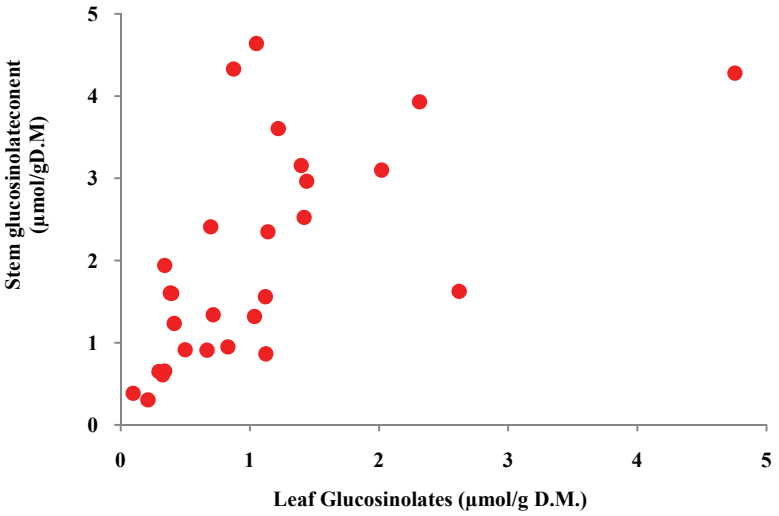

Figure 3. Total glucosinolate content in the leaves and the stems of 28 resynthesized lines

Lower correlations were observed between seed glucosinolate content and glucosinolates in stems (r^2= 0.47) and leaves (r^2=0.39), Figure 4.

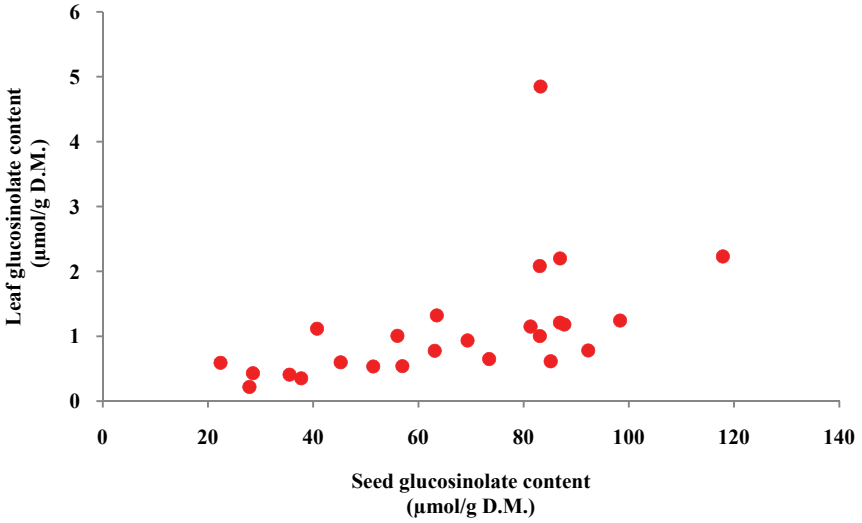

Figure 4. Total glucosinolate content in the seeds and leaves of 28 resynthesized rapeseed lines

DISCUSSION

Glucosinolate content in leaves and stems is low in comparison with the content in seeds. Also while the leaves are quite fragile material, which differ within the season, within and between the years, during their development, the amount of seed glucosinolates is higher. Major glucosinolate types were not always present in the different plant organs.

Jürges (1982) who did a comparable research on winter rapeseed cultivars before flowering measured leaf concentrations ranging from 1.0- 15.5 µmol/g D.M. According to Clossais-Besnard and Larher (1991), the concentration of glucosinolates in dry seeds is about five to ten times higher as in stems and leaves; however this is not always the case (Mithen 2004). Therefore manipulation of 0 and 00 lines with low and high seed glucosinolate content independently from the leaf glucosinolates (Mithen 2004) is rather challenging. Except for a daily and environmental variation (Rosa 1997), distribution of the glucosinolates varies depending on plant part, with both quantitative and qualitative differences among leaves, stems and seeds (Velasco et al. 2007). A low (< 4.8µmol/ g D.M.) total glucosinolate content in the leaves of winter rapeseed is observed.

A further explanation for the low glucosinolate content in the leaves and stems in comparison with the seeds could be found in the dilution of glucosinolates during plant growth (Clossais-Besnard and Larher 1991). This starts already after germination, where a mixture of enzymatic reactions causes the further turn-over of glucosinolates. Because of the existence of seed-specific glucosinolates, it is suggested that vegetative parts mainly provide precursors and that the final steps for glucosinolate synthesis occur in the seed (Clossais-Besnard and Larher 1991). Secondly while the tissue in the seeds is morphologically protected, a lower decomposition of instable glucosinolates types due to environmental reasons in the seeds as in the green material is caused. Thirdly a possible explanation could be differences in transport between the different plant organs. Transport properties of glucosinolates within *Brassica napus* L. are of interest as identification of the mechanism leading to lower levels obtained in specific tissues such as seeds (Brudenell et al. 1999). This is particularly observed for PRO, which is highest in the seeds and leaves of *Brassica napus* L.

The correlation between total seed and leaf glucosinolates is absent this is most probable caused by differences in biochemical reactions by different gene actions in the tissue of the green material as in the seeds. Seed glucosinolate concentrations cannot be used for indirectly determining the concentration of the glucosinolates in the leaves. A triangle shaped plot is formed in figure 3. This means that low seed

glucosinolate lines have low leaf glucosinolate content. High seed glucosinolate lines may have low or high leaf glucosinolate content.
Concerning the total amount of glucosinolates in the different plant tissues the correlations show a large heterogeneity of the concentrations of glucosinolates in the stems, leaves and seeds of the plants. For the relation of the total glucosinolate contents in the different plant organs, earlier articles on the presence or absence of correlations are until now rather contradictory (Jürges 1982). It is even suggested that weak correlations between seed and leaf glucosinolates content might be caused by the dependence of leaf glucosinolate content on environmental effects and growing stage (Schilling and Friedt 1991). This means that the concentrations of the alkenyle glucosinolates in the seeds cannot be used for indirectly determining the concentration of the glucosinolates in the leaves.

The study describes the genetic variation of alkenyle glucosinolates, indoles and phenyl glucosinolates occurring in low but measurable quantities. Genetic variation in leaves and stems of rapeseed is high for alkenyle glucosinolate types (PRO, GNA and GBN). Glucosinolate variability has been observed within leaves of the *Brassicaceae*, which are distinct for their alkenyle glucosinolate composition. An assumption is that a difference in gene action causes this methionine side chain elongation. This makes it possible to detail further for gene controlled variation in leaves within *Brassica napus* L. (Kroymann et al. 2000). In leaves of *Brassica napus* L., this is expressed in significant correlated levels of PRO and GNA (Gland et al. 1981). GBC, which is synthesized from tryptophane (Kutácek and Králová 1971) is the indole glucosinolate with the highest level. However, with levels lower than 0.5 µmol/g D.M, certain genotypes express higher levels as others. The causes of high glucobrassicin levels are possibly enzymatic and absence can be explained by a genetic block for direct glucosinolate synthesis from tryptophane (Kutácek and Králová 1971). Gluconasturtiin originating from phenyl glucosinolate is recognized too, as a minor glucosinolate form (Underhill 1965).

The role of glucosinolates within plant parts different from the seeds might therefore exhibit an influence on the competitive ability of the plant. Besides modern applications, such as the production of biogas indicates that there might be other fields of applications. Agronomic traits like seed yield, biomass and insect resistance requires simultaneous analysis of physiological components such as glucosinolates.

The resynthesized material of this study is however to be expected inferior to all other breeding material. Further assumptions would be that a selection on this unadapted material towards high biomass yielding cultivars, in which a high seed oil yield and other traditional important rapeseed seed quantity and quality traits have to be adjusted for an improved leaf composition.

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3 Factorial crosses of winter rapeseed (*Brassica napus* L.) to predict combining ability and heterosis of leaf glucosinolates

ABSTRACT

Glucosinolates are secondary components, which appear in the leaves, stems and seeds of winter rapeseed (*Brassica napus* L.). Except for maintaining a high genetic variation, the crop is useful in renewable energy and insect resistance applications. Until now, little is known about the general and specific combining ability of glucosinolate levels in the leaves and stems of winter rapeseed. Seven male sterile lines from a breeding company were test-crossed with 16 different resynthesized winter rapeseed lines to develop hybrids. The field experiments were located at two sites nearby Göttingen and Einbeck. The leaf glucosinolate content of the resynthesized lines (L239, H196, S3 and S14) ranged from 0.40 till 4.75 µmol/g dry matter. Five mother and 4 resynthesized father lines where tested for their general and specific combining ability effects. The cytoplasmatic male sterile lines ranged from 0.56 till 2.59 µmol/g dry matter. Glucosinolate levels of the crosses ranged between 0.30 and 3.53 µmol/g dry matter. The parental values were not significantly correlated with the general combining abilities, also the heterosis and the crosses were not significant correlated. The variance component of the specific combining ability (0.32) is remarkably higher than the variance components of the general combining abilities of the mothers (0.02) and of the fathers (0.12). This opens up perspectives towards a successful selection of cultivars with an altered glucosinolate profile in the leaves of *Brassica napus* L.

INTRODUCTION

Glucosinolates appear in sixteen families of dicotyledonous angiosperms. Glucosinolates are sulfur containing glycosides occurring in *cruceriferous* crops (Fahey et al. 2001). Complete glucosinolate profile and content information for leaf glucosinolates in breeding of winter rapeseed is scarce. The breeding of rapeseed for differentiated leaf glucosinolate content requires a broad genetic material. Nine different glucosinolate types are analyzed within the leaves of resynthesized lines of *Brassica napus* L. Currently winter rapeseed breeding has focused on reducing glucosinolate levels in the seeds. Complete glucosinolate profile and content information for leaf glucosinolates in breeding of winter rapeseed is rather scarce. Besides genetic influences, glucosinolates content in rapeseed is also influenced by environmental effects. As renewable energy is a valuable alternative energy resource, crop improvement with a focus on leaf glucosinolates offers prospects. Plant organs, such as leaves and stems are in this case of specific interest. Resynthesized winter rapeseed lines are known for their high genotypic variation. This variation is caused by the genetic improvement, based on the use of genetic variability from the interspecific cross between cabbage (*B. oleracea* L.) and turnip rapeseed (*B. rapa* L.).Though there is limited information, glucosinolates, more specifically their decomposition products might inhibit the production of biogas due to inhibiting effects on the bacteria. The glucosinolate-myrosinase system is known for its defense, caused by the production of isothiocyanates, thiocyanates and nitriles after glucosinolate hydrolysis. Resynthesized rapeseed is a valuable source for broadening the genetic base of rapeseed breeding (Becker et al. 1995). The specific objective is to determine the glucosinolate content of the stem and leaf glucosinolate values of crosses made between cultivars with a high biomass and resynthesized lines with a high genetic variation for the glucosinolate content. The closed related material is used and its resemblance is measured directly. The crosses between resynthesized lines and high biomass yielding cultivars are adequate material. This variation is based on the use of genetic variability from the interspecific cross between cabbage (*B. oleracea* L.) and turnip rapeseed (*B. rapa* L.). Maintaining genetic variation is the ultimate scope, a good start is given by observing small families of the crosses. Small families of winter rapeseed crosses are also a recommendation for breeders. In this way selection intensity is lowered and better controlled. This can help optimize selection in such a way, that traits such as leaf or seed glucosinolate content of hybrid breeding lines are higher or lower leveled. Combining ability estimates are useful for identifying superior parents for cultivar development (Fehr 1987). The general performance of a parent in different crosses would allow one to make a hybrid with desirable and predictable glucosinolate content. The number of inbred lines tested is higher than that using diallel crosses. However, selection of testers differentiating the analyzed inbred lines in respect of the interested trait; in this case leaf glucosinolates is often

a large problem (Bocianowski et al. 2009). Factorial crosses are principally useful to exploit the complementarity of the cross. Specifically, the high biomass lines are crossed with resynthesized lines to combine the high biomass productivity of the former with a large variation of glucosinolates in leaves of resynthesized lines.

Heterosis is a common phenomenon in organisms. Utilization of heterosis has become a major strategy for increasing productivity of crops. Generally, hybrids from two parents with a distant genetic background have high heterosis (Shen et al. 2006). Heterosis is used in all winter rapeseed hybrid breeding schemes except for the line breeding. Prerequisite for heterosis is the dominant inheritance of useful genes and the existence of these useful genes in both parental lines. Inbreeding effects and heterosis in rapeseed can be detected from the performance of the test crosses. In this study heterosis is referred to the increase in glucosinolate content. Heterosis is used in practical breeding for a wide spectrum of winter rapeseed through the production of hybrid varieties (Radoev 2007). Crossbreeding is principally applied to exploit breeding complementarities. In this situation a quite simple factorial crossing scheme has been applied to maximize the heterosis and looking for segregation inside the single breeding populations. The high potential breeding material can then be used for recurrent selection to further function as donor in breeding programs and introgression of desired genes. This is particularly useful when further enhancing genetic progress inside the resulting breeding lines.

MATERIAL AND METHODS

Field experiment

The test crosses from the resynthesized parent lines and male sterile lines were grown in a factorial design. Leaves and stems of the F1 plants were harvested with the objective to estimate the influence of the crosses on the different glucosinolate groups and types inside the winter rapeseed lines. Both parental lines and hybrids were tested in a one year trial at two locations. The term testcross hybrid is used to determine the F1 hybrid between the test crosses. Testcross evaluation of winter rapeseed after one growing cycle helps to estimate the genetic variance for leaf glucosinolates. Factorial crosses between four resynthesized parental lines and five sterile male lines were used. One cross is missing; missing value was estimated (figure 1). Because of a lack of seeds, the mother line h605800 was not analyzed for its leaf glucosinolate content.

Further 16 resynthesized lines (L122, H196, H231, H327, R75, H344, L239, R53, R59, R64, R73, S14, S3, S30, S31 and S4) from bag-isolated selfing seeds were grown.

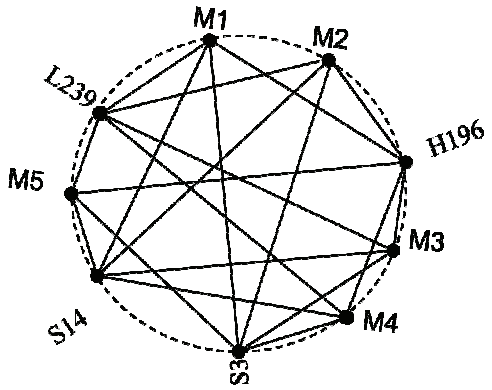

Figure 1 20 Factorial Crosses: 5 male sterile mothers x 4 fathers

Laboratory analysis

The leaf and stem samples were harvested in early spring season, cooled during transport and these samples were dried overnight at a temperature of 55°C. High Pressure liquid Chromatography with an UV detector was used for determining the glucosinolate content.

Statistical analysis

Treatments were the four x five factorial arrangements of the genotypes across two environments. The two factors were mothers and fathers of the crosses. The experiments are factorial including the same objective, namely the acquisition of genetic information about the parental crosses and on the leaf glucosinolate trait, in this case the effect of the father and mother on the cross. General (GCA) and specific combining abilities (SCA) were determined with an ANOVA done among hybrid combinations across all environments. A combined analysis was run based on the model: $Y_{ijk} = \mu + l_i + m_j + f_k + mf_{jk}$ with Yijk = observation of cross jk at location i, μ = general mean, l_i = effect of location i, m_j = effect of mother j, f_k = effect of father k, mf_{jk} = effect of the cross between mother j and father k.

RESULTS

Test crosses

Total seed glucosinolate content of the parent mother lines varied from 14.09 - 85.12 µmol/g D.M, having an average value of 43.53 µmol/g D.M. The major glucosinolate types in the mother lines are progoitrin (PRO), gluconapin (GNA) and glucobrassicanapin (GBN). The minor glucosinolate types are gluconapoleiferin (GNL), 4-hydroxyglucobrassicin (4OH), glucobrassicin (GBC),

4-Methoxyglucobrassicin (4-ME) and neo-glucobrassicin (NEO). The range of the glucosinolate content of the parental material is relatively high [0.32-4.75 µmol/g D.M.]. The cross of the mother line H605800 with R12 had the lowest leaf glucosinolate value (1.05µmol/g D.M.), whereas the cross with R75 had the highest leaf glucosinolate value (6.46 µmol/g D.M.). Similar values were reached for the stems. The cross with R75 reached a content of 10 till 14 µmol/g D.M. The lowest value was reached for H19 with a value of 1.54 µmol/g D.M. The higher values were mainly dominated by the main glucosinolate types, PRO [0.44-1.82], GNA [0.10-0.76] and GBN [0.20-3.07]. The cross of 1046-95-1aams with S3 (3.53µmol/g D.M.) and H196 (0.30µmol/g D.M.) had the highest and lowest glucosinolate content.
The leaf glucosinolate content of the testcrosses deviated from the original glucosinolate content of the parental lines. The mother line 1231-199-3cams had higher values for the glucosinolate types progoitrin (PRO), gluconapin (GNA) and glucobrassicanapin (GBN). Nasturtiin (NAS) has the highest value in the line 2097-95-Ams.

Combining ability comparison among different crosses based on the factorial design

Testcrosses are meaningful to determine the general combining abilities. Together with the specific combining ability theses coefficients serve as valuable breeding tools. Results represent main parental effects and are average values from two locations.

In general the father lines affected the glucosinolate content of the cross. The average mean value of 1231-1993 cams was similar with the other mother lines. Within the crosses there is a high difference. L239 crosses had a higher overall leaf glucosinolate content. The specific combining ability for the 5 mother lines and the 4 father lines is tabulated below (table 1).

Table 1 Variance components of the leaf glucosinolates

Source	DF	Mean squares	Var. Components	F-value
Locations	1	3.03	0.1	2.71
General Combining Ability Mothers	4	1.31	0.02	1.17
General Combining Ability Fathers	3	2.36	0.12	2.11
Specific Combining Ability	11	1.76	0.32	1.57
Rest	15	1.12	1.12	

The variance components of the mothers (0.02) and the fathers (0.12) were remarkably lower as the specific combining ability (0.32). Correlation coefficients of the general combining ability of the leaf glucosinolates versus the parental value (r^2=0.30) and for the crosses versus the mid-parent value (r^2=0.27) were n.s.

Correlation coefficients of the general combining ability of the leaf glucosinolates versus the parental value (r^2=0.30) and for the crosses versus the mid-parent value (r^2=0.27) were n.s.

Heterosis effects

Figure 2 shows 20 testcrosses with the highest leaf glucosinolates at the two locations, in Göttingen and in Einbeck. Overall heterosis ranged from -1.37 till 2.39µmol/g D.M. The crosses with the highest glucosinolate values are those with the resynthesized line S3 and L239. 1231-199-3cams was the mother line, which had the respective highest total leaf glucosinolate value, followed by 1108-102-2ms. Dominant glucosinolate types are PRO, GBN and GNA. The cross between L239 and the respective mother lines has an overall high positive heterosis effect [0.04-2.39 µmol/g D.M].

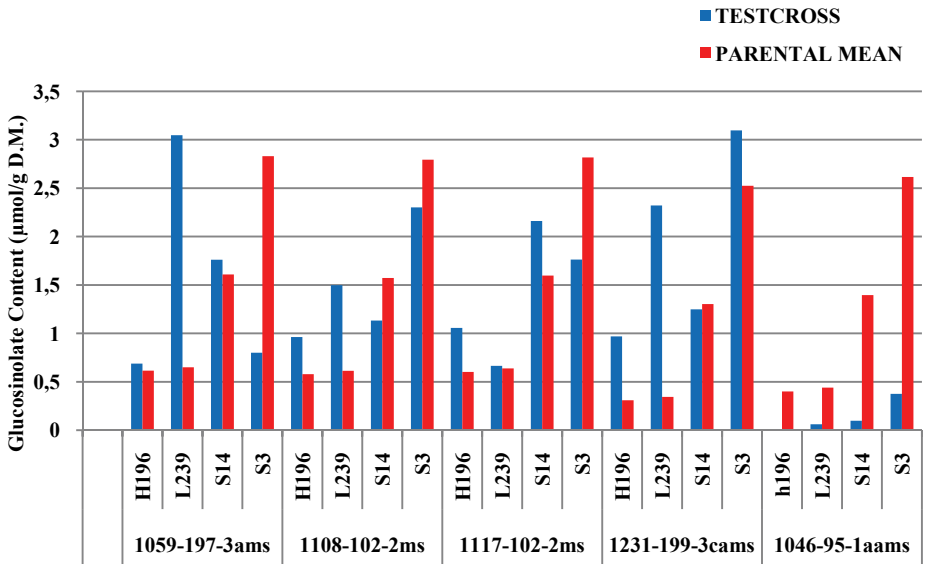

Figure 2. 20 Hybrids (male sterile lines x resynthesized lines), Einbeck, Göttingen
The hybrids between the resynthesized and the adapted high biomass winter rapeseed lines exhibit high heterosis levels for the glucosinolate content.

DISCUSSION

The mother lines chosen were high biomass yielding cultivars, not specifically selected for differentiating glucosinolate contents within their leaves. By comparing their glucosinolate levels within the leaves following conclusions can be drawn.

The parental mean, together with the heterosis effects exhibited large differences between the offspring and the mid-parent mean value, this exquisitely for the line S3, L239 and R53. The crossing experiment helps to see in which crosses the glucosinolate values are the highest.

The testcrosses show that there is a large variation for the leaf glucosinolate content at both locations. By this it is possible to determine the lines with high or low leaf glucosinolate content. The analysis of factorial crosses helps to understand the process of hybridizing. PRO, GNA and GBN are the dominant aliphatic glucosinolate types within the leaves.

Crosses with high and low leaf glucosinolate content resynthesized lines are tested for their combining effects. Specific combining abilities are higher as general combining abilities. The specific combining ability which is higher as the general combining ability offers perspectives to find eligible selection towards specific crosses with according towards the application, suited glucosinolate profiles in its leaves.

Further if a strong correlation between hybrid performance and general combining ability exists it is possible to predict hybrid performance from GCA effects (Qian et al. 2009). While in the case of the leaf glucosinolates n.s. Pearson correlation coefficients were found, this is not possible.

This process, whereof heterosis is a resulting effect, requires the occurrence of differences in alleles of the same gene. The original parental lines differed from the crosses in their total and individual glucosinolate content. The hybrids between the resynthesized and the adapted high biomass winter rapeseed lines exhibit relative high heterosis levels for the leaf glucosinolate content.

ACKNOWLEDGMENTS

The results are the outcome of a project funded by the German Federal Environmental Foundation. The authors express their gratitude to the KWS SAAT AG for establishing the field experiments.

4 Mapping quantitative trait loci that are responsible for the glucosinolate content in leaves of winter rapeseed (*Brassica napus* L.)

ABSTRACT

Leaf glucosinolates are secondary components, which occur in *Brassica napus* L. Glucosinolates might inhibit bacterial growth during fermentation and decrease methane production. In this experiment, 56 lines of the cross Sollux times Gaoyou were grown and analyzed in detail for their leaf glucosinolate to represent the segregation pattern of the glucosinolates. Less is known about quantitative trait loci of this leaf glucosinolate content. A map was constructed, compromising of 133 markers revealing the exact location of the QTL (Quantitative Trait Loci). These QTL are involved in glucosinolate biosynthesis. The markers are distributed on 19 linkage groups covering 1441cm. The linkage map is created to localize QTL for leaf glucosinolate content by interval mapping. QTL mapping of low and high total glucosinolate content is of special interest to learn more about the processes that control the glucosinolate content in the leaves of *Brassica napus* L.

INTRODUCTION

Glucosinolates and their breakdown products have been recognized for their effects on plant defense, human health, flavor and taste of *cruciferous* vegetables. Despite this importance, little is known about the regulation of the biosynthesis and degradation inside the plants (Lou et al. 2008). In *Brassica napus* L. glucosinolates are known for their toxic products that are produced after cleavage by the myrosinase enzyme that occurs in combination with the glucosinolates. The latest step after development of low seed glucosinolate cultivars was the development of mostly low indole glucosinolate content within the seeds (Chavadej et al. 1994). The myrosinase-glucosinolate defense system is known for the reactive products against insects (isothiocyanates, nitriles and thiocyanates) and fungal diseases (*Phoma lingam).* The gene family of seed glucosinolates is mapped previously by Uzunova et al. (1995) and Gül (2002). The construction of a dendrogram can help to specify the specific gene families. Important steps in the bioenzymic pathways that lead to glucosinolates are side-chain elongation and respective modification (Kliebenstein et al. 2001). For example until now little is known about the genes encoding the other types of glucosinolates such as the indoles. It is expected that quantitative trait loci are identified.

Intensive studies of the *Brassica* family and glucosinolate gene families were previously performed on Arabidopsis. Genetic polymorphism and loss-of-function mutations have been identified in other *Brassicaceae* (*Arabidopsis thaliana*). Whole genome associations are important for agronomical important traits in winter rapeseed. The collections have been genotyped and were evaluated for phenotypic traits in field trials over two locations.

QTL (Quantitative Trait Loci) mapping in segregating populations from biparental double haploid crosses is a well known breeding tool. Breeding of winter rapeseed, which intends to reduce glucosinolates in the leaves and stems, requires the knowledge on the location and presence of their respective genes in the genome. The mapping of quantitative traits is attributed to the interactions between two or more genes and their environment, the degree of association of a specific region on the genome to the inheritance of the trait of interest, including or excluding epistatic effects. How close two genes are situated beside each other is described by the use of a linkage map. The use of genetic marker information for mapping quantitative trait loci depends on assumptions about the distribution of the quantitative trait values (Doerge and Rebai 1996). Segregating double haploid populations, which have been quantified for leaf glucosinolates are rarely described. With the results of this study it is possible to conclude which marker based selection is helpful in revealing genetic

variation in the leaves of winter rapeseed. Identification of QTL (Quantitative trait loci), which are responsible for lines with high or low leaf glucosinolate content could further increase the efficiency of a marker-assisted selection (MAS) and enhance in this way genetic progress in detecting genes responsible for the glucosinolate content in the leaves.

MATERIALS AND METHODS

Glucosinolate analysis

The green material was freeze-dried and the samples were milled using liquid nitrogen, the glucosinolate profiles of stems, leaves and seeds were analyzed by HPLC (High Pressure Liquid Chromatography). After heating 200 mg of milled material twice for 10 minutes at 75°C; glucosinolates were extracted and hydroxylated using concentrates of both 70% and 10% methanol. After decantation the extract was passed through sephadex micro-columns. After rinsing the columns with 1 ml of water and addition of a sulphatase, these were incubated over night at 40 °C. The desulfo-glucosinolates were eluated by 500µl of water. An ultraviolet detector (190 - 400 nm) was used for peak detection. Glucosinolates are expressed in µmol/g dry matter (D.M.). For seed meal containing sinigrin, glucotropaeolin (200µl 6mM) was used as an internal standard. For leaf material, sinigrin (200µl, 6mM) was used as an internal standard (Spinks et al. 1984).

Linkage map construction

The construction of a linkage map requires a Mendelian segregating plant population, such as a double haploid population. Double haploid lines (DH) are desirable for genetic studies because fixed genotypes can be propagated indefinitely by sexual means. DNA markers (133) are accepted as potentially valuable tools for crop improvement in winter rapeseed. The technical method of how DNA markers are generated is not discussed, however the linkage map indicate the possible position and distance between markers and the most important use of linkage maps is to identify chromosomal locations containing genes and QTLs associated with traits of interest. Such maps may then be referred to as ‘QTL’. LOD (logarithm (base 10) of odds) scores are feasible to marker analyse. Reliable markers require accurate phenotypic data, different genetic backgrounds and independent verification, in order to develop reliable markers for MAS (Marker Assisted Selection). The doubled haploid population Sollux x Gaoyou (56 lines) was grown in 2009 in Göttingen and Einbeck. In the spring, fully expanded leaves were harvested. Leaf material per double haploid line per experiment was combined, freeze-dried and ground into a fine powder. The freeze-dried plant material was stored at -30°C for further glucosinolate

analysis. The glucosinolate content of each sample was calculated using the response factors referring to the internal standard (benzyl glucosinolate). The values were expressed as µmol g^{-1} dry weight (DW) of material. The peaks were identified by comparison with individual glucosinolate standards.

QTL analysis

Linkage analysis and map construction were carried out using QTLNetwork-2.0 (Yang et al. 2005) for the population grown in Göttingen the mapping function according to Kosambi was used. The linkage maps were drawn using MapChart 2.1 (Voorrips 2002). Linkage groups were assigned based on SSR (Single Sequence Repeated) markers that are also mapped in reference maps. The 19 linkage groups of *Brassica napus* L. were renamed L1-L19. In the regions of the putative QTL, the markers with the highest LOD values were taken as co-factors. The LOD thresholds for QTL significance were determined by permutation tests (1000 replications) with a genome-wide signifance level of P=0.05 for significant linkages; LOD=2.0 was used as a significant threshold.

RESULTS

The double haploid population in Göttingen and Einbeck shows a minimum and a maximum value for progoitrin, gluconapoleiferin, gluconapin 4-hydroxyglucobrassicin, glucobrassicanapin, glucobrassicin, nasturtiin, 4-Methoxyglucobrassicin, neo-glucobrassicin and the sum.

The total leaf glucosinolate content shows a minimum value for Göttingen of 1.23 µmol/g D.M. and a maximum value of 25.73 µmol/g D.M. The average value for Göttingen is 4.75 µmol/g D.M. and for Einbeck this is 6.51 µmol/g D.M.

The doubled haploid population is normally distributed and average leaf glucosinolate values over the two locations range from 0.54 till 17.61µmol/g D.M. (Figure 1).

The interaction between two or more genes and their environment, or the degree of association of a specific region on the genome to the inheritance of the trait of interest is not determined. Candidate gene hypothesizes the SSR (Single Sequence Repeated) markers HMR087c and HMR299b are linked with the 4-Hydroxy glucobrassicin marker interval. The double haploid lines included in this study reveal a prospect on the advantages of the use of the double haploid lines in the field experiments. The further phenotypical analysis of these lines can lead to the position of the main quantitative trait loci responsible for the expression of the glucosinolate trait in the different tissues.

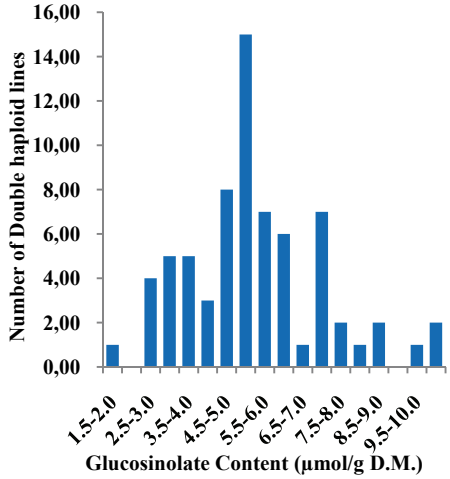

Figure 1. The distribution of the total leaf glucosinolate values of the double haploid population Sollux x Gaoyou

One of the DH lines (DH68) proved to have an extremely high 4OH (2.66 µmol/g D.M.) content. The 4-hydroxyglucobrassicin values are not normally distributed (at the p=0.1 level), therefore it is not possible to conclude that the candidate gene responsible for the production of the indole glucosinolate 4OH is found on a specifically locus inside this marker interval. The genes of the leaf glucosinolates that are shown are expressed in a late stage of maturity, namely during flowering time. Through quantitative trait loci analysis the inheritance of the leaf glucosinolates is investigated. The map interval of this specific glucosinolate is given on the framework map. One significant marker interval was found on chromosome 2 for 4-hydroxglucobrassicin.

The results of quantitative trait loci are usually presented in a map, which indicates the position and significance of the quantitative trait loci. Figure 2 shows the quantitative trait loci, with the largest effects on the glucosinolate content, this quantitative trait loci is situated on the second chromosome. The marker interval of 4-hydroxy-glucobrassicin is located above the critical value.

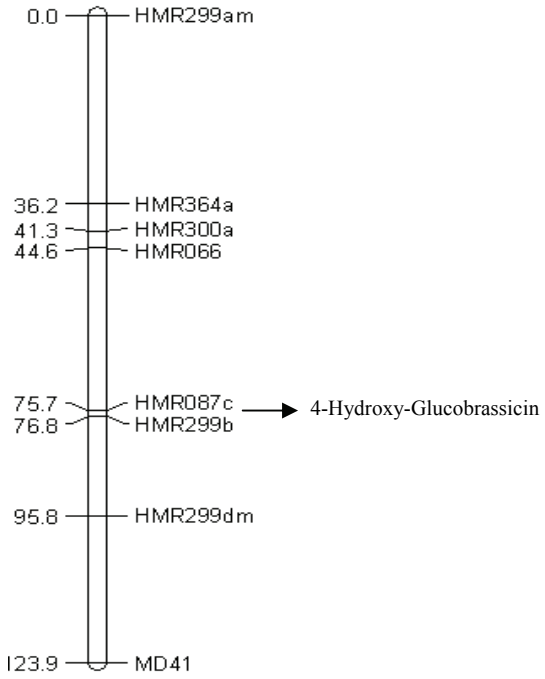

Figure 2. The framework map of linkage group N2, with the mapping interval of the indole glucosinolate 4-hydroxy-glucobrassicin QTL. The distance between the markers is given in Centimorgan (Kosambi 1944). Marker interval analysis where the quantitative trait locus of 4 hydroxy-glucobrassicin reaches the significant level.

Figure 2 shows that it is necessary to gain further information on the candidate genes, which might reveal whether a possible genetic block for the quantitatively important alkenyle glucosinolates can be exploited to reduce the total glucosinolate content in the green material of rapeseed. In most studies the heritability of the main group of glucosinolates namely alkenyles is investigated. With a combined search for a relation between the genetic action and the glucosinolate regulation in different tissues it can be proved whether the glucosinolate groups have different biosynthetic pathways and overall candidate genes.

DISCUSSION

Identification of the QTL (Quantitative trait loci), which are responsible for lines with a high or low leaf glucosinolate content can further increase the efficiency of a marker-assisted selection (MAS) and enhance in this way

progress in detecting genes that are related with glucosinolate biosynthesis. One marker interval was identified; however the interaction between two or more genes and their environment, or the degree of association of a specific region on the genome to the inheritance of the trait of interest is not determined. Further association analysis can lead to an identification of the candidate gene on this specific locus, which is related with this indole glucosinolate type. The quantitative trait loci, which was determined and used over SSR (Simple Sequence Repetitions) markers was determined and of interest, while one gene was determined that might be responsible for the genetical behavior of the glucosinolate molecules in the leaves of double haploid lines, if this genetic interaction is a prerequisite for further transformation in the glucosinolate biosynthesis it can create a large potential for distinctions for genes that already start in the embryo genetic process, this is a rather complex process. As the primary processes of the glucosinolate biosynthesis starts in the embryo, this can play a primary role. With the results of this study scientists might continue to use quantitative trait loci maps and markers that tag the genes of interest.

ACKNOWLEDGMENTS

The results are the outcome of a project funded by the German Federal Environmental Foundation. The authors express their gratitude to the KWS SAAT AG for establishing the field experiments.

5 Leaf, stem glucosinolates profiles and methane production of hybridized winter rapeseed

ABSTRACT

Winter rapeseed (*Brassica napus* L.) is one of the most important oilseed crops in the world. The biogas processing is considered as a relative new sustainable treatment in all its applications. Biogas contains much of the chemically bonded energy as methane and includes several advantages. Inhibiting components such as glucosinolates are particularly interesting. Different lines were evaluated for biogas in replicated field experiments in two environments, each in Germany. During the fermentation process, bacteria and biomass consisting partially of winter rapeseed substrate are mixed. High Performance Liquid Chromatography was used to evaluate the total glucosinolate content of the crosses of hybridized resynthesized winter rapeseed lines. The average methane production percentage of the investigated crosses was 53.10 %. The different glucosinolates types within rapeseed genotypes are low for their total content. A more detailed analysis to understand the effect on bacteria involved in the biogas fermentation processes is required. Based on the results a future selection of winter rapeseed lines for renewable energy applications opens up perspectives for winter rapeseed breeders. The energy recovery of the methane conversion process is relatively high.

INTRODUCTION

Winter rapeseed (*Brassica napus* L.), which seeds are used to produce oil, is not a regular crop. It is intensively cultivated for the food and beverage industry. A new application is the use of the whole crop for the production of biogas. However less is known about its biochemistry. The objective of this study was to investigate whether the green mass of winter rapeseed can be used as a substrate for biogas fermentation (Görisch and Helm 2006). Glucosinolates as sulfur containing components are reported to have an anti-bacterial and anti-fungal activity. Sulfur is mainly stored in sulfur containing proteins, with high amounts of cysteine and methionine. The sulfur cycle is the specific interest of this study and to relate glucosinolates, which are basically sulfur containing components, with methane and total biogas production. Further other important quality traits can become of major interest, this means sugar or ash content, which subsets the total of minerals available. Winter rapeseed is a demanding crop as far as the sulfur nutrition is concerned, for example, McGrath and Zhao (1996) state the need of 16kg of sulfur for ensuring the production of 1 metric ton of rapeseed plant seeds.

The efficiency of the winter rapeseed for its methane production has never been investigated before. Specifically less or no literature is available that describes the characteristics of winter rapeseed for biogas applications. Neither was data available from secondary components, which might influence the methane production. Winter rapeseed genotypes are analyzed for parameters relevant during biogas fermentation. Lignin content also influences the way of combustion. Rapeseed is known not only from the seeds, but also the stems are useful to produce biomass. Aside from a high biomass value for a sufficient biogas conversion (Ofori and Becker 2007), also an optimal composition i.e. lowered glucosinolate content in the leaves and stems might be required. It should be mentioned that the production of biogas is not a new technological achievement. In Germany biogas became important as an alternative source of energy, because of its decentralized approach. Thereby it is the result of a natural biological process, which is active over all times in swamps, seas and in the digestion tract of animals. Relatively new is the optimilisation of the biological processes under economical perspectives, ecological sustainable perspectives are often neglected, for this plant breeding can help by insisting on a long term selection towards a highly digestible crop. The system used for biogas production is an innovative mixed crop cultivation system which is of use as a pre-crop. In this way the arable land can be committed twice and the potential can be optimized. Fermentation, which occurs over aerobic and anaerobic decomposition processes, can be broken down into two processes. Aerobic decomposition (fermentation) will produce carbon dioxide, ammonia and some other gases in small quantities, heat in large quantities and a final product that can be used as a fertilizer. Anaerobic decomposition will produce methane, carbon dioxide, some hydrogen and other

gases in traces are produced by aerobic fermentation. The hydrolysis, acidification and methane construction of organic substances brought in the biogas fermentation unit is depending on the hydrolysis of the high molecular components. For this hydrolysis inside the biogas fermentation unit involves several bacteria responsible for the biogas reaction processes. These bacteria are responsible for the hydrolysis of the different main and rest fractions such as carbohydrates, proteins and fats. The main components for biogas fermentation are sugars and starches, whereas lignin is less decomposed. Due to its short history of domestication the genetic base of rapeseed is very narrow. Resynthesized rapeseed lines offer a wider genetic basis and can help to further select for suited energy crops. Besides glucosinolates also tannins, proteins and dry matter content are important parameters, which have to be identified in individual winter rapeseed genotypes.

The plant breeding objectives for an optimal harvesting time for winter rapeseed are earliness and dry matter content in spring time. The quality of the substrate is further influenced by inhibiting substrate components, such as tannins and glucosinolates. Methane production for each hectare should be rather high. This corresponds to the optimal harvesting period of whole the plant. Due to the large genetic variation breeding of resynthesized lines, there is a large potential. Besides a high green yield, and optimizing the growing conditions of the plant, selection should focus on lines that fit optimal environmental and local growing conditions.

Lines that are grown in the field have not been selected for the genetic variation of the leaf glucosinolates. The mass selection followed by between and within line selection maintains and selects for quality traits that can be used for lines conform those traits. Modern applications, such as renewable energy production based on biogas are challenging. While they combine conservative winter rapeseed selection methods, based on line selection with technical demanding settings found in the biogas combustion unit. The enlargement of the basic material, through inter-hybridization of the parental material, creates the possibility to capture those genes, originating from the mutations. Modern varieties are preferred at the base of this selection scheme, while wild and old varieties sustain traits that are not really accepted within modern applications, with biogas production with winter rapeseed as an example.

Crop rotation can be adjusted with energy crops such as *Brassica napus* L. Winter rapeseed is grown on the same area ever three to four year and this excludes an excessive proportion of maize in the crop rotation. Until now, plant breeding regularly improved the suitability of rapeseed for biogas extraction by increasing its dry matter; nutrient and protein content.

The production of biogas is blocked by the high sulfur content. The micro-biological access of these proteins, fats, starches and sugars are important indicators for the biogas production. The study of the influence of the methane production is depending on a complex number of variables like harvesting time, biomass production and to a minor extent minor component, which influence the biochemical processes during the storage of the substrate.

Renewable energy plants that are used for biogas fermentation are mainly interesting for their specific methane production and whether an influence on the quality of the product is there. Dry matter and organic matter are important parameters which have to determine first in order to have a simple but good overview over what can be achieved in the process of biogas production.

MATERIALS AND METHODS

Plant material and laboratory analysis

Leaves and stems of the resynthesized parental lines were harvested with the objective to estimate sulfur and nitrogen content of these winter rapeseed lines. The hybridized resynthesized lines used for hybridized rapeseed lines were developed from crosses between resynthesized rapeseed lines and high yielding biomass cultivars. The term testcross hybrid is used to determine the F1 hybrid between the test crosses. Both Resynthesized parental lines and hybridized lines were tested in a one year trial at two locations (Göttingen and Einbeck). In 2007 20 testcross hybrids were only grown in Göttingen. In 2008, 15 testcross hybrids, which were different from those grown in 2007, were grown in Göttingen and Einbeck and were analyzed for their glucosinolate content and methane percentage.

Except from the glucosinolate content and the methane percentage for these individual crosses, also the fermentation parameters of crosses were quantified. These are expressed in norm liter, total matter minus inorganic content, percentage methane from biogas, ash content (XA), sugar, oil content (XL), lignin content (ADL), acid soluble fraction (oADF). These parameters are quantified and except for biogas and methane, these values are expressed in %. Besides this the sulfur and nitrogen percentage of the resynthesized lines grown in 2008 is determined. The sulfur and nitrogen values were quantified using a Carbon Nitrogen Sulfur analyzer. The material originates from two different locations (Göttingen and Einbeck).

RESULTS AND DISCUSSION

The total sulfur and nitrogen content of the resynthesized lines is shown in table 1, having low nitrogen values for H344 and S14.

Table 1. The sulfur content of the resynthesized rapeseed lines

	N (% of DM content)			S (% of DM content)		
Line	**Leaves**	**Seeds**	**Stems**	**Leaves**	**Seeds**	**Stems**
S3	3.72	3.53	0.99	0.99	0.97	0.25
R59	3.85	3.93	0.80	0.79	0.79	0.19
G35	4.49	3.53	1.09	1.08	0.99	0.53
H344	3.2	3.64	1.00	0.73	1.11	0.32
R64	3.7	3.59	0.76	0.85	0.88	0.19
S14	3.45	3.31	1.13	1.21	1.09	0.31
Mean	3.73	3.59	0.96	0.94	0.97	0.30
LSD5	0,73	0.91	0.33	0.63	0.91	0.23

None of the resynthesized lines shows a highly significant difference for the sulfur content.

Minerals have an influence on combustion characteristics. The sulfur content in the ash value can be an issue of convenience for winter rapeseed breeders. Its composition and quantity of combustion residue are the primary factors determining whether or not a feedstock can be combusted effectively in a particular appliance. Sulfur is inhibiting while reacting with alkali metals to form alkali sulfates that stick to heat transfer surfaces. Sulfur can also form sulfates with calcium, but this is not as critical as the alkali reactions. The stems show the lowest content with a percentage of 0.19% in comparison with the leaves (0.99%). The sulfur content in the plant organs ranges from 0.19 till 1.5%. None of the resynthesized lines show a highly significant difference for the sulfur content.

A positive correlation for the nitrogen and the sulfur content in the leaves (0.69) as well as in the stems (0.75) was recorded.

A correlation between methane fermentation and leaf glucosinolate content and values for the experiment considering the biogas is given. In 2008 the correlation between biogas and glucosinolate content is low but present, (r^2=0.20). For the experiment in 2007 methane values didn't correlate (R^2 = 0.05) (Figure 1).

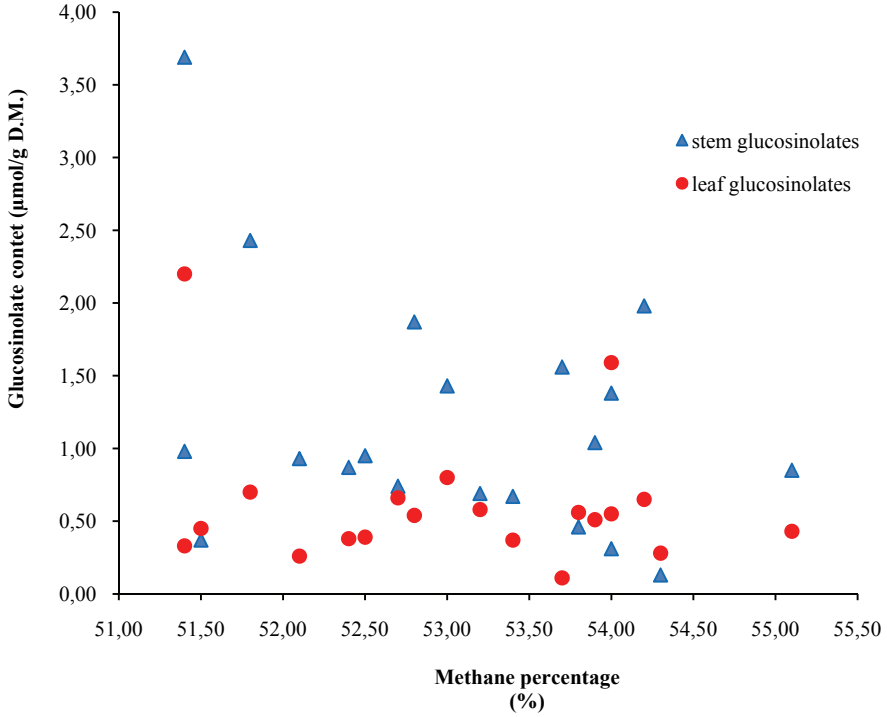

Figure 1. Demonstrates the absence of a correlation between the stems and the leaves and methane percentage for 20 crosses grown in 2007.

The highest biogas value is recorded for the cross R31 with 2097-95-ams, this in 2007. The lowest biogas value is recorded for the cross H219 – H605800 with a value of 523 NL ODM. In 2008, the correlation between biogas and glucosinolates is low, but present, (r^2=0.20).

For an experiment in 2007 methane values did not correlate (R^2 = 0.05) or only 5% of the total variation in methane production is related with the glucosinolate content within the stems and leaves (in µmol/g D.M.). Average methane production of the investigated crosses was 53.10%. The absolute content of the different glucosinolates types within rapeseed genotypes are low. There are correlations between the glucosinolate content of the stems or the leaves and the methane percentage. In 2007 the average biogas value for the crosses was 589 Norm Liter Organic dry matters (NL oDM). In 2008 the mean biogas values range from 477.52 till 694.00 norm liter. Methane percentages are remarkably higher in 2008 than in 2007; this is mainly depending on the type of the cross. In 2008, the biogas values

ranged from 477.52 till 693.50 [l/kg oDM]. Methane values in 2008 ranged from 275.38 till 396.71 [l/kg oDM].

The genetic correlation for the fermentation parameters and glucosinolates of crosses which were grown in 2008 are tabulated in table 2.

Table 2. Genetic correlations of leaf glucosinolates and biogas related traits

Genetic correlation (r_g)			
Trait	**Leaf Glucosinolates**		
	Alkenyles	**Indoles**	**Sum**
Oil content	0.19	-0.25	0.21
protein content	0.26	-0.03	0.09
Acid soluble fraction	0.26	-0.07	0.15
ash content	-0.04	0.06	0.12
Lignin content	-0.06	-0.28	-0.15
Methane	0.05	-0.05	-0.10

The total glucosinolate values of the stems and leaves are genetically not correlated with the methane percentage.

DISCUSSION

Conventional oilseed plants, such as winter rapeseed have reached a low meaningful value for breeding purposes for renewable energy or biomass applications. Some of the energy plants, for example winter rapeseed, let stand permanently high H_2S content in the biogas reactor, these energy crops are therefore less suited (Gehrig 2007). Breeders, which are interested in the breeding of winter rapeseed lines with adapted glucosinolate content for the biogas fermentation units can select over hybrid crossing schemes in order to improve traits such as the dry matter and the mineral content. Sulphur content and nitrogen are important biogas related parameters. Sulphur might inhibit bacterial growth, whereas nitrogen is the element that balances together with carbon the bacterial growth in the fermentation reactor. Sulphur and nitrogen content are lower in the stems, as in the seeds and the leaves. The comparison of the different other biogas related parameters such as proteins, lignin and acid soluble fraction is relatively important. Parameters such as dry matter and lignin content should not be underestimated for assessing the relevance of winter rapeseed inside the whole conversion process. In the first trial year there was general a non-correlation present with the methane production, this as well for the leaves as for the stems. Glucosinolates are for this matter not influencing the

methane percentages of the crosses. Although the results of 2008 indicate that the leaf glucosinolates do not influence the total methane production, the results of 2007 express this was the case. In 2008 the methane values depend on the type of the cross.

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6 General Conclusions

Drying temperature influences stability of glucosinolates. A general rule is to choose for short drying periods, with an as intensive air circulation. Secondly the stability of the different glucosinolate groups plays a role in the total recovery of glucosinolates. The output efficiency of indole glucosinolates is lower as for alkenyle glucosinolates. Most of the plants were harvested before flowering (April), which is a representative time period for material that is harvested for biogas production plant. In this stage alkenyle glucosinolates are the most dominant glucosinolate group. In an earlier stage of development it is possible that the indole glucosinolates can exceed the alkenyle glucosinolates, this while the rising phase of the alkenyle glucosinolates in the green mass occurs retarded, afterwards however the concentrations decline slowly, to remain at a lower level during the rest of their further development (Stephani 1985).

The triangle shape form of these plotted leaf glucosinolate values and the glucosinolate values of the seeds shows a large variation of the material. The relative high variation among and within the genotypes might occur due to different causes. Although the spring of 2007 was characterized by an unusual drought during the flowering, which led to an inhomogeneous flowering and seed kernel formation, it is not possible to state any conclusion for the role of the environment on the glucosinolate content. Stephani (1985) reported that the alkenyle glucosinolate concentrations in the vegetative mass are more than double as high as the values that are measured before flowering. The values that were found for resynthesized line S3 were in line with this result. The average value of the concentration of stem glucosinolates is higher than the glucosinolate concentrations in the leaves. This is characteristic for plants, which are in the flowering time, namely the glucosinolate content in the leaves is decreasing, whether the glucosinolate content of the stems is increasing (Bennert 1992).

The absence of a correlation and the confrontation with the often poor values for the leaf glucosinolate values in comparison with the higher seed glucosinolate values suggests independent genetic control for glucosinolate regulation in seeds and leaves. Some rare winter rapeseed lines may exist that confronts both these qualities in such a way that both a high resistance against insects and low seed glucosinolate values is achieved. Concerning the total amount of glucosinolates in the different plant tissues the correlations show a large heterogeneity of the concentrations of glucosinolates in the stems, leaves and seeds of the plants. For the relation of the total glucosinolate contents in the different plant organs, earlier reports on the presence or absence of correlations are until now rather contradictory (Jürges 1982). It is even suggested that weak correlations between seed and leaf glucosinolates content might be caused by the dependence of leaf glucosinolate content on environmental effects and growing stage (Schilling and Friedt 1991). This means that the concentrations of the alkenyle glucosinolates in the seeds

cannot be used for indirectly determining the concentration of the glucosinolates in the leaves. At least not for the higher seed values, for the relative low seed glucosinolate content there is most probable a weak correlation.

For some genotypes like “H61”, which is an outer value, a relative high glucosinolate levels in the seeds is shown (109.24µmol/g D.M.), whereas before flowering, a relative low amount of total glucosinolates is reached in the leaves (0.95µmol/g D.M). Correlation with methane percentage is not present. For the further relevance for breeding purposes concerning biogas it is valuable if the correlation between glucosinolate values and the methane percentage is present and whether individual glucosinolate types have their influence on total methane percentage produced from the crop residues.

7 Appendix

LEAF GLUCOSINOLATES FOR DOUBLE HAPLOIDS GROWN IN 2008 (µMOL/G D.M.)										
Nr.	**Pro**	**Gnl**	**Gna**	**4OH**	**Gbn**	**Gbc**	**Nas**	**4Me**	**Neo**	**Sum**
4	1.56	1.86	0.08	1.95	0.24	0.08	0.18	0.03	0.02	6.01
19	2.06	0.88	0.54	0.02	1.87	0.15	0.24	0.01	0.00	5.78
21	0.62	0.15	0.00	0.00	0.68	0.04	0.40	0.06	0.05	1.99
22	1.11	0.81	0.00	0.69	0.52	0.08	0.00	0.05	0.01	3.26
25	0.92	0.40	0.96	1.27	1.58	0.07	0.15	0.04	0.03	5.42
27	1.09	0.45	0.67	1.64	1.06	0.24	0.13	0.02	0.02	5.32
29	1.33	0.31	0.60	0.15	2.70	0.10	0.11	0.02	0.01	5.34
32	2.23	0.56	1.05	0.52	2.50	0.13	0.10	0.01	0.00	7.10
35	0.79	0.56	0.52	0.38	1.22	0.07	0.06	0.01	0.00	3.62
36	1.09	0.37	1.45	0.40	2.35	0.13	0.25	0.02	0.02	6.09
44	1.61	0.19	0.99	0.43	1.95	0.10	0.09	0.01	0.01	5.41
53	1.22	0.37	0.59	0.70	1.86	0.19	0.22	0.01	0.00	5.16
54	2.27	0.88	1.53	0.92	2.19	0.08	0.16	0.02	0.00	8.05
55	1.03	0.30	0.34	0.46	0.70	0.06	0.07	0.04	0.02	3.01
59	2.59	0.61	0.88	0.17	2.66	0.13	0.10	0.01	0.03	7.18
69	1.63	0.67	0.58	0.92	1.65	0.09	0.06	0.02	0.01	5.64
76	2.15	0.61	1.11	0.12	3.03	0.07	0.11	0.03	0.01	7.25
79	2.11	0.91	1.24	0.58	2.00	0.14	0.22	0.05	0.03	7.25
80	3.15	0.83	0.00	0.00	1.48	0.08	0.00	0.02	0.00	5.64
86	1.36	0.46	0.74	0.34	2.59	0.25	0.13	0.04	0.00	5.88
87	1.52	0.46	0.63	0.12	1.30	0.16	0.11	0.06	0.02	4.32
88	1.40	0.58	0.79	0.55	1.02	0.11	0.23	0.02	0.02	4.71
90	1.98	0.35	0.77	0.46	0.16	0.09	0.01	0.02	0.03	3.92
90	1.99	0.72	0.55	0.52	0.60	0.09	0.02	0.01	0.00	4.50
91	2.57	0.62	1.05	0.69	2.17	0.09	0.19	0.07	0.01	7.39
93	1.68	0.41	0.14	2.41	0.57	0.07	0.00	0.01	0.01	5.37
94	1.68	0.78	0.05	0.38	1.87	0.05	0.00	0.02	0.02	4.86
99	1.08	0.29	0.93	0.95	2.24	0.07	0.14	0.08	0.01	5.72
102	1.95	1.25	0.56	0.66	0.87	0.13	0.06	0.01	0.00	5.52
104	1.41	0.60	1.46	1.45	0.36	0.07	0.00	0.02	0.01	5.41
118	2.88	1.76	1.38	0.71	3.12	0.08	0.18	0.02	0.04	10.14
120	0.86	0.59	0.56	1.43	0.35	0.06	0.15	0.02	0.00	4.04
124	0.76	0.10	0.41	0.64	0.63	0.05	0.19	0.03	0.02	2.85
128	2.87	0.35	0.00	0.04	0.67	0.10	0.00	0.01	0.03	4.11
128	1.54	0.29	0.84	0.02	1.85	0.04	0.00	0.04	0.01	4.63
129	0.95	0.27	0.69	0.90	2.10	0.09	0.25	0.03	0.00	5.34
130	1.51	0.67	0.29	0.01	2.11	0.05	0.24	0.08	0.07	4.89
132	1.48	1.03	0.42	0.38	1.58	0.10	0.27	0.01	0.04	5.31
133	0.94	0.55	0.58	1.39	0.95	0.07	0.07	0.03	0.02	4.59
134	1.21	0.44	0.46	0.02	1.64	0.10	0.02	0.03	0.00	3.96
141	2.12	0.70	0.00	0.00	1.90	0.09	0.00	0.03	0.00	4.85
143	1.20	0.50	0.25	0.54	0.87	0.08	0.13	0.01	0.01	3.59
148	1.14	1.12	0.00	1.34	1.21	0.07	0.00	0.02	0.01	4.92
149	0.88	0.66	0.10	0.21	0.47	0.08	0.04	0.04	0.02	2.51
152	1.43	0.65	0.52	0.01	1.71	0.12	0.04	0.04	0.01	4.53
153	1.26	0.55	0.09	0.71	0.52	0.08	0.00	0.05	0.01	3.26
158	1.84	0.68	0.83	0.28	1.71	0.09	0.10	0.02	0.02	5.57
160	2.13	0.86	1.16	0.37	1.80	0.07	0.09	0.02	0.01	6.50
163	1.13	0.21	1.66	1.19	1.69	0.08	0.11	0.03	0.01	6.10
164	1.24	0.68	2.82	1.39	0.07	0.10	0.17	0.01	0.00	6.49
165	1.21	0.88	0.16	0.89	0.38	0.11	0.00	0.05	0.01	3.69

SEED GLUCOSINOLATES (µmol / g D.M.) of LINES GROWN IN 2007											
Line	**Pro**	**Sin**	**Gnl**	**Gna**	**4OH**	**Gbn**	**Gbc**	**Nas**	**4Me**	**Neo**	**Sum**
ESKESEHIR	54.60	2.30	0.03	17.91	3.35	0.25	0.08	0.19	0.17	0.04	78.92
EXPRESS	10.82	0.26	0.00	3.10	4.92	0.82	0.33	0.44	0.07	0.02	20.78
GAOYOU	20.14	0.78	0.00	7.46	3.67	0.00	0.25	0.00	0.14	0.03	32.48
NIKOS	7.60	0.06	0.02	8.39	7.77	0.58	0.96	0.44	0.04	0.02	25.89
SOLLUX	44.70	1.04	0.00	22.15	4.27	2.47	0.05	0.48	0.25	0.01	75.42
G2	26.58	0.02	7.42	12.26	8.36	2.76	0.20	0.43	0.16	0.12	58.30
G35	36.68	0.13	0.00	7.44	3.18	0.00	0.23	0.08	0.11	0.07	47.91
H10	44.03	5.23	0.20	9.24	5.40	0.35	0.70	0.64	0.17	0.11	66.07
H111-2	23.41	0.13	0.04	25.45	9.12	1.37	0.39	0.00	0.14	0.05	60.10
H128	3.05	6.85	0.00	0.78	3.04	0.00	1.01	0.52	0.37	0.05	15.68
H149	46.45	0.44	2.72	11.72	3.59	0.95	0.18	0.60	0.26	0.06	66.97
H19	10.11	1.13	0.23	2.33	4.67	0.26	0.31	0.18	0.13	0.05	19.41
H196	26.72	1.72	1.60	8.72	4.38	0.26	0.27	0.57	0.13	0.02	44.39
H219	14.32	1.28	0.00	3.57	3.70	0.26	0.62	0.00	0.25	0.04	24.04
H327	40.11	0.98	0.00	8.07	4.77	0.43	0.66	0.14	0.19	0.05	55.40
H344	46.06	1.67	0.23	16.01	4.92	2.62	0.22	0.64	0.24	0.11	72.73
H357	44.55	0.24	0.24	16.56	3.88	1.77	0.24	0.07	0.13	0.06	67.74
H54	49.22	1.85	0.08	21.62	4.63	0.00	0.14	0.00	0.14	0.06	77.75
H61	73.76	2.04	0.00	27.31	4.90	0.00	0.40	0.66	0.13	0.05	109.24
L239	25.63	0.53	0.63	9.83	5.18	0.95	0.41	0.23	0.27	0.04	43.71
R28	20.54	0.17	0.70	4.79	3.88	0.00	0.22	0.32	0.22	0.03	30.94
R31	37.61	0.62	0.09	15.28	5.04	2.79	0.49	0.43	0.24	0.05	62.64
R53	19.60	0.13	3.33	2.09	2.36	0.00	0.67	0.54	0.32	0.02	29.05
R64	23.16	7.36	2.40	7.34	3.80	1.51	0.43	0.08	0.34	0.07	46.48
R73	35.53	6.85	0.16	12.32	2.82	1.07	0.15	0.10	0.26	0.07	59.34
R75	8.77	2.23	0.08	2.69	3.47	0.03	0.20	0.00	0.13	0.01	17.61
R76	24.20	0.92	0.79	16.72	5.02	1.09	0.40	0.19	0.29	0.06	49.67
RS 13/6	42.13	0.79	0.32	17.91	4.13	0.62	0.17	0.27	0.23	0.05	66.63
S14	45.54	0.61	1.01	8.44	4.63	2.38	0.19	0.02	0.27	0.07	63.17
S15	49.58	0.33	0.00	16.11	3.58	0.69	0.25	0.06	0.26	0.04	70.92
S17	57.46	0.97	0.10	16.13	5.20	1.45	0.71	0.00	0.29	0.06	82.36
S2	28.84	0.29	0.86	32.05	2.18	0.00	0.19	0.01	0.29	0.04	64.76
S3	39.93	2.66	0.25	14.64	4.32	0.84	0.24	0.37	0.15	0.07	63.49
S30	45.10	0.16	0.57	10.35	4.84	3.10	0.35	0.14	0.15	0.07	64.83
S31	54.60	2.30	0.03	17.91	3.35	0.25	0.08	0.19	0.17	0.04	78.92
MEAN	33.75	1.57	0.69	12.42	4.47	0.91	0.35	0.26	0.20	0.05	54.68
MIN.	3.05	0.02	0.00	0.78	2.18	0.00	0.05	0.00	0.04	0.01	15.68
MAX.	73.76	7.36	7.42	32.05	9.12	3.10	1.01	0.66	0.37	0.12	109.24

LEAF GLUCOSINOLATES (µmol/g D.M.) OF RESYNTHESIZED LINES GROWN IN 2007								
Line	**Pro**	**Gnl**	**Gna**	**4OH**	**Gbn**	**Gbc**	**Nas**	**Sum**
ESKESEHIR	1.08	0.00	0.12	0.03	0.30	0.03	0.00	1.56
EXPRESS	0.54	0.04	0.10	0.16	0.00	0.03	0.03	0.90
GAOYOU	0.27	0.02	0.05	0.01	0.00	0.01	0.00	0.36
NIKOS	0.88	0.00	0.43	0.16	0.00	0.03	0.31	1.82
SOLLUX	3.06	0.00	1.78	1.30	3.76	0.01	0.00	9.91
G2	0.15	0.00	0.25	0.07	0.00	0.02	0.04	0.54
G35	0.20	0.00	0.11	0.03	0.00	0.04	0.03	0.42
H10	0.14	0.00	0.18	0.02	0.00	0.03	0.07	0.44
H111-2	0.34	0.00	0.17	0.82	0.44	0.09	0.06	1.92
H128	0.10	0.00	0.08	0.03	0.00	0.77	0.00	0.98
H149	2.37	0.00	0.67	0.14	0.68	0.27	0.06	4.20
H19	0.17	0.00	0.00	0.02	0.00	0.04	0.03	0.25
H196	0.26	0.00	0.07	0.00	0.12	0.01	0.00	0.47
H219	0.24	0.00	0.04	0.04	0.00	0.02	0.07	0.42
H327	0.59	0.00	0.04	0.14	0.12	0.07	0.00	0.96
H344	0.24	0.00	0.08	0.07	0.00	0.02	0.06	0.46
H357	0.18	0.00	0.18	0.00	0.00	0.13	0.00	0.49
H54	0.05	0.00	0.04	0.37	0.00	0.12	0.22	0.80
H61	0.26	0.00	0.28	0.07	0.00	0.18	0.03	0.81
L239	0.21	0.00	0.12	0.05	0.13	0.00	0.00	0.51
R28	0.64	0.00	0.30	0.11	0.18	0.08	0.17	1.48
R31	0.77	0.07	0.37	0.00	0.00	0.05	0.00	1.25
R53	0.16	0.00	0.04	0.00	0.00	0.05	0.00	1.25
R64	0.23	0.00	0.02	0.02	0.00	0.05	0.54	0.89
R73	1.26	0.00	0.02	0.02	0.02	0.00	0.05	0.75
R75	0.12	0.00	0.00	0.02	0.00	0.12	0.12	1.92
R99	0.12	0.03	0.62	0.02	0.00	0.01	0.09	0.24
S14	0.65	0.03	0.62	0.02	0.04	0.06	0.24	1.13
S15	0.79	0.00	0.26	0.07	0.00	0.08	0.14	1.08
S17	0.29	0.00	0.26	0.07	0.00	0.08	0.14	1.34
S2	0.39	0.00	0.23	0.02	0.00	0.02	0.00	0.66
S3	3.84	0.00	0.73	0.60	0.33	0.06	0.13	5.69
S30	0.36	0.00	0.00	0.17	0.15	0.21	0.10	1.00
MEAN	0.63	0.00	0.24	0.15	0.19	0.09	0.09	1.40
MIN.	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.24
MAX.	3.84	0.07	1.78	1.30	3.76	0.77	0.54	9.91

LEAF GLUCOSINOLATES OF RESYNTHESIZED LINES GROWN IN 2008										
LINE	**PRO**	**GNL**	**GNA**	**4OH**	**GBN**	**GBC**	**NAS**	**4ME**	**NEO**	**SUM**
S3	2.00	0.00	1.65	0.03	0.75	0.11	0.25	0.04	0.02	4.85
S30	0.17	0.03	0.06	0.01	0.02	0.03	0.15	0.03	0.03	0.54
S31	0.16	0.03	0.17	0.01	0.09	0.09	0.09	0.01	0.02	0.65
H196	0.13	0.02	0.04	0.02	0.00	0.10	0.08	0.01	0.01	0.41
L239	0.15	0.00	0.05	0.01	0.08	0.08	0.04	0.01	0.02	0.43
R53	0.09	0.00	0.02	0.02	0.05	0.31	0.58	0.03	0.01	1.12
R59	0.24	0.00	0.81	0.00	0.71	0.05	0.07	0.01	0.01	1.90
G56	0.43	0.00	0.19	0.02	0.14	0.09	0.08	0.01	0.03	1.00
H4	0.07	0.00	0.01	0.02	0.00	0.01	0.05	0.02	0.04	0.22
H231	0.18	0.38	0.01	0.03	0.03	0.11	0.11	0.06	0.03	0.93
H327	0.30	0.11	0.48	0.04	0.42	0.28	0.42	0.08	0.11	2.23
H357	0.10	0.03	0.05	0.03	0.59	0.30	0.84	0.08	0.06	2.08
R1	0.20	0.37	0.27	0.04	0.23	0.06	0.20	0.05	0.05	1.48
G2	0.13	0.14	0.00	0.03	0.03	0.04	0.09	0.05	0.02	0.53
G35	0.06	0.31	0.00	0.02	0.02	0.01	0.14	0.03	0.01	0.61
H19	0.08	0.00	0.01	0.02	0.16	0.03	0.14	0.08	0.07	0.59
H344	0.24	0.04	0.37	0.02	0.39	0.04	0.13	0.01	0.02	1.24
L122	0.46	0.01	0.15	0.05	0.42	0.03	0.05	0.01	0.14	1.32
L314nc	0.13	0.00	0.08	0.02	0.01	0.03	0.16	0.10	0.06	0.60
R12	0.13	0.00	0.00	0.02	0.05	0.03	0.06	0.04	0.03	0.35
R19	0.38	0.00	0.21	0.02	0.18	0.04	0.10	0.04	0.03	1.01
R28	0.43	0.05	0.22	0.00	0.24	0.31	0.17	0.11	0.06	1.58
R64	0.11	0.28	0.11	0.02	0.04	0.02	0.11	0.05	0.04	0.78
R73	0.19	0.29	0.09	0.03	0.00	0.06	0.07	0.01	0.05	0.78
R75	0.14	0.32	0.25	0.00	0.22	0.09	0.10	0.02	0.01	1.15
S14	0.63	0.34	0.23	0.01	0.81	0.07	0.06	0.04	0.00	2.20
S15	0.27	0.39	0.19	0.01	0.14	0.04	0.11	0.01	0.02	1.18
S16	0.20	0.45	0.02	0.01	0.10	0.07	0.29	0.04	0.02	1.21
MEAN	0.24	0.09	0.19	0.02	0.19	0.08	0.14	0.04	0.03	1.03
MIN.	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.22	0.00
MAX.	0.45	1.65	0.05	0.81	0.31	0.84	0.23	0.14	4.85	0.45

STEM GLUCOSINOLATES (µmol/g d.M.) OF RESYNTHESIZED LINES GROWN IN 2007								
LINE	**PRO**	**GNL**	**GNA**	**4OH**	**GBN**	**GBC**	**NAS**	**SUM**
ESIKESEHIR	4.28	0.00	0.23	0.06	0.35	0.03	0.00	4.95
EXPRESS	0.79	0.00	0.39	0.05	0.00	0.05	0.10	1.37
GAOYOU	0.73	0.00	0.58	0.05	0.00	0.04	0.06	1.46
NIKOS	3.13	0.00	2.12	0.07	0.00	0.04	0.36	5.73
SOLLUX	9.61	0.00	4.17	0.67	7.16	0.11	0.00	21.72
G2	1.10	0.04	1.28	0.04	0.09	0.08	0.00	2.64
G35	0.44	0.00	0.21	0.07	0.09	0.01	0.05	0.87
H10	0.06	0.00	0.09	0.05	0.00	0.02	0.08	0.31
H111-2	1.39	0.00	0.28	0.24	0.85	0.03	0.19	2.98
H128	2.23	0.10	0.80	0.06	0.00	0.09	0.34	3.61
H149	3.52	0.14	0.72	0.19	1.23	0.09	0.05	5.93
H19	0.10	0.00	0.05	0.00	0.00	0.02	0.03	0.20
H196	0.16	0.00	0.04	0.00	0.00	0.03	0.00	0.23
H219	0.33	0.00	0.08	0.07	0.00	0.02	0.00	0.51
H327	0.43	0.00	0.08	0.07	0.00	0.00	0.00	0.58
H344	0.59	0.22	0.42	0.37	0.00	0.00	0.00	1.61
H357	0.26	0.00	0.43	0.00	0.00	0.10	0.00	0.79
H54	0.13	0.00	0.19	0.18	0.00	0.01	0.07	0.59
H61	0.50	0.07	1.46	0.51	0.00	0.17	0.00	2.71
L239	0.45	0.00	0.09	0.04	0.15	0.01	0.00	0.74
R28	0.36	0.00	0.27	0.09	0.00	0.00	0.00	0.72
R31	*	*	*	*	*	*	*	*
R53	0.32	0.00	0.33	0.00	0.00	0.08	0.45	1.18
R64	0.73	0.03	0.30	0.06	0.00	0.00	0.00	1.12
R73	2.80	0.00	1.35	0.08	0.00	0.04	0.00	4.28
R75	0.10	0.00	0.03	0.00	0.00	0.01	0.03	0.16
R99	0.30	0.00	0.24	0.04	0.10	0.04	0.12	0.84
S14	2.75	0.00	1.48	0.08	0.00	0.08	0.00	4.40
S15	1.20	0.00	0.30	0.08	0.00	0.03	0.08	1.69
S17	3.58	0.00	2.48	0.10	0.00	0.08	0.25	6.49
S2	0.67	0.00	0.27	0.07	0.00	0.00	0.00	1.01
S3	5.64	0.00	0.39	0.47	0.06	0.08	0.20	6.84
MEAN	0.97	0.14	0.23	0.32	0.12	0.22	2.01	0.97
MIN.	0.08	0.00	0.00	0.03	0.04	0.05	0.33	0.08
MAX.	3.50	0.45	0.93	1.03	0.36	0.54	4.65	3.50

LEAF GLUCOSINOLATES OF DOUBLE HAPLOIDS AND THEIR MOTHER LINES (µmol/g D.M.)										
Double haploid Lines, M=Mansholt, S=Samourai										
M x S	**Pro**	**Gnl**	**Gna**	**4OH**	**Gbn**	**Gbc**	**Nas**	**4Me**	**Neo**	**Sum**
124	0.04	0.02	0.18	0.00	0.22	0.07	0.00	0.03	0.02	0.58
158 7.4.9	0.06	0.05	0.07	0.01	0.01	0.03	0.02	0.02	0.01	0.29
589 4.4.7	0.00	0.06	0.03	0.00	0.00	0.02	0.00	0.23	0.06	0.40
265	0.04	0.17	0.16	0.01	0.17	0.11	0.16	0.04	0.01	0.87
769	0.11	0.03	0.01	0.02	0.13	0.09	0.05	0.05	0.02	0.50
264	0.09	0.00	0.12	0.00	0.19	0.03	0.00	0.03	0.02	0.48
1036	0.16	0.08	0.05	0.02	0.17	0.09	0.01	0.02	0.01	0.60
258	0.01	0.04	0.04	0.05	0.06	0.07	0.11	0.07	0.00	0.44
1400 9.4.9	0.56	0.03	0.43	0.04	0.32	0.05	0.13	0.04	0.01	1.61
Cytoplasmatic male sterile mothers										
1059-197-3Ams	0.11	0.00	0.02	0.02	0.04	0.02	0.27	0.02	0.05	0.56
1231-199-3CAms	1.04	0.00	0.82	0.02	0.38	0.12	0.17	0.03	0.01	2.59
1046-95-1AAms	0.14	0.02	0.03	0.03	0.01	0.08	0.14	0.02	0.02	0.48
DH2527-94ms	0.15	0.01	0.13	0.01	0.12	0.08	0.06	0.01	0.01	0.60
2097-95-Ams	0.14	0.01	0.04	0.03	0.05	0.06	0.53	0.04	0.01	0.90
1108-102-2ms	0.16	0.00	0.28	0.01	0.31	0.06	0.05	0.01	0.01	0.89
1117-102-2ms	0.16	0.08	0.05	0.02	0.17	0.09	0.01	0.02	0.01	0.60
Standard cultivar										
Express	0.15	0.00	0.41	0.02	0.36	0.03	0.08	0.02	0.02	1.09
Statistics										
Mean	0.24	0.09	0.19	0.02	0.19	0.08	0.14	0.04	0.03	1.03
Min.	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.22
Max.	2.00	0.45	1.65	0.05	0.81	0.31	0.84	0.23	0.14	4.85

TESTCROSSES GROWN IN 2007 IN GÖTTINGEN										
Testcross		**Leaf glucosinolate content (µmol/g D.M.)**								
Mother	**Father**	**Pro**	**Gnl**	**Gna**	**4OH**	**Gbn**	**Gbc**	**Nas**	**4Me**	**Sum**
	R28	0.24	0.00	0.03	0.01	0.00	0.01	0.04	0.00	0.37
	H128	1.49	0.00	0.29	0.24	0.04	0.06	0.03	0.02	2.20
	H344	0.41	0.00	0.11	0.04	0.06	0.05	0.02	0.00	0.70
	R73	0.48	0.00	0.05	0.03	0.00	0.01	0.08	0.01	0.66
	S30	0.30	0.00	0.12	0.08	0.00	0.00	0.02	0.01	0.55
	R31	0.36	0.00	0.03	0.03	0.00	0.00	0.10	0.01	0.56
	H19	0.31	0.05	0.00	0.00	0.00	0.01	0.09	0.08	0.58
	S14	0.93	0.00	0.27	0.06	0.20	0.01	0.08	0.02	1.59
2097-	**R64**	0.21	0.00	0.05	0.02	0.00	0.02	0.04	0.02	0.45
95ams	**H327**	0.31	0.00	0.00	0.00	0.00	0.01	0.14	0.00	0.51
	G35	0.43	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.54
	R53	0.17	0.00	0.00	0.00	0.00	0.03	0.04	0.02	0.26
DH2527-	**H196**	0.18	0.00	0.05	0.00	0.00	0.01	0.00	0.00	0.28
94ms	**S3**	0.35	0.00	0.00	0.02	0.00	0.00	0.15	0.03	0.65
	H54	0.28	0.00	0.00	0.03	0.00	0.00	0.06	0.00	0.38
	H357	0.07	0.00	0.00	0.00	0.00	0.00	0.03	0.01	0.11
	S2	0.49	0.00	0.16	0.06	0.00	0.01	0.04	0.03	0.80
	G2	0.19	0.00	0.08	0.01	0.00	0.01	0.03	0.00	0.33
	H219	0.22	0.00	0.00	0.05	0.07	0.00	0.05	0.01	0.39
H605800	**S17**	0.25	0.00	0.08	0.01	0.00	0.00	0.06	0.01	0.43
MEAN		0.38	0.00	0.07	0.03	0.02	0.01	0.06	0.01	0.62
MIN.		0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11
MAX.		1.49	0.05	0.29	0.24	0.20	0.06	0.15	0.08	2.20
TESTCROSSES GROWN IN 2008										
Testcross (2008)		**Leaf glucosinolate content (µmol/g D.M.)**								
Mother	**Father**	**Pro**	**Gnl**	**Gna**	**4OH**	**Gbn**	**Gbc**	**Nas**	**4Me**	**Sum**
DH2527-	**S3**	0.24	0.54	0.19	0.01	0.42	0.04	0.14	0.03	1.68
94ms	**L239**	0.27	0.63	0.10	0.01	0.24	0.07	0.26	0.02	1.70
	S30	0.15	0.47	0.13	0.02	0.15	0.05	0.34	0.09	1.48
H605800	**R64**	0.77	0.58	0.20	0.00	0.43	0.05	0.09	0.04	2.19
	S15	0.61	0.61	0.07	0.01	0.23	0.06	0.24	0.02	1.88
	S16	0.32	0.54	0.04	0.01	0.14	0.09	0.16	0.02	1.31
	R73	0.44	0.65	0.07	0.01	0.15	0.13	0.19	0.01	1.65
	H19	0.24	0.59	0.08	0.01	0.13	0.07	0.11	0.01	1.23
	H357	0.69	0.66	0.07	0.00	0.26	0.22	0.26	0.04	2.23
	R19	1.08	0.62	0.23	0.01	0.41	0.10	0.25	0.01	2.70
	G2	0.41	0.46	0.09	0.01	0.28	0.11	0.07	0.00	1.42
	H327	*	*	*	*	*	*	*	*	*
	R1	0.31	0.65	0.04	0.00	0.11	0.11	0.16	0.01	1.42
	H344	1.51	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.51
MEAN		0.30	0.61	0.15	0.02	0.31	0.08	0.13	0.03	1.66
MIN.		0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.30
MAX.		0.93	1.28	0.73	0.37	1.55	0.28	0.38	0.10	3.80

TESTCROSSES GROWN IN 2008									
Testcross (2008)		**Stem glucosinolate content (µmol/g D.M.)**							
Mother	**Father**	**Pro**	**Gnl**	**Gna**	**Gbn**	**Gbc**	**Nas**	**4Me**	**Sum**
1046-95-1AAms	R59	0.99	1.00	0.23	0.71	0.08	0.17	0.01	3.19
	R53	0.20	0.53	0.01	0.08	0.10	0.18	0.01	1.12
	S30	0.29	0.56	0.00	0.09	0.07	0.21	0.01	1.24
	L239	1.19	1.26	0.02	0.39	0.20	0.38	0.02	3.46
	H196	1.38	0.51	0.20	0.75	0.12	0.27	0.01	3.28
	S4	0.10	0.02	0.00	0.02	0.13	0.00	0.01	0.40
	S14	1.47	0.60	0.27	1.05	0.15	0.14	0.00	3.71
	S3	1.76	1.22	0.17	0.63	0.13	0.52	0.01	4.44
	S31	0.74	0.46	0.31	0.39	0.12	0.15	0.02	2.19
1059-197-3ams	S3	0.64	0.50	0.05	0.05	0.11	0.28	0.00	1.63
	H196	0.24	0.34	0.01	0.28	0.07	0.13	0.06	1.13
	L239	0.30	0.75	0.11	0.19	0.18	0.14	0.01	1.68
	S14	0.92	0.64	0.29	0.86	0.12	0.32	0.06	3.21
1108-102-2ms	L239	0.55	1.22	0.07	0.35	0.11	0.26	0.02	2.58
	R53	0.33	0.66	0.05	0.26	0.09	0.16	0.01	1.56
	R59	0.84	0.58	0.18	0.58	0.08	0.04	0.01	2.38
	H196	0.38	0.38	0.06	0.25	0.09	0.13	0.01	1.30
	S3	0.81	0.57	0.25	0.51	0.10	0.08	0.01	2.34
	S4	0.51	0.60	0.15	0.46	0.16	0.16	0.01	2.08
	S14	0.52	0.63	0.16	0.33	0.10	0.08	0.00	1.84
	S30	0.23	0.62	0.00	0.09	0.07	0.08	0.00	1.12
	S31	0.15	0.32	0.08	0.15	0.06	0.06	0.00	0.84
1117-102-2ms	L239	0.32	0.00	0.00	0.44	0.00	0.00	0.06	0.88
	H196	0.26	1.25	0.04	0.08	0.11	0.22	0.02	1.96
	S14	0.48	0.00	0.10	0.17	0.08	0.00	0.00	0.89
	S3	0.82	1.19	0.21	0.37	0.11	0.09	0.02	2.81
	R53	0.38	0.99	0.07	0.22	0.07	0.14	0.00	1.88
	L122	1.81	1.13	0.28	1.46	0.17	0.37	0.02	5.31
1231-199-3cams	H196	0.41	0.04	0.09	0.09	0.06	0.12	0.00	0.82
	L239	0.91	0.68	0.13	0.28	0.22	0.16	0.00	2.39
	S14	0.59	0.38	0.08	0.25	0.05	0.12	0.01	1.49
	S3	1.01	0.00	0.26	0.43	0.07	0.22	0.00	1.98
2097-95-ams	R73	0.76	0.60	0.05	0.20	0.05	0.04	0.01	1.77
	R64	0.75	0.39	0.04	0.36	0.11	0.21	0.01	1.89
	H344	1.16	0.39	0.45	0.88	0.11	0.11	0.00	3.10
DH2527-94ms	H196	0.42	0.43	0.04	0.00	0.23	0.12	0.22	0.03
	S3	0.91	0.17	0.22	0.00	0.40	0.09	0.11	0.00
	L239	0.64	0.60	0.03	0.00	0.25	0.13	0.16	0.01
	S30	0.47	0.35	0.08	0.01	0.12	0.09	0.17	0.01

TESTCROSSES GROWN IN 2008										
Testcross (2008)		**Stem glucosinolate content (µmol/g D.M.)**								
		Pro	**Gnl**	**Gna**	**4OH**	**Gbn**	**Gbc**	**Nas**	**4Me**	**Sum**
H605800	**R64**	1.38	1.15	0.34	0.00	0.79	0.08	0.12	0.04	3.90
	S15	0.78	0.60	0.14	0.01	0.20	0.06	0.17	0.00	1.96
	S16	0.52	0.54	0.06	0.00	0.17	0.14	0.25	0.00	1.68
	R73	0.67	0.65	0.07	0.00	0.11	0.16	0.17	0.01	1.84
	H19	0.29	0.59	0.12	0.01	0.16	0.11	0.18	0.00	1.46
	H357	0.77	0.64	0.09	0.00	0.26	0.22	0.25	0.02	2.25
	R19	1.10	0.62	0.17	0.00	0.36	0.11	0.28	0.01	2.65
	G2	0.52	0.46	0.09	0.00	0.34	0.12	0.14	0.00	1.67
	H327	0.90	0.00	0.15	0.00	0.42	0.11	0.24	0.00	1.82
	R1	0.56	0.63	0.14	0.00	0.18	0.12	0.12	0.01	1.76
MEAN		0.76	0.57	0.15	0.01	0.40	0.12	0.16	0.01	2.07
MIN.		0.10	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.40
MAX.		3.88	1.26	1.64	0.07	3.17	0.58	0.52	0.10	5.31

BIOGAS FERMENTATION PARAMETERS FOR 2007									
Mother	**Father**	**Biogas Norm liter**	**Methane Norm liter**	**Methane**	**oADF**	**Dry matter**	**XA**	**ADL**	**XL**
		organic D.M.	**organic D.M.**	**%**	**%**	**%**	**%**	**%**	**%**
2097-95ams	**R28**	660	352	53.40	39.90	16.39	8.76	2.65	1.69
	H128	567	291	51.40	32.20	20.71	8.27	3.25	2.10
	H19	626	333	53.20	41.10	16.22	9.26	3.59	1.83
	H327	611	329	53.90	37.40	15.48	9.99	3.39	1.91
	H344	574	297	51.80	35.40	17.42	8.53	3.61	1.84
	R31	694	373	53.80	39.80	14.70	10.94	3.72	1.63
	R64	563	290	51.50	39.00	16.38	10.54	3.30	1.98
	R73	565	298	52.70	39.90	17.07	9.94	4.88	1.73
	S14	627	339	54.00	38.70	16.73	9.40	2.86	1.69
	S30	566	306	54.00	41.30	18.68	8.47	4.46	1.70
dh2527-94ms	**G35**	600	317	52.80	36.40	16.10	8.68	3.28	1.55
	H196	624	339	54.30	40.60	18.87	7.77	6.16	1.56
	R53	492	256	52.10	/	18.88	7.80	3.48	1.76
	S3	608	330	54.20	37.00	18.29	7.95	4.16	1.90
H605800	**G2**	550	287	52.20	37.30	19.28	9.85	3.73	1.49
	H219	523	275	52.50	39.50	16.93	9.15	4.23	1.69
	H357	665	357	53.70	36.00	14.93	9.84	3.31	2.13
	H54	554	290	52.40	34.60	20.88	8.97	4.40	1.93
	S17	584	322	55.10	40.80	15.51	9.83	3.72	1.63
	S2	525	278	53.00	39.00	17.90	8.99	4.65	1.76
MEAN		588.9	312.95	53.10	38.21	17.37	9.15	3.84	1.78
MIN.		492	256	51.40	32.20	14.70	7.77	2.65	1.49
MAX.		694	373	55.10	41.30	20.88	10.94	6.16	2.13

BIOGAS FERMENTATION PARAMETERS FOR 2008, GÖTTINGEN AND EINBECK											
Mother	**Father**	**Dry matter %**	**XL %**	**oADF %**	**ADL %**	**XA %**	**ADF %**	**N %**	**XP %**	**Biogas [l/kg oTS u.NB]**	**Methane %**
1108-102-2ms	**H196**	12.42	1.32	38.50	3.96	11.95	38.50	2.17	13.29	510.46	53.95
	S31	12.80	2.41	40.50	4.50	11.59	40.50	2.03	12.73	501.36	61.76
H605800	**H357**	13.12	1.84	43.60	4.63	12.15	43.60	1.75	10.86	477.52	64.26
	L341nc	15.57	2.03	41.70	4.13	9.45	41.70	2.31	14.42	549.32	61.84
1046-95-1AAms	**S31**	14.30	2.70	36.80	3.40	10.91	36.80	2.39	15.02	626.98	53.70
	R59	12.65	2.27	43.10	3.73	11.89	43.10	1.76	11.12	658.21	60.16
DH2527-94ms	**S30**	13.92	2.33	42.10	4.03	10.87	42.10	2.17	13.69	505.59	54.84
	H196	13.94	2.18	42.50	4.36	11.48	42.50	1.66	10.52	515.49	58.55
2097-95-Ams	**R73**	13.36	2.05	39.90	3.40	10.42	39.90	2.25	13.74	646.69	59.68
1046-95-1AAms	**Akela**	12.72	2.33	41.20	3.50	12.00	41.20	2.12	13.09	687.74	56.58
2097-95-Ams	**R64**	13.60	2.70	39.30	4.49	12.10	39.30	2.13	13.39	693.50	57.88
H605800	**G2**	13.15	2.26	38.90	3.94	11.56	38.90	2.19	13.69	541.26	65.67
1059-197-3Ams	**H196**	14.62	2.18	44.60	5.02	10.01	44.60	1.70	10.81	531.20	57.57
	S3	14.34	2.42	36.90	3.19	10.80	36.90	2.23	13.76	568.83	58.65
DH2527-94ms	**S3**	15.08	2.93	36.80	3.74	10.07	36.80	2.72	17.05	549.29	54.77

SEED GLUCOSINOLATES FOR DOUBLE HAPLOID LINES GROWN IN GÖTTINGEN (2008, µMOL/G D.M.)											
Dh	**Pro**	**Sin**	**Gnl**	**Gna**	**4OH**	**Gbn**	**Gbc**	**NAS**	**4Me**	**Neo**	**Sum**
3	43.77	0.00	0.08	17.49	2.77	4.64	0.23	0.45	0.60	0.07	70.11
4	57.81	0.00	0.10	31.26	4.35	3.50	0.31	0.71	0.65	0.07	98.80
6	75.45	1.94	0.08	24.26	4.17	1.29	0.32	0.64	0.50	0.09	108.76
8	59.54	0.34	0.07	22.42	4.46	4.10	0.69	0.59	0.54	0.07	92.83
9	38.11	0.00	0.00	14.91	3.51	0.02	0.15	0.45	0.31	0.08	59.69
10	51.85	0.00	0.00	22.44	4.11	3.09	0.25	0.49	0.24	0.08	82.57
11	28.63	0.53	0.00	15.14	4.01	1.15	1.21	1.21	0.35	0.05	52.29
12	60.04	0.25	0.00	19.00	3.34	2.42	0.31	0.36	0.29	0.12	86.15
15	41.46	0.03	0.00	16.55	3.29	2.21	0.27	0.39	0.21	0.08	64.51
16	48.55	0.47	0.00	22.92	4.08	3.19	0.35	0.51	0.26	0.05	80.40
17	42.84	0.00	0.00	15.38	3.90	2.21	0.28	0.45	0.31	0.09	65.48
18	54.57	0.00	0.00	18.88	3.75	3.44	0.32	1.30	0.58	0.09	82.93
19	36.93	0.00	0.00	14.42	4.61	2.14	0.43	0.51	0.42	0.08	59.58
20	40.20	0.05	0.00	21.74	4.20	3.32	0.31	0.46	0.57	0.06	70.94
21	54.55	0.00	0.00	15.64	3.28	3.54	0.32	0.43	0.86	0.08	78.70
22	31.82	0.00	0.08	16.14	3.98	3.43	0.24	0.46	0.33	0.12	56.60
25	35.84	0.00	0.06	14.04	4.20	3.59	0.34	0.43	0.42	0.07	59.00
26	33.52	0.00	0.00	11.20	4.95	1.26	0.63	0.51	0.26	0.08	52.42
27	46.96	0.00	0.00	19.70	3.68	1.63	0.46	0.38	0.35	0.06	73.21
28	47.63	0.00	0.00	15.05	3.23	1.50	0.33	0.33	0.29	0.10	68.46
29	41.39	0.16	0.00	18.09	3.42	1.39	0.26	0.32	0.38	0.04	65.45
30	45.14	0.02	0.00	12.29	2.95	2.03	0.14	0.31	0.22	0.08	63.18
31	39.48	0.05	0.00	15.54	3.14	2.13	0.19	0.36	0.42	0.14	61.45
32	54.99	0.04	0.00	13.21	3.89	1.80	0.21	0.33	0.19	0.07	74.75
33	39.25	0.00	0.00	17.31	3.38	1.81	0.29	0.30	0.60	0.05	62.99
35	51.95	0.00	0.00	19.30	3.43	1.70	0.18	0.30	0.42	0.06	77.35
37	40.95	0.00	0.00	17.65	3.52	2.96	0.18	0.32	0.34	0.06	65.98
38	45.34	0.12	0.00	25.13	3.50	3.22	0.18	0.32	0.57	0.06	78.44
44	36.25	0.00	0.04	17.01	3.71	2.53	0.27	0.37	0.37	0.07	60.62
45	43.41	0.00	0.04	21.20	3.53	2.55	0.21	0.31	0.22	0.05	71.52
46	58.91	1.45	0.11	16.72	5.20	1.87	0.82	0.42	0.25	0.10	85.87
47	42.99	0.08	0.00	14.51	4.56	2.23	0.55	0.41	0.37	0.12	65.86
48	40.95	0.00	0.04	17.08	3.59	3.13	0.21	0.32	0.43	0.10	65.85
49	64.37	0.00	0.06	22.02	5.88	3.31	0.69	0.52	0.28	0.13	97.30
50	4.91	0.16	0.09	58.24	4.45	4.19	0.30	0.40	0.34	0.02	73.11
52	44.76	0.03	0.00	13.98	4.04	3.46	0.50	0.42	0.35	0.13	67.67
53	43.10	0.00	0.00	16.48	4.82	2.08	0.36	0.48	0.36	0.08	67.79
102	47.84	0.82	1.84	13.41	4.52	2.88	0.49	0.48	0.50	0.09	72.88
103	47.87	0.03	0.00	22.86	3.64	3.56	0.30	0.20	0.51	0.19	79.18
104	56.32	0.02	0.00	16.04	3.79	2.84	0.30	0.41	0.55	0.10	80.41
105	49.61	0.05	0.00	18.07	3.71	4.52	0.22	0.37	0.42	0.08	77.09
106	44.74	0.00	0.00	15.02	4.36	1.91	0.38	0.47	0.41	0.11	67.43
109	59.22	0.05	0.00	14.09	2.91	3.42	0.16	0.33	0.46	0.11	80.76
111	48.28	0.17	0.13	5.82	2.49	4.82	0.18	0.31	0.30	0.08	62.61
112	45.23	0.05	0.00	13.44	4.27	2.23	0.91	0.49	0.28	0.09	67.01
113	41.43	0.02	0.00	13.77	4.16	2.53	0.60	0.47	0.27	0.07	63.33
114	38.80	0.00	0.00	11.71	4.08	1.63	0.71	0.46	0.25	0.07	57.73
115	67.55	0.00	0.00	18.71	7.72	1.78	1.16	0.85	0.36	0.14	98.30
117	54.15	0.02	0.00	17.26	5.18	2.14	0.43	0.60	0.28	0.08	80.19

SEED GLUCOSINOLATES FOR DOUBLE HAPLOID LINES GROWNN EINBECK (2008, µMOL/G D.M.)												
Dh	**Pro**	**Sin**	**Gnl**	**Gna**	**4OH**	**Gbn**	**Eru**	**Gbc**	**Nas**	**4Me**	**Neo**	**Sum**
3	55.41	0.39	0.00	23.10	3.41	5.61	0.02	0.36	0.91	0.59	0.11	89.89
4	42.35	0.30	0.00	25.52	3.14	2.30	0.02	0.29	0.85	0.28	0.09	75.15
6	57.74	3.06	0.00	23.25	4.16	1.71	0.02	0.40	0.94	0.44	0.08	91.82
8	46.05	0.12	0.00	24.08	3.90	1.65	0.03	0.41	0.93	0.32	0.09	77.58
9	51.95	0.18	0.00	31.55	3.08	2.43	0.01	0.25	0.64	0.45	0.04	90.58
10	62.55	0.16	0.00	25.92	3.84	3.99	0.02	0.32	0.86	0.38	0.08	98.13
11	38.65	0.15	0.02	20.09	3.15	2.11	0.02	0.32	0.70	0.34	0.07	65.64
12	59.13	0.29	0.00	23.42	3.57	3.65	0.02	0.35	0.75	0.42	0.10	91.69
15	48.15	0.21	0.00	22.04	3.43	2.15	0.03	0.52	0.76	0.27	0.08	77.64
16	42.93	0.22	0.00	22.06	3.71	4.28	0.03	0.43	0.79	0.29	0.07	74.79
17	39.31	0.18	0.00	17.62	3.44	2.22	0.02	0.28	0.71	0.24	0.04	64.05
18	63.54	0.35	0.10	23.81	3.94	3.34	0.03	0.47	0.87	0.56	0.08	97.07
19	48.42	0.27	0.02	21.31	4.54	4.04	0.03	0.47	0.89	0.61	0.08	80.69
20	52.22	0.00	0.00	25.86	3.25	4.10	0.00	0.43	0.80	0.71	0.10	87.47
21	67.26	0.00	0.00	18.08	3.54	2.69	0.00	0.37	0.52	0.74	0.09	93.29
22	43.84	0.00	0.06	19.69	5.18	2.00	0.00	0.82	0.83	0.53	0.09	73.04
25	34.44	0.00	0.14	16.56	3.19	2.48	0.00	0.32	0.63	0.42	0.09	58.27
26	45.03	0.00	0.06	17.05	4.00	1.95	0.00	0.46	0.75	0.35	0.07	69.72
27	51.59	0.00	0.00	23.52	3.25	1.40	0.00	0.22	0.46	0.32	0.02	80.78
28	39.98	0.00	0.06	18.16	2.78	1.35	0.00	0.24	0.41	0.25	0.04	63.26
29	75.26	0.00	0.11	32.49	6.48	1.94	0.00	0.48	0.89	0.54	0.04	118.22
30	57.90	0.00	0.16	27.55	4.45	1.78	0.00	0.24	0.68	0.42	0.02	93.21
31	48.24	0.00	0.13	20.97	4.24	1.87	0.00	0.25	0.59	0.51	0.03	76.82
32	70.80	0.00	0.09	30.26	5.56	2.60	0.00	0.27	0.73	0.42	0.06	110.78
33	64.63	0.00	0.09	33.15	5.07	2.45	0.00	0.30	0.60	0.66	0.04	106.98
35	38.04	0.00	0.09	17.21	3.17	1.40	0.00	0.24	0.57	0.37	0.06	61.15
36	55.57	0.00	0.04	29.06	2.20	1.83	0.00	0.11	0.26	0.42	0.03	89.52
37	42.52	0.00	0.06	21.34	2.94	2.22	0.00	0.24	0.40	0.31	0.02	70.05
38	41.90	0.00	0.00	22.09	2.81	2.66	0.00	0.25	0.51	0.61	0.06	70.88
44	31.07	0.00	0.07	26.30	2.54	2.19	0.00	0.19	0.35	0.25	0.02	62.97
45	54.74	0.00	0.07	21.64	3.06	2.45	0.00	0.29	0.46	0.29	0.05	83.06
46	55.34	0.00	0.03	16.60	3.73	1.47	0.00	0.33	0.79	0.26	0.06	78.60
47	40.05	0.00	0.07	20.99	4.65	1.45	0.00	0.60	0.56	0.41	0.05	68.82
48	40.80	0.00	0.17	18.60	3.34	2.93	0.00	0.25	0.46	0.51	0.06	67.12
49	47.95	0.00	0.00	17.96	3.81	1.61	0.00	0.33	0.40	0.17	0.09	72.32
50	16.82	0.00	0.10	42.55	3.60	2.78	0.00	0.38	0.63	0.36	0.06	67.28
52	48.27	0.00	0.03	19.99	3.45	1.90	0.00	0.62	0.59	0.42	0.15	75.43
53	43.44	0.00	0.00	21.96	4.03	1.27	0.00	0.32	0.45	0.34	0.05	71.86
54	57.21	1.57	0.15	37.64	3.62	2.47	0.22	0.27	0.68	0.44	0.05	104.32
55	41.91	0.47	0.00	17.66	3.16	2.60	0.00	0.33	0.69	0.22	0.06	67.10
56	59.20	1.17	0.17	20.13	3.60	3.04	0.01	0.54	0.74	0.27	0.11	88.99
58	65.69	0.73	0.00	25.32	3.86	4.42	0.04	0.39	0.90	0.42	0.05	101.82
59	46.09	0.51	0.05	24.55	3.02	2.93	0.01	0.22	0.66	0.27	0.07	78.40
60	46.87	0.48	0.00	22.92	2.79	3.16	0.01	0.19	0.63	0.28	0.09	77.41
61	44.95	2.01	0.07	25.66	3.28	3.65	0.11	0.27	0.78	0.35	0.07	81.21
63	38.17	1.59	0.28	44.53	3.72	5.48	0.09	0.48	0.80	0.46	0.06	95.65
65	5.05	0.25	0.00	71.57	2.17	5.64	0.01	0.21	0.55	0.27	0.02	85.75
66	48.83	1.16	0.09	24.87	3.38	3.38	0.11	0.31	0.74	0.38	0.06	83.31
67	42.70	1.51	0.29	28.55	2.90	2.54	0.07	0.32	0.75	0.37	0.07	80.06
68	41.16	0.88	0.10	21.15	3.34	2.97	0.01	0.62	0.79	0.45	0.05	71.52
69	61.44	1.63	0.14	18.47	3.49	3.29	0.03	0.85	0.74	0.44	0.08	90.61
70	57.19	0.85	0.00	33.04	3.22	3.83	0.02	0.34	0.72	0.56	0.05	99.82

Seed glucosinolates for the double haploid lines grown in 2008 in Einbeck (µmol/g D.M.)												
Dh	**Pro**	**Sin**	**Gnl**	**Gna**	**4OH**	**Gbn**	**Eru**	**Gbc**	**Nas**	**4Me**	**Neo**	**Sum**
71	39.92	1.32	0.23	23.55	3.71	2.28	0.00	0.45	0.92	0.16	0.06	72.60
72	57.93	2.11	0.29	24.13	4.47	4.17	0.12	0.46	1.18	0.53	0.08	95.48
73	50.32	1.30	0.10	25.64	3.98	3.24	0.07	0.35	0.90	0.33	0.07	86.31
74	52.20	1.27	0.13	27.79	3.72	5.38	0.11	0.28	0.83	0.55	0.07	92.32
75	40.82	0.56	0.00	19.21	3.18	2.60	0.00	0.34	0.73	0.23	0.06	67.74
76	46.49	0.61	0.00	22.05	2.08	3.06	0.00	0.26	0.54	0.27	0.04	75.40
79	57.00	0.70	0.00	25.37	2.45	2.40	0.00	0.41	0.44	0.29	0.09	89.15
80	64.83	0.76	0.00	24.04	2.84	5.16	0.02	0.39	0.61	0.44	0.05	99.12
81	67.25	0.96	0.00	27.65	2.46	4.15	0.05	0.38	0.63	0.35	0.05	103.93
82	60.90	0.91	0.00	19.81	3.51	3.28	0.02	0.55	0.73	0.25	0.09	90.04
83	27.94	0.44	0.00	34.08	3.41	3.42	0.03	0.26	0.82	0.31	0.07	70.79
85	60.05	0.94	0.00	28.76	4.67	4.93	0.02	0.99	0.93	0.31	0.06	101.67
86	59.52	1.97	0.16	25.79	4.55	6.06	0.05	0.55	0.99	0.51	0.06	100.21
87	58.99	1.26	0.14	25.84	3.34	4.26	0.04	0.48	0.68	0.32	0.06	95.41
88	49.22	0.64	0.00	23.57	3.54	2.39	0.02	0.36	0.80	0.37	0.08	80.97
89	44.19	0.68	0.00	17.43	2.44	1.89	0.01	0.32	0.62	0.29	0.06	67.92
90	79.07	1.91	0.37	29.37	3.29	3.21	0.04	0.34	0.60	0.52	0.11	118.83
91	45.62	1.15	0.10	19.95	3.64	2.29	0.02	0.51	0.78	0.40	0.07	74.52
93	42.99	0.32	0.00	18.89	2.87	3.65	0.02	0.41	0.50	0.32	0.05	70.02
94	40.79	0.70	0.00	21.66	3.23	3.05	0.02	0.43	0.55	0.38	0.05	70.85
95	68.42	0.61	0.14	25.04	4.04	6.80	0.02	0.37	0.66	1.26	0.05	107.43
96	68.85	0.79	0.04	29.53	3.87	3.64	0.02	0.25	0.61	0.34	0.05	107.99
98	55.58	0.65	0.09	27.19	3.26	2.80	0.02	0.19	0.53	0.31	0.05	90.67
99	57.23	0.65	0.11	25.67	3.35	2.84	0.02	0.21	0.55	0.29	0.05	90.97
101	43.70	0.95	0.09	17.01	3.43	3.02	0.01	0.39	0.57	0.22	0.04	69.43
102	31.45	0.00	0.02	16.69	2.88	1.61	0.00	0.19	0.37	0.39	0.06	53.67
103	31.83	0.00	0.02	20.34	2.98	1.54	0.00	0.19	0.32	0.28	0.05	57.55
104	44.09	0.00	0.00	16.85	2.93	1.32	0.00	0.12	0.31	0.24	0.06	65.91
105	42.78	0.00	0.07	16.69	2.48	3.25	0.00	0.19	0.42	0.43	0.07	66.38
106	39.54	0.00	0.07	14.72	3.18	1.22	0.00	0.19	0.35	0.25	0.06	59.59
109	44.61	0.00	0.00	12.89	2.43	1.96	0.00	0.10	0.26	0.25	0.11	62.61
111	42.26	0.00	0.00	19.24	3.09	1.38	0.00	0.14	0.38	0.34	0.11	66.94

SEED GLUCOSINOLATES FOR THE DOUBLE HAPLOID LINES GROWN IN EINBECK (2008, µMOL/G D.M.)												
Dh	**Pro**	**Sin**	**Gnl**	**Gna**	**4OH**	**Gbn**	**Eru**	**Gbc**	**Nas**	**4Me**	**Neo**	**Sum**
112	37.40	0.00	0.02	13.32	3.33	1.12	0.00	0.39	0.40	0.28	0.07	56.33
113	41.35	0.00	0.02	17.12	3.67	1.32	0.00	0.42	0.37	0.31	0.04	64.62
114	54.15	0.00	0.00	22.08	5.04	1.46	0.00	0.63	0.48	0.35	0.04	84.23
115	63.36	0.25	0.16	22.19	5.26	3.05	0.05	0.53	0.80	0.46	0.11	96.23
117	44.75	0.34	0.10	17.56	4.01	2.28	0.04	0.22	0.43	0.21	0.10	70.04
118	64.17	1.42	0.09	25.49	4.25	2.76	0.07	0.34	0.64	0.33	0.07	99.62
119	50.17	1.49	0.00	26.83	2.64	2.09	0.07	0.20	0.64	0.16	0.03	84.34
120	44.27	0.00	0.27	11.92	4.47	3.98	0.04	0.51	0.96	0.50	0.13	67.05
121	41.50	0.00	0.06	16.43	4.11	3.01	0.00	0.42	0.55	0.57	0.03	66.70
122	28.42	0.00	0.09	16.81	3.70	0.70	0.00	0.25	0.55	0.30	0.02	50.84
123	31.83	0.00	0.09	14.72	3.01	2.21	0.00	0.21	0.55	0.42	0.15	53.18
124	23.91	0.00	0.10	34.91	3.94	0.83	0.00	0.22	0.58	0.24	0.03	64.76
126	56.51	0.00	0.00	20.45	3.49	1.32	0.00	0.23	0.45	0.29	0.06	82.80
127	49.53	0.00	0.05	16.07	3.70	2.46	0.00	0.40	0.52	0.38	0.05	73.16
128	30.10	0.00	0.13	17.85	2.24	1.57	0.00	0.15	0.42	0.31	0.05	52.81
129	32.07	0.00	0.16	17.64	2.51	1.68	0.00	0.16	0.35	0.37	0.04	54.98
130	40.40	0.00	0.04	24.89	2.59	3.15	0.00	0.14	0.39	0.25	0.04	71.90
132	35.61	0.00	0.15	15.88	2.59	1.56	0.00	0.25	0.41	0.26	0.09	56.81
133	32.14	0.00	0.19	19.32	2.30	1.69	0.00	0.10	0.30	0.35	0.05	56.44
134	37.77	0.00	0.16	14.26	3.36	1.37	0.00	0.35	0.43	0.17	0.05	57.91
137	67.32	0.58	0.07	19.16	3.03	2.36	0.00	0.21	0.43	0.22	0.10	93.49
138	50.74	0.36	0.00	18.78	2.89	2.45	0.01	0.15	0.64	0.31	0.09	76.41
120	53.06	0.89	0.00	17.48	3.85	3.22	0.01	0.33	0.83	0.35	0.13	80.17
141	42.59	0.38	0.00	17.66	3.23	3.00	0.01	0.26	0.72	0.30	0.17	68.32
142	60.70	0.13	0.00	26.63	4.38	4.19	0.01	0.25	0.56	0.39	0.09	97.32
143	50.44	0.13	0.04	21.56	3.99	1.95	0.02	0.24	0.59	0.23	0.15	79.33
144	47.70	0.49	0.00	31.41	4.65	1.71	0.02	0.41	0.62	0.30	0.07	87.39
145	48.79	0.46	0.00	28.51	3.55	1.64	0.01	0.30	0.49	0.24	0.06	84.07
146	38.59	0.24	0.00	14.77	4.15	1.87	0.03	0.36	0.91	0.26	0.13	61.32
147	48.02	0.33	0.06	26.24	4.02	1.93	0.03	0.39	0.72	0.22	0.09	82.05
148	46.52	0.29	0.03	29.04	4.60	3.84	0.02	0.33	0.60	0.50	0.11	85.89
149	50.72	0.35	0.00	32.38	3.83	3.98	0.02	0.28	0.57	0.65	0.10	92.87
150	47.61	0.31	0.00	22.18	3.40	2.12	0.01	0.24	0.47	0.29	0.06	76.70
151	38.84	0.25	0.00	21.52	3.20	1.87	0.02	0.23	0.45	0.16	0.07	66.61
152	48.09	0.34	0.00	24.31	4.31	2.88	0.02	0.45	0.58	0.64	0.09	81.70
153	73.89	0.56	0.10	25.44	4.47	3.90	0.02	0.64	0.58	0.70	0.09	110.38
154	64.28	0.37	0.13	22.51	3.70	4.49	0.02	0.31	0.65	0.43	0.12	97.01
155	38.37	0.24	0.00	22.11	3.14	2.03	0.02	0.26	0.56	0.18	0.08	66.99
156	39.28	0.91	0.09	26.21	3.84	2.28	0.02	0.25	0.57	0.25	0.09	73.80
157	32.95	0.32	0.00	19.25	3.45	1.84	0.00	0.33	0.63	0.26	0.05	59.10
158	45.51	0.44	0.00	24.87	3.21	3.17	0.07	0.35	0.57	0.49	0.06	78.75
159	38.73	1.89	0.00	20.70	3.61	2.41	0.11	0.28	0.76	0.31	0.07	68.87
160	56.23	0.57	0.00	18.25	3.21	2.51	0.02	0.48	0.71	0.45	0.12	82.55
161	51.94	0.54	0.00	24.09	3.05	2.83	0.02	0.24	0.57	0.38	0.08	83.75
162	45.70	0.94	0.12	22.99	4.00	2.69	0.05	0.29	0.68	0.37	0.07	77.91
163	46.84	0.48	0.00	21.72	2.47	1.88	0.00	0.19	0.47	0.19	0.09	74.33
164	70.04	0.74	0.23	22.38	3.70	2.46	0.05	0.51	0.69	0.79	0.14	101.73
165	56.58	0.61	0.00	25.18	3.50	3.32	0.07	0.29	0.66	0.45	0.12	90.76
167	44.97	0.25	0.00	20.75	3.44	1.79	0.00	0.35	0.68	0.31	0.07	72.60
164	70.04	0.74	0.23	22.38	3.70	2.46	0.05	0.51	0.69	0.79	0.14	101.73
168	34.56	0.47	0.00	19.64	2.82	1.74	0.00	0.18	0.57	0.29	0.07	60.35

SEED GLUCOSINOLATES FOR THE DOUBLE HAPLOIDS GROWN IN 2008 IN EINBECK (µMOL/G D.M.)												
DH Nr.	**Pro**	**Sin**	**Gnl**	**Gna**	**4OH**	**Gbn**	**Eru**	**Gbc**	**Nas**	**4Me**	**Neo**	**Sum**
169	51.09	0.55	0.00	20.47	3.46	3.19	0.00	0.37	0.79	0.46	0.08	80.46
171	44.20	0.54	0.00	24.30	3.84	2.71	0.01	0.46	0.82	0.18	0.06	77.12
172	42.91	0.47	0.00	20.50	2.58	2.04	0.00	0.19	0.54	0.29	0.13	69.65
173	40.27	0.92	0.00	16.51	3.41	1.96	0.06	0.39	0.69	0.22	0.09	64.52
195	66.35	0.30	0.04	28.57	4.48	3.65	0.03	0.42	0.57	0.25	0.05	104.73
196	44.96	0.99	0.00	20.62	3.83	3.12	0.01	0.49	0.40	0.35	0.06	74.82
198	55.77	0.00	0.03	27.96	4.31	2.12	0.03	0.32	0.70	0.37	0.07	91.68
199	52.33	0.12	0.03	21.31	3.75	4.14	0.03	0.27	0.65	0.38	0.05	83.07
200	41.28	0.87	0.06	21.86	3.21	2.98	0.01	0.16	0.47	0.37	0.04	71.33
201	42.40	0.36	0.08	19.71	3.21	3.59	0.01	0.22	0.41	0.47	0.05	70.51
202	44.84	0.00	0.04	24.65	3.08	2.72	0.02	0.17	0.40	0.20	0.07	76.19
203	50.53	0.00	0.03	17.65	3.61	3.43	0.02	0.31	0.62	0.33	0.10	76.64
204	44.50	0.21	0.06	20.93	4.02	2.99	0.03	0.30	0.60	0.41	0.06	74.11
205	44.91	0.32	0.03	22.20	3.75	2.50	0.02	0.29	0.54	0.40	0.10	75.05
206	58.71	0.65	0.00	25.09	3.52	4.07	0.04	0.29	0.65	0.65	0.04	93.71
213	51.95	0.66	0.10	14.77	5.07	5.58	0.02	0.81	0.79	0.83	0.08	80.67
215	61.57	0.68	0.07	18.11	4.17	2.41	0.02	0.26	0.73	0.26	0.06	88.33
218	42.12	0.52	0.00	20.36	2.58	3.22	0.01	0.32	0.50	0.38	0.04	70.04
219	44.27	0.52	0.10	20.94	3.47	2.39	0.01	0.29	0.62	0.30	0.04	72.97
241	67.73	0.00	0.04	24.22	5.06	3.94	0.05	0.42	0.83	0.51	0.09	102.87
242	51.42	0.20	0.07	22.94	3.26	2.20	0.02	0.18	0.50	0.26	0.09	81.15
243	53.99	0.29	0.11	20.97	4.59	3.10	0.03	0.36	0.65	0.32	0.07	84.49
245	49.41	0.00	0.00	25.21	3.00	2.53	0.00	0.39	0.35	0.27	0.05	81.21
247	67.17	0.31	0.09	23.47	4.04	3.92	0.03	0.38	0.55	0.48	0.12	100.56
248	46.37	0.09	0.06	20.31	3.53	2.44	0.02	0.32	0.59	0.37	0.05	74.14
249	56.53	0.24	0.06	24.69	3.33	5.30	0.03	0.28	0.56	0.40	0.12	91.54
250	43.68	0.53	0.09	21.37	4.19	2.60	0.03	0.33	0.65	0.27	0.10	73.82
251	60.88	0.02	0.00	19.80	4.07	4.19	0.03	0.25	0.47	0.38	0.07	90.17
252	42.92	0.02	0.03	19.66	3.38	1.88	0.01	0.20	0.45	0.23	0.06	68.85
253	46.84	0.00	0.08	19.06	3.66	4.61	0.05	0.40	0.64	0.61	0.06	76.00
254	56.28	0.22	0.09	20.05	3.65	5.15	0.03	0.38	0.59	0.58	0.05	87.07
256	48.45	0.59	0.00	29.03	2.57	2.61	0.03	0.24	0.66	0.30	0.08	84.55
258	46.22	0.38	0.06	29.08	4.45	2.61	0.03	0.38	0.68	0.48	0.05	84.40
260	70.50	0.33	0.09	28.96	4.07	4.42	0.03	0.61	0.59	0.39	0.09	110.09
263	48.76	0.03	0.00	14.25	3.13	2.01	0.00	0.23	0.35	0.28	0.11	69.15
247	67.17	0.31	0.09	23.47	4.04	3.92	0.03	0.38	0.55	0.48	0.12	100.56
SOLLUX	57.91	0.71	0.10	21.88	3.79	3.11	0.02	0.29	0.56	0.29	0.08	88.74
SOLLUX	61.75	0.03	0.00	23.17	4.70	3.41	0.03	0.31	0.53	0.27	0.08	94.28
GAOYOU	32.85	0.00	0.07	15.93	2.98	2.05	0.02	0.29	0.49	0.27	0.05	55.00
GAOYOU	43.26	0.08	0.08	22.28	3.94	3.33	0.03	0.40	0.64	0.44	0.06	74.54

LEAF GLUCOSINOLATES FOR THE DOUBLE HAPLOIDS GROWN IN 2008 (µMOL/G D.M.)										
Nr.	**Pro**	**Gnl**	**Gna**	**4OH**	**Gbn**	**Gbc**	**Nas**	**4Me**	**Neo**	**Sum**
4	1.56	1.86	0.08	1.95	0.24	0.08	0.18	0.03	0.02	6.01
19	2.06	0.88	0.54	0.02	1.87	0.15	0.24	0.01	0.00	5.78
21	0.62	0.15	0.00	0.00	0.68	0.04	0.40	0.06	0.05	1.99
22	1.11	0.81	0.00	0.69	0.52	0.08	0.00	0.05	0.01	3.26
25	0.92	0.40	0.96	1.27	1.58	0.07	0.15	0.04	0.03	5.42
27	1.09	0.45	0.67	1.64	1.06	0.24	0.13	0.02	0.02	5.32
29	1.33	0.31	0.60	0.15	2.70	0.10	0.11	0.02	0.01	5.34
32	2.23	0.56	1.05	0.52	2.50	0.13	0.10	0.01	0.00	7.10
35	0.79	0.56	0.52	0.38	1.22	0.07	0.06	0.01	0.00	3.62
36	1.09	0.37	1.45	0.40	2.35	0.13	0.25	0.02	0.02	6.09
44	1.61	0.19	0.99	0.43	1.95	0.10	0.09	0.01	0.01	5.41
53	1.22	0.37	0.59	0.70	1.86	0.19	0.22	0.01	0.00	5.16
54	2.27	0.88	1.53	0.92	2.19	0.08	0.16	0.02	0.00	8.05
55	1.03	0.30	0.34	0.46	0.70	0.06	0.07	0.04	0.02	3.01
59	2.59	0.61	0.88	0.17	2.66	0.13	0.10	0.01	0.03	7.18
69	1.63	0.67	0.58	0.92	1.65	0.09	0.06	0.02	0.01	5.64
76	2.15	0.61	1.11	0.12	3.03	0.07	0.11	0.03	0.01	7.25
79	2.11	0.91	1.24	0.58	2.00	0.14	0.22	0.05	0.03	7.25
80	3.15	0.83	0.00	0.00	1.48	0.08	0.00	0.02	0.00	5.64
86	1.36	0.46	0.74	0.34	2.59	0.25	0.13	0.04	0.00	5.88
87	1.52	0.46	0.63	0.12	1.30	0.16	0.11	0.06	0.02	4.32
88	1.40	0.58	0.79	0.55	1.02	0.11	0.23	0.02	0.02	4.71
90	1.98	0.35	0.77	0.46	0.16	0.09	0.01	0.02	0.03	3.92
90	1.99	0.72	0.55	0.52	0.60	0.09	0.02	0.01	0.00	4.50
91	2.57	0.62	1.05	0.69	2.17	0.09	0.19	0.07	0.01	7.39
93	1.68	0.41	0.14	2.41	0.57	0.07	0.00	0.01	0.01	5.37
94	1.68	0.78	0.05	0.38	1.87	0.05	0.00	0.02	0.02	4.86
99	1.08	0.29	0.93	0.95	2.24	0.07	0.14	0.08	0.01	5.72
102	1.95	1.25	0.56	0.66	0.87	0.13	0.06	0.01	0.00	5.52
104	1.41	0.60	1.46	1.45	0.36	0.07	0.00	0.02	0.01	5.41
118	2.88	1.76	1.38	0.71	3.12	0.08	0.18	0.02	0.04	10.14
120	0.86	0.59	0.56	1.43	0.35	0.06	0.15	0.02	0.00	4.04
124	0.76	0.10	0.41	0.64	0.63	0.05	0.19	0.03	0.02	2.85
128	2.87	0.35	0.00	0.04	0.67	0.10	0.00	0.01	0.03	4.11
128	1.54	0.29	0.84	0.02	1.85	0.04	0.00	0.04	0.01	4.63
129	0.95	0.27	0.69	0.90	2.10	0.09	0.25	0.03	0.00	5.34
130	1.51	0.67	0.29	0.01	2.11	0.05	0.24	0.08	0.07	4.89
132	1.48	1.03	0.42	0.38	1.58	0.10	0.27	0.01	0.04	5.31
133	0.94	0.55	0.58	1.39	0.95	0.07	0.07	0.03	0.02	4.59
134	1.21	0.44	0.46	0.02	1.64	0.10	0.02	0.03	0.00	3.96
141	2.12	0.70	0.00	0.00	1.90	0.09	0.00	0.03	0.00	4.85
143	1.20	0.50	0.25	0.54	0.87	0.08	0.13	0.01	0.01	3.59
148	1.14	1.12	0.00	1.34	1.21	0.07	0.00	0.02	0.01	4.92
149	0.88	0.66	0.10	0.21	0.47	0.08	0.04	0.04	0.02	2.51
152	1.43	0.65	0.52	0.01	1.71	0.12	0.04	0.04	0.01	4.53
153	1.26	0.55	0.09	0.71	0.52	0.08	0.00	0.05	0.01	3.26
158	1.84	0.68	0.83	0.28	1.71	0.09	0.10	0.02	0.02	5.57
160	2.13	0.86	1.16	0.37	1.80	0.07	0.09	0.02	0.01	6.50
163	1.13	0.21	1.66	1.19	1.69	0.08	0.11	0.03	0.01	6.10
164	1.24	0.68	2.82	1.39	0.07	0.10	0.17	0.01	0.00	6.49
165	1.21	0.88	0.16	0.89	0.38	0.11	0.00	0.05	0.01	3.69

CULTIVAR	FORM	QUALITY	BREEDER	COUNTRY	1. Permission
NIKOS	FORAGE RAPESEED	00	EURO Grass	Netherlands	2000
EXPRESS	WINTER RAPESEED	00	NPZ	Germany	1993
SOLLUX	WINTER RAPESEED	++	ZG Winterraps	Germany (DDR)	1973
GAOYOU	CHINESE CULTIVAR	++	Zhejiang Agric. Univ.	China	1990
ESKISEHIR	WINTER RAPESEED	/	/	Turkey	/
LIRAJET	WINTER RAPESEED	00	DSV	Germany	1989
ZHONGSHUANG 9	CHINESE CULTIVAR	/	/	China	/
PERKO	TURNIP RAPESEED	++	KWS	Germany	1969
LARGO	TURNIP RAPESEED	00	SW Seed	Sweden	2002
LEMBKES NORMAL	WINTER RAPESEED	++	Lembke	Germany	/
MOSA	FORAGE RAPESEED	00	Joordens	Netherlands	2001
DH SAMOURAI	WINTER RAPESEED	00	Serasem	France	1991
MARUCA	WINTER RAPESEED	+0	NPZ	Germany	2002
DH MANSHOLTS	WINTER RAPESEED	++	Mansholt	Netherlands	1899
BRISTOL	WINTER RAPESEED	00	Cargill	France	1991

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SUMMARY

Brassica napus L.*,* a plant that belongs to the *Brassicaceae* family is one of the main oil crops. Inside its seeds glucosinolates appear to be the main secondary glycoside, sugar containing component with sulfur containing bindings. Plant breeders have tried to lower the glucosinolate levels of seeds of rapeseed so that the high-protein seed meal remaining after oil extraction can be used as animal food. Products such as the nitriles and isothiocyanates are toxic components. Some of the glucosinolate types or alkenyles have negative effects in animal nutrition such as progoitrin. Very little is known about the function and roles of glucosinolates in the vegetative tissue of flowering winter rapeseed. The decomposition products are possibly inhibiting for bacteria in fermentation processes, caused by the very reactive side chains, released after cleavage with the myrosinase enzyme. The main objectives were to explore and define the genetic basis of the synthesis of glucosinolates in the green matter of oilseed rape and their effect on biogas production, to investigate the genetic variation of the glucosinolate contents and pattern in the vegetative tissue in classical breeding material and resynthesized rapeseed lines, to acquire information that determines the influence of the glucosinolate content and pattern in the green matter on the biogas production and finally to develop and characterize a quantitative trait loci map based on a mapping population from a cross. Causes of genetic variation are diverse, from so called mutations towards differences in chromosome number, whether this genetic variation is potentially available for certain secondary components such as the glucosinolates. More recently developed varieties with low glucosinolate levels in seeds but high glucosinolate levels in leaves are more resistant to pests and still provide a protein-rich seed residue for animal feeding. Winter rapeseed resynthesized parents and testcrosses with high biomass yielding lines were tested under different environments. Besides this a double haploid population from a cross between an exotic line (Gaoyou) and a cultivar were grown and analyzed for their glucosinolate content. These alternative crosses are made to find out more about differences in essential acting key genes that reveal the sequences behind exotic lines competing and interfering with local breeding forms. The glucosinolate content within the leaves does not correlate with the seed glucosinolates; also there is no correlation between methane, leaf and stem glucosinolates. Attention is focused on the leaf glucosinolates to identify those quantitative trait loci, which are situated in the genome and which are responsible for the glucosinolate content within the leaves. The determination of genetic variation of leaf, stem and seed glucosinolates in resynthesized winter rapeseed lines is a rather exceptional step in plant genomics. Although this study does not go into detail in the molecular level, a small jumping-leap is taken when the location of the traits is estimated. Further genetic studies are necessary to develop appropriate breeding strategies to reduce or increase leaf, stem and seed glucosinolates in winter rapeseed lines. Segregating populations of winter rapeseed lines should be tested in

the future for their glucosinolate content in the leaves, stems and seeds. The polymorphisms for the occurrence of leaf stem and seed glucosinolates might be explained by their phenotypical differences, but also by the different functioning of these specific plant parts. Whether the number of genes involved in leaf glucosinolates is different from those involved in the functioning of the seed glucosinolates is not known. The higher the genetic variation for the resulting winter rapeseed breeding lines however, the better their persistence in different environments, where for example insect resistance is required.