GLUCOSINOLATES & BEYOND

Proceedings | 3rd International Glucosinolate Conference 2014

12th October – 15th October 2014 | Wageningen | The Netherlands

Guusje Bonnema en Ruud Verkerk

WAGENINGEN UR
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Proceedings of the 3rd International Glucosinolate Conference 2014

“Glucosinolates and Beyond”

Wageningen University

Editors:
Guusje Bonnema
Ruud Verkerk

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Welcome

Welcome to the third international glucosinolate conference: Glucosinolates and beyond.

Dear colleagues,

On behalf of the organizing committee, we have the pleasure to welcome you to Wageningen, The Netherlands, to attend the 3rd international glucosinolate conference **"Glucosinolates and Beyond"**. This conference is a follow-up of previous conferences held in Jena, Germany (2006) and Elsinore, Denmark (2009).
We succeeded in attracting over 100 participants, from 23 countries in Europe, Asia, USA and Africa, and sponsors from Dutch graduate schools, research organizations and companies.

The mission of Wageningen University and Research Center is: "to explore the potential of nature to improve the quality of life". Wageningen UR combines the strengths of research institutes and the university in the fields of plant science, agrotechnology and food science, environmental science, animal science and the social sciences. These different disciplines, and the interactions and synergy between disciplines, are also reflected in the breadth of research focused on glucosinolates.

During this conference we aim at bringing together scientists that are active in diverse areas of glucosinolate research in order to exchange scientific knowledge and ideas.

To stimulate exchange between the disciplines molecular genetics, evolution and ecology, food science, agronomy and human nutrition, we chose four plenary sessions and invited keynote speakers in the respective disciplines and poster sessions introduced by poster flash presentations. This, together with the excursion Tuesday afternoon, where we will take you on a tour of the Arboretum, the city of Wageningen or the Wageningen University campus, followed by a dinner in restaurant O Mundo, in the historical building "Hotel de Wereld", should lead to new friendships and collaborations between scientists from different disciplines, to strengthen world-wide glucosinolate research.

We wish you a very inspiring conference and a pleasant stay in Wageningen.

Guusje Bonnema & Ruud Verkerk

on behalf of the Scientific Organizing Committee

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General Information

Abstracts
These Proceedings contain abstracts of keynote speakers, followed by abstracts of oral presentations, and finally poster abstracts. All posters are arranged in alphabetical order of the presenting author. The organising committee does not take any responsibility for scientific of typographical errors.

Oral presentations
All presentations are plenary and will be held in the Bosrand room, Hotel and Conference Centre De Wageningsche Berg

Posters
All posters are enlisted in alphabetical order in this book. During the whole conference they will be on display and can be found in the Arboretum room.
To enlarge the attention of all participants, poster flash presentations are included in the program. Presenters can briefly highlight their work on one single slide in a plenary session.

Excursions and Conference dinner

Tuesday 14th October 15.30-22.30 hrs

For attending an excursion please subscribe at the registration desk on site

1. Tour Wageningen Campus
During this visit you have the opportunity to have a quick glance around Wageningen Campus, where expertise and talent come together. Scientists and students carry out globally pioneering research here or train to become professionals who will make breakthroughs in knowledge and technology in the future. Students will guide you around Wageningen Campus.

Transportation by bus between Conference Centre de Wageningsche Berg and Wageningen Campus is arranged.

Date and Time: Tuesday 14th October 15.30 – 17.30 hrs, Wageningen

2. Tour Wageningen Centre
During this visit you have the opportunity to have a quick glance around the old Centre of Wageningen. There are traces of an early medieval settlement on the mountain (Wageningsche Berg). Presumably people first settled in the foothills of the Western-Veluwe massif in the 12th century.
The defence works were modernized early in the 17th century, when the double moat became one single and wider moat. The walls were strengthened by martial ramparts in six places.
Towards the end of the 17th century members of the influential Torck family were responsible for numerous changes in and around the castle.

Lubbert Adolf Torck (1687-1758), mayor of Wageningen and squire of Rosendael, also ordered the construction of three major (and luxurious) buildings with apartments for rent. Two of these still exist, the most important one being the former administrative centre of the Wageningen University. It now forms the nucleus of the prestigious new housing development built by the well-known Belgian architect Charles Vandenhove.

The excursion will start and end at Conference Centre De Wageningsche Berg and will be on foot. To reach Wageningen Centre starting from Hotel de Wageningsche Berg, we will first walk through the botenical garden “Arboretum Bel Monte”. This botanical garden manages special collections of the genera *Rhodendron* and the *Rosaceae,* with most important representatives the roses, flowering cherries, ornamental apples, - pears, hawthorns and rowans.
Date and Time: Tuesday 14th October 15.30 – 17.30 hrs, Wageningen

3. Conference Dinner Hotel De Wereld
All participants are invited to join the conference dinner. A great opportunity to network and socialize with other participants while enjoying a delicious dinner.

Hotel de Wereld in Wageningen was the site of the capitulation of the German troops in the Netherlands on 5 and 6 May 1945, and the end of German occupation during World War II.

On 6 May 1945, the German general Blaskowitz surrendered to the Canadian General Charles Foulkes, which ended the Second World War in the Netherlands. Prince Bernhard, acting as commander-in-chief of the Dutch Interior Forces, attended the meeting as well. The Generals negotiated the terms of surrender in Hotel de Wereld.

This fact is remembered annually by a Remembrance ceremony May the 4th and a Liberation festival May the 5th.

Transportation by bus between Conference Centre de Wageningsche Berg and Hotel De Wereld is arranged.

Date and Time: Tuesday 14th October 19.00-22.30 hrs, Wageningen

Lunches, coffee / tea breaks

All coffee/tea breaks will be served in Arboretum Room

All conference lunches will be served in Restaurant Belmonte

On Tuesday 14th October dinner will be served in the restaurant of Hotel de Wereld.

Sponsors

The organizing committee gratefully acknowledges the generous support of the following sponsors:

The Royal Netherlands Academy of Arts and Sciences (KNAW)

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Program

Program

** all oral presentations are in the Bosrand Room*
** all posters are on display in the Arboretum- and Bosrand Room*

Sunday, October 12, 2014

15:30	Registration
17:30	Welcome *Chair: Guusje Bonnema and Ruud Verkerk*
17.30-17.45	Conference Opening ***Tiny van Boekel*** *Dean of Education, Wageningen University*
17:45–18:30	Glucosinolates and beyond: From mustard oil bomb to functional food ***Keynote: Nicole van Dam, iDiv Halle-Jena-Leipzig and RU Nijmegen***
18:30-20:00	Drinks & Bites

Monday, October 13, 2014

Session 1:	**Molecular and genetic aspects of glucosinolates** *Chair: Guusje Bonnema and Eric Schranz*
9:00-9:40	Pathway and transport processes in specialized metabolism – using glucosinolates as case study ***Keynote: Barbara Ann Halkier, Department of Plant and Environmental Sciences, University of Copenhagen, Denmark***
9:40-10:00	Novel insights into the function of Arabidopsis R2R3-MYB transcription factors regulating aliphatic glucosinolate biosynthesis ***Yimeng Li***
10:00-10:20	The glucosinolate breakdown product Indole-3-Carbinol acts as an auxin antagonist in roots of *Arabidopsis thaliana* ***Ella Katz***
10:20-10:40	Indole glucosinolate modification in arabidopsis ***Juergen Kroymann***
10:40-11:10	**Coffee Break**
11:10-11:30	Evolution and biochemistry of specifier proteins involved in glucosinolate hydrolysis ***Ute Wittstock***
11:30-11:50	Glucosinolate transport processes in Arabidopsis thaliana ***Hussam Hassan Nour-Eldin***

11:50-12:10	Beyond glucosinolates: the biosynthesis of indolic phytoalexins in cruciferous plants ***Erich Glawischnig***
12:10-12:30	Specific regulation of glucosinolate biosynthesis by conjoint activity of MYB and bHLH transcription factors ***Henning Frerigmann***
12:30-13:45	**Lunch**
13:45-14:15	**Poster flashes**
Session 2:	**Application of glucosinolates in agro- and food systems** *Chair: Nicole van Dam and Tom de Jong*
14:15-14:55	Improving the formation of dietary glucosinolates in agro- and food systems ***Keynote: Monika Schreiner, Institut für Gemüse- und Zierpflanzenbau, Grossbeeren, Germany***
14:55-15:15	Quantification and Identification of Glucosinolates by HPLC and LC–ESI–MS in Brassicaceae Plants ***Sun-Ju Kim***
15:15-15:35	Glucosinolates and amides in maca *(Lepidium meyenii)* during traditional postharvest drying practices ***Eric Cosio***
15:35-16:00	**Coffee Break**
16:00-16:20	The exploitation of white cabbage for the soil phytoremediation and biofumigation - overview of the results of the project AGROBIOKAP ***Agnieszka Bartoszek***
16:20-16:40	A metabolomics approach to identify factors influencing glucosinolate thermal degradation rates in Brassica vegetables ***Kristin Hennig***
16:40-17:00	Biofumigant plants and materials as bio-bases in plant management and protection ***Luca Lazzeri***
17:00-17:20	Towards a more accurate assessment of health potential of glucosinolates containing vegetables ***Edoardo Capuano***
17:20-17:40	Metabolic profiles in sprouts of Brassicaceae vegetables ***Yuji Sawada***
17:40-19:00	**Refreshments and Poster session**

Tuesday, October 14, 2014

Session 3:	**Glucosinolates and human health** *Chair: Ruud Verkerk and Tom de Jong*
9:00-9:40	Glucosinolates and human health ***Keynote: Richard Mithen, Institute of Food Research, Norwich Research Park, UK***
9:40-10:00	Enzymatic degradation of Brassica glucosinolates and bioactivity of formed epithionitriles ***Franziska S. Hanschen***
10:00-10:20	Tuscan black kale: from seeds to grams of highly pure glucoraphanin ***Gina Rosalinda De Nicola***
10:20-10:40	In vivo formation and bioavailability of isothiocyanates from glucosinolates in broccoli as affected by processing conditions ***Teresa Oliviero***
10:40-11:10	**Coffee Break**
11:10-11:30	Interaction of isothiocyanates and glucosinolates with the Ah receptor as their chemopreventive potency ***Ahmad Faizal Abdull Razis***
11:30-11:50	Allyl isothiocyanate inhibits actin-dependent intracellular transport in Arabidopsis thaliana ***Atle M. Bones***
11:50-12:10	Human intervention study investigating conversion of encapsulated glucoraphanin to isothiocyanates, by gut microbiota and genotype ***Lee Kellingray***
12:10-12:30	Selective cytotoxicity of isothiocyanates from Brassicales plants on human liver cancer cells and underlying mechanisms ***Evelyn Lamy***
12:30-13:45	**Lunch**
13:45-15:30	**Poster flashes and Poster session**
15:30-22:30	**Excursion and Dinner**

Wednesday, October 15, 2014

Session 4:	**Ecology and evolution of glucosinolates** *Chair: Eric Schranz and Arjen Biere*
9:00-9:40	Pathway function influences complex traits and fitness in plant populations ***Keynote: Thomas Mitchell-Olds, Duke University, Biology Department, USA***
9:40-10:00	The glucosinolates-myrosinase system in Ochradenus baccatus: Ecology, biochemistry and physiology ***Yoram Gerchman***
10:00-10:20	Phyllotreta flea beetles utilize host plant defense compounds to create their own glucosinolate-myrosinase system ***Franziska Beran***
10:20-10:40	Natural genetic variation in growth and metabolite regulatory roles of Allyl glucosinolate in Arabidopsis thaliana ***Marta Francisco***
10:40-11:10	**Coffee Break**
11:10-11:30	Glucosinolate biodiversity screening with distinction of isomers reveals evolutionary innovations and structure-dependent reactivity ***Niels Agerbirk***
11:30-11:50	Toxicity and detoxification of glucosinolates in generalist insect herbivores ***Daniel Giddings Vassao***
11:50-12:10	ER bodies contain a novel class of myrosinases and are functionally coordinated with indole glucosinolates. ***Ryohei Thomas Nakano***
12:10-12:30	Glucosinolate-myrosinase defence system modified oilseed rape MINELESS plants in response to above- and below-ground herbivores ***Ishita Ahuja***
12:30-12:45	**Closing / Perspective Conference**
12:45-14:00	**Lunch and Departure**

Oral presentations

Opening Session
Glucosinolates and Beyond

Sunday, October 12th 2014

Keynote

Nicole M. van Dam, German Centre for Integrative Biodiversity Reserach (iDiv)

Halle-Jena-Leipzig, Germany

Nicole M. van Dam started her scientific career with an MSc of Wageningen University. After obtaining her PhD degree at Leiden University, the Netherlands, she was a post-doc at University of California Riverside (USA) and at the Max-Planck Institute for Chemical Ecology in Jena, Germany, respectively. In 2000 she returned to the Netherlands and worked as senior research scientist at the Netherlands Institute of Ecology (NIOO-KNAW). Since that time, she has studied the ecological role of glucosinolates in plant-herbivore interactions. After obtaining a full professorship at Radboud University Nijmegen, she recently got appointed as research group head at the brand new German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig. Her research on glucosinolates is mainly driven by her fascination for the great chemical diversity within the class and their versatility as (induced) plant defence compounds both above and below the ground. With her current research she specifically wants to resolve the question whether root and shoot glucosinolate profiles, and the allocation of glucosinolates within these organs, reflect optimal defence patterns.

Glucosinolates and beyond: From mustard oil bomb to functional food

Nicole M. van Dam, on behalf of the organizing committee
German Centre for Integrative Biodiversity Reserach (iDiv)
Halle-Jena-Leipzig, Germany

Glucosinolates are a structurally diverse and fascinating group of plant secondary compounds. The presentations at this conference evidence the extent of glucosinolate research. The topics range from fundamental research on their biosynthesis and evolutionary origin to practical applications in human health an agriculture. In this opening presentation, we aim to summarize recent trends and developments in glucosinolate research that have emerged since the last conference in Denmark (2009) and to identify some pertinent questions to guide future research.

Session 1.
Molecular and genetic aspects of glucosinolates

Monday, October 13th 2014

Keynote

Barbara Ann Halkier, University of Copenhagen, Denmark

Head of DynaMo Center of Excellence
My research is focused on glucosinolates as model specialized metabolites to study cellular and organismal biology

Glucosinolates are amino acid-derived natural plant products found in the order Brassicales, which includes the economically important oilseed rape, vegetables such as broccoli and the model Arabidopsis. Glucosinolates are hydrolyzed by myrosinases to produce isothiocyanates and nitriles, which have a wide range of biological activities. For plants, glucosinolates protect against herbivore and microbial attacks, and is implicated in host-plant recognition by specialized predators. For humans, glucosinolates have received increased attention as cancer-preventive agents and potential biopesticides.

In my research group, we apply pathway and transport engineering of glucosinolates to improve human nutrition and to increase disease resistance of crops. In addition, we use the glucosinolates in Arabidopsis thaliana as a unique model system for studying cellular and organismal biology. The goal is to uncover the molecular interactions facilitating the dynamic changes in glucosinolates levels that are generated in response to developmental cues as well as abiotic and biotic stresses.

The current research focuses on:
- Bioengineering of glucosinolates in different host organisms
- Investigation of the presence of glucosinolate metabolon
- Identification of the glucosinolate transporter complement
- Regulation of glucosinolate transporters
- USER technology

1.1 Pathway and transport processes in specialized metabolism – using glucosinolates as case study

Barbara Ann Halkier

DNRF DynaMo Center of Excellence, Department of Plant and Environmental Sciences, Faculty of Science, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg C, Denmark

Glucosinolates are interesting compounds to study as their presence in the model plant Arabidopsis has made them the model specialized compounds both from a perspective of basic science and applied biotechnology. Towards our goal of engineering the biosynthetic pathway of the cancer-preventive glucoraphanin into microbial organism, we study how the pathway is orchestrated. Is the glucosinolate pathway organized in metabolons to ensure that substrates are efficiently channeled into and through the pathway? Our progress in understanding the orchestration of the pathway and in engineering the glucoraphanin pathway into a microbial host will be presented.

Upon long-distance transport, a given metabolite has to cross multiple membrane barriers as it exits source tissues, is loaded into the phloem 'transport highway', exits this and enters sink tissues. At the subcellular level, transport processes are important to ensure efficient channeling of intermediates between compartments and to ensure proper storage of end products to prevent feedback inhibition. Increasing attention is given to transport processes of specialized metabolites, of which little is known. We have recently identified the first glucosinolate transporter, a plasma-membrane localized importer that is essential for seed loading. We have as a goal to identify all the players in the glucosinolate transporter complement. Our progress in reaching this goal will be presented.

1.2 Novel insights into the function of Arabidopsis R2R3-MYB transcription factors regulating aliphatic glucosinolate biosynthesis

Y. Li[1,3], Y. Sawada[1], A. Hirai[1], M. Sato[1,2], A. Kuwahara[1,2], X. Yan[3], M.Y. Hirai[1,2,*]

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2. JST, CREST, 4-1-8 Honcho, Kawaguchi, 332-0012 Japan
3. Alkali Soil Natural Environmental Science Center, Northeast Forestry University; Key Laboratory of Saline-Alkali Vegetation Ecology Restoration in Oil Field, Ministry of Education, Harbin, 15040 China

Aliphatic glucosinolates (AGSLs) are a group of sulfur-containing secondary metabolites. AGSLs are biosynthesized from methionine, and positively regulated by transcription factors MYB28, MYB29, and MYB76 in *Arabidopsis* [1-5]. Mutual transcriptional regulation among these *MYB* genes makes it difficult to elucidate their individual function simply by analyzing knock-out mutants or ectopically overexpressing lines of these genes. In this study, we constructed transgenic lines expressing each MYB gene driven by its own promoter in the *myb28myb29* background, in which the expression of the endogenous *MYB28, MYB29,* and *MYB76* was repressed resulting in no AGSL accumulation. In leaves, transgenic *MYB28* expression activated AGSL biosynthetic genes and restored accumulation of AGSLs with short side-chains. Transgenic *MYB29* expression activated the same biosynthetic pathway, but induction of the genes involved in side-chain elongation was weaker than that by MYB28, resulting in a weaker recovery of AGSLs. Neither *MYB28* nor *MYB29* recovered long-chain AGSL accumulation. *MYB76* was considered to require both *MYB28* and *MYB29* for its normal level of expression in leaves, and could not activate AGSL biosynthesis on its own. Interestingly, the accumulation in seeds of long- and short-chain AGSLs was restored by transgenic expression of MYB28 and MYB76, respectively. Under sulfur deficiency, expression levels of *MYB29* and *MYB76* were positively correlated with sulfur concentration. Expression level of *MYB28* in the wild type was slightly elevated under mild sulfur deficiency, and maintained at a basal level under extreme sulfur deficiency. On the other hand, in the absence of MYB29 and MYB76, expression level of MYB28 was apparently increased under mild sulfur deficiency. This study illustrated how the individual MYBs work in regulating AGSL biosynthesis when expressed alone under normal transcriptional regulation.

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Omics-based identification of Arabidopsis Myb transcription factors regulating aliphatic glucosinolate biosynthesis. Proc Natl Acad Sci USA 104, 6478-6483

2. Gigolashvili, T, Yatusevich, R, Berger, B, Muller, C, Flugge, U. (2007)
The R2R3-MYB transcription factor HAG1/MYB28 is a regulator of methionine-derived glucosinolate biosynthesis in Arabidopsis thaliana. Plant J 51, 247-261

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A systems biology approach identifies a R2R3 MYB gene subfamily with distinct and overlapping functions in regulation of aliphatic glucosinolates. PLoS One 2: e1322

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HAG2/MYB76 and HAG3/MYB29 exert a specific and coordinated control on the regulation of aliphatic glucosinolate biosynthesis in Arabidopsis thaliana. New Phytol 177, 627-642

5. Malitsky, S, Blum, E, Less, H, Venger, I, Elbaz, M, *et al.* (2008)
The transcript and metabolite networks affected by the two clades of Arabidopsis glucosinolate biosynthesis regulators. Plant Physiol 148, 2021-2049

1.3 The glucosinolate breakdown product Indole-3-Carbinol acts as an auxin antagonist in roots of Arabidopsis thaliana

Ella Katz[1], Melkamu G. Woldemariam[2], Sophie Nissani[1], Ben Shai[3], Marcelo Ehrlich[3], Georg Jander[2] and Daniel A. Chamovitz[1]

1. Department of Molecular Biology and Ecology of Plants, Tel Aviv University, Israel
2. Boyce Thompson Institute for Plant Research, Ithaca, NY, USA
3. Department of Cell Research and Immunology, Tel Aviv University, Israel

Indole-3-Carbinol (I3C) is a phytochemical that is produced endogenously in cruciferous vegetables as one of the breakdown products of indol-3-methylglucosinolate. In Arabidopsis thaliana I3C is synthesized upon tissue rupture and deters herbivores. In mammals, I3C has anti-carcinogenic properties and tested positive as a chemopreventive agent in several short-term bioassays. So far little is known about the effects of I3C on plant cells. Identifying the specific pathways and cellular processes in which I3C is involved will be a starting point for further understanding the molecular basis of I3C action and detoxification. We found that treatment of Arabidopsis with exogenous I3C inhibits root elongation, reduces auxin signaling in the root apical meristem, and effects overall plant development. One hour following I3C treatment of seedling root tips about 300 genes are differently regulated, including genes that are known to be activated by auxin. Further experiments showed that I3C acts as an auxin antagonist, both at the molecular level and the physiological level. Thus our results suggest that I3C is a novel modulator of auxin signaling and may contribute to the cessation of plant growth in response to insect herbivory.

1.4 Indole Glucosinolate modification in Arabidopsis

M. Pfalz, M. Mukhaimar, M. Paupière, M. Shehadi, M. Ouassou and J. Kroymann

Laboratoire d'Ecologie, Systématique et Evolution, Université Paris-Sud/CNRS, Campus Orsay, Bâtiment 360, F-91405 Orsay, France

Indole glucosinolates, derived from tryptophan, play important roles in plant – parasite interactions. We are dissecting the molecular genetic architecture of indole glucosinolate biosynthesis in Arabidopsis. We have identified two families of genes involved in indole glucosinolate modification. These genes encode cytochrome P450 monooxygenases of the CYP81F subfamily [1, 2], and indole glucosinolate O-methyltransferases (IGMTs) [2], respectively. CYP81Fs catalyze hydroxylation reactions at the glucosinolate indole ring, and the resulting hydroxy intermediates serve as substrates for subsequent methoxylation, carried out by IGMTs. We present and discuss our recent findings, and focus in particular on the question whether different gene family members are functionally redundant or have distinct roles in modification reactions.

References

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The gene controlling the *Indole Glucosinolate Modifier 1* QTL alters indole glucosinolate structures and aphid resistance in *Arabidopsis. Plant Cell* 21, 985-999.

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Metabolic engineering in *Nicotiana benthamiana* reveals key enzyme functions in *Arabidopsis* indole glucosinolate modification. *Plant Cell* 23, 716-729.

1.5 Evolution and biochemistry of specifier proteins involved in glucosinolate hydrolysis

F. Gumz[1], D. Eisenschmidt[2], E. Schulze[2], J. Kuchernig[1], J. Krauße[3], W. Brandt[2] and U. Wittstock[1]

1. Institute of Pharmaceutical Biology, Technische Universität Braunschweig, Mendelssohnstr. 1, 38100 Braunschweig, Germany
2. Leibniz Institute of Plant Biochemistry, Weinberg 3, 06120 Halle (Saale) , Germany
3. Helmholtz Centre for Infection Reasearch, Inhoffenstr. 7, 38124 Braunschweig, Germany

Structural diversity of the glucosinolate-myrosinase system arises from the variation of both glucosinolate biosynthesis and glucosinolate breakdown. Specifier proteins have a key function in controlling the outcome of glucosinolate breakdown and thereby the biological impact of the glucosinolate-myrosinase system. Phylogenetic analysis of nitrile specifier proteins (NSPs), epithiospecifier proteins (ESPs), and thiocyanate forming proteins (TFPs) from a range of Brassicaceae has suggested a common ancestor of these proteins with an early split between ESPs and NSPs and at least two independent origins of TFPs from ESPs [1]. In order to better understand the structural and functional changes associated with this diversification, we have generated molecular models and used them in docking studies with glucosinolate aglucons as likely substrates. In conjunction with mutational analysis, molecular modeling predicted specifier proteins to adopt a six-bladed propeller fold with a metal-ion-containing active site [2]. For experimental structure elucidation, we have expressed the TFP from penny cress (Thlaspi arvense) heterologously and purified it to homogeneity for crystallization. We have recently obtained a high resolution X-ray structure which confirmed some of the predictions and is presently being used for the refinement of our molecular models, for the elucidation of specifier protein reaction mechanisms and for docking studies with myrosinase.

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Evolution of specifier proteins in glucosinolate-containing plants. BMC Evolutionary Biology 12:127

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Molecular models and mutational analyses of plant specifier proteins suggest active site residues and reaction mechanism. Plant Molecular Biology 84:173-188

1.6 Glucosinolate transport processes in *Arabidopsis thaliana*

H.H. Nour-Eldin[1], M.E. Jørgensen[1], D. Xu[1], S.R. Madsen[1], T.G. Andersen[1], C.E. Olsen[1] and B.A. Halkier[1]

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Historically, seeds have represented the only known sink for glucosinolate transport. Recently, we identified the glucosinolate transporters GTR1 and GTR2 and showed that they are essential for glucosinolate accumulation in seeds. In planta characterization of these high-affinity glucosinolate transporters is revealing that transport processes play many more roles in controlling glucosinolate distribution both within and between tissues. We have combined micro- and standard grafting techniques together with glucosinolate analyses of mutant plant dissections to identify hitherto unknown source sink relationships and transport barriers in the glucosinolate transport pathway. We show that i) glucosinolates can be transported bi-directionally between rosette and roots ii) that glucosinolates are likely transported through plasmodesmata within leaves to establish the strategically important leaf-edge accumulation iii) that GTR1 and GTR2 activity within seeds is essential for glucosinolate accumulation in seeds iv) that the very high glucosinolate content in S-cells stems to a large degree from transport of glucosinolates from other tissues and v) that cauline leaves represent important sources for stem glucosinolate content. Importantly, we showed that indole glucosinolates can also be transported between roots and rosettes but that this transport is not inhibited in the gtr1 gtr2 double knockout plant. This indicated the existence of an additional plasma membrane glucosinolate importer specific for indole glucosinolates. Furthermore, our investigations indicate the existence of vacuolar glucosinolate transporters and plasmamembrane exporters, which remain to be identified at the molecular level. Additionally, our data indicate the existence of a mechanism potentially capable of regulating biosynthesis in source tissues by relaying the glucosinolate status in the sink tissue.

1.7 Beyond glucosinolates: the biosynthesis of indolic phytoalexins in cruciferous plants

T. Müller[1], S. Mucha[1], A. Chapman[1], C. Götz[1], M. Choudhary[1], C. Böttcher[2], R. Morbitzer[3], T. Lahaye[3] and E. Glawischnig[1]

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In the Brassicaceae, pathogen infection induces the biosynthesis of a range of indolic phytoalexins [1]. Camalexin is the major phytoalexin of *Arabidopsis thaliana*. Its biosynthetic pathway branches off indole glucosinolate biosynthesis by the conversion of indole-3-acetaldoxime (IAOx) to indole-3-acetonitrile (IAN) catalysed by the closely related cytochrome P450 monooxygenases CYP71A12 and CYP71A13 [2], which are localized in tandem. We have characterized their kinetic parameters and their specific product spectra and demonstrated by Co-IP that CYP71A13 forms homodimers and physically interacts with CYP71B15 (PAD3), the essential bifunctional enzyme of the pathway [3,4]. *Cyp71a12 cyp71a13* double knockout lines were created by using TALE-nucleases. After induction, these plants produce only traces of camalexin in contrast to the corresponding single knockouts.

After induction, in Arabidopsis also derivatives of indol-3-carbaldehyde (ICHO) and indole-3-carboxylic acid (ICOOH) accumulate in total amounts similar to camalexin [4,5]. We show that they are synthesised also via indole-3-acetonitrile (IAN), involving Aldehyde Oxidase 1 (AAO1) [5]. To study an alternative model for indolic phytoalexin biosynthesis we introduced *Thellungiella salsuginea (=Eutrema salsugineum)*. The major phytoalexins in this species are 1-methoxybrassinin and wasalexin, probably derivatives of indole glucosinolates [1]. To identify biosynthetic genes we are currently following a comparative transcriptomics approach.

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1.8 Specific regulation of glucosinolate biosynthesis by conjoint activity of MYB and bHLH transcription factors

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The MYB34, MYB51, and MYB122 transcription factors are known to regulate indolic glucosinolate (IG) biosynthesis in *Arabidopsis thaliana.* We determined the distinct regulatory potential of MYB34, MYB51 and MYB122 by analysis of the accumulation of IGs in different parts of plants and upon treatment with plant hormones. It could be shown that MYB34, MYB51, and MYB122 act together but control distinctly the biosynthesis of I3M in shoots and roots, and act specifically downstream of abscisic acid, salicylic acid, jasmonate (JA) or ethylene [1]. The *myb34 myb51 myb122* triple mutant is devoid of IGs, indicating that these three MYB factors are indispensable for IG production under standard growth conditions [1].

As R2R3 MYB transcription factors are usually known to act in concert with other regulatory proteins, we sought for putative interaction partners of MYB51. A yeast two-hybrid screen revealed bHLH05/MYC3, a member of subgroup IIIe of the bHLH transcription factor family to be an interaction partner of MYB51 [2]. Other close homologues of bHLH05 and members of the IIIe subgroup, bHLH04/MYC4, bHLH06/MYC2 and bHLH28/MYC5 also demonstrated interactions with all six R2R3-MYBs regulating glucosinolate (GSL) biosynthesis in pull-down experiments *in vitro* and BiFC analysis *in vivo*. Single *bhlh mutants* retained GSL levels that were similar to those in wild-type plants, indicating functional redundancy between bHLH04, bHLH05 and bHLH06. However, the triple *bhlh04/05/06* mutant was almost depleted in the production of GSL and the expression of GSL biosynthetic genes was significantly reduced. Unlike *bhlh04/06* and *bhlh05/06* mutants, the double bhlh04/05 mutant was strongly affected in the production of GSL, pointing to a special role of bHLH04 and bHLH05 in the production of GSL in the absence of JA. Simultaneous gain-of-function of MYB and bHLH proteins had an additive effect on GSL biosynthesis, as demonstrated by the analysis of the double MYB34-1D bHLH05D94N mutant, which produces twice as much IGs as *MYB34-1D* and 20-fold more than *bHLH05D94N* and Col-0. Remarkably, the amino acid substitution D94N in bHLH05D94N causes constitutive activation of bHLH05 due to impaired interaction with JASMONATE-ZIM DOMAIN (JAZ) proteins, mimicking JAZ degradation or JA treatment responses. Our study revealed bHLH factors as novel regulators of GSL biosynthesis and contributes to the better understanding of the specific role of different bHLH proteins [2].

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Session 2. Applications of glucosinolates in agro- and food systems

Monday, October 13th 2014

Keynote

Prof. Monika Schreiner

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Monika Schreiner obtained her Master of International Agricultural Development and her PhD at the Technische Universität Berlin. She worked as a Postdoctoral Scientist at the Leibniz-Institute of Agricultural Engineering Bornim before becoming the head of the Department of Quality at the Leibniz-Institute of Vegetable and Ornamental Crops Großbeeren/Erfurt and Professor at the Leibniz Universität Hannover. Her major research interests focus on the study of secondary plant metabolites – particularly glucosinolates – and their functions aiming to optimize the concentration and composition of certain protective glucosinolates.

Her research emphasis on targeted elicitor applications used to modify the biosynthesis and degradation process of certain, favoured glucosinolates underlined by corresponding gene expression studies. As glucosinolate respond extremely structure-dependent to elicitor applications and also the various functional effects of glucosinolates are tremendously structure-dependent, enabling by targeted elicitor treatments the generation of plant-based food products with a desired glucosinolate profile.

2.1 Improving the formation of dietary glucosinolates in agro- and food systems

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Inverse associations between *Brassica* vegetable intake and chronic diseases have been demonstrated in numerous epidemiological studies. Glucosinolates have been indicated to be responsible for these observed protective effects. However, only for certain individual glucosinolates evidence has been found that their hydrolysis products induce functional effects.

Recently, a number of plant physiological studies demonstrated the potential to affect concentration and composition of glucosinolates in many Brassicales species. In order to optimize the concentration and composition of certain protective glucosinolates, targeted chemical, physical and biological elicitor applications – in relation to ecophysiological and (onto)genetic effects – are used to modify the biosynthesis and degradation process of certain, favored glucosinolates by triggering distinct changes in the glucosinolate metabolism [e.g. 1, 2].

As glucosinolates respond extremely structure-dependent to elicitor applications [e.g. 1, 3] and also the various functional effects of glucosinolates are tremendously structure-dependent [e.g. 4, 5], targeted elicitor treatments enable the generation of plant-based food products with a desired glucosinolate profile.

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2.2 Quantification and Identification of Glucosinolates by HPLC and LC–ESI–MS in Brassicaceae Plants

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Recently *Brassica* vegetables renewed interest as a fresh food because of their relatively high protein, phytochemicals contents and high dry matter digestibility. The family contains well-known species such as *Brassica oleracea* (broccoli, cabbage, cauliflower, etc.), *B. rapa* (turnip, Chinese cabbage, etc.), *B. napus* (rapeseed, etc.), *Raphanus sativus* (common radish) and many others. These vegetables have a potential pharmaceutical effect including anti-cancer agents because of the presence of sulphur containing glycosides, glucosinolates (GSLs). Recently about 200 different types of GSLs are identified and characterized in wide groups of plants [1]. They are classified into three major groups such as aliphatic, indolyl and aromatic GSLs based on their functional group of amino acids precursor methionine, phenylalanine and tryptophan, respectively. Individual GSLs in Brassicaceae plants were lyophilized, and extracted by 70% boiling methanol at 70°C. The crude extract was applied to a mini-column packed with DEAE-Sephadex A-25. GSLs were desulfated by adding a solution of aryl sulfatase to the column. After the overnight reaction at room temperature, desulfo-GSLs were eluted with ultra-pure water. GSLs were analyzed by HPLC and LC–ESI–MS equipped with Inertsil ODS-3 column at 227 nm [2]. Total sixteen glucosinolates [eleven aliphatic: glucoiberin, progoitrin, glucoraphanin, sinigrin, glucoalyssin, gluconapoleiferin, gluconapin, glucocochlearin, glucobrassicanapin, glucoerucin, glucoraphasatin; four indolyl: 4-hydroxyglucobrassicin, glucobrassicin, 4-methoxyglucobrassicin, neoglucobrassicin; one aromatic: gluconasturtiin] were separated and identified. Individual GSLs were quantified with the external standard sinigrin based on its HPLC area and relative response factors [3].

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2.3 Glucosinolates and amides in maca *(Lepidium meyenii)* during traditional postharvest drying practices

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Maca, Lepidium meyenii Walpers (Brassicaceae), is an annual herbaceous plant native to the high plateaus of the Peruvian central Andes. Its underground storage hypocotyls have been a traditional medicinal agent and dietary staple since pre-Columbian times. Reported properties include energizing and fertility-enhancing effects. Published reports have focused on the glucosinolates and benzylalkamides (macamides) present in dry hypocotyls as the main bioactive components [1]. Macamides are secondary amides formed by benzylamine and a fatty acid moiety, with varying substitution patterns, hydrocarbon chain lengths and degree of unsaturation [2]. Although it has been assumed that they are usually present in fresh undamaged tissues, analyses show them to be essentially absent from them. However, hypocotyls dried by traditional Andean postharvest practices contain up to 800 µg.g-1 dw (2.3 µmol.g-1 dw) of macamides. In this report we have followed the generation of macamides and their putative precursors during nine-week traditional drying trials at 4200 m altitude and in ovens under laboratory conditions. Freeze-thaw cycles in the open field during drying result in tissue maceration and release of free fatty acids from storage and membrane lipids up to levels of 1200 µg.g-1 dw (4.3 µmol.g-1 dw). Endogenous metabolism of the isothiocyanates generated from glucosinolate hydrolysis during drying results in maximal benzylamine values of 4300 µg.g-1 dw (40.2 µmol.g-1 dw). Pearson correlation coefficients of the accumulation profiles of benzylamine and free fatty acid to that of macamides showed good values of 0.898 and 0.934 respectively, suggesting that glucosinolate and lipid hydrolysis provide sufficient substrate for amide synthesis, possibly by way of reverse fatty acid amide hydrolases (FAAH) working in reverse [3]. Understanding the as yet unrecognized relevance of the traditional post-harvest metabolism of this Inca crop should help develop products with improved chemical profiles.

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2.4 The exploitation of white cabbage for the soil phytoremediation and biofumigation - overview of the results of the project AGROBIOKAP

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The project AGROBIOKAP was aimed at recognising the possibility of white cabbage (*Brassica oleracea* var. *capitata* f. *alba*) utilisation for sustainable agriculture. The proposed approach involves two-phase technology dedicated to lessen the environmental burden of chemical pollutants, as well as final treatment of contaminated biomass. The first phase consists in growing cabbage on the soil that requires recultivation to extract contaminating heavy metals in the process of phytoremediation. The results of phytotron experiments extrapolated to field conditions suggested that white cabbage may be capable of removing up to about 360 g/ha of Cd and/or about ten times more Zn when grown on soils strongly contaminated by these elements. In brassica plants, chemical stress is known to stimulate the biosynthesis of glucosinolates which are precursors of isothiocyanates and indoles exhibiting strong biocide activity. Therefore, the plants grown on contaminated soil can represent the enriched source of these antibiological phytochemicals. Our studies demonstrated that the exposure of white cabbage on heavy metals increased the biosynthesis mainly of sinigrin, a precursor of allyl isothiocyanate which is ranked among the most effective natural pesticides. In the second phase of the proposed technology, the contaminated plants are used as a raw material for production of natural pesticide preparation that can be employed for ecological crop protection in the process of biofumigation, at the same time constituting green manure. The remaining contaminated biomass can be utilized in biogas production. The experimental installation revealed that cabbage biomass enhanced biogas production when combined with fermented municipal sludge. Apparently, this increased efficiency occured because cabbage sulfurorganic compounds was able to bind present in the municipal waste heavy metals that otherwise would inhibit enzymatic reactions. In the approach proposed, only the final residual contaminated biomass requires specialistic, most expensive disposal. The results of the project AGROBIOKAP demonstrated that phytoremediation-biofumigation cycle involving white cabbage may be inexpensive and easy to apply technology for sustainable agriculture.

Project AGROBIOKAP co-financed by the European Union from the European Fund for Regional Development within the framework of the Operational Program for an Innovative Economy 2007-2013

2.5 A metabolomics approach to identify factors influencing glucosinolate thermal degradation rates in Brassica vegetables

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Thermal processing of Brassica vegetables can lead to substantial loss of potential healthpromoting glucosinolates (GLs). The extent of thermal degradation of a specific GL varies in different vegetables, possibly due to differences in the composition of other metabolites within the plant matrices. An untargeted metabolomics approach followed by random forest regression was applied to identify metabolites associated to thermal GL degradation in a segregating Brassica oleracea population. Out of 413 metabolites fifteen were associated with the glucobrassicin degradation, six with the glucoraphanin degradation and two with both GLs. Among these twenty-three metabolites three were identified as flavonols (one kaempferol- and two quercetin-derivatives) and two as GLs (4 methoxyglucobrassicin, gluconasturtiin). Twenty quantitative trait loci (QTLs) for these metabolites, which were associated with glucoraphanin and glucobrassicin degradation, were identified on linkage groups C01, C07 and C09. Two flavonols mapped on linkage groups C07 and C09 and co-localize with the QTL for GL degradation determined previously.

2.6 Biofumigant plants and materials as bio-based in plant management and protection

Luca Lazzeri[1], Lorenzo D'Avino[1], Lorena Malaguti[1], Susanna Cinti[1], Roberto Matteo[2], Nerio Casadei[1], Laura Righetti[1], Eleonora Pagnotta[3] and Luisa Ugolini[1]

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More than 20 years have gone since the first experiences on the application of Brassicaceae plants in crop management and defense (Kirkegaard & Matthiessen, 2004) and today this technique is known as the biofumigation technique and applied at a commercial level in several countries across the world,. After the application of biofumigant and catch crops green manure selected for their glucosinolate qualitative and quantitative content, a great innovation for this chain was represented from the definition of the technology for defatted brassicaceae oilseed meals as bio-based materials in which the glucosinolate hydrolysis process can be modified for improving the efficacy of the system. In fact, defatted oilseed meals can be applied alone or in combination with biofumigant green manure plants, but, at the same time represent also the starting materials for new application fields beyond pre-planting treatments viz in the post-harvest defense.

Liquid new materials were defined as based on a vegetable oil emulsion in water with the addition of defatted seed meals suitably formulated afterwhich can be sprayed directly onto foliage (Rongai *et al.*, 2008) or distributed by drip irrigation (Lazzeri *et al.*, 2013) as treatments for pathogen and pest containment. Their application has determined a biostimulant effect on the plants enlarging to unexplored potential for biofumigation application. The synergic application of these natural compounds, year after year, can maximize the biofumigation efficacy and can offer the farmer a natural option in plant cultivation and management as a partial or total alternative to conventional inputs in agriculture.

In these last few years, the European Community has dedicated great attention to the use of chemicals in industry and agriculture. EC Regulation No. 1907/2006 concerning the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) implemented a first procedure of control and registration of chemicals. In addition, regarding the use of pesticide, the EU issued the Directive 2009/128/EC that strongly encourages non-chemical methods in agriculture. These decisions led to more restricted limits for the registration of new pesticides and the phase-out of conventional products characterized by a high environmental impact, for instance substances that deplete the ozone layer (UNEP, 2006), or for their biocidal properties. These decisions are in line with a growing interest in reducing pollution by a decrease of CO2 (Directive 28/2009/CE on renewable energy sources) and for food safety (EC Regulation 10/2001 on articles intended to come into contact with food). The biofumigation products derived from the Italian experiences can be an option in this direction following the strategic lines reported in the EU communication, based on the development of a sustainable biorefinery approach in the production of bio-based materials (Lazzeri et al., 2011), including those for agricultural uses.

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2.7 Toward a more accurate assessment of health potential of glucosinolates containing vegetables

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At present, glucosinolates (GLS) content and myrosinase (MYR) activity of many vegetable species has been investigated along with the relevant source of their variability and the effect of industrial processing and domestic cooking on their stability. Based on that knowledge, optimisation of processing/cooking conditions in order to retain as much GLS and MYR activity as possible is already feasible. We suggest here that it is not so much the amount of residual GLS and MYR activity in the vegetable that has to be maximized as the amount of beneficial breakdown products that are bioavailable for the body upon consumption. For that reason, future research should focus on 1) the fate of GLS during digestion (stability, type of breakdown products formed under different conditions), 2) the short- and long-term role of the human microbiota on GLS bioconversion, 3) the effect of processing/cooking on food matrix structure/composition and 4) the modulating effect of the latter on the GLS fate and the total amount of beneficial breakdown products that are absorbed along the gastrointestinal tract. By integrating all this information with the effect of cooking/ processing conditions on GLS content and MYR activity, the beneficial effect of GLS-containing vegetables may be more accurately assessed.

2.8 Metabolic profiles in sprouts of Brassicaceae vegetables

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Glucosinolates (GSLs) in Brassicaceae vegetables have great potential for promoting human health, and the cost-effective production of bioactive phytochemicals have attracted attention among agricultural and breeding companies. As a success story, the high content of glucoraphanin in young sprouts (3 days after germination) of broccoli has been reported [1], and the functional sprouts with higher health-promoting activity has achieved a high market price. Thus, metabolic profiles in the sprouts of Brassicaceae plants are important information for breeding of value-added vegetables.

In this study we elucidated metabolic profiles of the sprouts of four Brassicaceae vegetables: kale, broccoli, garden cress and radish. Previously we established a practical metabolomics methodology based on the high-sensitivity detection by selected reaction monitoring (SRM) of LC-QqQ-MS, which we named “widely targeted metabolomics” [2-4]. Using this methodology, ca. 500 metabolites were detected and 170 including GSLs, amino acids, and flavonoids, were selected based on the signal to noise ratio (S/N>10) for further analyses. We confirmed high GSL contents in these sprouts with high reproducibility between independent experiments. Additionally, co-accumulation between all pairs of metabolites in metabolic profiles were tested based on the Pearson’s correlation coefficient (PCC), and significant co-accumulation patterns were detected (PCC > 0.8). To expand the number of detectable GSLs by our analysis, we are establishing a few hundred SRMs for GSLs, which will promote metabolic profiling and breeding of Brassicaceae vegetables.

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Session 3.
Glucosinolates and human health

Tuesday, October 14th 2014

Keynote

Prof. Richard Mithen

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Richard Mithen leads the Food and Health strategic programme at the Institute of Food Research at the Norwich Research Park, UK. His research on glucosinolates over many years has focussed the genetic regulation of glucosinolate accumulation in plants, their role in mediating plant-herbivore and plant-pathogen interactions, and how they may maintain and promote human health. He developed high glucoraphanin broccoli cultivars, commercialised under the tradename 'Beneforte' broccoli, which have been used in a series of human intervention studies. The current focus of his research is exploring how high glucoraphanin broccoli may reduce the risk of aggressive prostate cancer. This research involves human clinical studies with men with localised prostate cancer, and research with model systems to elucidate the biological activity of glucosinolate degradation products with an emphasis on redox regulation of signalling pathways and downstream effects on metabolism.

3.1 Glucosinolates and human health

Richard Mithen

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There are two major bodies of evidence that dietary intake of glucosinolates may have positive effects upon human health. Firstly, a large number of observational studies have associated diets that are rich in cruciferous vegetables with reduction in risk of cancer and cardiovascular disease. Secondly, a plethora of animal and cell-based studies have provided evidence that glucosinolate degradation products can have beneficial effects, and described many possible molecular mechanisms. Both types of evidence have flaws: high dietary intake of cruciferous vegetables is often correlated with other lifestyle factors and these vegetables contain many other chemical entities in addition to glucosinolates, and the majority of animal studies use levels of glucosinolate degradation products far in excess of what can be achieved through dietary consumption. More convincing evidence is required from human intervention studies. I briefly describe the development of high glucoraphanin (HG) broccoli through introgression of a novel Myb28 allele from the wild species *Brassica villosa,* and the use of HG broccoli in a series of human intervention studies. The results of these studies suggest that glucoraphanin can affect metabolism in a manner that is consistent with reduction in risk of cancer and other forms of chronic disease. I suggest that this is due to the modulation of cellular redox state and its effect on the activity of redox sensitive proteins, notably PTEN and AMPK, that regulate central pathways of metabolism. I conclude with a brief description of an on-going longer term human intervention study to investigate whether a glucoraphanin-rich diet can reduce the risk of prostate cancer progression.

3.2 Enzymatic degradation of Brassica glucosinolates and bioactivity of formed epithionitriles

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Consumption of glucosinolate (GLS)-rich *Brassica* vegetables such as broccoli, radish, or cabbage is often linked with a reduced risk for cancer [1, 2]. However, not the GLS itself but their breakdown products, most of all the isothiocyanates (ITC), are made responsible for health-promoting effects. Upon hydrolysis, next to ITC also nitriles or epithionitriles (EPTs) can be formed. The latter derive from alkenyl GLS but only in presence of epithiospecifier protein (ESP) [3]. However, their occurrence in food plants as well as their potential bioactivity is widely neglected, so far.

Therefore, in the present study, enzymatic degradation of GLS in *Brassica* vegetables was studied. Hydrolysis products were quantified by GC-MS, GLS by UHPLC-DAD. Additionally, biological effects of 1-cyano-2,3-epithiopropane (CETP) on selected human liver cancer (HCC) cell lines (HepG2, Huh7, and Hep3B) have been investigated. Plant extracts containing CETP as well as pure CETP were used for the experiments.

The majority of the *Brassica* vegetables tested are producers of nitriles or EPTs as main breakdown products and not of ITC. Especially Brussels sprouts and savoy cabbage were very rich in CETP, containing up to 0.8 µmol/g fresh weight.
As determined *in vitro* by the WST-1 assay, DNA content analysis, caspase3/7 activity, and PI staining of the cells, pure CEPT had an impact on HCC cells in a dichotomous way. It adversely affected cells already at nM concentrations but unspecific cell killing in terms of necrosis was observed only at 100-fold higher concentrations. Controlled cells death was not a relevant mechanism triggered by CETP in our studies Presence of plant matrix increased CETP-based toxicity.

The results obtained might raise concerns about EPT-mediated noxious effects in context with human *Brassica* vegetable consumption. Further research is needed to evaluate bioavailability, metabolism and effects of EPTs *in vivo*.

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3.3 Tuscan black kale: from seeds to grams of highly pure glucoraphanin

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Glucosinolates (GLs) are bio-relevant chemical tags in the botanical order Brassicales, in which cruciferous vegetables represent the largest family [1]. Methionine-derived GLs, which constitute the largest class of GLs, display an additional thio-function – namely sulfide, sulfoxide or sulfone – in ω-position of their aglycon chain [2]. Among those, glucoraphanin (4-methylsulfinylbutyl GL) is the precursor of natural sulforaphane ((R)-1-isothiocyanato-4-methylsulfinylbutane), known as a potent chemopreventive agent [3]. In the context of our continuous program concerning the purification of GLs, we have identified Tuscan black kale (Brassica oleracea L., ssp. acephala DC, var. sabellica L.) seeds as an appropriate vegetable source, alternative to broccoli seeds, for gram-scale production of glucoraphanin. The defatted Tuscan black kale seed meal contained a high percentage (5.1% w/w) of glucoraphanin which was also the most abundant (80% of the total GL content) among a limited number of only five GLs. Those data fulfilled the starting conditions to make it a remarkable candidate amongst brassica vegetables for an efficient purification through a simple procedure that allowed us to obtain large quantities of glucoraphanin with a high purity level. The straightforward availability of both glucoraphanin and homogeneous myrosinase (E.C. 3.2.1.147) purified from Sinapis alba L. seeds, has allowed us to investigate the possible neuroprotective role of in situ generated R-sulforaphane in animal experimental models of multiple sclerosis and Parkinson's disease [4,5].

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3.4 In vivo formation and bioavailability of isothiocyanates from glucosinolates in broccoli as affected by processing conditions

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Food processing can reduce the concentration of glucosinolates and myrosinase (MYR) and therefore reducing the formation of isothiocyantes in processed vegetables. Glucosinolates are stable during mild heat treatments, although their content may be reduced by leaching into the cooking water upon boiling [1]. MYR is often inactivated even during mild heat treatment. As a consequence, vegetables are mainly consumed with no MYR activity [2].

The aim of this study was to investigate the effect of residual MYR activity in differently processed broccoli on sulforaphane (SR) formation, bioavailability and excretion in human volunteers.

Methods. Five different broccoli products were obtained with similar glucoraphanin (GR) content, yet different MYR activity. Excretion of SR conjugates in urine was determined in 15 participants after ingestion of the broccoli products. The bioavailability was calculated as cumulative amount (µmol) of excreted SR conjugates divided by the consumed GR amounts (µmol).

Results. A reduction of 80% of MYR in the product did not cause differences in the SR bioavailability compared to the product with 100% MYR. The product in which MYR was completely inactivated led to the lowest bioavailability (10%). A residual MYR of 2% in the product gave an intermediate bioavailability (17%). The excretion half-lives of SR conjugates were comparable for all the products (2.5h on average), although the maximum excretion peak times were clearly shorter when the residual MYR was higher (2.3-6.1h).

Conclusion. For the first time, the effect of residual MYR activity on isothiocyanate bioavailability was systematically and quantitatively studied. Processing conditions have a large effect on the kinetics and bioavailability of ITC's from broccoli.

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3.5 Interaction of isothiocyanates and glucosinolates with the Ah receptor as their chemopreventive potency

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The aryl hydrocarbon (Ah) receptor is a cytosolic transcription factor involved increasingly in many patho-physiological processes [1], so that antagonists of the Ah receptor imply chemopreventive potency. It regulates carcinogen-metabolising enzymes, for example the CYP1 family of cytochromes P450 and quinone reductase, which play an essential role in the biotransformation of many chemical carcinogens [2]. Using the CALUX assay it was established that phenethyl isothiocyanate, erucin and sulforaphane, are such antagonists. These isothiocyanates were poor ligands to the Ah receptor and weak inducers of CYP1A1 mRNA levels when incubated in precision-cut rat liver slices. They effectively antagonised, however, in a non-competitive manner, the activation of the receptor by the avid ligand benzo[a]pyrene. In studies involving intact glucosinolates, glucoraphanin was more potent antagonist of the Ah receptor than glucoerucin. Furthermore, phenethyl isothiocyanate, erucin and sulforaphane suppressed, in concentration-dependent manner, the benzo[a]pyrene-mediated rise in rat hepatic CYP1A1 mRNA levels in rat slices, in concordance with studies reporting that these isothiocyanates antagonise the benzo[a]pyrene-mediated increase in the O-deethylation of ethoxyresorufin in both rat and human precision-cut liver slices [3], as well as in human mammary tumour cell line Mcf7 [4], where sulforaphane inhibited benzo[a]pyrene-mediated CYP1A2 induction. Thus, it can be inferred that isothiocyanates are effective antagonist of the Ah receptor, and this potential may contribute to their established chemopreventive activity.

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3.6 Allyl isothiocyanate inhibits actin-dependent intracellular transport in Arabidopsis thaliana

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Volatile allyl isothiocyanate (AITC) derives from biodegradation of the glucosinolate sinigrin and has been associated with growth inhibition of several plants including the model plant Arabidopsis thaliana. However, the underlying mechanisms of this feature are poorly investigated in plants. In this study we present evidence of an AITC-induced inhibition of actin-dependent intracellular transport in A. thaliana. A transgenic line of A. thaliana expressing yellow fluorescent protein (YFP)-tagged actin filaments was used to show attenuation of actin filament movement by AITC. This appeared gradually in a time- and dose-dependent manner and resulted in actin filaments appearing close to static. Further we employed four transgenic lines with YFP-fusion proteins labelling either the Golgi apparatus endoplasmic reticulum (ER), vacuoles or peroxisomes to demonstrate an AITC-induced inhibition of actin-dependent intracellular transport of or in these structures consistent with the decline in actin filament movement. Furthermore, morphologies of actin filaments, ER and vacuoles appeared aberrant following AITC-exposure. However, AITC-treated seedlings of all transgenic lines tested displayed morphologies and intracellular movements similar to that of the corresponding untreated- and control-treated plants following over night incubation in an AITC-absent environment, indicating that AITC-induced decline in actin-related movements is a reversible process. These findings provide novel insights into the cellular effects by which AITC inhibits plant cell growth and expose clues to the physiological significance of the glucosinolate-myrosinase system.

3.7 Human intervention study investigating conversion of encapsulated glucoraphanin to isothiocyanates, by gut microbiota and genotype

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Glucosinolates (GSLs) are hydrolysed to isothiocyanates (ITCs) by the plant enzyme myrosinase. However, when cruciferous vegetables are cooked the myrosinase enzyme is denatured which results in intact GSLs passing into the colon where they are converted to ITCs and other products by the gut microbiota [1]. There may be significant differences between individuals in the ability of their gut microbiota to metabolise glucosinolates to isothiocyanates. This may, in turn, influence any health benefits that can be obtained from consuming cruciferous vegetables or glucosinolates as a dietary supplement [2, 3]. To explore individual variation in the metabolism of glucoraphanin, we undertook a human dietary study with purified glucoraphanin, the predominant glucosinolate from broccoli. 68 healthy participants followed a glucosinolate-free diet for three days and then consumed 100 mg of purified glucoraphanin in the form of a capsule. Each participant provided a blood sample for genotyping and a faecal sample for characterisation of their gut microbiota. The extent of glucoraphanin metabolism was quantified through the analysis of metabolites within a 24 hour urine sample provided by the participants [4]. The extent of metabolism of glucoraphanin to ITC metabolites was normally distributed with a mean of 10.86% ± 8.38%, and a range of 0.23% to 52.83%. The role of GSTM1 genotype and the gut microbiota profile, which may explain some of this variation, is currently being explored [5].

3.8 Selective cytotoxicity of isothiocyanates from Brassicales plants on human liver cancer cells and underlying mechanisms

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Therapeutic selectivity is one of the most important considerations in cancer chemotherapy. The design of therapeutic strategies to preferentially kill malignant cells while minimizing harmful effects to normal cells thereby depends on our understanding of the biological differences between cancer and normal cells. Isothiocyanates from Brassicales plants are classified as compounds which possess both potent protective and therapeutic properties against carcinogenesis in animals and human cells. Various molecular mechanisms are discussed as responsible for the chemopreventive/ chemotherapeutic actions of isothiocyanates which are characterized by a high oral bioavailability. However, information on their tumor-selective cytotoxicity is very limited so far.

We will demonstrate here that isothiocyanates selectively induce growth arrest and apoptosis in human liver cancer as well as tumor initiating cells while unaffecting the healthy human liver. Thereby we focused on the question whether abrogation of telomerase is involved in the cytotoxic response induced by isothiocyanates and tried to clarify the relevance of the tumor suppressor p53 in this context. Telomerase is differentially expressed in liver cancer cells but not in normal, well differentiated hepatocytes and strongly protects cancer cells from apoptosis.

The findings presented by us could provide an important approach in understanding selective growth inhibition of human liver cancer cells by isothiocyanates.

Session 4.
Ecology and evolution of glucosinolates

Wednesday, October 15th 2014

Keynote

Thomas Mitchell-Olds

Duke University, USA

Thomas Mitchell-Olds received his Ph.D. from the University of Wisconsin, followed by an NIH postdoc in human genetics. He is a former director of the Max-Planck Institute of Chemical Ecology in Jena, Germany. At Duke University he is the Newman Ivey White Distinguished Professor in the Department of Biology and Institute for Genome Sciences & Policy. His research examines genetic variation in plant populations, focusing on insect resistance in relatives of Arabidopsis, and on drought tolerance in rice for poor farmers in Asia and Sub-Saharan Africa.

4.1 Pathway function influences complex traits and fitness in plant populations

Thomas Mitchell-Olds

Duke University, USA

Although many studies provide examples of evolutionary processes such as balancing selection or deleterious polymorphism, the relative importance of these processes for phenotypic variation is unclear. To understand the evolutionary forces that influence complex trait variation, we focus on the glucosinolate pathway, which influences resistance to insects and pathogens, and provides a well-characterized pathway for evolutionary and ecological functional genomics. Ecological measurements of selection indicate that this polymorphism is influenced by spatially heterogeneous natural selection. Finally, we examine the relationship between flux and protein polymorphism in this pathway, where we find that flux control is focused in the first enzymatic step, and that flux control of these defensive phenotypes is robust across environmental treatments.

4.2 The glucosinolates-myrosinase system in Ochradenus baccatus: Ecology, biochemistry and physiology

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The glucosinolates-myrosinase system is common in members of the Brassicales order and is well established in its ability to reduce herbivory. *Ochradenus baccatus* is a desert plant that belongs to the Resedaceae family (the Brassicales order). *Ochradenus baccatus* is exceptional among desert plants as it harbors fleshy fruits. These fruits attract a wide verity of organisms, seed predators as well as seed dispersers. We have demonstrated that *O. baccatus* fruits have a unique seed-pulp myrosinase-glucosinolates partition; the fruit pulp is very rich in glucosinolates while the seeds contain the myrosinase activity [1]. We found that this separation promotes seed dispersal by predominant seed predating rodents such as *Acomys cahirinus* [1] but not in a related species, *A. russatus* [2]. A third rodent, *A. minous*, whose habitat does not include *O. baccatus*, was able to circumvent this defense system by eating only the seeds [2,3]. In good agreement with the behavioral observations, when activated pulp (i.e. fruit mash) was served, *A. russatus*, the seed predator, showed physiologically high tolerance levels to the *O. baccatus* defense mechanism, while, *A. cahirinus*, the seed disperser, was the most negatively affected. To-date we are completing the picture by performing biochemical analysis of the *O. baccatus* glucosinolates and the myrosinase, so far we have found a novel glucosinolate and high temperature resistance of the enzyme.

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4.3 Phyllotreta flea beetles utilize host plant defense compounds to create their own glucosinolate-myrosinase system

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Isothiocyanates formed upon enzymatic hydrolysis of glucosinolates are well-known defense compounds against herbivores produced in plants of the order Brassicales. Several lepidopteran generalists detoxify these highly reactive compounds by conjugating them with glutathione [1], while specialists evolved adaptations allowing them to circumvent isothiocyanate formation [2]. Several insects were shown to sequester glucosinolates from their host plants. This adaptation strategy is challenging in particular for chewing insects because the spatial separation of glucosinolates and the activating enzyme myrosinase is abolished during feeding. We investigate host plant adaptation in *Phyllotreta* flea beetles which are cosmopolitan pests of *Brassica* crops. We previously observed that *P. striolata* adults are able to produce volatile alkenyl isothiocyanates and examined how these toxic metabolites are formed in this insect. To test whether *P. striolata* adults sequester glucosinolates from their food plants, we performed feeding assays with host plants differing in their glucosinolate profile and traced the accumulation of plant glucosinolates using HPLC. We found that the beetles selectively sequestered mainly alkenyl glucosinolates up to 1.75% of their body weight, but only low amounts of aromatic and indolic glucosinolates [3]. Myrosinase activity was readily detectable in crude beetle protein extracts and the corresponding enzyme was partially purified using FPLC. By combining proteomics and transcriptomics we identified six candidate proteins in the active fraction which were predicted to belong to glycoside hydrolase family 1 and have β-glucosidase activity. We cloned and heterologously expressed these six candidate genes but in myrosinase activity assays, only one of these heterologously expressed enzymes hydrolyzed the substrate allyl glucosinolate. The major substrates of the identified beetle myrosinase were aliphatic glucosinolates which were hydrolyzed with at least 4-fold higher efficiency than aromatic and indolic glucosinolates and β-O-glucosides [3]. Our results reveal the convergent evolution of a glucosinolate myrosinase system in *P. striolata* that enables this herbivore to use glucosinolate hydrolysis products for its own purposes.

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4.4 Natural genetic variation in growth and metabolite regulatory roles of Allyl glucosinolate in Arabidopsis thaliana

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Glucosinolates (GSLs) play an important role in plant as biotic and abiotic stress response mediators inducing complex defense strategies networks [1-2]. However, the physiological significance of GSL sensing in plants is not completely understood. Thus, we initiated an investigation of the effects of exogenous Allyl GSL on plant development. To identify suitable genetic screening conditions we initially tested seven *A. thaliana* accessions with different GSL profile. Plants were feed with 50 µM Allyl GSL in MS media with different sucrose concentrations. Results showed that the inclusion of Allyl GLS within the media lead to increased biomass of most accessions with increasing effects as sucrose increased. HPLC verified that all the accessions were able to take up the Allyl GSL from the media and this transport was also dependent upon the sucrose concentration. To elucidate the potential mechanism by which Allyl GSL can affect biomass changes in Arabidopsis we increased the study to a survey of a 96 *A. thaliana* natural accessions [3-5]. Results showed that growth was highly heritable and that natural Arabidopsis accessions have significant variation for the effect of Allyl GSL upon seedling growth. The accessions displayed both positive and negative response in growth. In addition to growth, the exogenous Allyl also altered endogenous GSL accumulation with different effects across the Arabidopsis accessions. There was also an interaction of GSL profile and growth with the Allyl treatment having stronger effects on growth for the genotypes which predominantly display C3 GSL than those with C4 GSL. To better understand how the different GSL may combine to relate with growth responses, we performed regression analysis with all individual GSL traits. This resulted in a model where variation in the response of eight GSL traits, seven aliphatics and one indolic, explained 43% of the variability plant growth response to exogenous Allyl GSL. 8-methylsulfinyloctyl GSL responses to Allyl were the most strongly correlated with growth responses. In conclusion, it appears that Allyl GSL has the capacity to differentially affect plant growth and metabolite content of Arabidopsis accessions dependent upon the environment and endogenous GSL genetic variation. Further Genome-wide association studies will help to elucidate the regulatory network and candidate genes controlling growth response variation to exogenous Allyl GSL.

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4.5 Glucosinolate biodiversity screening with distinction of isomers reveals evolutionary innovations and structure-dependent reactivity

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Although HPLC-MS analysis has greatly advanced glucosinolate analysis, distinction of isomers is an inherent challenge of the technique [1, 2]. The challenge is even higher in evolutionary studies including wild species, in which previously unknown isomers are often encountered [3, 4, 5]. Recent advances in specific glucosinolate analysis using NMR and MS2 detection after HPLC separation are presented, and the importance of isomer distinction in evolutionary studies is illustrated with recent submitted and/or unpublished results. The examples include several novel isomeric glucosinolates and formation of different classes of hydrolysis products from isomeric glucosinolates: isothiocyanates, oxazolidine-2-thiones and previously unknown thiazolidine-2-ones.

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4.6 Toxicity and detoxification of glucosinolates in generalist insect herbivores

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While the negative effects of glucosinolate-derived hydrolysis products towards herbivore growth have been well-documented, the metabolism and the mechanisms underlying the bioactivities of these compounds *in vivo* are not completely understood. Due to their reported health benefits to humans, many studies of the metabolic fate of glucosinolate-derived isothiocyanates (the major products of glucosinolate hydrolysis, including in the model plant *A. thaliana*) were performed in mammal systems, with metabolic studies in insects being generally restricted to specialist herbivores and/or to non-quantitative pathway elucidation. Using radiolabeled 4-methylsulfinylbutyl (4msob) glucosinolate, the major glucosinolate in *A. thaliana* Col-0, we have quantitatively determined that the mercapturic acid pathway starting with conjugation to glutathione is the main route of isothiocyanate metabolism in generalist insect herbivores such as *Spodoptera littoralis*, the Egyptian cotton leafworm [1]. Unexpectedly, the extent of isothiocyanate detoxification differed greatly among the species we examined, with only traces of mercapturic acid metabolites being observed in certain other lepidopteran larvae. Detoxification of isothiocyanates via this pathway, however, is metabolically costly, and we have observed drastic changes in primary metabolism after exposure to 4msob-isothiocyanate, including in sugar, amino acid and protein metabolism. These toxic effects can be directly observed for example as a depletion of intracellular glutathione (and its precursor amino acid Cys) due to metabolic losses during isothiocyanate conjugation and excretion, with these in turn correlating with lower body protein levels and imbalances in cellular redox homeostasis. Additionally, we have examined the individual and combined effects of alkyl- and indolic-glucosinolates on insect larval development over one generation. Surprisingly, although growth rates were negatively affected by dietary glucosinolate levels, we have found that the amounts of glucosinolates normally present in *A. thaliana* Col-0 leaves are not sufficient to directly cause increased larval mortality. The negative effects of glucosinolates on earlier instar growth were more pronounced than on later instars; in the latter we saw a reversal of these negative effects. Thus, glucosinolates do not seem to directly influence insect survival/mortality, but their direct negative effects on insect fitness (e.g. via slower growth rates and longer development cycles) remain to be examined in more detail. Additionally, the experimental identification and localization of the molecular factors that mediate these effects will help shed light on the *in vivo* mode(s) of action of these molecules in insect pests.

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4.7 ER bodies contain a novel class of myrosinases and are functionally coordinated with indole glucosinolates

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The endoplasmic reticulum (ER) bodies are ER-derived organelles thought to function in plant defense. These are detectable only in a certain group of plant species restricted to the order Brassicales. ER bodies in Arabidopsis thaliana contain large amounts of β-glucosidases, for which little is known about their in planta substrates and physiological function(s). Here we show that PYK10/BGLU23, the most abundant β-glucosidase in *A. thaliana* ER bodies, is potent to hydrolyze an indole glucosinolate (IG) that represents a well-known family of Brassicales secondary metabolites considered as phytoanticipins. Phylogenetic analysis including a comparison of putative substrate binding/catalytic amino acid residues revealed that 16 out of 47 β-glucosidases in *A. thaliana* might have similar activity, defining a novel clade of myrosinases. Furthermore, the co-occurrence of these components (ER bodies and IGs) appears to be subjected to coordinated regulation as evidenced by the gene co-expression network. This idea is also supported by the fact that ER bodies and IGs taxonomically co-occur in Brassicaceae. Overall, our results imply a convergent evolution of two independent clades of myrosinases, a part of which are functionally coordinated with ER bodies and IGs to constitute a Brassicaceae-specific defense machinery.

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4.8 Glucosinolate-myrosinase defence system modified oilseed rape MINELESS plants in response to above- and below-ground herbivores

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After being attacked by insect herbivores, the glucosinolates in plant tissues are hydrolysed by the enzyme myrosinase (EC 3.2.1.147) into a variety of degradation products, which can deter further herbivory, well-known as glucosinolate-myrosinase defence system and also described as “the mustard oil bomb”[1, 2]. Using genetic cell ablation strategy, transgenic oilseed rape *(Brassica napus) MINELESS* plants have been produced [3, 4] for cv. Westar designated as the wild-type. *B. napus MINELESS* plants with high glucosinolate levels, low myrosinase activity and glucosinolate-myrosinase hydrolysis products are currently being applied as a model to study the role of glucosinolate-myrosinase defence system against some of the above- and below-ground herbivores. The above-ground herbivores include: cabbage moth *(Mamestra brassicae)* and cabbage looper *(Trichoplusia ni)*, while below-ground herbivores include: cabbage and turnip root flies *(Delia radicum and Delia floralis)*. M. brassicae larvae after feeding on *MINELESS* seedlings showed higher reduction in number and stunted growth compared to the wild-type. The glucosinolates indol-3-yl-methyl (glucobrassicin), 4-hydroxy-indol-3-yl-methyl (4-hydroxyglucobrassicin) and 1-methoxy-indol-3-yl-methyl (neoglucobrassicin) increased in *MINELESS* seedlings after M. brassicae feeding. M. brassicae feeding significantly increased the levels of indole-3-yl-methylnitrile, and total glucosinolate-myrosinase hydrolysis products in *MINELESS* seedlings. Insect herbivory by larvae of *T. ni enhanced* production of glucobrassicin, 4-methoxy-indol-3-yl-methyl (4-methoxyglucobrassicin) and neoglucobrassicin in leaves of *MINELESS* plants. Besides, larvae of both M. brassicae and T. ni insect herbivores induced genes belonging to the glucosinolate biosynthesis pathway. Larvae of D. radicum and D. floralis showed higher weight on day 10 after feeding on roots of *MINELESS* plants compared to the wild-type. For day 10, *MINELESS* plants showed higher weights for above-ground biomass for both *D. radicum* and *D. floralis* herbivory, but for below-ground biomass *MINELESS* showed slightly lower weights than the wild-type. Larvae of both flies caused induction of glucosinolate glucobrassicin in above-ground biomass of *MINELESS* plants after feeding on roots for days 4, 10 and 14. Levels of glucosinolates have been reported to be altered by feeding activities of different insect species [5]. The results from these investigations with MINELESS plants seems to be very interesting in terms of analysing the role of glucosinolates and the glucosinolate-myrosinase defence system in response to above-ground/below-ground herbivores as well as the generalists and specialists. Similar studies in future with *MINELESS* plants can also provide highly applicable knowledge for agriculture and novel integrated pest management tools that could increase the environmental and economic sustainability of Brassica crops in the face of insecticide-resistant pest populations.

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Poster presentations abstracts in alphabetic order

P.01 Glucosinolate biosynthesis and regulation of sulfur assimilation in Brassicaceae as affected by foliar sulfur nutrition

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Glucosinolates are a group of secondary sulfur-compounds mainly found in *Brassica* species. They may function in plant defense against insects, herbivory and pathogens and have anti-carcinogenic properties [1]. The content of glucosinolates varies strongly between species, cultivars, developmental stage and may be affected by the plant sulfur supply/status [2]. From previous finding it was obvious that *Brassica* species are able to utilize foliar absorbed sulfur gases, viz. H_2S or SO_2, which replace sulfate taken up by the root as sulfur source for growth [3]. In the current study the impact of foliar sulfur deposition and sulfate nutrition on glucosinolate biosynthesis and regulation of sulfur assimilation pathway was investigated in two Brassica species characterized by a high (*B. juncea*) and low (*B. rapa*) glucosinolate content. 10-day-old seedlings were grown on a 25% Hoagland nutrient solution containing 0.5 mM sulfate for 3 days and subsequently transferred to fresh 25% Hoagland solution at 0 mM sulfate (-S) or 0.5 mM sulfate (+S) and exposed to 0.25 µl l^{-1} H_2S or SO_2 for 7 days. At an ample sulfate supply, exposure of both species to foliar sulfur nutrition hardly affected the total content and composition of glucosinolates. H_2S or SO_2 exposure resulted in a slight decrease in expression of APS reductase, whereas that of ATP sulfurylase, APS kinase (is involved in synthesis of glucosinolates) and sulfite reductase remained unaffected. Sulfate-deprivation of plants resulted in a decreased biomass production and glucosinolate content. The expression of APS reductase was strongly enhanced in sulfate-deprived plants, while that of APS kinase and ATP sulfurylase was slightly up-regulated and sulfite reductase hardly changed. When sulfate-deprived plants simultaneously exposed to H_2S or SO_2 the glucosinolate content remained lower than that of sulfate-sufficient plants but the composition became similar to sulfate-sufficient plants. Moreover, the expression of APS reductase was partially down-regulated, whereas expression of APS kinase, ATP sulfurylase and sulfite reductase remained unaffected. It was obvious from the current study that the glucosinolate content was more or less determined and only slightly affected by the sulfur status of the plant.

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P.02 Glucosinolate metabolism in the striped flea beetle, *Pyllotreta stiolata*: feeding experiments with isotope-labelled glucosinolates

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The striped flea beetle, *Pyllotreta striolata*, is a specialist herbivore of crucifers and selectively sequesters glucosinolates from its host plants, activating them by an endogenous beetle myrosinase. Interestingly, the adult beetles sequester glucosinolates from their diet with differing specificities, showing an increased preference for aliphatic glucosinolates [1]. However, the mechanisms of glucosinolate sequestration and metabolism used by this insect are not yet fully understood. To investigate how this beetle metabolizes glucosinolates, we performed feeding studies with three different ^{14}C-labelled compounds: 4-methylsulfinylbutyl glucosinolate (4MSOB-GLS), 4-hydroxybenzyl glucosinolate (sinalbin), and allyl glucosinolate (sinigrin). Labelled glucosinolates were incorporated into detached leaves of the *Arabidopsis thaliana myb28myb29* double knock-out mutant, which lacks endogenous methionine-derived glucosinolates, and fed to adults (40 beetles per leaf). After two days, feces and beetles were collected and extracted. Samples were analyzed by fractionation via HPLC and radioactivity was measured with a liquid scintillation counter. The nitrile derived from hydrolysis of 4MSOB-GLS was found to be excreted, whereas the intact 4MSOB-GLS and its other hydrolysis product, 4MSOB isothiocyanate (ITC), were detected in the body, confirming our previous finding that the 4MSOB-GLS is sequestered [1]. The presence of the 4MSOB nitrile suggests the existence of a nitrile specifier protein, as in *Pieris* spp. *P. striolata* sequesters very little sinalbin when feeding on *Sinapis alba.* But, when it fed on the *A. thaliana* leaves spiked with the labelled sinalbin, a considerable amount of the intact sinalbin was found in the body, suggesting that plant-specific factors affect sequestration. In addition, sinalbin was also excreted into the feces without modification. When labelled sinigrin was fed, allyl glutathione (allyl-GSH) was identified in the beetle's body, but not in the feces. This compound is a known metabolite of the sinigrin hydrolysis product, allyl-ITC, in generalist herbivores. However, only 19 % of the total radioactivity was recovered from the body and feces, possibly due to loss of the highly volatile allyl-ITC. In conclusion, this study gave initial clues on the metabolism of three major glucosinolates by *P. striolata*. Further investigations on how detoxification interacts with sequestration in the striped flea beetle and related species will shed light on the novel aspects of successful host adaptation in this group of insects.

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P.03 Rat gut microbial metabolism of the anticancer glucosinolate glucoraphaninis influenced by feeding broccoli

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Glucoraphanin (GRP), the major glucosinolate present in broccoli, can undergo hydrolysis to form the anticancer isothiocyanates (ITC) sulforaphane and erucin In plant tissue, this reaction is catalyzed by the enzyme myrosinase (EC 3.2.1.147), not present in mammalian cells. We previously identified by a BLAST search, potentially similar thioglucosidases in two components of the microbiota, lactobacilli and bifidobacteria (Lai *et al.*, 2010). Yet most studies of microbial metabolism ex vivo show little or no ITC formation. Here we tested the hypothesis that consumption of broccoli for up to 2 weeks can cause a change in rat caecal microbiota, seen as significantly enhanced metabolism of GRP to ITC. This prolonged broccoli feeding increased ITC formation up to 5-fold. A diet containing only the vegetable matrix of the broccoli powder, rich in fiber but devoid of glucosinolates, did not enhance metabolism of GRP by microbiota.

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P.04 Co-occurrence of glucosinolates and saponins in *Barbarea* spp. (Brassicaceae) and resistance to diamondback moth

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Glucosinolates are plant secondary metabolites used in plant defense. For insects specialized on Brassicaceae, such as the diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae), glucosinolates act as "fingerprints" that are essential in host plant recognition. Some plants in the genus *Barbarea* (Brassicaceae) contain, besides glucosinolates, saponins that act as feeding deterrents for *P. xylostella* larvae and prevent their survival on these plants [1-3]. Removal of leaf epicuticular waxes with gum arabic has shown that glucosinolates, but not saponins, occur on the leaf surface of some Barbarea plants in concentrations that are sufficient to be perceived by ovipositing *P. xylostella* [4]. Glucosinolate concentrations on the abaxial and adaxial leaf surfaces of *Barbarea* spp. can be very different. Nevertheless, these differences in glucosinolate concentrations between abaxial and adaxial leaf surfaces do not result in significant differences in oviposition preference by *P. xylostella*. Within a Barbarea plant, however, *P. xylostella* moths tend to lay more eggs per leaf area on young leaves, which contain higher concentrations of glucosinolates and saponins than older leaves [5]. Co-occurrence of a high content of glucosinolates and saponins in *Barbarea* leaves can protect them against P. xylostella and other herbivores.

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P.05 Effect of food chain production residues on the glucosinolate profile of *Brassica rapa* ssp. *chinensis*

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The effect of food chain residues on the content of glucosinoltes in *Brassica rapa* ssp. *chinensis* (pak choi) was investigated in this study. Pak choi is a fast growing green leafy vegetable, which is very frequently consumed in Asian countries and in steadily rising quantities in Europe. As with other plants, the amount of bioactive substances is known to depend on several parameters, including genotype [1], growing conditions, developmental stage and soil characteristics. Agricultural residues and remains of the food production chains are regard as an alternative to chemical fertilizers. We used residues of the food production chain of coffee, aronia and hop and were particularly interested if these can modulate the glucosinolate concentrations in pak choi.
Five or 10% of the soil was replaced by organic residue. The sprouts were grown for 10 days on soil or soil mixtures in glasshouses during summer 2013.
To assess differences in the concentrations of the glucosinolates the lyophilized samples were analyzed by UHPLC after a slightly modified method previously reported [1].
We observed significant changes in concentrations of aliphatic, indole and aromatic glucosinolates. Strongest effects were found in samples grown on 10% soil mixtures. The concentrations of aliphatic and aromatic glucosinolates decreased if grown on soil with organic residues. In most samples the concentrations of indole glucosinolates decreased with one exception if pak choi was grown on soil fortified with coffee residue. Indole glucosinolates can be induced by methyl jasmonate [2] and will be of great interest to investigate if the observed changes are due to changes in the jasmonate signaling pathway.

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P.06 Stability of glucosinolates in innovative broccoli-based vegetarian bars

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Advances in nutrition research during the past few decades with aspects of growing urbanization and consumer awareness recommend an increasing contribution of vegetarian diets for improving human health by reducing the risk of degenerative diseases. In this context, *Brassicaceae* is a vegetable family that is highly appreciated due to its bioactive compounds i.e. glucosinolates (GLSs), but it is consumed mostly after processing. However, food processing can result in various changes in the content of GLSs. In this project, innovative broccoli-based vegetarian bars had been developed. The effect of cooking methods on the GLSs content has been investigated. Primary results indicated that the effect of cooking methods varies, whereas the effect of steaming, microwaving, and backing were lower than stir-frying which reduced the GLSs content considerably. Interestingly, the possibility of producing broccoli-based vegetarian diets as ready-to-use and ready-to-eat bars could provide a promising approach for improving traditional meals and potential benefits for human health. Indeed, studying the change of GLSs during processing is shown to be valuable to understand the impact of processing and to optimize the thermal processing conditions for health benefits of those compounds.

P.07 Evaluation of Glucosinolate Variation in a Collection of Turnip (Brassica rapa) Germplasm by the Analysis of Intact and Desulfo glucosinolates

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Glucosinolates (GLS) are secondary metabolites occurring in cruciferous species. These compounds are important for plant defense, human health, and the characteristic flavor of *Brassica* vegetables. In this study, the GLS in tubers from a collection of 48 turnip (*Brassica rapa*) accessions from different geographic origin were analyzed. Two different methods were used: desulfo GLS were analyzed by HPLC-PDA, and intact GLS were analyzed by accurate mass LC-MS. For most GLS, desulfo and intact signals correlated well and the analytical reproducibility for individual GLS was similar for both methods. A total of 11 different GLS were monitored in the turnip tubers, through both intact and desulfo GLS analysis methods. Four clusters of accessions could be clearly distinguished based on GLS composition of the turnip tuber. Clustering based on tuber GLS differed markedly from a previously published clustering based on leaf GLS.

P.08 Glucosinolates in Rocket Salad Germplasm & Commercial Varieties: Correlations with Human Sensory Perceptions, Preferences & Genetics

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Rocket crops are becoming increasingly popular with growers, consumers and researchers alike [1]. Previous studies have identified variability of glucosinolate traits between varieties [2], however few of which have made a direct comparison between those commercially available and those in underutilised germplasm collections. We propose that by screening underutilised rocket cultivars for glucosinolate concentration and sensory attributes, new and more nutritious varieties can be developed and marketed to consumers.

A comprehensive analysis of 19 gene bank and 16 commercial varieties of rocket (*Eruca sativa, Eruca vesicaria, Diplotaxis tenuifolia*) was conducted by Liquid Chromatography-Mass Spectrometry to determine glucosinolate content. Concentrations were estimated using an external calibration curve of sinigrin hydrate. 12 glucosinolate compounds were positively identified across all samples tested using ion data presented in journal literature [3]; none of the compounds discriminated between species. The compounds identified were: 4-hydroxyglucobrassicin, glucotrapaeolin, glucolepiidin, glucoraphanin, glucoiberverin, glucosativin, DMB, glucoalyssin, glucoerucin, glucoraphenin, diglucothiobeinin and glucoibarin.

Concentrations of glucosinolates varied significantly between accessions, and on average, were higher in the underutilised gene bank accessions than the commercially available varieties. Six gene bank accessions and one commercial variety of *Eruca sativa* were selected for further study based on the varying glucosinolate concentrations (e.g. low, medium, high), and presented to a panel of 96 untrained consumers. Each was asked to evaluate their sensory perceptions of different attributes of rocket leaves: bitterness, sweetness, hotness, pepperiness and overall liking. These data were used in Principal Component Analyses (PCA) to determine any significant correlations with glucosinolate content.

Like many *Brassicaceae* vegetables, some individuals have an intense dislike for rocket due to the suspected bitter attributes of glucosinolate and isothiocyanate compounds [4]. A buccal swab sample was taken from consenting volunteers and their genotype determined by Quantitative-PCR Single Nucleotide Polymorphism analysis of the TAS2R38 thiourea taste receptor gene. The genotype of volunteers was then correlated using PCA analysis to determine any link between preference of rocket taste and bitterness perception.

The results from our experiments are now being actively utilised by collaborating plant breeders at Elsoms Seeds Ltd. (Spalding, Lincolnshire, UK) to produce new breeding lines of rocket, and ultimately, new varieties of enhanced nutritive and sensory quality. Similarly, our research is actively identifying untapped, niche markets of rocket consumers, and advising collaborating product developers at Bakkavor Group Ltd. (Bourne, Lincolnshire, UK).

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P.09 Glucosinolates in the Endemic Plants of the Tribe Alysseae (*Brassicaceae*) Wild-Growing in Croatia

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A significant group of plants found in the Croatian native flora belongs to the tribe *Alysseae* which comprises seven genera: *Alyssoides, Alyssum, Aurinia, Berteroa, Clypeola, Degenia* and *Fibigia*. The genus *Aurinia* comprises 12 taxa among which *Aurinia sinuata* (L.) Griseb. and *A. leucadea* (Guss.) C. Koch represent two endemic species wild-growing in Croatia [1]. These plants can be found in mountainous areas of Central and Southern Europe, Russia and Turkey. *Degenia velebitica* (Degen) Hayek and *Fibigia triquetra* (DC.) Boiss. ex Prantl are rare Croatian stenoendemic species representing tertiary relicts. Natural area of *D. velebitica*, the only species of the genus *Degenia*, is restricted to only several localities on the South and Mid Velebit massif. F. triquetra, sometimes confused with *D. velebitica*, is one of two species in the genus *Fibigia*, wild-growing on rocky grounds of Southern Dalmatia [1].

As molecular tags of the order Brassicales, glucosinolates (GLs) were characterized and quantified in different plant parts (flower, leaf, stem, and seed) according to the ISO 9167-1 official method based on the HPLC analysis of desulfo-GLs and by GC/MS analysis of the volatile fractions obtained after enzyme hydrolysis. With high contents ranging from 9.9 to 135.4 μmol/g of dried material in different plant parts - especially in the seeds (over 4.0% w/w with the highest, 6.1% w/w in *F. triquetra*) - those *Alysseae* are found to represent a good GL source. The major GLs found in the seeds of investigated plants were the following: glucoalyssin (GAL) and gluconapin (GNA) in *A. leucadea*; GAL and glucobrassicanapin (GBN) in A. *sinuata*; glucoberteroin (GBE) in *D. velebitica*; glucoerucin (GER), GNA and glucoraphanin (GRA) in *F. triquetra*. Our results and previous reports [2], on *Aurinia* species, D. velebitica and *F. triquetra* chemistry, as well as other plants of the tribe *Alysseae* [3], suggest that those endemic species represent appropriate sources for GLs bearing a C-4 and/or C-5 olefinic aglycon chain (GNA, GBN) and/or a thiofunctionalized chain (GER, GBE, GRA, GAL).

Keywords: glucosinolates, Aurinia sinuata, Aurinia leucadea, Degenia velebitica, Fibigia triquetra, HPLC, GC-MS.

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P.10 Glucosinolate-myrosinase system in Mediterranean plant - Capparis ovata Desf.

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Glucosinolate-myrosinase system; an interesting and important defense system in plants that has been extensively studied and recent researches have been focused on to the elucidation of its components potential to be used for human health including cancer treatment and as biodegradable pathogen deterrent molecules [1]. Glucosinolates are among the major secondary metabolites in *Arabidopsis thaliana* and found almost in all plants belonging to the order *Brassicales*. We have identified glucosinolate-myrosinase system in the Mediterranean plant *Capparis ovata* (caper) and tried to understand what are the similarities and discrepancies between caper and the other plants' glucosinolate-myrosinase system. Therefore we analyzed the glucosinolate content and myrosinase enzyme activity in different organs of *C. ovata* such as leaf, shoot, flower bud, flower and seed [2]. It was interesting to see that *Capparis* myrosinase had very high constituent activity and stimulated more with high ascorbic acid content as we compare these results to those of *Arabidopsis thaliana* and *Brassica* sp. Glucocapparin, glucoiberin, progoitrin, epiprogoitrin, sinigrin, gluconapin, glucosinalbin, and glucobrassicin were among the glucosinolates which were extracted and quantified from the leaves, seeds, flowers, flower buds, and young shoots of *C. ovata*. The major glucosinolate was glucocapparin, which accumulated to values of 39.35±0.09 and 25.56±0.11 μmol g^{-1} dry weight in seed and leaves respectively [2]. Glucocapparin is among the rare glucosinolates that occurs relatively at low levels in other *Brassica* sp. Myrosinase activity was detected in seeds, leaves, flowers, and flower buds and the highest enzyme activities were found in flower buds and seeds. The myrosinase protein migrated as a single band with a molecular weight of 65 kDa on SDS-PAGE [2]. *C. ovata* (caper) is an economically important plant due to its flower buds' edibility and usage as a medical plant therefore understanding the glucosinolate-myrosinase system and its components in this plant have provided information for further studies.

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P.11 Identification of QTL controlling glucosinolate contents in leaf, stem and seed in a B. napus DH-Population

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The development of low erucic acid varieties and subsequently the introduction of low seed glucosinolate (GSL) content through the polish cultivar 'Bronowski' were two milestones in the breeding of *B. napus* as oil crop. While the inheritance of seed GSL content has been studied well due to its importance, only a few quantitative trait loci (QTL) mapping studies with the focus on GSL contents in vegetative tissues exist. Here, we used a *B. napus* DH-Population derived from the cross of the resynthesized line 'L16' and the cultivar 'Express' to identify regions in the genome which are possibly involved in GSL metabolism. In a field trial we measured individual GSLs in leaf, stems and seeds in order to investigate the tissue-specificity of GSL-QTL. Applying Composite interval mapping we found a total of 115 QTL related to individual or groups of GSL. 49 from the 115 QTL belonged to GSL traits from seeds, 31 QTL from leaf GSL traits and 35 QTL from stem GSL. 36 of the 49 QTL in seeds were mapped in linkage groups (number of QTL) A03 (13), C02 (11) and C09 (12). Linkage groups A03 and C09 also had the highest number of QTL in vegetative tissue (A03 15 and C09 14 QTL). Comparison of the QTL positions by trait between tissues generally showed more overlapping regions between leaf and stem than between seed and leaf/stem. Nevertheless, some of the mapped QTL were trait specific and detected only in leaf, stem or seed. The phenotypic correlation for the sum of GSL contents was high (> 0.94) between stem and leaf, but lower (~0.52) between vegetative tissues and seeds. These correlations support the mapping results by underlining that leaf and steam as vegetative tissue are more similar than seed and vegetative plant parts and the underlying genetic structure is more similar.

P.12 Capture the pungency of horseradish products (Armoracia rusticana); study on glucosinolates and myrosinase.

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Armoracia rusticana (Horseradish, *Brassica* family) is a root characterized by a pungent taste and smell that is released from glucosinolates (GLSs) hydrolysis. GLSs are hydrolyzed by the endogenous enzyme myrosinase (MYR) upon cells damage, producing volatile breakdown products, such as isothiocyanates (ITCs) [1]. ITCs are also known to have anticarcinogenic properties, thus their formation has a health benefit for consumers [2].

Horseradish is widely consumed freshly grated or mixed with vinegar as sauce. Due to its very long growing season, horseradish is only available for few months a year, and the storage affects pungency of horseradish.

The aim of this study is to investigate how different horseradish preparations and storage temperatures affect the GLSs, MYR and ITCs levels.

Horseradish was collected from Basilicata (Italy) and investigated as (1) grated and (2) grated with vinegar and olive oil (sauce). Both products were stored for two months at 4°C and -20°C. GLSs, MYR and ITCs were measured in the untreated, fresh prepared and stored samples. No MYR activity was detected in the sauce, probably due to the low pH of vinegar (pH 2.8). Whereas in grated radish 10% MYR activity was retained upon two weeks of storage at 4°C. The storage temperature that most preserved the MYR activity was -20°C with 50% of retention after two months.

Sinigrin, the most abundant GLS in horseradish [1], show the highest stability storing the samples at -20°C, regardless the type of product. The sinigrin degradation was explained by the hydrolytic activity of MYR. In fact, in samples in which MYR was still active and in which the conditions were favorable for the hydrolysis, the sinigrin degradation was higher.

For the allyl-ITC, which are the ITC of the sinigrin, very low levels were detected in all samples regardless the storage temperatures, probably because these compounds were lost during the grating of the radish due to their volatility. A supplementary experiment was conducted to evaluate the taste and flavor of the graded horseradish during storage, to associate the low levels of allyl-ITC detected with the pungency of the samples. The sensory test (6 participants) showed that the storage severely affected the pungency of the samples, especially by storing at 4°C.

This work shows that the storage decreases the release of the allyl-ITCs reducing the healthy effect and also its taste and flavor. New researches should be conducted to optimize the preparation of horseradish to preserve the sensory attributes and its biologically active compounds upon storage.

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P.13 Glucosinolate metabolism by bacterial enzymes

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Plant myrosinases are normally responsible for transforming glucosinolates (GSLs) into the more bioactive isothiocyanates (ITCs) that are known to have chemoprotective properties. However, during cooking these plant enzymes are inactivated so the metabolism of GSLs to their corresponding ITCs is dependent on the myrosinase-like enzymes produced by human gut bacteria. Many bacteria including *Bifidobacterium* spp [1], *Bacteroides thetaiotaomicron* [2], *Lactobacillus agilis* R16, *Enterococcus casseliflavus* CP1 and *Escherichia coli* VL8 [3] were reported to show GSL degrading ability, but in contrast to the plant and aphid derived enzymes the bacterial enzymes are largely uncharacterised. This study aims to provide a better understanding of how human gut bacteria perform these transformations and which enzymes are playing crucial roles in gut bacterial glucosinolate metabolism. Genomic DNA from two isolates, *Lactobacillus agilis* R16 and *Enterococcus casseliflavus* CP1, was extracted and sequenced. Putative myrosinase genes and genes encoding putative methylsulfinylalkyl reductases were identified. As all reported myrosinases from plant or cabbage aphids are members of the Glycosyl Hydrolase 1 (GH1) family, one glucosidase gene (*gh44*) from *Enterococcus casseliflavus* CP1 belonging to GH1 family and showing the highest identity with Brevicoryne brassicae myrosinase (aphid myrosinase) was selected, in addition to the 3 methylsulfinylalkyl reductase genes identified in the *Lactobacillus agilis* R16 genome. The reductases were suggested to be responsible for bioconversion of 4-methlysulfinlyalkyl glucosinolates to 4-methylthiobutyl glucosinolates [4] and serve an alternative pathway for bacteria that are not able to metabolise methylsulfinylalkyl GSLs directly. The genes were amplified, cloned and expressed with a His-tag in *Escherichia coli* and Ni-NTA purified proteins were used to determine enzyme activities. One of the purified reductase proteins showed activity to transform 4-methlysulfinlyalkyl GSL into 4-methylthioalkyl GSL. Although preliminary enzyme activity assays performed with crude protein extracts of gh44 showed the enzyme is potentially active, Ni-NTA purified gh44 protein failed to show myrosinase activity. However, the effect of the His-tag has not yet been established. The study will further focus on characterisation of these enzymes and other GSL degrading bacteria isolated from the human gut.

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P.14 Glucosinolates, some innovative biochemical, chemical and analytical methodologies

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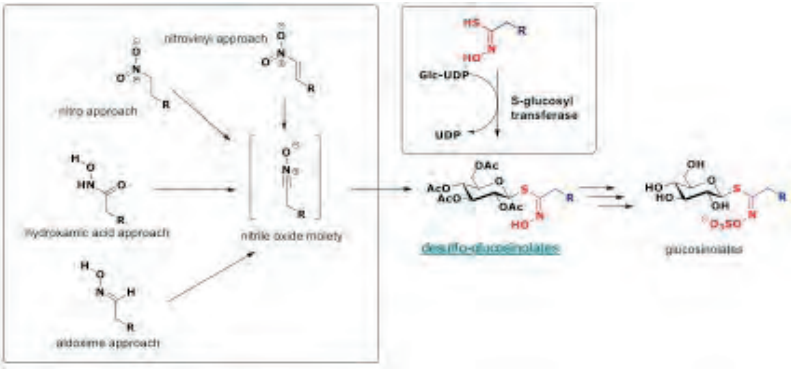
The Brassicale order of the plant kingdom contains 16 different families. [1] A major part of these are found in our daily diet mainly within crucifers, which show a significant chemotaxonomy marked by the presence of glucosinolates - secondary metabolites with a thiosaccharidic structure. These "thioglucosides" have three features in common: a β-D-glucopyranosyl unit, an anomeric O-sulfated thiohydroximate function (both structures are invariant) and a variable side chain according to plant species. Glucosinolates (GLs) – more than 130 molecules characterized – are associated in plants to myrosinase (E.C.3.2.1.147), an atypical glucohydrolase able to transform these substrates into isothiocyanates. The latter molecules show diverse biological activities and are implicated in plants defense mechanism. [1]
Our team is involved in the biosynthesis, synthesis, degradation and analysis of glucosinolates either chemically or enzymatically. [2] As the GL content in plants is usually low, purification and characterization are challenging tasks especially when biological assays are required. The chemical synthesis and the biochemical synthesis are alternatives to prepare the molecules. Various approaches have been designed in our laboratories to access the different needed GLs natural or artificial. [3]



We will disclose our recent results in these different topics on glucosinolates chemistry, biochemistry and analysis.[4]

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P.15 Yield and Quality Attributes Comparison of Various Accessions of Brassica napus L.

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Rapeseed (*Brassica napus* L.) is a famous oil crop and is vastly grown in Canada, India, China and European countries for its nutritive value regarding protein and oil contents. A field study was conducted to assess the agronomic, yield and quality parameters of rapeseed under field conditions during winter season 2013. Ten accessions were compared with UAF-11, an approved potential yield and oil producing cultivar. Crop was sown on flat land with 0.15 m and 0.50 m plant to plant and row to row distance, respectively. Data were recorded using standard procedure. UAF-11 exhibited results having least i.e. 75.33 and 91.67 days to 50% and 100% flowering respectively while Accession-028192 showed maximum i.e. 109.67 days taken to 50% flowering and Accession-028181 took maximum (137.67) days to 100% flowering. Same results were observed in case of days to 50% and 100% maturity. High variability was observed regarding plant height with a minimum height (79.93 cm) of UAF-11 and maximum height (201 cm) of Accession-028189. Accession-028190 showed maximum number of branches per plant (12.133), siliques per raceme (60), siliqua length (92.347 mm), seeds per siliqua (19.733), seed yield per plant (36.433 g) and seed yield (3.06 t ha-1) while UAF-11 and Accession-028182 showed maximum results for 1000-seed weight (4.8167 g) and stem girth (27.707 mm), respectively. Accession-028192 exhibited minimum number of branches per plant (5.2), seeds per siliqua (7.33), seed yield per plant (1.234 g) and seed yield (1.413 t ha-1) while Accession-028181 showed minimum values for pod length (31.993 mm), pods per raceme (16.267) and 1000-seed weight (2.5667 g). For stem girth, minimum value was exhibited by UAF-11 i.e. 16.387 mm. Among quality parameters, Accession-028190 exhibited maximum values, 46.9 % and 24.8 % for oil and protein contents, respectively while Accession-028192 had maximum level (36.1 µM g-1) of glucosinolates and minimum levels of oil (38.3%), protein (19.7%) and glucosinolates (7.67 µM g-1) contents were recorded in Accession-028181, Accession-028184 and Accession-028190, respectively. After analysis and comparison of accessions with a latest approved cultivar UAF-11, Accession-028190 is recommended as a potential candidate for the approval to be cultivated under field condition on the basis of better seed and oil yield accompanied with low glucosinolates contents.

P.16 Bioactive compound retention as a function of kale cooking: boiling vs. steaming

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Kale is a leafy green vegetable belonging to the Brassicaceae family with interesting potential health-promoting value, due to its richness in bioactives such as glucosinolates, phenolics, and carotenoids [1]. Kale leaves represent a characteristic ingredient in traditional recipes in Italy, such as soups or filling of pastries. Boiling in water is a common way to prepare kale leaves [2]. Nevertheless, this process may lead to significant phytochemical losses, mainly because of leaching and thermal degradation. An alternative cooking method could be represented by steaming [3, 4]. The object of the present study is to compare the effect of boiling and steaming as cooking methods on the retention factors (RF) of kale leaf bioactives.
Three kale populations (Italian, Portuguese, and Turkish) were obtained from an experimental trial established in Cesena (Italy). Two cooking methods (boiling and steaming), and four cooking times (5, 10, 15, and 20 min) were selected as experimental theses. Samples of kale and cooking water were analyzed for their contents in glucosinolates, phenolics, carotenoids and chlorophylls.
Boiling caused significant glucosinolate and phenolic losses. RF decreased from 1.0 to 0.2-0.1 mg mg-1 in the first 5 minutes of boiling and did not change for longer cooking times. Steaming did not cause significant phenolic losses, while for glucosinolates RF initially decreased at a slow rate, to reach values very similar to that determined in boiled samples after 20 minutes. Including phenolic detected in cooking waters, RF determined both for boiling and steaming were not significantly different. Considering glucosinolates lost in water, RF in boiled kale were about twice higher than RF calculated for the leaves only, at the same cooking time. Moreover, steaming led to a faster decrease in chlorophyll RF in comparison to boiling. After 20 min, RF ranged from 0.6 to 0.7 mg mg-1 for chlorophyll a and b in boiled samples, and from 0.1 to 0.4 mg mg-1 in steamed leaves.
Neither boiling nor steaming caused a chemical degradation of phenolics; these compounds proved to be less sensitive to heat-induced chemical changes in comparison to chlorophylls and glucosinolates. A remarkable migration of hydrophilic bioactives occurred from leaves to cooking waters during boiling. Steaming led to a faster chemical degradation of glucosinolates and chlorophylls in kale samples in comparison to boiling, owing to the high exposure of leaves to oxygen and chemical degradation.

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P.17 Metabolic differences in chromosome substitution lines of Arabidopsis thaliana received by Reverse Breeding

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Traditionally, hybrid seeds are produced by crossing selected inbred lines. This is currently the case for most commercial cultivars of fruit vegetables such as tomato, cucumber, bell pepper and melon. A recent method for haploid production in the model plant *A. thaliana* [1] enabled us to provide a proof of concept for an alternative strategy: Reverse Breeding, a new approach that simplifies meiosis such that homozygous parental lines can be generated from a vigorous hybrid individual [2]. We silenced DMC1, which encodes the meiotic recombination protein DISRUPTED MEIOTIC cDNA1, in hybrids of *A. thaliana*, so that non-recombined parental chromosomes segregate during meiosis [3]. We then converted the resulting gametes into adult haploid plants, and subsequently into homozygous diploids, so that each contained half the genome of the original hybrid. From 36 homozygous lines, we were able to identify a complete set of chromosome-substitution lines. Those lines are currently being analyzed for metabolic differences by LC-MS. Based on first initial results, conclusions can be drawn about the metabolic impact of targeted chromosome substitutions. Among others, several aliphatic as well as indolic glucosinolates have been identified to decrease in one of the chromosome substitution lines. The genetic differences between the parental lines at the AOP locus [4] can explain those metabolic changes in the aliphatic glucosinolate profile.

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P.18 Removing glucosinolates to create food security in the Sahel

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Since decades, malnutrition and food insecurity are problems throughout the Sahel region. At the same time a great variety of local wild perennials are able to grow naturally in this region. They are adapted to the harsh dry climate and able to cope with the variation in rainfall. Those perennials provide very nutritious food, but many of them contain glucosinolates. Trees like *Boscia senegalensis, Boscia salicifolia, Maerua crassifolia, Cadaba farinosa* and *Crateva adansonii* all belong to the Capparaceae family, whose members usually contain glucosinolates. All five are part of a long and deep food tradition in the Sahel although their use has declined in the 20th century.
Research was done on how to process the seeds of the Boscia senegalensis tree, locally called Hanza. These seeds can be used as staple food. However, in order to produce Hanza suitable for human consumption, the seeds must be soaked in water for up to seven days to remove bitter components. Different experiments have been done on debittering strategies for the seeds. In cooperation with the Radboud University of Nijmegen, the type of glucosinolate responsible for the bitterness, and its breakdown products have been researched. Waste water from Hanza has been tested as irrigation on a number of different plants.

After testing 4 types of cold water debittering systems, none of them proved to save water compared to the traditional method. In the warm debittering line it was discovered that too high temperatures blocked the bitter taste inside the seeds.

Chemical analysis showed a high amount of glucocapparine in the raw seeds, but only traces of them were found back in the debittered seeds or the wastewater. [1] The glucocapparine was metabolized into methylisothiocyanate and other elements. [2]

The waste water is bitter and toxic to some plant species. Hanza wastewater from different soaking days (from day 1 as well as from other days) applied to *Bauhinia rufescens* trees and *Bougainvillea* species caused wilting symptoms within a few hours. On the other hand, young *Maerua crassifolia* and *Balanites aegyptiaca* trees showed no negative reactions. These species stayed green and kept on growing well.

Further research is needed to find out how to eliminate the methylisothiocyanate and other compounds from the wastewater. This could allow the recycling of Hanza wastewater and decrease its water footprint, which could be of major importance to creating scalable, water-efficient and ecological food production systems in the Sahel.

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P.19 Effects of domestic processing on the glucosinolate content of watercress

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Background: Watercress (*Nasturtium officinale*) belongs to the family of Brassicaceae. It is a particularly rich source of glucosinolates that can be converted to their subsequent isothiocyanates after their metabolism. The most abundant isothiocyanate in watercress is phenethyl isothiocyanate (PEITC) and has been attributed several anticancer effects like induction of apoptosis, inhibition of angiogenesis and metastasis in cancer cells. Watercress components have been also been shown to possess anti-genotoxic properties which result in attenuated DNA damage in lymphocytes. Domestic processing of Brassica vegetables significantly affects the bioavailability of glucosinolates and their corresponding isothiocyanates. The main aim of this study was to evaluate the effects of culinary processing in the glucosinolate content of watercress.

Methodology: Fresh watercress was processed using seven different preparation methods: boiling, microwaving, steaming, chopping and a watercress smoothie. The effect of cooking time on the phytochemical content was also examined. Glucosinolates were quantified by liquid chromatography with tandem mass spectrometric detection (LC-MS).

Results: Preliminary results indicate that boiling has a detrimental effect on the concentrations of glucosinolates in watercress with their levels decreasing by about 60% compared to the raw vegetable. In contrast microwaving and steaming for a short period of time increase slightly the availability of glucosinolates. Surprisingly, the watercress smoothie is completely devoid of gluconasturtiin, the PEITC glucosinolate precursor.

Conclusions: Domestic processing methods like microwaving and steaming, that maintain a certain level of myrosinase activity in watercress can potentially be beneficial for the bioavailability of glucosinolates during cooking. On the other hand, boiling of watercress should be avoided if high levels of glucosionaltes need to be maintained in the vegetables. Since elevated consumption of Brassica vegetables has been inversely associated with lower cancer risk it is only reasonable that we adapt to proper domestic preparation methods for these vegetable in order to ingest the highest glucosinolate levels possible.

P.20 Effects of added ingredients on glucosinolates thermal degradation in Broccoli (Brassica oleracea L. var. italica)

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Vegetables of the *Brassicaceae* family are characterized by the presence of glucosinolates. Broccoli (*Brassica oleracea* L. var. *italica*) contains high levels of glucoraphanin, a precursor of the isothiocyanate sulphoraphane, a bioactive compound known to have protective effects against carcinogenesis [1].
Recently a great interest was addressed at the study of the effect of cooking systems on glucosinolates retention [2]. Moreover, the degradation kinetic of such compounds has been investigated by mathematical modelling. However, both kinetics and cooking studies have been performed applying thermal treatments on a single type of vegetable whereas, in real cooking, many other ingredients are mixed in the pan, making the cooking process much more complex [3].
The aim of this study is to investigate the effect of added ingredients on glucosinolate degradation during heating. In order to mimic the preparation of domestic recipes, 5 different systems were studied. The broccoli batch used in this study was previously shortly heated to inactivate the myrosinase, a plant endogenous enzyme that hydrolyzes glucosinolates. The five systems were prepared by adding or not to broccoli single other ingredients: potato starch, corn starch, lentil proteins, freeze-dried onion, using two broccoli/other ingredient ratios (1:9 and 1:1). Water was added to each system, to obtain the same value of aw (0.99), in order to rule out the aw effect of glucosinolates degradation [4]. Two temperatures (90 °C and 100 °C) were tested and the glucosinolates concentration was monitored during heating.
The results of this experiment, shows that in the systems containing onion the glucoraphanin degradation was lower, especially at the ratio1:9.
Then a second set of experiments was conducted to better investigate the effect of the onion ingredient. Three broccoli/onion ratios were used (1:9, 3:7, 1:1), and the kinetic of glucosinolate degradation was investigated at 100 °C. Glucoraphanin showed the highest degradation rate in the broccoli without onion system, whereas in the system with the highest onion content, glucoraphanin showed to lowest degradation rate. In the case of lower amount of onion we observed still a protective effect but only during the initial phase of heating.
This first study demonstrated that onion could play an important role in the thermal degradation of glucosinolates. However, further studies are necessary to explore deeply the mechanisms involved and detect compounds responsible of this effect.

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P.21 The crystal structure of Thlaspi arvense thiocyanate - forming protein and its interaction with myrosinase

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On tissue disruption, glucosinolates are hydrolyzed by myrosinases to instable aglycons which rearrange spontaneously to isothiocyanates. Isothiocyanates serve as defense compounds to fight back insects, bacteria, fungi and nematodes. In the presence of specifier proteins glucosinolate breakdown is redirected to products like epithionitriles, thiocyanates and simple nitriles by unknown mechanisms. All specifier proteins known to date possess kelch motifs indicating that they have a β-propeller tertiary structure. To affect the outcome of myrosinase-dependent glucosinolate breakdown, specifier proteins need physical proximity to myrosinase, and presumably interact with myrosinase [1]. The aim of our study was to provide a basis for investigations on specifier protein mechanisms by experimental structure elucidation and in vitro interaction studies with myrosinase.
A homogenous preparation of recombinant *Thlaspi* arvense thiocyanate-forming protein (TaTFP) was obtained after heterologous expression in *E. coli* and purification by affinity, anion exchange and size exclusion chromatography. This preparation of 11 mg/ml TaTFP was subjected to a crystallization screen using the hanging-drop method, and crystals were obtained after one round of optimization. The structure was solved by molecular replacement resulting in a high resolution (1.5 Å) three-dimensional structure of TaTFP. The structure confirms that the predicted kelch domains form a six-bladed β-propeller. Each blade contains four β-strands and together they are assembled around a central channel. The ring structure is closed with one N-terminal ($\beta_6$4) and three C-terminal ($\beta_6$1 to $\beta_6$3) strands.
As specifier proteins do not seem to form stable complexes with myrosinase [1,2], we used a photoactivatable crosslinker (Mts-Atf-Biotin) to detect a possible transient interaction between myrosinase and TaTFP. Myrosinase was isolated form Sinapis alba seeds and labeled with the crosslinker via disulfide bonds to the Mts-moiety. Upon incubation with purified recombinant TaTFP, the Atf-moiety was activated by UV-light to react with C-H and unsaturated carbon chains at 11.1 Å distance. DTT treatment was used to release the Mts-moiety from myrosinase. Upon detection of the biotin residue of the linker, the TaTFP band became visible on Western blots indicating successful label transfer and an interaction between TaTFP and myrosinase at 11.1 Å. The interaction was independent of glucosinolate substrate and ferrous ions.
In conclusion, we have obtained the first experimentally determined structure of a member of the specifier protein family and provided first evidence for a transient interaction of specifier proteins with myrosinases. This information will now be used to elucidate specifier protein interaction sites with myrosinases and their catalytic mechanism.

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P.22 Quantitative analysis on expression patterns of genes involved in aliphatic glucosinolates biosynthesis in radish plants (Raphanus sativus L.) during their Development

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We have generated the radish homozygous doubled haploid (DH) lines through microspore culture using a donor plant (designated as DP). The aliphatic glucosinolates(GSLs) content in the DH lines were compared with the donor plant, and two lines were designated as high (HH-042915) and low (LL-040901) genotypes. In this study, the expression levels of key genes involved in the aliphatic GSLs biosynthesis were examined through quantitative real-time PCR analysis. The RNA samples were isolated from radish leaves and roots during their growth, that is 2, 4, 8, and 12 weeks after planting. The expression of *RsMAM3*, an enzyme involved in side chain elongation, was highest in the leaves of HH-042915 at 8 weeks, followed by DP and LL-040901. The expression of *RsMAM3* was low from 2 weeks leaves in all genotypes, however, in the root of DP at 8 weeks the expression it was the highest. The expression of two Cytochrome P450 family (*RsCYP79F1* and *RsCYP83A1*), which is involved in the core structure formation, were differently regulated. The expression of *RsCYP79F1* (in the leaves and roots), and *RsCYP83A1* in the roots showed similar to *RsMAM3*. The expression pattern of *RsFMOgs-ox1,* an enzyme for side chain modification, was highest in the leaves of HH-042915 at 2 weeks after planting. In contrast, the highest expression of RsFMOgs-ox1 was observed in LL-040901 in the root sample at all stages. In conclusion, the expression of *RsMAM3, RsCYPs* and *RsFMOgs-ox1* in the leaves, but not in the roots, was positively correlated with the aliphatic GSLs contents.

P.23 Formation of enzymatic hydrolysis products of glucosinolates in 19 Arabidopsis thaliana accessions is modulated by different NSPs and ESP.

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Plants have evolved several mechanisms to defend themselves against pests and pathogens. The secondary plant metabolites specific for the order Brassicales, the glucosinolates (GS) represent part of this defense system. Their definite enzymatic degradation products account for various bioactive effects [1] and contribute to the characteristic smell and flavor of *Brassica* vegetables as well. Besides the toxic effects on herbivores and plant pathogens, also health promoting properties on humans after consumption of *Brassica* vegetables such as broccoli have been reported [2]. In this regard especially the formation of isothiocyanates is presumed to be a good contribution to health benefits.

The production of specific degradation products is strongly dependent on several factors [3]: (i) the presence of myrosinase, a β-thioglucosidase hydrolyzing the GS after cell disruption resulting in formation of an instable aglucon; (ii) the chemical structure of the glucosinolate side chain; (iii) the presence of modifying proteins that influence further degradation of the intermediary aglucon; (iv) the pH value; (v) the presence of iron ions.

Arabidopsis thaliana is a wide-spread and well characterized model plant with a variety of ecotypes known. In this approach 19 accessions have been selected that diverge in their origin, phenotype, as well as in their GS profile. These accessions are part of the multiple reference genomes and transcriptomes for A. thaliana [4] and available genome information can be easily implemented. The selected ecotypes accumulate next to several alkenyl GS, hydroxyalkyl, methylthioalkyl, and methylsulfinylalkyl GS in their leaves. We further characterized for the first time all 19 different ecotypes with focus on the degradation of their different GS profiles. Dependent on the enzyme composition the ecotypes varied in their ability to form isothiocyanates, nitriles and epithionitriles. The formation of GS hydrolysis products was further correlated with gene expression profiles of myrosinases and different specifier proteins in order to study their interaction in the enzymatic degradation pathway. GS were analyzed by UHPLC-DAD, enzymatically formed breakdown products were identified and quantified by GC-MS, and expression profiling was done using qRT-PCR.

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P.24 Cyanide detoxification by β-cyanoalanine synthase in the glucosinolate specialist Pieris rapae

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Pieris rapae, the Small Cabbage White, is a specialised herbivore that feeds on plants defended by the glucosinolate-myrosinase system. Larvae of *P. rapae* have adapted to their food plants by expressing a gut nitrile-specifier protein (NSP), which redirects glucosinolate breakdown to form nitriles. Aliphatic nitriles are excreted with the faeces. Nitriles of phenylalanine-derived glucosinolates are further metabolised in the larvae leading to the formation of cyanide and we found that *P. rapae* larvae are exceptionally tolerant to cyanide [1]. The aim of our study is to test the relevance of cyanide detoxification enzymes for host plant adaptation in Pieridae.

Commonly known enzymes of cyanide detoxification, β-cyanoalanine and rhodanese, are believed to play a role in cyanide detoxification in insects, but have not been identified at a molecular level. We have used a PCR approach with degenerate primers to isolate three cDNAs encoding proteins with 32-34% identity to known β-cyanoalanine synthases from *Caenorhabditis elegans*. Enzyme characterisation after heterologeous expression in *E. coli* and purification showed that all three cDNAs encode β-cyanoalanine synthases. Together with metabolite and enzymatic analyses of *P. rapae* this shows that β-cyanoalanine synthases play a role in cyanide detoxification in *P. rapae*.

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P.25 Identification of genome regions causal for rapeseed glucosinolate content

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The main target of the NuGGET consortium is the rapid molecular identification of genes that are causal for traits with high agronomic impact. To achieve this, the consortium adapted the SHOREmap methodology (Schneeberger et al. 2009) that was originally developed for the model organism *Arabidopsis thaliana* for use in various crop plants. The principle behind this approach is the identification of causal mutations by crossing homozygous parents, pooling the DNA of phenotypically identical F2 plants, deep sequencing of the pooled DNA, and analysing the allele frequency by using a SNP marker table. By following up variations of the SHOREmap approach, we applied the concept of "variant frequency mapping" to *Brassica napus* (rapeseed) for seed glucosinolate (GSL) levels. The GSL content in rapeseed is an oligogenic trait which is controlled by at least 3-5 loci with larger impact, which makes identification of the causative genes more difficult than in monogenic cases. For the molecular identification of genomic loci controlling this oligogenic trait we deeply sequenced pools selected from segregating populations. A large F2 population was established from a cross of a high and a low GSL content genotype by the breeders that joined NuGGET. Both parents are used in current breeding programs. Two pools of extreme genotypes were built based on phenotypic evaluation of the seed GSL content in the F2 individuals. DNA from these pools was sequenced, mapped to a reference sequence and evaluated for polymorphisms. By analysing changes in allele frequencies between the two pools, we were able to detect five to six regions with a putative influence on the GSL content. These regions are of varying explanatory power, but are in concordance to data published for species of the Brassicaceae. The performed gene annotation in the respective regions points on promising, and in some cases on already expected, candidate genes. We have started the development of molecular markers deduced from the allele frequency analysis. First markers are currently tested in diverse *Brassica napus* individuals with varying seed GSL contents.

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P.26 An enzyme-coding RNA induces transgenerationally inherited phenotypes

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Plant survival in challenging environments is dependent on tight control of plant defense and reproduction. Two phenotypes, which are critical for defense and reproduction in *Arabidopsis*, are glucosinolates and timing of onset of flowering. Investigations of natural variation of glucosinolate profiles and flowering time have revealed several loci involved in regulatory networks controlling these phenotypes. In this study, we investigate the regulatory role of a gene in such a locus, which interestingly does not encode a transcription factor, but an enzyme in the glucosinolate biosynthetic pathway.

In order to investigate the regulatory capacity, we introduced the enzyme to two different accessions that do otherwise not express the gene and observed that the gene has a regulatory role in Gie-0, and not Col-0. In Gie-0, a significant change in the glucosinolate profile was accompanied by an 8 days earlier onset of flowering. In order to get more insight into how a biosynthetic gene can mediate these regulatory changes at the molecular level, two other versions of the genes were generated and introduced into the different backgrounds, one encoding an enzymatically non-functional protein and the other an un-translatable RNA. Both versions of the gene were able to regulate glucosinolate profiles and timing of flowering in Gie-0 showing that the RNA is the molecular entity sufficient to regulate both phenotypes dependent on the genetic background.

Regulatory RNAs have several potential molecular mechanisms including the ability to guide epigenetic changes. Thus, we investigated whether this RNA provides a mechanistic link to transgenerationally inheritance of phenotypes that can be induced *in planta* upon environmental stimuli. Analysis of the plants segregating without the transgene from a parent carrying the regulatory RNA revealed an epigenetic memory independent of the expression of the RNA. The plants not expressing the RNA showed the same change in glucosinolate profile and the same shift in flowering time as their siblings expressing the regulatory RNA. This shows that the RNA is able to regulate glucosinolate biosynthesis and flowering time by inducing epigenetic changes. Our study demonstrates that a glucosinolate biosynthetic gene encodes not only an enzyme but also a regulatory RNA. The RNA feedback controls its own pathway and also links to other phenotypes allowing for coordination of defense and reproduction.

P.27 Effects of glucosinolates and isothiocyanates on the development, physiology and chemistry of generalist herbivorous larvae

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Some small insect herbivores feed successfully on plants containing glucosinolates, despite their diverse array of harmful and deterrent breakdown products. Isothiocyanates (ITCs) are considered to be the most toxic glucosinolate-derived metabolites and are typically thought to be responsible for the reduced growth and delayed development observed in several generalist insects feeding on glucosinolate-producing plants. Some insects detoxify ITCs via conjugation with glutathione (GSH), but a large amount of ITCs remains unmodified [1] and may lead to the observed ill-effects in the insects.

We compared the effects of aliphatic and indolic glucosinolates, which form distinct ITC and non-ITC hydrolysis products, against two generalist chewing herbivores in a lab set-up. The development of larvae of *Spodoptera littoralis* (African cotton leafworm) and *Mamestra brassicae* (cabbage moth) was investigated from hatching until adult emergence while the larvae were reared on Arabidopsis thaliana Col-0 and the glucosinolate-deficient mutants *myb28myb29* (deficient in aliphatic glucosinolates), *cyp79B2cyp79B3* (deficient in indolic glucosinolates), and *myb28myb29cyp79B2cyp79B3* (deficient in both aliphatic and indolic glucosinolates). Comparing growth and instar durations, we found that both types of glucosinolates alone negatively affect larval development, but in combination their effect is significantly stronger. Interestingly, the impact of the different glucosinolate classes differs between the two investigated generalist species. To our surprise, the negative effects of the glucosinolates on insect weights were inverted for pupae and adults.

Furthermore, in order to understand the mechanisms of ITC toxicity, we determined how these compounds disturb the biochemistry and metabolism of *S. littoralis*. We investigated changes in physiological processes and chemistry of different body tissues after feeding the aliphatic 4-methylsulfinylbutyl-ITC (sulforaphane) in an artificial diet. The most typical effect is the decrease of GSH in the midgut tissue and hemolymph, likely due to losses by conjugation to ITC during detoxification. As a consequence, the levels of free amino acids are altered, in particular that of cysteine. Secondly, a characteristic symptom of ITC intoxication is a reduction in protein levels in the integument. Lastly, we found that energy metabolism and respiration are perturbed by ITC consumption. In combination, these effects contributed to the reduced performance of generalist insect herbivores feeding on glucosinolate-/isothiocyanate-containing diets.

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P.28 Identification and characterization of AtGTR3, an indole-specific glucosinolate transporter responsible for controlling root/shoot indole glucosinolate distribution

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Glucosinolates (GLS) are essential defense compounds and especially indole GLS have recently received substantial attention in a plant-pathogen context [1, 2]. Two GLS transporters, NPF2.10 (AtGTR1) and NPF2.11 (AtGTR2), are responsible for translocation of GLS to the seeds and for shaping the root/shoot profile of aliphatic GLS by phloem and xylem mediated bi-directional transport[3, 4]. Intriguingly, the root-shoot profile of indole GLS was not determined by AtGTR1 and AtGTR2 as evidenced by grafting experiments with stocks and scions from biosynthetic null and transport mutants [3]. This indicates the existence of additional GLS transporters in *A. thaliana* specific for indole GLS. We have identified a transporter responsible for controlling the indole GLS root/shoot profile *in planta* by employing a functional genomics approach in *Xenopus laevis* oocytes. Characterization of the identified transporter by Two Electrode Voltage Clamp (TEVC) electrophysiology and LCMS based uptake assays in *X. laevies* shows high-affinity active uptake of indole GLS in contrast to aliphatic GLS which were not actively transported. Micrografting of GLS transporter and biosynthesis knockout mutants demonstrate a role for this newly identified transporter in shaping the root/shoot indole GLS profile. The physiological role of an indole GLS specific transporter is discussed in the context of AtGTR1 and AtGTR2.

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P.29 Do Brassica vegetables have a prebiotic effect on our gut microbiota?

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Brassicas are sulphur-rich plants, characterised by the presence of glucosinolates (GLSs), and commonly consumed in our regular diet. GLSs are hydrolysed by the plant enzyme myrosinase to form breakdown products, such as isothiocyanates (ITCs). ITCs are involved in plant defence, and numerous studies have indicated that they have potential health benefits to humans, such as the prevention of various cancers (Author, 2009), (Author, 2007). When Brassica vegetables are thoroughly cooked, the myrosinase enzyme is inactivated and the conversion of GLSs to ITCs is dependent on bacterial myrosinase-like enzymes originating from our gut microbiota. Although studies have shown that bacteria from a range of phyla have the ability to hydrolyse GLSs, bacterial myrosinase-like enzymes have yet to be fully characterised (Author, 2013). Our recent studies show that some bacteria from the human gut microbiota have the ability to convert GLSs to their reduced analogues (Author, 2012), (Author, 2014).

In this study, the impact of broccoli leachate on the structure of the human gut microbiota was explored in vitro. Fresh faecal slurry was added to a myrosinase-inactivated broccoli leachate media and cultured for 12 hours at 37°C, under anaerobic conditions. An aliquot was taken to re-inoculate fresh media, which was then cultured as before, and this was repeated for a maximum of seven cycles. Glucosinolate content was analysed by HPLC, ITC and ITC metabolites were measured using liquid chromatography tandem mass spectrometry (LC-MS/MS), and any modulation of the bacterial communities were determined using 454 pyrosequencing of the 16S rDNA gene.

Results indicated that there was very little GLS hydrolysis by any of the human microbiotas tested, whilst HPLC analysis showed that all but one of the microbial communities converted glucoraphanin to its reduced analogue, glucoerucin. The composition of the gut bacteria was altered due to leachate exposure with significant increases in the proportions of *Lactobacilli*. The reduction of glucoraphanin to glucoerucin, and the increase in *Lactobacilli* was not observed in the control media. Further analysis has identified two species of Proteobacteria that are able to reduce glucoraphanin to glucoerucin, and the dominant *Lactobacillus* was identified as *Lactobacillus fermentum*. These results raise the question of whether a diet rich in Brassica may impart additional health benefits by increasing the numbers of beneficial gut bacteria.

P.30 Relationship between conversion rate of glucosinolates to isothiocyanates / indoles and genotoxicity of different Brassica parts

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Most of studies on the characterisation of glucosinolates (GLs) and their breakdown products in *Brassicaceae* species focus on the edible parts of these plants. However, other products e.g. dietary supplements may be produced also from non-edible parts such as seeds or roots. Biological activity of these products depends not that much on quantitative and qualitative GLs composition, but is strictly determined by the conversion rate to isothiocyanates (ITC) and indoles, often regarded as the most important chemopreventive agents. In the case of dietary supplements, which contain bioactive phytochemicals in doses far exceeding normal intake, the knowledge about the composition of bioactive compounds in different plant parts is particularly important in the view of the recent results revealing the genotoxicity and mutagenicity of break-down products of some GLs.
The aim of this study was to evaluate the conversion rate of GLs to bioactive ITC and indoles in plant parts of chosen Brassica species in relation to their biological activity. For this purpose, the composition of GLs, ITC and indoles were determined, as well as activity of myrosinase, cytotoxicity against human colon cancer cell line (HT29) as a model of alimentary tract, mutagenicity in Ames test and the ability to modify DNA in a cell-free system using restriction analysis.
The GL conversion rate to ITC and indoles was found to differ significantly not only between Brassica species but also between individual parts of the plant. Our results reveal that this directly affects biological activity of investigated material. The highest efficiency of conversion was observed for edible parts of plants – more than 70 %, while in sprouts it was less than 1 %, though myrosinase activity did not differ. Finally, our results confirm previous reports [1] that mainly indolic GLs and their derivatives are responsible for the ability of extracts obtained from Brassica species to induce DNA covalent modification.

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P.31 Field evaluation of 16 biofumigation crops for the control of P. penetrans and V. dahliae

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This project concentrates on Biofumigation as an alternative for chemical soil disinfestation against soil pathogens like nematodes and fungi. Biofumigation is a biological control technique in which specific (green manure) crops (mainly crucifers) are grown and incorporated into the soil. By incorporating these crops, the contents and breakdown products diffuse through the soil and can help to control pathogens and improve soil health. Within this project 16 different crops were tested in a field experiment. The impact of the crops on nematodes (*Pratylenchus penetrans*), fungi (*Verticilium dahlia*e) as well as a cash crop (potato) grown afterwards were tested in a randomized block design. Until now it seems that most crops can be seen as a host plant for nematodes, and that the supposed population decrease due to the biofumigation effect does not compensate this negative effect. However, on the quantity and quality of the potatoes, there was an overall positive effect of most biofumigation crops. If the biofumigation crops and the techniques to grow and to incorporate them improve, biofumigation can become a new alternative to chemical control and can help to improve soil health.

P.32 Cytotoxic potential of nitriles resulting from glucosinolate degradation in a human in vitro model

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Glucosinolates are secondary metabolites present in plants of the *Brassicaceae* family (e.g. broccoli, garden cress, horseradish). Hydrolysis by the plant endogenous enzyme myrosinase results in a range of products such as isothiocyanates, nitriles, epithionitriles or thiocyanates. So far, isothiocyanates are the most intensively studied breakdown products and are nowadays well recognized for their pleiotropic effects against cancer. Besides enzymatic degradation, glucosinolate breakdown can also be mediated thermally giving mainly rise to the formation of nitriles [1]. Knowledge about their toxicological potential is poor, though. Therefore, the aim of this study was to systematically investigate the toxic potential of nitriles on human liver cells in vitro. Based on this, structure activity relationship analysis to identify structural features in nitriles that are associated with cytotoxicity was carried out.

Determination of cytotoxicity was done using the WST-1-assay. The latter is based on the ability of intact cells to cleave the tetrazolium salt WST-1 to formazan by their mitochondrial succinate-tetrazolium-reductase system. Nitriles with aliphatic and aromatic side chains as well as (methylthio) alkylnitriles were investigated using concentrations ranging from 0.3-30 mM in a logarithmic dilution.

Treatment of HepG2 cells with aromatic nitriles and (methylthio)alkylnitriles showed a significant reduction of cell viability only at the highest concentration tested. The cytotoxic potential of the two aromatic nitriles was similar with an IC_{50} of 19.95 mM (phenylacetonitrile) and 18.21 mM (3-phenlypropanenitrile), respectively. Using sulfur-containing nitriles, a structure dependent increase in cytotoxicity was observed with increasing chain length, with the lowest IC_{50} at 8.46 mM for 7-(methylthio)heptylnitrile. It was suggested that a double bond in an aliphatic chain structure has no relevant effect on the cytotoxicity of the investigated compounds. Insertion of, a sulfur or aromatic ring leads to increased cytotoxicity against human liver cells. Based on the present in vitro data, no indications are given that nitriles pose a risk under conditions relevant for food consumption.

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P.33 High-throughput quantitative profiling of Glucosinolates in Brassica leaf extracts with ion pairing LC-MS/MS

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Glucosinolates are secondary metabolites in plants which can act as defence chemicals in Brassicaceae family [1] but are also involved in flavour and taste aspects in edible Brassica. They are also precursors to isothiocyanates formed when the β-D thioglucopyranoside bond is hydrolysed by the myrosinase enzyme. Isothiocyanates are biologically active with health benefits [2] and they also participate in directing insect-plant interactions (stimulative as feeding cue for specialized insects and toxic for non-adapted insects).

We highlight the challenges faced to quantify intact glucosinolates in brassica leaves extracts and emphasize the importance of a simple sample processing method for large scale study. Simplification of sample preparation is advantageous for high throughput profiling.
A simple "one vial" sample processing method is developed to cope with a large number of samples. The tissues are firstly blended with water, and the aliquots placed in cryovials and immediately snap-frozen. At this point, the frozen samples are stable enough where they can be either stored in freezers or shipped in dry ice. As the homogenated samples are immediately frozen until required, there was no need for pre-treatment with chemicals for enzyme deactivation during extraction in water [3]. This easy procedure also replaces laborious steps of snap freezing the tissues in liquid nitrogen and subsequent grinding [4]. Prior to analysis, methanol is added to the frozen sample followed by rapidly heating until boiling to ensure the myrosinase are fully deactivated and any dissolved protein are precipitated.

Separation and identification of 9 glucosinolate (Sinigrin, Gluconapin, Progoitrin, Glucotropaeolin, Glucoerucin, Glucoiberin, Gluconasturtiin, Glucoraphanin, Glucobrassicin) is achieved with LC-MS-MS, using tributyl amine and ammonium acetate as the volatile ion pair modifier. Multi reaction monitoring scan mode is used to detect the diagnostic fragment ion of m/z 97 as a result of the direct loss of the sulphate moiety from the glucosinolate molecule. The LOD for the glucosinolates are respectively 0.006 µg/g, 0.008 µg/g, 0.03 µg/g, 0.03 µg/g, 0.001 µg/g, 0.007 µg/g, 0.003 µg/g, 0.02 µg/g and 0.003 µg/g.

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P.34 Secondary metabolite profiling of Brassica oleracea varieties

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Brassica oleracea var. sabellica is of great cultural importance in northern Germany - so-called "Grünkohl-Fahrten" celebrating its harvest are common social events in the second half of winter. The present study will focus on the distinction of secondary metabolite profiles in "Grünkohl" varieties leaf material in regard to glucosinolate (GS) composition and lutein concentrations. Both of these substances are known to be of huge medical interest. While autolytic breakdown products of GS are known to exhibit protective activities against cancer (as reviewed by Cartea and Valesco 2008), lutein is discussed to prevent cardiovascular and eye diseases (as reviewed by Calvo 2005) Quantitative Realtime PCR (qRT-PCR) will provide insights in the expression patterns of these metabolites in response to low temperature conditions.

A – Glucosinolate composition: Polymorphisms in a limited number of conserved loci are known to determine intraspecific variability in aliphatic GS content and distribution among *Brassicaceae* (e.g. Kliebenstein 2009). Alleles at the loci GS-ELONG, GS-ALK, GS-OH and GS-OX will be determined for more than 50 different *B. oleracea* varieties to allow the estimation of their GS contribution from a molecular point of view.

B – Lutein content: Two key enzymes modulate the expression of α-carotenoids such as lutein (controlled by εLCY or ε-cyclase) and ß-carotenoids, e.g. zeaxanthin (controlled by βLCY or β-cyclase). The relevance of these genes is evident because genetic variation in εLCY explained about 60 % of the variation in lutein and ß-carotenoids in maize (Harjes et al. 2008). Sequence information of εLCY will be generated and correlations between genetic polymorphisms and intraspecific lutein concentration differences will be uncovered.

C – qRT-PCR analyses: It has been shown that GS content is highly dependent on temperature. Interestingly, GS contents increase at 12 °C as well as at 32 °C relative to temperatures around 22 °C (Charron & Sams 2004). As "Grünkohl" has always been known as an autumn vegetable, this study aims at uncovering the best harvesting conditions for *Brassica oleracea var. sabellica*, intraspecific variations and genetic basis of temperature dependent differences in GS and lutein concentrations.

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P.35 Quantitative trait loci (QTL) for leaf glucosinolates under salt stress in Brassica napus

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Salinity is one of the abiotic stresses that threat the crop production globally. Glucosinolates are secondary metabolites characteristic for the *Brassicaceae* family and the genetic control of seed glucosinolates, in particular in oilseed rape *B. napus*, is well known. Compared to seed glucosinolates much less is known about leaf glucosinolates. Glucosinolates and their degradation products are known for their influence on plant resistance to insects and pathogens and for their anticancer effect [1]. Their role in plant response to abiotic stress is hardly investigated, whereas effects of salt stress on the glucosinolate content have been reported for example in broccoli and *Thellungiella salsuginea* [2, 3].

The objectives were to analyse the effect of salinity on leaf glucosinolate profile and concentration and to map QTL controlling the variation of leaf glucosinolate content and composition in a *B. napus* doubled haploid (DH) population. The mapping population with 138 DH lines has been derived from the cross between Mansholts Hamburger Raps (high erucic acid content and high seed glucosinolates) and Samourai (low erucic acid and low seed glucosinolates content) [4]. The parental lines and the mapping population were grown in soil under semi-controlled greenhouse conditions (20 °C at day and 15°C at night). Salt stress was applied beginning at 20 days after sowing and the NaCl concentration was increased up to 200mM. Control and treated plants were harvested after two weeks of salt treatment and the glucosinolate composition was determined by high pressure liquid chromatography.

A large variation in glucosinolate content and composition has been observed between the parental lines and within the mapping population both in the control and under salt stress. Mansholts showed an increase in the total glucosinolate content under salt stress, whereas Samourai exhibited a reduction in glucosinolate content. Several QTL have been identified for single components and for total glucosinolates content under both growth conditions. Interstingely, under salinity glucoraphanin and glucobrassicin were increased markedly, both have positive effect against cellular damage and cancer development. The mapped QTL are distributed on several linkage groups, especially on linkage groups A9, C2 and C9, where QTL for seed glucosinolates were mapped earlier in many populations. Moreover, we found QTL on other genomic regions which are leaf glucosinolate specific. Our results can contribute to better understand the variation of leaf glucosinolates and the interaction between glucosinolates and salinity and might help to identify the causal genes which are involved in this interaction.

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P.36 Influence of genotype and climate on glucosinolates in kale (Brassica oleracea var. sabellica)

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Kale was shown to have high concentrations of structurally different; antioxidative flavonoid glycosides[1,2] that responded structure dependent to temperature and global radiation.[3] Furthermore, kale is a promising source of glucosinolates (GLS) that have anticarcinogenic potential. The aim of this study was to determine the concentration and composition of these phytochemicals in kale, dependent on the genotype and their interaction with temperature and global radiation to develop crop management strategies for kale with a distinct health potential.
Eight kale cultivars, comprising hybrid and traditional cultivars, were grown in two subsequent field experiments and harvested four times at four-week intervals from October to January. The main glucosinolates in kale were the short chain aliphatic GLS: 3-methyl-sulphinylpropyl GLS (glucoiberin), 2-propenyl GLS (sinigrin) and 4-methyl-sulphinylbutyl GLS (glucoraphanin). The genotypic variation revealed that the traditional cv. Halbhoher grüner Krauser had high concentrations of glucoiberin while the hybrids cv Arsis and cv Frostara were rich in sinigrin and glucoraphanin, respectively, both glucosinolates showing an anticarcinogenic potential.[4]
Although the temperature and global radiation decreased in our experiment from October to January the highest total GLS concentrations were found in kale harvested in December and January. Concomitantly, the highest concentrations of the aliphatic GLS glucoiberin, sinigrin and glucoraphanin as well as of the indolyl GLS 3-indolylmethyl GLS (glucobrassicin) and 4-hydroxy-3-indolylmethyl GLS (4-methoxy-glucobrassicin) were in December and January.
The data reveal that beside high concentrations of quercetin glycosides[3] that have a high antioxidant activity[2] a high anticarcinogenic potential due to high concentrations of GLS can be achieved in December and January. A late harvest of kale in winter results in a customer-oriented quality production.

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P.37 How to visualize the orchestration of glucosinolate biosynthesis?

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Glucosinolates (GLS) are known to respond in abundance to environmental conditions and attack. Therefore, the biosynthetic pathways of these specialized metabolites serve as a fruitful model system for studying the dynamics of pathway organization. The GLS pathways involve several subcellular compartments. Moreover, different types of GLS are synthesized partly by pathway-specific specialized, and partly by more promiscuous enzymes. Preliminary data suggest that specific interactions between involved proteins may play a role in GLS biosynthesis.

To study the location of the biosynthetic machinery at the whole plant as well as the cellular level, we have developed a toolset of fluorophore-tagged biosynthetic enzymes stably expressed in the native host *Arabidopsis* under the control of their native promoters. This toolset will furthermore be used to study the subcellular spatial organization of the enzymes and to investigate protein-protein interactions by *in vivo* FRET/FLIM measurements. In parallel, we utilize a transient expression system in *Nicotiana benthamiana* to express the entire GLS biosynthetic machinery, allowing us to further investigate subcellular organization, protein-protein interactions and to measure the efficiency of GLS biosynthesis. The ability to include the entire pathway and thus quantify GLS production is a major advantage compared to in vitro methods or yeast-based analyses.

Together, these tools allow for investigation of protein dynamics in living plant cells. We thus aim at elucidating the GLS biosynthetic pathway organization and its plasticity in plant development and stress response by employing these techniques.

P.38 Identification and characterization of glucosinolate biosynthetic and breakdown related genes in Brassica oleracea

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Brassica oleracea comprises many important vegetable crops, which are high in protein and carotenoids, and contain diverse glucosinolates (GSLs), whose hydrolysis products function as unique phytochemicals for plant defence against fungal and bacterial pathogens and have potent anticancer properties. In order to elucidate the glucosinolate biosynthetic pathway in *B. oleracea*, this study identified 102 GSL biosynthetic and 22 breakdown related genes from previous studies and analyzed their expression in 16 subspecies of *B. oleracea*. Concentration of GSLs and their hydrolysis products were determined through a HPLC analyses in these subspecies and compared with expression profile of biosynthetic and breakdown related genes. A considerable number of genes were found to show specificity to the GSLs and their products, and then characterized their functions through an *in silico* study. Thus, the identified genes are predicted to be potential molecular markers specific for GSLs and might be potential resources for developing high valued *Brassica* vegetables.

P.39 The effects of deviation of glucosinolate profiles in plant defense syndromes on herbivores

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Plants have various defense strategies against herbivores with multiple defenses being utilized. In "plant defense syndrome" hypothesis raised by Agrawal [1], these multiple defenses can be grouped into several syndromes. Brassicaceae species have evolved the greatest variety of glucosinolate (GSL) as defensive chemical compounds against herbivores [2]. To understand the ecological function of this variation in GSLs, the defensive effects of each GSL on specialist and generalist herbivores were well investigated [3, 4]. However whether defense syndrome is observed among Brassicaceae species, and its relationship with GSL profile are unknown. In this study, a clear "defense syndrome triangle" [1] was shown by analyzing various defense traits including GSL profiles among 12 species (11genera) of brassicaceous plants. The defence strategies of plant species were divided into three categories; "nutrition and defense", "low nutrition quality with physical defense" and "tolerance and escape". It is also revealed that the GSL profiles were different depending on these defense strategies. Plants taking "nutrition and defense" strategy possessed high concentration of long-chained aliphatic GSLs, and plants with "low nutrition quality with physical defense" had intermediate concentration of short-chained aliphatic GSLs. Plants with strategy of "tolerance and escape" showed low concentration of GSLs. These results imply that the distribution pattern of GSLs among plant species also has some kind of syndromes corresponding the plant defense syndrome as a whole. In addition, the herbivore adaptation tested by feeding experiment using two types of herbivores, *Pieris rapae* (Lepidoptera Pieridae) as specialist herbivore and Eri silkmoth *Samia cynthia* ricini (Lepidoptera: Saturniidae) as generalist, showed differential effects of these defense strategies. Although Eri silkmoth grew well by feeding on the plants with "tolerance and escape" strategy, the growth rates were decreased when feeding on plans with "nutrition and defense" and "low nutrition quality with physical defense" strategies, which contain aliphatic GSLs. So that, we could apparently say that Eri silkmoth growth showed adequate match to the defense strategies. On the other hand, the specialist herbivore P. rapae growth showed no differences between plant strategies. These results suggested that the variety of GSLs among plant species cause different effects on the performance of divergent herbivores, which showed the ecological function of variations of GSLs.

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P.40 Myrosinase Activity of Different Cabbage Varieties Grown Under Controlled Environment

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Cabbage (*Brassica oleracea*), like other Brassica vegetables, contains a group of thioglucosides called glucosinolates and myrosinase enzymes (thioglucoside glucohydrolase EC 3.2.3.1) [1]. Glucosinolates (GLSs) and myrosinase enzymes coexist in separate compartments in the plant, while glucosinolates exist in the vacuoles of cells [2], myrosinase enzymes are localised inside the myrosin cells. When plant tissue is damaged as a result of processing or mastication, glucosinolates are hydrolysed by myrosinase enzymes to yield several hydrolysis products. These hydrolyses products are principally responsible for the health properties and sensory characteristics of *Brassica* vegetables [3] . Epidemiological studies have shown that the consumption of brassica vegetables reduces the risks of cardiovascular diseases, cancer and a recent report found that they possess a cytoprotective effect against tissue damage associated with oxidative stress [4]. The myrosinase activity of several brassica vegetables has been investigated but most studies on cabbage myrosinase have been limited to few varieties of two genera namely; *Brassica oleracea var. oleracea* and *Brassica oleracea var. capitata*. However, little information is available on the myrosinase activity of other commonly consumed varieties such as *Brassica oleracea var. acephala* and *Brassica oleracea var. tronchuda*. It is with this in mind that the current study focused on determining the myrosinase activity of 18 accessions of 6 cabbage genera (black kale, red, savoy, white, wild and tronchuda). Cabbage seeds were planted in loam-based soils under controlled environment (22 oC and 14 oC, day and night temperature respectively; 60 % humidity and 16 hour photoperiod) and the leaves harvested after 14 weeks of planting. Plants were immediately placed in ice after harvesting and then frozen at -80 oC before lyophilising. The coupled enzymatic procedure was used to measure myrosinase activity [5]. This procedure depends on the glucose released from the reaction between myrosinase enzyme and the substrate (Sinigrin). Myrosinase activity was significantly different ($p<0.05$) between genera and between accessions with the exception of the wild cabbage genus where no significant difference ($p>0.05$) was observed within the three accessions studied. The results showed large variations in myrosinase activity between accessions of the same genus for the cultivated cabbage. The result obtained can be useful in the selection of cabbage varieties with maximum health benefits as myrosinase plays an important role in the health promoting properties of brassica vegetables. The glucosinolate content and myrosinase stability of the cabbage accessions are currently being studied.

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P.41 Eruca sativa and Industrial crops: a wealth of active ingredients for the health-food industry

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Growing evidences demonstrate that a correct Mediterranean Diet is associated with a significant reduction in total mortality [1]. The dominant component of this diet as predictor of lower mortality are moderate consumption of alcohol, low intake of meat and meat derivatives, high consumption of vegetable, fruits, nuts and olive oil. Among vegetables, Brassicaceae family, in the past called Crucifer, maybe particularly beneficial as reported in several clinical trials or epidemiological studies [2,3]. Glucosinolates (GLs) are claimed to be the active components of cruciferous vegetables responsible for many beneficial effects *in vitro, in vivo,* and in human. Since 1992, the year that glucosinolates of broccoli were identified, more than 1000 studies have been conducted to determine the mechanisms and biological activities of sulforaphane and its precursor, glucoraphanin. When ingested sulforaphane has an half-life of ~2h, while little ideas are on the slow hydrolysis sustained by microbiota at intestinal level. Recent studies are trying to elucidate the metabolism of sulforaphane *in vivo* and it seems clear that the sulfoxide sulforaphane is in part converted in the liver as well as in the gut microflora in its tioether analogue erucin [4]. *Eruca sativa* seeds are the main holders of glucoerucin, and its leaves contain also glucoraphanin, high levels of vitamin C, flavonoids, nitric oxide and phenols which together contribute to the beneficial effects obtained by their sustained and controlled intake. The concentration of these molecules in food crops was continuously lowered to moderate pungent taste and distinctive aroma which is not always appreciated by the mass of consumers. Non-food crops, such as oleaginous industrial crops of cruciferous family, can guarantee up to 100 micromoles/gr of glucosinolates at seed level, that improve after defatting.

In this work 4 species of rocket seeds from the Brassicaceae collection of CRA-CIN [5] were analyzed and one of them was selected to produce pressure defatted flours under controlled conditions in order to obtain a valid product for the enrichment of functional bakery products. The development of an extraction method for the assessment of GLs in complex matrices has allowed to verify that the path taken led to prototypes interesting both at the level of taste and concentrations of active substances per portion.

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P.42 Regulatory Network of Secondary Metabolism in Brassica rapa: Insight into the Glucosinolate Pathway

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Brassica rapa studies towards metabolic variation have largely been focused on the profiling of the diversity of metabolic compounds in specific crop types or regional varieties, but none aimed to identify genes with regulatory function in metabolite composition. Here we followed a genetical genomics approach to identify regulatory genes for six biosynthetic pathways of health-related phytochemicals, i.e carotenoids, tocopherols, folates, glucosinolates, flavonoids and phenylpropanoids. Leaves from six weeks-old plants of a Brassica rapa doubled haploid population, consisting of 92 genotypes, were profiled for their secondary metabolite composition, using both targeted and LC-MS-based untargeted metabolomics approaches. Furthermore, the same population was profiled for transcript variation using a microarray containing EST sequences mainly derived from three Brassica species: B. napus, B. rapa and B. oleracea. The biochemical pathway analysis was based on the network analyses of both metabolite QTLs (mQTLs) and transcript QTLs (eQTLs). Colocalization of mQTLs and eQTLs lead to the identification of candidate regulatory genes involved in the biosynthesis of carotenoids, tocopherols and glucosinolates. We subsequently focused on the well-characterized glucosinolate pathway and revealed two hotspots of co-localization of eQTLs with mQTLs in linkage groups A03 and A09. Our results indicate that such a large-scale genetical genomics approach combining transcriptomics and metabolomics data can provide new insights into the genetic regulation of metabolite composition of Brassica vegetables.

P.43 A novel mechanism to specifically regulate MYB transcription factor localization

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Specific gene expression in time and space is regulated by transcription factors (TFs). Of the transcription factors in Arabidopsis, 339 are MYB and 162 are bHLH (basic Helix-Loop-Helix) TFs. Glucosinolate synthesis is tightly controlled by a clade of six R2R3 domain MYB TFs regulating the biosynthesis machinery (1, 2). Mechanical stimuli like wounding increase expression of these MYBs which in turn leads to distinct changes in glucosinolate profiles and reduces insect herbivory. The individual MYBs induce production of different classes of glucosinolates, which is thought to be due to their specific binding to DNA elements through the R2R3 domain. While the R2R3 domain is highly conserved among the six MYBs, the C-terminal domain is highly variable. Thus, we propose that MYB specificity could be modulated by specific protein-protein interactions (PPIs) and post-transcriptional modifications rather than by DNA binding specificity.
Recently, all six MYBs have been shown to interact with the three bHLH TFs MYC2, 3 and 4, which are involved in jasmonate signaling, suggesting that the MYBs possess protein-protein interaction (PPI) domains (3). To learn more about the influence of the DNA binding and the PPI domains on the synthesis of specific glucosinolates, we have generated stable plants expressing chimeric MYB proteins in myb knock-out backgrounds and determined the relative abundance of glucosinolate classes for each chimeric MYB. Under the hypothesis that binding partners provide additional functional specificity, we identified membrane-bound partners that can retain the MYBs in the cytoplasm until rapid activation of the glucosinolate pathway is required. We observed specific modulation of the MYBs' localization by those specific interactors providing first evidence for differential regulation of the MYBs at the protein level. The interaction may mimic a membrane tethered transcription factor (MTTFs), providing specificity in the response and thus increasing performance towards pathogens and herbivores.

Key words: MYB transcription factor, membrane anchored, triggered response.

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P.44 Function of a glutathione-S-tranferase in Arbaidopsis immunity and glucosinolate metabolism

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Induced defense responses in plants usually involve biosynthesis of antimicrobial metabolites and their targeted secretion at the site of pathogen contact. Our former study on the model plant *Arabidopsis* revealed a novel pathogen triggered metabolism pathway for indole glucosinolates [1]. This pathway requires at least two enzymatic components: CYP81F2 P450 monooxygenase and PEN2-myrosinase. CYP81F2 is essential for the pathogen induced accumulation of 4-methoxyindol-3-ylmethyl glucosinolate, which in turn is activated by PEN2 for antifungal defense. In addition, our former analysis suggested contribution of glutathione to the PEN2/CYP81F2-defence pathway [1]. This finding prompted us to investigate in detail the mechanisms underlying this putative glutathione immune function. Here we report on the *Arabidopsis* glutathione-*S*-transferase that is crucial for the pathogen triggered indole glucosinolate metabolism. We provide evidence that this particular glutathione transferase constitutes an indispensable component of the PEN2/CYP81F2 immune pathway and mediates resistance towards biotrophic, hemibiotrophic and necrotrophic fungal pathogens.

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P.45 Metabolism and bioavailability of the benzyl glucosinolate glucotropaeolin in humans investigated by LC-ESI-MS/MS

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The aromatic benzyl glucosinolate glucotropaeolin is the major glucosinolate in nasturtium (Indian cress; *Tropaeolum majus* L) and occurs almost exclusively in these Brassicales species [1]. The potential of this plant can be regarded as being the source of the bioactive breakdown product benzyl isothiocyanate (BITC) [2]. A reliable and sensitive LC-ESI-MS/MS method for the quantification of BITC metabolites in human plasma and urine was developed for the first time. In this study, the following BITC metabolites have been considered: BITC-glutathione, BITC-cysteinylglycine, BITC-cysteine, and BITC-*N*-acetyl-L-cysteine. The assay development included: (I) synthesis of BITC conjugates acting as reference substances; (II) sample preparation based on protein precipitation and solid-phase extraction; (III) development of a quantitative LC-MS/MS method working in the multiple-reaction monitoring mode; (IV) validation of the assay; (V) investigation of the stability and the reactivity of BITC conjugates *in vitro*; (VI) application of the method to samples from a human pilot study (n=4).

The lower limits of quantification were in the range of 21-183 nM depending on analyte and matrix, whereas the average recovery rates from spiked plasma and urine were approximately 85% and 75%, respectively. After consumption of nasturtium, containing 1000 µM glucotropaeolin, quantifiable levels of BITC-NAC, BITC-Cys, and BITC-CysGly were found in human urine samples. Maximum levels in urine were determined 4 h after the ingestion of nasturtium. With regard to the human plasma samples, all metabolites were determined obtaining individual distributions. These results will help to understand the bioavailability of BITC in dietary and its effects on human health. Moreover, the assay can be applied for further clinical pharmacologic studies of BITC metabolites.

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P.46 Anti-carcinogenic effect of glucosinolate degradation products from Brassica local varieties

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The anti-carcinogenic effect of the glucosinolates-hydrolyzed derivatives, *isothyocyanates* (ITCs), has been extensively proven, essentially for *sulforaphane* [1]. However, just traces of glucoraphanin (the glucosinolate precursor of sulforaphane) are found in most *Brassica* crops [2]. Little is known about the putative anti-carcinogenic effect of ITCs derived from other glucosinolates. Three ITCs (ITC-MBG1, ITC-MBG2, and ITC-MBG3) derived from the most abundant glucosinolates identified in *Brassica* vegetable crops grown in Galicia (Northwestern Spain) were assayed to test the *in vitro* effect on survival and proliferation of cultured PC-3 and DU-145 human prostate cancer cells in the presence or in absence of the anti-cancer drug docetaxel. The effect of each ITC was evaluated at four doses and three incubation times.

In general terms, the three ITCs showed a remarkable anti-carcenogenic effect, in most cases even higher than that observed with docetaxel alone. The ITC-MBG1 inhibited cell growth in a dose and time-dependent manner against both PC-3 and DU-145 cells. The effect of this ITC was higher than that observed for the docetaxel, showing both compounds a synergistic effect, suggesting that the ITC-MBG1 could make the cancer cells more susceptible to this chemotherapic. ITC-MBG1 induced apoptosis mechanism in both cell types.

The ITC-MBG2 inhibited the cell growth of the PC-3 but not so for the DU-145 cell lines. The growth inhibition of the PC-3 cells was dose and time-dependent but only has a higher effect than docetaxel after 72 h of incubation. The synergistic effect of these two compounds was partial and data indicate that apoptotic mechanisms are not involved in this inhibition.

The performance of the ITC-MBG3 was similar to that observed for the ITC-MBG1. This compound inhibit the growth of both cell types in a dose-dependent manner, but in this case the maximal effect was achieved at 24h and does not increase with longer incubation times. The highest concentration (2 and 4 µM) showed higher inhibition than docetaxel. There is also a synergistic effect when ITC-MBG3 is incubated along with docetaxel. Similar to ITC-MBG1, apoptotic mechanisms are involved in the inhibitory effect of the ITC-MBG3.

These preliminary results indicated that ITCs from Brassica local varieties could have a remarkable effect inhibiting prostate cancer cells proliferation and survival, and they have the potential to be used as chemotherapics in the future. Nevertheless, further investigation is required to understand the mechanistic pathways, effects and implications of the adjuvant therapy with these ITCs.

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P.47 Evaluation of the Glucosinolate Degradation in Tropaeolum Based Plant Powders

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The breakdown products of various glucosinolates (GS) are associated with beneficial health effects. Therefore, extracts and plant powders derived from different kinds of GS containing *Brassicaceae* are marketed as dietary supplements and claimed as antimicrobial preparations. However, in most cases the GS as such show no bioactivity, but their degradation products e.g. isothiocyanates (ITC) do. As several preparations for human consumption are based on *tropaeolum* (*Tropaeolum majus* L.) the aim of the present work was to investigate the fate of GS in different powders and dietary supplements containing parts of this plant. For all samples the total GS content was measured by LC-UV. Moreover, the amount of the main hydrolysis products (i.e. ITC and nitriles) was determined by GC-MS. The results show strong variability for the formation of ITC after 30 minutes of aqueous incubation at 37°C between the different *tropaeolum* preparations. Even for plant powders that were claimed to be identical in one case, the GS were vastly degraded into the ITC, in another case the GS dominatingly remained intact. The results for different dietary supplements for *tropaeolum* alone or in combination with other herbal components are presented. Additionally, the effect of heat during storage and the application additional myrosinase on the formation of degradation products is investigated.

The results indicate that a declaration restricted to the GS content and composition of the herbal ingredients is not sufficient for the characterization of *tropaeolum* based plant powders and preparations thereof.

P.48 A randomised controlled trial to investigate the bioavailability of phytochemicals and minerals from broccoli soups

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In order to explore the contribution of glucosinolates to the health promoting effects of cruciferous vegetables, we have developed broccoli cultivars which have enhanced levels of 4-methylsulphinylbutyl glucosinolate (glucoraphanin). This has been achieved through the introgression of a novel Myb28 allele from the wild species B.villosa into an elite broccoli genetic background. Expression of a single $Myb28^{villosa}$ allele, as in Beneforte broccoli, results in a three-fold increase in glucoraphanin, while expression of two $Myb28^{villosa}$ alleles, as in Beneforte Extra, result in a seven fold increase. In this manner, the role of glucoraphanin as opposed to other chemical components of broccoli on biomarkers of health can be assessed in human dietary intervention studies. To facilitate these studies, we have developed broccoli soups from an allelic series of broccoli genotypes. The soups provide a practical solution for delivering glucoraphanin in long term dietary intervention studies. When consumed, glucoraphanin is metabolised to the isothiocyanate sulforaphane by the activity of the gut microbiota. We are undertaking a randomized, double-blinded, three-phase crossover trial to measure the bioavailability of SF and other metabolites from the three soups. The results of this study will inform the use of these soups in long term intervention studies.

P.49 Divergent selection for the major glucosinolates in kale (Brassica oleracea var acephala)

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Divergent mass selection has been widely used in plant breeding as it can generate groups of individuals that share the same genetic background but with extreme values for a particular trait. We report here the first results of direct divergent selection for glucosinolate (GSL) content in order to develop *Brassica oleracea* genotypes with divergent concentration of the three major GSL, sinigrin (SIN), glucoiberin (GIB), and glucobrassicin (GBS). Divergent selection program was started in 2006 by using seeds of a local kale population and then after three selection cycles, six plant genotypes were obtained which had high or low concentration of the major GSL. Selection was carried out in leaves. The aims of this study were to determine if the three divergent selections were successful in leaves and then, to study how each divergent selection affected the GSL content in other organs such as flower buds and seeds.

For the aliphatic GSL (SIN and GIB), differences among cycles of the divergent selections were observed for all of organs under study (leaves, flower buds and seeds). After three cycles, GSL concentration in leaves and flower buds were significantly different than the original cycle (C0). Significant and positive simple linear regression coefficients for SIN concentration were observed in leaves (R^2=0.9684), flower buds (R^2=0.8810) and seeds (R^2=0.6889). In the case of GIB divergent selection, significant and positive simple linear regression values were also found in leaves (R^2=0.9311), flower buds (R^2=0.8889) and seeds (R^2=0.6068).

For the indolic GSL, GBS, differences among cycles of the divergent selection were also found in the three organs under study. Flower buds showed the best response to increase or to reduce of the GSL content. Significant and simple linear regression coefficients were found in the three organs, leaves (R^2= 0.6574), flower buds (R^2= 0.9835) and seeds (R^2= 0.9677).

We can conclude that it is possible to modify the GSLs concentration with a classical divergent mass selection program in leaves. Furthermore, we can observe that there is a correlated response in other organs as flower buds and seeds, indicating a similar genetic regulation in the different tissues. In addition, we are studying the relationships between the modifications of these three major GSLs with other GSLs present in kales to increase our knowledge on the GSL biosynthesis pathway. These genotypes provide an excellent source of variation for future studies about the effect of GSLs in different biological processes.

P.50 Nasturtium and its degradation product BITC inhibit the inflammatory response of human immune cells

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Nasturtium *(tropaeolum majus)* from the plant order *Brassicales* is used in traditional medicine and as herbal medicinal product for the treatment of bacterial infections of the upper air tract and urinary bladder [1, 2]. It contains substantial amounts of benzylglucosinolate (benzyl GLS) besides flavonoids and carotenoids. This pro-drug and consequently its degradation product benzyl isothiocyanate (BITC) is made accountable for the therapeutic efficacy of nasturtium. In studies of animal cell cultures or permanent cancer cell lines, the anti-inflammatory potential of BITC has already been demonstrated but no information was so far available on human immune cells [3, 4, 5]. This study consequently aimed to investigate the anti-inflammatory potential of nasturtium and the degradation products of benzyl GLS on primary human peripheral mononuclear cells (PBMC). We focused here on the metabolism of arachidonic acid either via the cyclooxygenase (COX) route to prostaglandin E2 (PGE2) or via the lipoxygenase route to leukotriene B4 (LTB4). These are key aspects in the inflammatory process resulting in symptoms like fever and pain.
The release of tumor necrosis factor alpha (TNF-alpha) was also investigated. Enzyme immunoassays were applied to quantify the formation of PGE2 and LTB4 as well as TNF-alpha. Interference with the COX signalling pathway was determined by immunoblotting. Hydrolysis products were quantified by GC-MS, GLS by UHPLC-DAD.
We found that water extracts of nasturtium strongly interact with the proinflammatory pathways in PBMC which are triggered by bacteria upon release of lipopolysaccharide (LPS) from their membrane. These effects could be partly but not solely attributed to isothiocyanates, as demonstrated with benzyl ITC but not benzyl nitrile.
In conclusion, this study provided for the first time a rationale for the anti-inflammatory efficacy of nasturtium as determined in primary human PBMC. It can be hypothesized that inhibition of LPS-activated ERK1/2 and downstream signalling could partly account for this observation.

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P.51 Testing optimal defense theory: Root and shoot glucosinolate allocation patterns in seven plant species

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We examined glucosinolates (GSLs) concentrations and profiles in shoots (leaf lamina, petiole, and stem or hypocotyl) and root (main, lateral, and fine roots) of different species of Brassicaceae (*Brassica rapa, Brassica nigra, Brassica oleracea, Barbarea vulgaris, Sinapis alba, Nasturtium officinale*) and in *Tropaeolum majus* (Tropaeolaceae). Ten plants of each species were grown in the greenhouse. The plants were harvested and separated into the different organs, freeze-dried, and analyzed for their glucosinolate content by HPLC.
Overall, the stem and the main root had the highest concentrations of total GSLs, whereas in most species, the lamina and fine roots had the lowest concentrations. GSLs concentrations of *N. officinale* (a wetland species) did not differ between shoot organs. Indole GSLs, which may be less effective defenses, were relatively higher in concentration in the fine roots.
These results suggest that both aboveground and belowground plant species with GSLs show optimal defense allocation patterns: tissues that contribute the most to a plant's fitness and have the highest probability of being attacked are the best defended[1,2]. This is consistent with our earlier findings that herbivory on the main root is often lethal whereas feeding on fine roots has little effect on plant performance[3,4].

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P.52 Postharvest application of glucosinolate-derived allyl-isothiocyanate in kiwifruit: effect on grey mould and fruit quality

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Grey mould decay, caused by Botrytis cinerea Per: Fr., is a common postharvest disease on kiwifruit that can cause significant fruit losses during storage. Kiwifruit ca be stored over a 4-5 month period, maintaining temperature at values from -0.8 to 0.5 °C, relative humidity (RH) at 92-95% and ethylene concentration below 0.02 ppm. Controlled atmosphere storage (1.8-2 % O_2 and 3-4.5 % CO_2), delaying fruit softening, could extend storage up to 5-6 months with low impact on fruit quality [1]. However, controlled atmosphere seems to favour fungal infection even if the appearance of symptoms was delayed [2]. In the past fungicides were widely applied to control grey mould occurrence in cold storage, but they contribute to the development of resistant strains. Moreover, the need of control means harmless for human health and environment increased the interest for approaches alternative to traditional fungicides. The use of natural compounds with antimicrobial activity, such as glucosinolate (GL)-derived isothiocyanates (ITCs), showed to be a promising in postharvest control of several fruit pathogens [3, 4].
Recently, allyl isothiocyante (AITC) demonstrated to inhibit *B. cinerea* in *in vitro* assays [5]. In the same work vapours of AITC produced from Brassica meal in a concentration of 0.1 mg L^{-1} showed to successfully contain fungal development in *in vivo* postharvest treatments on two naturally infected 'Tecla' and 'Monterey' strawberries, without affecting fruit phenolic content and antioxidant capacity. In the present study the effect of similar treatments, was evaluated on 'Hayward' kiwifruit. Fruit artificially inoculated were treated at 0.8 mg L^{-1} AITC concentration and stored in controlled atmosphere for 5 months. At the end of storage the incidence of infection, AITC residues and the main quality and nutritional parameters of fruit were evaluated and compared with the untreated control stored under the same conditions. Surprisingly, despite the *B. cinerea* inhibitory activity of AITC previously evidenced [5], treated kiwifruit showed to be more susceptible to fungal infection than the control, probably due to the effect of the AITC on the fruit's cell wall. Indeed flesh firmness, polyphenol and ascorbic acid content and antiradical capacity values were lower in treated fruit with respect to the control. Therefore, AITC use in postharvest treatments has shown to have a potential in that depends not only on the target pathogen but also on the host fruit and/or storage conditions.

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P.53 Biological effects of glucosinolate hydrolysis products on Arabidopsis thaliana

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The enzymic decomposition of glucosinolates by myrosinases can lead to a variety of hydrolysis products, depending on the presence or absence of special cofactors such as additional proteins, metal ions and pH [1]. The biological effects of glucosinolate hydrolysis products, particularly isothiocyanates (ITCs), have been well documented in several systems, including human cells and lab animals [2] as well as insect pests [3]. That glucosinolate hydrolysis products also can exert biological effects on plants is substantiated for example by reports of the role of ITCs in biofumigation [4] and the herbicidal effects by direct exogenous application of ITCs [5]. To be able to better understand and characterize the bioactivity of glucosinolate hydrolysis products in plants, we have established a standardized in vitro system and investigated the dose-dependent response of Arabidopsis thaliana to several ITCs and nitriles by assessing different growth parameters. Results from these assays which have revealed marked differences in the plant response to various glucosinolate hydrolysis products tested in this study will be presented and discussed.

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P.54 HPLC screening of sprout glucosinolates from commercial broccoli cultivars related to the germination time

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The last years, many researchers are focused to find vegetable resources rich in phytonutrients that can prevent cancer. One representative resource is represented by broccoli cultivars, used in diet as sprouts or mature form. Beside other phytonutrients (vitamin C, phenolic compounds etc.), glucosinolates (GLS) are the main components found in broccoli, and their hydrolysis products are good inducers of enzymes which metabolize various xenobiotics and protect against cellular mechanisms which induce cancer development. Our aim was to make a screening related to the content of GLS from broccoli sprouts obtained from two different producers (BRC1-producer Lobelia II, Chrzanów Poland and BRC2 -producer PlantiCo,Zielonki Poland) depending on the germination time, in order to select the suitable germination time to provide the highest concentration of bioactive compounds. The HPLC-PDA was applied to determine the content of GLS. In the first sample, BRC1, the content of GLS was recorded at 3, 5, 7 and 9 days of germination and in the case of BRC2, after 7 and 9 days of germination (it started germination later). The content of total and individual GLS varied between two samples, and also, the germination time. Glucoraphanin (precursor of the anticarcinogenic sulforaphane) were the predominant GLS in both samples (BRC1and BRC 2) and their content decreased during germination. The highest level of glucoraphanin in the sample BRC1 was recorded in the third day of germination (1.20µmol/g dw), then significantly decreases during germination, after 9 days the level being decreased by 49.24%. Between 7 and 9 days of germination, in the case of BRC2 sample, the level of GRA decreases with 32.25%. The aliphatic GLS were the most predominant group in both sprout samples, while the highest level of GLS was recorded in the first sprout samples. On the other hand, the indolic glucosinolates were predominant in mature tissue of broccoli (70.76% vs. 29.24%, indolic respectively aliphatic) [1], but in the sprouts the levels of aliphatic GLS was predominant, decreasing during germination days (84.25% vs. 75.98%, after 3 and 9 days, respectively).Our data are in agreement with many recent publications [2,3,4]. For the consumer, the first days of germination are the best to provide to body the highest concentrations of glucosinolates as precursors of anticarcinogenic compounds.

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P.55 Integrated analysis of metabolites, transcripts and proteins gives insight into regulation of plant defense chemistry

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Plants frequently encounter attacks from herbivores and pathogens. One way by which plants cope with these threats is by producing chemical defense compounds, such as glucosinolates (GLS). However, GLS are metabolically costly, so plants have to decide between allocating their resources to growth or chemical defense. This dilemma is balanced by maintaining a relatively low basal level of GLS, which increases upon attack. The decision of channeling metabolic energy into chemical defense, rather than growth is in part a result of hormonal regulation, with jasmonates inducing the defense response, and gibberellins promoting growth. We are studying the immediate effects of these hormones on the GLS profile of *Arabidopsis thaliana* as this will allow us to elucidate which regulatory mechanisms determine the flux through the GLS pathway. In order to do this, we will combine methodologies which are able to reliably and accurately quantify metabolites, transcripts and proteins. While robust quantification methods are well-established for metabolites and transcripts, accurate quantification of proteins has previously been impossible to perform without raising protein-specific antibodies, and is even then difficult to perform in a high-throughput manner. Recent advances in mass spectrometry-based protein-identification now allow for the development of reliable protein quantification assays, otherwise known as selected reaction monitoring. We utilize such assays to quantify GLS-associated proteins in a high-throughput manner. By combining the methodologies we are able to accurately monitor the GLS pathway on several molecular levels simultaneously. Integrating the analysis of transcript, protein and metabolite under different conditions, will allow us to gain insight into the contributions of the individual regulatory levels which control metabolic flux during environmental changes.

P.56 Breeding for resistance to root flies (*Delia radicum*) in cabbage, Brassica oleracea, using genomic approaches

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Cabbage root flies constitute the most damaging biotic factor in cabbage vegetable production in Western Europe and North America. Root fly larvae (maggots) damage the root system of cabbage plants early in the season which may result in plant mortality or serious growth retardation. The threat to cabbage crops posed by root flies has recently become acute due to the legislative ban by the European Union of the major chemical insecticide to control cabbage root flies. The presence of antibiosis type of resistance against the larvae was evaluated in wild *Brassica* species using greenhouse and field assays. In 2012, 95 accessions belonging to 18 *Brassica*-species have been screened in a no-choice field test. Fifteen accessions were selected as putatively resistant, which were then tested against root fly under greenhouse conditions in spring 2013. Three accessions that showed a low average number of eclosed flies and a low average individual fly dry weight are considered as highly resistant. We further evaluated the most resistant accessions using two different root fly populations. Individual plants that showed strong resistance have been crossed with susceptible plants in order to generate a mapping population. We next aim to perform detailed analyses of larval behaviour, development and survival on the most promising accessions. Preliminary tests of larval feeding preference suggested that freshly hatched larvae are able to make choices between resistant and susceptible plants as food sources.

P.57 Pak Choi Fed to Mice: Formation of DNA Adducts and Influence on Xenobiotic-Metabolizing Enzymes

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1-Methoxy-indol-3-ylmethyl (1MOI3M) glucosinolate, a secondary plant metabolite in *Brassica* species, and its degradation product N-methoxy-indole-3-carbinol are mutagenic in *S. typhimurium* strains after activation by myrosinase and human sulphotransferase (SULT) 1A1, respectively [1, 2]. Both compounds react with DNA and proteins after administration as individual substances to mice [3, 4]. The aim of the present study was to investigate the effects of 1MOI3M as part of a plant matrix in order to compare them with previous experiments using isolated compounds.
Male FVB/N mice were fed a diet containing 1.2% lyophilized pak choi sprouts and an extract of intact glucosinolates prepared from methyl jasmonate-treated pak choi sprouts [5] for a maximum of 8 days. After day 1, 2, 4, and 8 subgroups of animals (n=4) were sacrificed by cervical dislocation. To evaluate the persistence of DNA adducts, two subgroups were fed a semisynthetic diet for further 8 or 16 days (+8 and +16 follow up) and then sacrificed. Blood and organs (liver, lung, kidney, and intestinal tissues) were taken and frozen immediately in liquid nitrogen. DNA adducts were determined by UPLC-ESI-MS/MS using stable isotopic-labeled internal adduct standards. Additionally, the influence of this glucosinolate-enriched diet on the activity of selected xenobiotic-metabolizing enzymes was investigated.

We observed a continuous accumulation of 1MOI3M DNA adducts formed in the intestine, liver, kidney and lung during 8 days of treatment. After cessation of the pak choi diet the DNA adducts decreased rapidly in jejunum, caecum, and colon, but persisted in liver, lung and kidney. The activity of several xenobiotic-metabolizing enzymes was weak induced by the pak choi diet. After 4 days of continuous feeding with pak choi diet, 7-ethoxy- and 7-methoxyresorufin-O-dealkylase activities (characteristic for cytochromes P450 1a1 and 1a2, respectively) as well as NAD(P)H:quinone oxidoreductase 1 and thioredoxin reductase activity were enhanced slightly ($p < 0.05$) compared to the pooled control groups. After cessation of the diet, the activity levels decreased to reach the levels of untreated groups. On the contrary, the activity of the toxifying enzyme, Sult1a1, was unaffected by the treatment.

Our results and recent published literature demonstrate that 1MOI3M glucosinolate forms DNA adducts in mice. DNA adducts are able to trigger mutations and therefore indicate a possible cancer risk. Further studies to investigate the mechanistic effects of 1MOI3M glucosinolate and its degradation products as well as the effects of main aliphatic glucosinolates in Brassica species, e.g. 2-hydroxy-3-butenyl and 3-butenyl glucosinolate, are needed.

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P.58 Effect of Glucosinolate Composition on Verticillium Spread in Arabidopsis

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Verticillium species are destructive vascular wilt fungi with worldwide distribution, causing severe losses in crop yield and quality. While a large body of physiological and biochemical alterations in the host are reported, the cellular mechanisms of pathogen resistance are still not fully clarified. Here we report on the application of the Arabidopsis MAGIC (Multiparent Advanced Generation Inter-Cross) population to study genotype-specific responses of the Verticillium syndrome. Arabidopsis and other Brassicaceae produce glucosinolates, secondary compounds with biocidal properties. The hydrolysis products of glucosinolates are formed upon tissue disruption and are known to suppress fungal growth. Screening the effect of volatile glucosinolate hydrolysis products of all nineteen MAGIC population parent lines on Verticillium growth identified 2-propenyl isothiocyanate as fungitoxic using *in vitro* studies [1]. To understand the effect of glucosinolate patterns on fungal spread *in planta*, we selected Arabidopsis accessions with a divergent glucosinolate composition and subjected those lines to inoculation studies. Alterations in accumulation of glucosinolates and their respective hydrolysis products were determined and related to fungal growth which was measured via qPCR. Protein expression of epithiospecifier protein ESP upon pathogen attack was studied with Western Blotting. The results and possible implications of these analyses on plant-pathogen interactions are presented here.

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P.59 Transport processes in exudation of glucosinolates from Arabidopsis root to the rhizosphere

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Rhizosphere microbial communities influence the physiology and development of plants. Plants use root exudates as chemical cues to monitor and interact with these microbial communities for defence and responses to biotic and abiotic stress [1]. The hydrolysis products of the sulphur-containing secondary metabolites, glucosinolates are toxic to a wide range of microorganisms including soil-borne pathogens. Production of exogenous tyrosine-derived p-hydroxybenzyl glucosinolate in *Arabidopsis* by introduction of CYP79A1 from sorghum lead to specific changes in the active microbial community on the roots but also in the rhizosphere of the transgenic plant compared to wildtype [2]. This indicates the well maintained glucosinolates profile in the rhizosphere is important for plant to interact with the microbial communities. Although induction of exudation of glucosinolates by hormones has been described in *Brassica rapa* [3], the molecular mechanisms of release of glucosinolates and their breakdown products in the natural environment remain unknown. Here, we use mutants of the Arabidopsis GTR transporters [4] to investigate the transport processes required for glucosinolate homeostasis in the rhizosphere. Using sand-growing *Arabidopsis*, we show through analysis of glucosinolates and their breakdown products in shoot, root and root exudation, that GTR1, GTR2 and GTR3 play key specific roles in release of glucosinolates into the rhizosphere. The role of the different GTRs in glucosinolate root exudation will be discussed.

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P.60 Functional identification of genes involved in the biosynthesis of 1-methoxy-indol-3-ylmethyl glucosinolate of *Brassica rapa* ssp. *chinensis*

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Brassica vegetables contain a class of secondary metabolites, the glucosinolates (GS), which determine their characteristic flavor and smell. While some of the respective degradation products of specific GS are recognized as health promoting substances for humans, recent studies also show evidence that namely the 1-methoxy-indol-3-ylmethyl GS (1MOI3M) might be deleterious by forming characteristic DNA adducts [1].
In order to identify genes involved in indole GS accumulation we initially established the leafy Brassica vegetable pak choi (*Brassica rapa* ssp. *chinensis*) as a suitable organism of investigation. Especially in this species very high concentration of 1MOI3M could be induced by application of jasmonic acid or methyl jasmonate [2]. In a subsequent effort we discovered differentially expressed genes in a comparative microarray analysis using the 2 x 104k format *Brassica* Array covering the 95k *Brassica* unigene set. The so identified gene candidates, coding for specific family members of either cytochrome P450 monooxygenases (CYP) or O-methyltransferases (OMT), were compared to available sequences and gene expression data from the *Arabidopsis* AtGenExpress effort. In addition, available *Arabidopsis* knock out mutants of the respective candidate gene homologs were subjected to a comprehensive examination of their GS profiles. The absence of 1MOI3M in one of the cyp-mutants confirmed the exclusive involvement of cytochrome P450 monooxygenase CYP81F4 in 1MOI3M biosynthesis. Further evidence comes from an approach where GS profiles have been measured in different accessions of the multiple reference genomes and transcriptomes for A. *thaliana* [3]. Among all analyzed accessions we found a single ecotype (Wu-0) that does not contain 1MOI3M. Genome information revealed that only in this ecotype a single nucleotide insertion in the coding sequence of *CYP81F4* produces a frame shift thus introducing a premature stop. The analysis of differences in mutants and ecotypes in the selected OMT did not show such specificity.
Two isoforms coding for CYP81F4 in the *Brassica rapa* genome were identified and functional characterization was performed using expression analysis and heterologous complementation of the respective *Arabidopsis* mutant [4]. These new identified *Brassica* genes and their functional attribution to their metabolic role in indole GS biosynthesis will contribute to develop new genetic tools for breeding vegetables with improved GS composition.

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P.61 Genetic dissecting the loci accounting for seed glucosinolates in Brassica carinata and its comparison with other Brassica species

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Glucosinolates, a group of sulfur-containing glucose derivative, occur as secondary metabolites characteristic for the Brassicaceae with complex biological functions, especially with resistance to plant diseases and insect pests. *Brassica carinata* (BBCC, 2n = 34) is one of the three tetraploid species of *Brassica* oilseed crops, with a high content of glucosinolates in plants, desirable biotic and abiotic resistance traits. We analyzed the glucosinolate content in the seeds of the YW DH genetic mapping population of B. *carinata*, in two spring-cropped and two winter-cropped environments. With a high density genetic map, dozens of loci controlling glucosinolates has been identified and compared with those identified in the related C-genome contained species, such as B. napus and B. oleracea. These results will provide information for analyzing species-specific loci controlling glucosinolates in Brassica crops.

Key words: Brassica carinata, glucosinolates, QTL, comparative mapping

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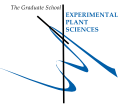

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