



BRASSICA2012

**6TH INTERNATIONAL SYMPOSIUM ON BRASSICA
AND 18TH CRUCIFER GENETICS WORKSHOP**

"EXPLOITATION OF BRASSICA DIVERSITY FOR IMPROVING AGRICULTURE CHAINS"

CATANIA 12 - 16 NOVEMBER 2012



DISPA

Dipartimento di Scienze delle
Produzioni Agrarie e Alimentari



ISHS

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Joao Silva Dias – Chair of Brassica Working Group

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Editors

Ferdinando Branca

Alessandro Tribulato

Welcome to the ISHS Brassica 2012 Symposium

Brassicas are widely grown around the world for many uses, as popular vegetable crops, oil production, and fodder, and their use is increasingly expanding to novel applications including nutraceutical, bioenergy, and biofumigation, among other uses.

There has been an increased interest in several species belonging to the Brassicaceae family and many researchers around the world are now exploiting Brassica genome information for crop development and improvement. The growth of genetic and genomic resources is supporting the improvement and diversification of traditional Brassica crops, with applications for specific traits as well as exploiting new species that can be introduced as novel crops.

The world economic crisis requires new solutions for building up a sustainable new economy and in this sense brassicas could offer several options for processing and produce innovation by its large diversity.

When I accepted to organize the ISHS Brassica 2012 Symposium I was sure Sicily would have been a great location to invite researchers involved in brassicas to discuss how to exploit the great diversity found around the world in general and in our islands in particular. The Brassicaceae diversity expressed in Sicily has recently interested researchers because it represents an important area of domestication in the Mediterranean region expressing a crucial primary gene pool.

The historical, naturalistic, social and gastronomic aspects of Sicily will provide an interesting backdrop for discussions and debate towards individuating, delineating and consolidating new research directions for future agriculture innovation.

On behalf of the International Society for Horticultural Science (ISHS) and the Department of Agricultural and Food Science (DISPA) of Catania University I cordially welcome you to the ISHS Brassica 2012 Symposium.

Ferdinando Branca

Convener

Co-ordinator of the Scientific Committee

SCIENTIFIC SESSIONS PROGRAM

MONDAY 12th NOVEMBER

08:30 **Registration of participants, poster display and full text submission**

10:00 **Opening Session**

Nicola S. - Chair of the ISHS Vegetable Section

Silva Dias J. - Chair of the ISHS Brassica Working Group

Cosentino S.L. – Director of DISPA and President of the Organising Committee

Branca F. - Brassica 2012 Convener

Session A1 - Crucifer Genetic Workshop

11:30 *Edwards D.* - Characterising Diversity in the Brassica Genomes (**Keynote lecture**)

12:00 *Zou J., Long Y., Meng J.** - Breeding a Dynamic Population of New Type *Brassica napus* Containing Unique Elite Genes from Oilseed *Brassica* Subgenomes Via Genome Selection (**Keynote lecture**)

12:30 Poster vision and discussion

13:30 Lunch

Session A2 - Crucifer Genetic Workshop

15:00 *Hammer K.*, Laghetti G., Pignone D.* - The Wild and the Grown - Remarks on *Brassica* (**Keynote lecture**)

15:30 *Cheng F., Mandáková T., Wang T., Lysak M.A.** - Reconstructing Ancestral Genome(s) of the Mesohexaploid Ancestor of *Brassica rapa* and Tribe *Brasiceae*

15:45 *Tang S.*, Preuss R., Robertson M., Buit T., Wiggins M., Gingera G., Zhao J., Ripley V., Meyer D., Thompson S., Channabasavaradhya C.* - SNP-Based High Density Map of *Brassica napus* L.

16:00 *Mason A.S.*, Cowling W.A., Nelson M.N.* - Meiotic Segregation is Initiated by Recent and Ancient Homoeologous Regions in the Haploid Genomes of *Brassica* Interspecific Hybrids

16:15 Coffee break

Session A3 - Crucifer Genetic Workshop

16:45 *Parkin I.*, Koh C., Clarke C., Navab Z.H., Sharpe A.* - Exploring the *Brassica* B Genome at the Whole Genome Sequence Level

17:00 *Diederichsen E.*, Konietzky S., Karlovsky P., Soquet-Juglard D., Häffner E.* - Identification of Resistance Loci to *Verticillium longisporum* in *Arabidopsis* and *Brassica*

17:15 *Suay L., Zhang D., Eber F., Jouy H., Lodé M., Huteau V., Coriton O., Szadkowski E., Leflon M., Martin O.C., Falque M., Jenczewski E., Paillard S., Chèvre A.M.** - How Homologous Recombination is Boosted in Oilseed Rape

- 17:30 *Xiao D., Zhao J., Basnet R.K., Pino del Carpio D., Bucher J., Bonnema G.** - Genetic Architecture of Flowering Time Variation in *Brassica rapa*: a Genetical Genomics Approach
- 17:45 Poster vision and discussion

TUESDAY 13th NOVEMBER

Session B1 - Genetic Diversity Conservation and Use

- 08:30 *von Bothmer R.* - Is There a Need for the Svalbard Global Seed Vault and Are Our Genetic Resources Safe for the Future? Examples in *Brassica* (**Keynote lecture**)
- 09:00 *McClung C.R.*, Aston T.L., Brock M.T., Dechaine J., Ewers B.E., Greenham K.M., Guadagno C.R., Khan S.L., Lou P., Weinig C., Xie Q, Yarkhunova Y.* - As Easy as ABC: Applying the Circadian Clock Model Developed in *Arabidopsis* to Brassica Crops
- 09:15 *Teakle G.*, Walley P., Moore J., Stevenson S., Carder J., Collier R., Buchanan-Wollaston V., Hand P., Pink D., Barker G.* - Genetic Diversity in Brassica Crops and Wild Relatives
- 09:30 *Artemyeva A.M.*, Budahn H., Bonnema G., Chesnokov Y.V.* - Association Mapping of Agronomically Important Traits in *Brassica rapa* L.
- 09:45 *Cervenski J.*, Takac A., Popovic V., Glogovac S.* - Possibility of Exploitation of Serbian Local Varieties and Landraces of Cabbages: Case of "Futoski cabbage" from Futog Region
- 10:00 *Chen B.*, Xu K., Gao G., Yan G., Li J., Wu X.* - Analysis of Phenotypic Diversity in Chinese Collection of *Brassica rapa* L.
- 10:15 Coffee break

Session B2 - Genetic Diversity Conservation and Use

- 10:45 *Silva Dias J.* - History and Preliminary Evaluation of Galega Kale Landraces
- 11:00 *Allender C.*, De Cesare M.* - Genetic Diversity in Brussels Sprouts and Conservation of F1 Hybrids in Genebank Collections
- 11:15 *Branca F.*, Ragusa L., Tribulato A., Lo Scalzo R., Picchi V.* - Evaluation of Sicilian Wild *Brassica* Species (n=9) for Glucosinolate Profile and Antioxidant Compounds
- 11:30 *Brullo S.*, Brullo C., Giusso del Galdo G., Fichera G., Branca F., Ilardi V., Salmeri C.* - Taxonomic Implications of Seed Morphology and Seed Coat Sculptures in *Brassica* Sect. *Brassica*
- 11:45 **ISHS BUSINESS MEETING**
- 13:00 Poster vision and discussion
- 13:30 Lunch

Session C1 - Biotechnology and Breeding

- 15:00 *Möllers C.*, Schatzki J., Ecke W., Becker H.C.* - Genetic Variation and Inheritance of Secondary Seed Dormancy in Winter Oilseed Rape
- 15:15 *De Jonge J.*, Kodde J., Immink R., Angenent G., De Groot C., Groot S.* - Environmental and Genetic Factors Controlling Shoot Apical Meristem Arrest in *Brassica oleracea*

- 15:30 *Dduan Y., Shi J., Zhang L.*, Zhang M., He Q.* - Expression of Transcriptional Factors and Structural Genes of Anthocyanin Biosynthesis in Purple-Heading Chinese Cabbage
- 15:45 *Niemann J.*, Wojciechowski A., Jędryczka M., Kaczmarek J., Książczyk T., Janowicz J.* - Interspecific Hybridization as a Tool for Broadening the Variability of Useful Traits in Rapeseed (*Brassica napus* L.)
- 16:00 *Rihan H.Z.*, Al-Issawi M., Al-Shamari M., Al-Swedi F., Fuller M.P.* - The Effect of Molybdenum on Artificial Seed Production, Cold Tolerance and Up-Regulation of CBF/DREB1 of *Brassica oleracea* var *botrytis*
- 16:15 Coffee break

Session C2 - Biotechnology and Breeding

- 16:45 *Zhang L.*, Yu N., Zheng C., Hui M., Xu X., Niu N.* - Inheritance of Cytoplasm Male Sterility and SCAR Markers Linked to Fertile Gene in Radish
- 17:00 *Zhang L., Ji X.H., Wang YG.*, Feng H.* - Mapping of a Quantitative Trait Locus for the Bolting Trait in *Brassica rapa* under Vernalizing Conditions
- 17:15 *Zuluaga D.L., Prinzenberg A.E.*, Graham N., van Ommen Kloeke A.E.E., Klinder A., Wagstaff C., Verkerk R., Aarts M.A.E.* - Overexpression of MYB29 Transcription Factor Increases the Beneficial Effects for Agriculture and Human Health of *Brassica oleracea* Plants
- 17:45 Poster vision and discussion

WEDNESDAY 14th NOVEMBER

Session D1 - Nutraceuticals and Processing

- 08:30 *Mithen R.* - High Glucoraphanin Broccoli – the Development of Beneforte™ Broccoli and Evidence of Health Benefits (**Keynote lecture**)
- 09:00 *Lola-Luz T.*, Gaffney M., Hennequart F.* - Enhancement of Phenolic Compounds, Flavonoids and Isothiocyanate Content of Broccoli (*Brassica oleraceae* var *italica*) in Response to Seaweed Applications
- 09:15 *Lim Y.P.*, Park S.U., Kim S.Y., Park J.T., Chung S.O., Sung Y.J., Lee G.J., Hur Y.K., Jo M.H., Kim T.I., Lee E.M.* - Nutriomic Approach on Chinese Cabbage Breeding
- 09:30 *Neugart S.*, Fiol M., Schreiner M., Rohn S., Zrenner R., Kroh L. W., Krumbein A* - The Influence of Temperature on the Efficiency of Moderate UV-B Treatment on the Flavonoid Profile in Kale (*Brassica oleracea* var. *sabellica*) and the Expression of Key Enzymes in Flavonoid Biosynthesis
- 09:45 *Steindal A.L.H.*, Mølmann J.B., Bengtsson G.B., Johansen T.J.* - Effects of Day Length and Temperature on Health Related Compounds in Broccoli (*Brassica oleracea* var. *italica*)
- 10:00 Coffee break

Session D2 - Nutraceuticals and Processing

- 10:30 Wiesner M.*, Zrenner R., Krumbein A., Glatt H., Schreiner M. - Genotypic Variation of the Glucosinolate Profile in Pak Choi (*Brassica rapa* ssp. *chinensis*)
- 11:45 Ragusa L.*, Tribulato A., Picchi V., Lo Scalzo R., Branca F. - Variability in Antioxidant Compounds of Seeds and Sprouts of Broccoli (*Brassica oleracea* L. var. *italica*) and Rocket (*Eruca sativa*) in Relation to Temperature and Germination Stage
- 11:00 Bengtsson G.B.*, Velasco Pazos P., Schreiner M., Mølmann J., Johansen T.J., Steinberg A. L. H., Cartea E., Krumbein A., Lea P., Skaret J., Hagen S., Seljåsen R. - Sensory Quality and Health-Related Substances of Broccoli Grown at Different Latitudes

Session E - *Isatis* Workshop

- 11:15 Galletti S.*, Bagatta M., Ragusa L., Argento S., Branca F., Iori R. - Nutraceutical Values of Woad (*Isatis tinctoria* L.) Flower Buds of Ecotypes from Sicily, Italy
- 11:30 Guarnaccia P.*, Pinio M., Testa G., Branca F. - Woad (*Isatis tinctoria* L.): an Innovative Crop for the Mediterranean Agro-Industrial System
- 11:45 Parkin I.*, Kagale S., Robinson S. - Capturing the Leaf Transcriptome of *Isatis tinctoria* L.
- 12:00 Spagna G., Todaro A., Palmeri R.* - Natural indigo from *Isatis tinctoria* L. for the valorisation of sicilian crops
- 12:15 Poster vision and discussion
- 13:30 Lunch

Session F - Agronomy

- 15:00 Calabrese N.*, Carito A. - Agronomical Evaluation of Broccoli Raab Genotypes for Fresh Cut and Processing Products
- 15:15 Adzic S.*, Prodanovic S., Girek Z., Pavlovic N., Cvikic D., Pavlovic S., Zdravkovic J. - Effect of the Application of Gibberellic Acid at Different Stages of the Late Head Cabbage Rosette on Vernalization and Seed Yield
- 15:30 Grønbaek M.*, Kidmose U., Kristensen H.L. - Cultivars and Fertiliser Strategies Affect the Concentration of Glucosinolates in Curly Kale (*Brassica oleracea* L. var. *sabellica* L.)
- 15:45 Lazzeri L.*, Malaguti L., Cinti S., De Nicola G.R., Ugolini L., Bagatta M., Matteo R., Casadei N., D'Avino L. - The *Brassicaceae* Biofumigation System for Plant Cultivation and Defence. An Italian Twenty Year Experience of study and Application
- 16:00 Gilardi G.*, Gullino M.L., Garibaldi A. - Success and Failures of Biofumigation with Brassicas
- 16:15 Nicoletto C.*, Santagata S., Sambo P. - Effect of Compost Application on Qualitative Traits in Cabbage
- 16:30 Coffee break

Session G - Pests and Diseases

- 17:00 Sigillo L.*, Senape V., Serratore G., Bravi R. - *Fusarium oxysporum* f. sp. and *Xanthomonas campestris* Patovars: Two Important Seed-Borne Pathogens of Wild Rocket Fourth Range Production in the Southern Italy

- 17:15 *Kaczmarek J.**, *Brachaczek A.*, *Kasprzyk I.*, *Sulborska A.*, *Karolewski Z.*, *Jędryczka M.* - Why to Study the Concentration of the Ascospores of *Leptosphaeria maculans* and *L. biglobosa* ?
- 17:30 *Velasco P.**, *Lema M.*, *Alonso I.*, *Cartea M.E.* - Antibiotic Effect of Glucosinolates and Derivatives on Different *Brassica* Diseases
- 17:45 *Godinho J.S.**, *Balesdent M.H.*, *Pereira E.M.*, *Dias J.S.* - Molecular Characterization of Portuguese Isolates of *Leptosphaeria maculans* Using PCR- ISSR and RAPD Markers
- 20:00 **Social dinner**

THURSDAY 15th NOVEMBER

Session C3 - Biotechnology and Breeding

- 08:30 *Bartoszek A.* - The Innovative Exploitation of Brassica Vegetables in the Health Quality Food Production Chain. From Field to Fork (**Keynote lecture**)
- 09:00 *Yokoi S.**, *Furuyama Y.*, *Takahashi M.*, *Aoki W.*, *Takahashi Y.*, *Ebina S.*, *Mimida N.*, *Takahata Y.* - QTL Analysis of Bolting Time in Radish (*Raphanus sativus* L.) Based on SNP Markers
- 09:15 *Domblides E.**, *Domblides A.**, *Zayachkovskaya T.*, *Bondareva L.*, *Startsev V.* - PCR-Based Identification of Cytoplasm Types in Male Sterile Accessions of *Brassicaceae*
- 09:30 *Yanaeva D.A.**, *Khovrin A.N.*, *Tereshonkova T.A.* - Creating a Source Material for Breeding Radish for Greenhouses
- 09:45 *Yan G.**, *Chen B.*, *Xu K.*, *Gao G.*, *Lu P.*, *Wu X.*, *Li F.*, *Li J.* - Gene Expression Profiling of Developing Seeds in *Brassica napus* L. at Different Nitrogen Levels
- 10:00 *Wang C.*, *Shi S.**, *Wu J.* - Useful Method for Distinguishing the Ploidy of Tube Plantlets Regenerated from Microspore Culture in *Brassica napus* L.
- 10:15 *Bondareva L.**, *Startsev V.* - Development of Heterosis Hybrids of White Head Cabbage Through Self-Incompatibility and Cytoplasmic Male Sterility
- 10:30 Coffee break

Session H - Clubroot

- 11:00 *Hatakeyama K.**, *Suwabe K.*, *Tomita R.N.*, *Kato T.*, *Nunome T.*, *Fukuoka H.*, *Matsumoto S.* - Molecular Cloning of *Crr1a*, a Gene for Resistance to Clubroot Disease (*Plasmodiophora brassicae* Woronin) in *Brassica rapa* L.
- 11:15 *Gravot A.*, *Wagner G.*, *Lariagon C.*, *Delourme R.**, *Manzanares-Dauleux M.J.* - Deciphering the Functional Typology of Resistance QTL through Metabolomics
- 11:30 *Jonsson A.**, *Wallenhammar A.C.*, *Dixelius C.* - Long-Term Soil Data Sets Reveal Shifts in Populations of *Plasmodiophora brassicae*
- 11:45 *Lewis M.**, *Wakeham A.*, *Kennedy R.* - A Molecular Based Seedling Assay System to Study the Germination and Primary Infection by *Plasmodiophora brassicae*
- 12:00 *Peng G.**, *Lahlali R.*, *Pageau D.*, *Hwang S.F.*, *Hynes R.K.*, *Anderson K.*, *McDonald M.R.*, *Gossen B.D.*, *Strelkov S.E.*, *Turkington T.K.*, *Yu F.Q.*, *Falk K.C.*, *Boyetchko S.M.*

- McGregor L., Hupka D., Geissler J.* - Crop Rotation and Cultivar Resistance Are Effective for Control of Clubroot on Canola while *Bacillus subtilis* Biofungicides Are Not
- 12:15 *Ramchiary N., Im S.B., Piao Z., Lim Y.P.** - Use of Genetics and Genomics Approaches to Fine Map the Clubroot Resistance “CRb” Locus in *Brassica rapa*
- 12:30 *Jing J., Chen J., Zhang T., Piao Z.** - QTL Mapping of Isolate-Specific Resistance to *Plasmodiophora brassicae* in *Brassica rapa*
- 12:45 *Schwelm A.*, Fogelqvist J., Dixelius C.* - First Glance at the Genome of the Club Root Pathogen *Plasmodiophora brassicae*
- 13:00 Poster vision and discussion
- 13:30 Lunch

Session A4 - Crucifer Genetic Workshop

- 15:00 *Li X., Ramchiary N., Dhandapani V., Choi S.R., Hur Y., Nou I., Yoon M.K., Lim Y.P.** - Quantitative Trait Loci Mapping in *Brassica rapa* Revealed the Structural and Functional Conservation of Genetic Loci Governing Morphological and Yield Component Traits in the A, B, and C Sub-Genomes of *Brassica* Species
- 15:15 *Tollenaere R., Hayward A., Bayer P., Dalton-Morgan J., Campbell E., McLanders J., Lorenc M., Manoli S., Stiller J., Raman R., Raman H., Edwards D., Batley J.** - Identification and Characterisation of Candidate Blackleg Resistance Genes in *Brassica napus* Using Next Generation Sequencing
- 15:30 *Kagale S.*, Koh C., Bollina V., Clarke C., Sharpe A., Parkin I.* - Genome Sequencing and Analysis of the Emerging Oilseed Crop *Camelina sativa*
- 15:45 *Artemyeva A.M.*, Rudneva E.N., Volkova A.I., Kocherina N.V., Chesnokov Y.V.* - Identification of QTLs Determined Black Rot Resistance and Morphological Characters in *Brassica rapa*
- 16:00 Poster vision and discussion
- 16:30 Coffee break
- 17:00 **Closing session** Concluding Remarks

FRIDAY 16th NOVEMBER

Technical Tour

08:30 - Departure

Arrival - late afternoon

LIST OF CONTRIBUTIONS

KEYNOTE LECTURES

The Innovative Exploitation of Brassica Vegetables in the Health Quality Food Production Chain.
From Field to Fork

Bartoszek A.

Is There a Need for the Svalbard Global Seed Vault and Are Our Genetic Resources Safe for the Future? Examples in Brassica

von Bothmer R.

Characterising Diversity in the *Brassica* Genomes

Edwards D.

The wild and the Grown – remarks on Brassica

Hammer K., Laghetti G., Pignone D.*

High Glucoraphanin Broccoli - the Development of Beneforte™ Broccoli and Evidence of Health Benefits

Mithen R.

Breeding a Dynamic Population of New Type *Brassica napus* Containing Unique Elite Genes from Oilseed Brassica Subgenomes via Genome Selection

*Zou J., Long Y., Meng J.**

SESSION A - CRUCIFER GENETIC WORKSHOP

Oral presentations

Reconstructing Ancestral Genome(s) of the Mesohexaploid Ancestor of *Brassica rapa* and Tribe *Brasiceae*

*Cheng F., Mandáková T., Wang T., Lysak M.A.**

Exploring the *Brassica* B genome at the Whole Genome Sequence Level

Parkin I., Koh C., Clarke C., Navab Z.H., Sharpe A.*

SNP-Based High Density Map of *Brassica napus* L.

Tang S., Preuss R., Robertson M., Buit T., Wiggins M., Gingera G., Zhao J., Ripley V., Meyer D., Thompson S., Channabasavaradhya C.*

Meiotic Segregation is Initiated by Recent and Ancient Homoeologous Regions in the Haploid Genomes of *Brassica* Interspecific Hybrids

Mason A. S., Cowling W. A., Nelson M.N.*

Identification of Resistance Loci to *Verticillium longisporum* in *Arabidopsis* and *Brassica*
*Diederichsen E.**, *Konietzky S.*, *Karlovsky P.*, *Soquet-Juglard D.*, *Häffner E.*

How Homologous Recombination is Boosted in Oilseed Rape
Suay L., *Zhang D.*, *Eber F.*, *Jouy H.*, *Lodé M.*, *Huteau V.*, *Coriton O.*, *Szadkowski E.*, *Leflon M.*,
Martin O.C., *Falque M.*, *Jenczewski E.*, *Paillard S.*, *Chèvre A.M.**

Genetic Architecture of Flowering Time Variation in *Brassica rapa*; a Genetical Genomics
Approach
Xiao D., *Zhao J.*, *Basnet R.K.*, *Pino del Carpio D.*, *Bucher J.*, *Bonnema G.**

Quantitative Trait Loci Mapping in *Brassica rapa* Revealed the Structural and Functional
Conservation of Genetic Loci Governing Morphological and Yield Component Traits in the A, B,
and C Sub-Genomes of *Brassica* species
Li X., *Ramchiary N.*, *Dhandapani V.*, *Choi S.R.*, *Hur Y.*, *Nou I.*, *Yoon M.K.*, *Lim Y.P.**

Identification and Characterisation of Candidate Blackleg Resistance Genes in *Brassica napus*
Using Next Generation Sequencing
Tollenaere R., *Hayward A.*, *Bayer P.*, *Dalton-Morgan J.*, *Campbell E.*, *McLanders J.*, *Lorenc M.*,
Manoli S., *Stiller J.*, *Raman R.*, *Raman H.*, *Edwards D.*, *Batley J.**

Genome Sequencing and Analysis of the Emerging Oilseed Crop *Camelina sativa*
*Kagale S.**, *Koh C.*, *Bollina V.*, *Clarke C.*, *Sharpe A.*, *Parkin I.*

Identification of QTLs Determined Black Rot Resistance and Morphological Characters in *Brassica*
rapa L.
*Artemyeva A.M.**, *Rudneva E.N.*, *Volkova A.I.*, *Kocherina N.V.*, *Chesnokov Y.V.*

Poster presentations

Agrobacterium Mediated TransforBalkayamation and integration of stress Genes into Cauliflower
(*Brassica oleracea* var. *botrytis* L.)
*Al-Swedi F.**, *Al-Shamari M.*, *Rihan H.Z.*, *Lane S.*, *Fuller M.P.*

Structural and Functional Divergence of the ABI1 Homeologous Genes in *Brassica napus*
*Babula-Skowrońska D.**, *Ludwików A.*, *Cieśla A.*, *Jędrzycka M.*, *Sadowski J.*

Brassica rapa Genome Assembly v2.0: an Improved Genome of *Brassica rapa*
Cheng F., *Wang J.*, *Wu J.*, *Wang Z.*, *Zhao X.*, *Liu B.*, *Lin K.*, *Wang X.**

QTL Analysis of Morphological Traits Using Different Population in Chinese Cabbage (*Brassica*
rapa ssp. *pekinensis*)
Choi S.R., *Ramchiary N.*, *Li X.*, *Dhandapani V.*, *Yu X.*, *Lim Y. P.**

Crosses of Rapeseed-Radish Disomic Addition Line D with *Brassica rapa* L. to Evaluate the Effect of the Radish Chromosome in Pakchoi Background

Ding Y., Budahn H. , Schrader O., Li L., Bao M.*

Transcript Analysis of Genes Relating to Seed Oil Content in *Brassica napus* L.

Doi H. , Akaba-Takahashi M., Yamada-Akiyama H., Yokoi S., Takahata Y.*

Mapping Root and Flowering Time QTL in *Brassica napus*: Evidence For Pleiotropy

Fletcher R.S. , Mullen J.L., McKay J.K*

Isolation and Expression Analysis of an OguCMS-Related AT-Hook DNA Binding Protein, BoMF2, in Cabbage Flowers

*Guo Y., Zhang L., Liu H., Jian Y., Kang J.**

Chromosomal Assignment of Radish Linkage Groups Using a Complete Set of Disomic Radish-Rapeseed Chromosome Addition Lines

Hashida T., Nakatsuji R., Budahn H. , Schrader O., Peterka H., Fujimura T, Kubo N., Hirai M.*

Morphological Variation of Resynthesized *Brassica napus* after Interspecific Hybridization

Karim Md.M. , Nukui S., Okazaki K.*

Radish Genetic Map Constructed by High-Density EST-Based Markers with Scaffold Sequences

Kitashiba H. , Li F., Kawanabe T., Fukushima A., Nishio T.*

Genome-wide Identification, Classification and Expression Analysis of Genes Encoding Putative Fasciclin-Like Arabinogalactan Proteins in Chinese cabbage (*Brassica rapa* L.)

*Li J., Wu X. **

The Mesopolyploid History of Australian and New Zealand Crucifers

*Mandáková T., Joly S., Mummenhoff K., Heenan P.B., Lysak M.A.**

Whole-Genome Triplication and Species Radiation in the Southern African Tribe *Heliophilleae* (*Brassicaceae*)

*Mandáková T., Mummenhoff K., Al-Shehbaz I.A., Mucina L., Mühlhausen A., Lysak M.A.**

Gene Expression Diversity in *Brassica* C Genome Crops and Wild Relatives Assessed Using RNA-Seq

Moore J. , Teakle G., Walley P., Stevenson S., Carder J., Collier R., Buchanan-Wollaston V., Hand P., Pink D., Barker G.*

Use of Natural Variability to Identify and Validate the Genetic Determinants of Oil Content in Rapeseed (*Brassica napus* L.)

Nesi N.*, Bouchet A. S., Kergoat R., Confolent C., Baron C., Clouet V., Daligault J., Laperche A.

Intertribal Hybridization between *Brassica oleracea* L. and *Eruca sativa* L.

Nothnagel T., Budahn H.*, Schrader O., Klocke E.

Identification of Metabolic QTLs for Glucosinolate Content in *Brassica oleracea*

Sotelo T, Cartea M.E., Francisco M, Soengas P. *

Genetics of Epidermal Cell Characters in *Brassica rapa* L.

Takahashi Y.*, Yokoi S., Takahata Y.

Production and Characterization of the Chromosome Addition Lines of Auto- and Alloplasmic *Brassica napus* with Single B Genome Chromosome of *Brassica juncea*

Takashima M.*, Bang S. W., Kaneko Y.

High-Throughput Single Nucleotide Polymorphism (SNP) Discovery and Validation in *Brassica napus* L.

Tang S.*, Wiggins M., Preuss R., Rider K., Ripley V., Gingera G., Stol C., Buyyarapu R., Ye L., Arora K., Elango N., Mansfield T., Evans C., Rounsley S., Meyer D., Thompson S., Channabasavaradhya C.

Developing a Set of SNP Markers to Study Genetic Diversity in a *Brassica* A Genome Diversity Collection

Tanhuanpää P.*, Tanskanen J., Manninen O.

Effects of Suppression of Msh1 on Mitochondrial Genome in *Brassica napus*

Terao Y.*, Imamura J.

Changes in Gene Expression of Harvested Cabbage in Response to Mechanical Wound Stress

Thammawong M.*, Umehara H., Yoshida M., Soga A., Kaneta T., Nakamura N., Ito Y., Nakano K., Shiina T.

Comparative Genome Analysis, Identification of Flowering Pathway and Development of Genome-Wide Molecular Markers Based on Genome Sequence in *Brassica juncea*

Yang J., Zhao X, He H., Qi X., Zhang M.*

Genetic Dissection and Fine Mapping of Downy Mildew Resistance at Various Developmental Stages in *Brassica rapa*

Yu S.*, Zhang F., Zhang S., Zhou W., Yu Y., Zhang D., Zhao X., Wang W.

High-Throughput Discovery of Chloroplast and Mitochondrial DNA Polymorphisms in *Brassicaceae* Species by ORG-EcoTILLING

Zeng C.L., Wang G.Y., Wang J.B., Yan G.X., Chen B.Y., Xu K., Li J., Gao G.Z., Wu X.M.*

Combined QTL Mapping and Transcriptomic Approach to Unravel the Genetics of Turnip Formation in *Brassica rapa* ssp. *campestris*

Zhang N.*, Lin K., Raynoud R., Wang S., Habtemariam H., Sun Y., Menano T., Bucher J., Wu J., Cheng F., Liu B., Wang X., Bonnema G.

SESSION B - GENETIC DIVERSITY, CONSERVATION AND USE

Oral presentations

As Easy as ABC: Applying the Circadian Clock Model Developed in Arabidopsis to Brassica Crops

McClung C. R.*, Aston T. L., Brock M. T., Dechaine J., Ewers B. E., Greenham K. M., Guadagno C. R., Khan S. L., Lou P., Weinig C., Xie Q., Yarkhunova Y.

Genetic Diversity in Brassica Crops and Wild Relatives

Teakle G.*, Walley P., Moore J., Stevenson S., Carder J., Collier R., Buchanan-Wollaston V., Hand P., Pink D., Barker G.

Association Mapping of Agronomically Important Traits in *Brassica rapa* L.

Artemyeva A.M.*, Budahn H., Bonnema G., Chesnokov Y. V.

Possibility of Exploitation of Serbian Local Varieties and Landraces of Cabbages: Case of "Futoški cabbage" from Futog region

Cervenski J.*, Takac A., Popovic V., Glogovac S.

Analysis of Phenotypic Diversity in Chinese Collection of *Brassica rapa* L.

Chen B.*, Xu K., Gao G., Yan G., Li J., Wu X.

History and Preliminary Evaluation of Galega Kale Landraces

Silva Dias J.

Genetic Diversity in Brussels Sprouts and Conservation of F1 Hybrids in Genebank Collections

Allender C.*, De Cesare M.

Taxonomic Implications of Seed Morphology and Seed Coat Sculptures in *Brassica* Sect. *Brassica*

Brullo S.*, Brullo C., Giusso del Galdo G., Fichera G., Branca F., Ilardi V., Salmeri C.

Evaluation of Sicilian Wild *Brassica* Species (n=9) for Glucosinolate Profile and Antioxidant Compounds

Branca F.*, Ragusa L., Tribulato A., Bagatta M., Lo Scalzo R., Picchi V.

Poster presentations

Studies on Enrichment of Varieties Assortment at Autumn Cabbage *Brassica oleracea* L. var. *capitata*

Ambarus S., Brezeanu C., Brezeanu P.M.*

Evaluation of Variation of Kale (*Brassica oleracea* var. *acephala*) Populations Collected from Black Sea Region, Turkey

Balkaya A., Yanmaz R.*

In Progress Activities of the Brassica Working Group of the European Cooperative Programme for Plant Genetic Resources (ECPGR)

Branca F., Bas N., Artemyeva A., De Haro A., Maggioni L.

Bio-Morphological Characterization of the ECPGR Brassica Working Group Collection

Branca F., Chiarenza G.L., Argento S., Ragusa L.

Diversity of Kales Growing in Europe as Basis for Crop Improvement

Branca F., Ragusa L., Poulsen G., Maggioni L., von Bothmer R., Tribulato A.

Genetic Relationship of Sicilian Wild and Cultivated *Brassicaceae* Using Five SSR

Branca F. Tribulato A., Chiarenza G. L., Di Gaetano C., Calì F.*

S-SAP Markers - A Valuable Tool for Genetic Distance Analysis in *Brassica rapa* L.

Budahn H., Artemyeva A. M., Chesnokov Y. V.**

Evaluation of a Possible Breeding between *Brassica napus* Cultivar and *Sinapis arvensis* in the Field

Buonamici A., Labriola M., Paffetti D., Tomaselli V., Travaglini D., Bottalico F., Balducci E. Materassi A., Fasano G., Vettori C.*

Preservation of Verzeznis Turnip

Capone F., Valent E., Cattivello ., Dalla Costa L.

Korea *Brassica* Resource Bank

*Kim J.A., Lee D. H., Lim Y.P.**

Development and Application of a Simple Sequence Repeat Panel for the Fingerprinting of Canola (*Brassica napus*) Cultivars

Li F., Xu K., Song WL., Chen BY., Gao GZ., Wu XM.*

Assessment of Genetic Diversity in Iberian Landraces of *Brassica oleracea* by Molecular Markers

Lopes V.R., Barata A.M., Nunes E., Cartea E., Soengas P., Allender C. , Bas N.*

Signals of Inter-Crossing between Leafy Kale Landraces and *Brassica rupestris* in South Italy
Maggioni L., von Bothmer R., Poulsen G., Bagger Jorgensen R., Andersen B.A., Branca F.

Using Controlled Deterioration Test on Determining Seed Vigour Performances of Cabbage
(*Brassica oleracea* L. var. *capitata*) Seed Lots
Öztürk B., Balkaya A.*

Genetics of Low Temperature Response of Photosynthesis and Growth in *Brassicaceae*
Prinzenberg A.E.* , Snel J., van der Schoor R., Harbinson J., Aarts M.G.M.

Ethnobotanical Uses of *Brassicaceae* in Sicily
Romano D.* , Parisi G., Toscano S., Scuderi D.

Provenance-Related Seed Heterogeneity and Germination in *Brassica insularis* Moris
Santo A.* , Mattana E., Grillo O., Bacchetta G.

The Use of *Brassicaceae* by the Ancient Romans
Toscano S.* , Scuderi D., Tribulato A., Romano D.

SESSION C - BIOTECHNOLOGY AND BREEDING

Oral presentations

Genetic Variation and Inheritance of Secondary Seed Dormancy in Winter Oilseed Rape
Möllers C.* , Schatzki J., Ecke W., Becker H.C.

Environmental and Genetic Factors Controlling Shoot Apical Meristem Arrest in *Brassica oleracea*
De Jonge J.* , Kodde J., Immink R., Angenent G., De Groot C. Groot S.

Expression of Transcriptional Factors and Structural Genes of Anthocyanin Biosynthesis in Purple-
Heading Chinese Cabbage
Dduan Y., SHI J., Zhang L.* , Zhang M., He Q.

Interspecific Hybridization as a Tool for Broadening the Variability of Useful Traits in Rapeseed
(*Brassica napus* L.)
Niemann J.* , Wojciechowski A., Jędryczka M., Kaczmarek J., Książczyk T., Janowicz J.

The Effect of Molybdenum on Artificial Seed Production, Cold Tolerance and Up-Regulation of
CBF/DREB1 of *Brassica oleraceae* var *botrytis*
Rihan H.Z.* , Al-Issawi M., Al-Shamari M., Al-Swedi F. and Fuller M.P.

Brassica rapa ssp. *chinensis* (Pak Choi) as Model Plant to Identify Genes Involved in Indole
Glucosinolate Biosynthesis
Zrenner R.* , Wiesner M., Krumbein A., Schreiner M.

Inheritance of Cytoplasm Male Sterility and SCAR Markers Linked to Fertile Gene in Radish
Zhang L. *, Yu N., Zheng C., Hui M., Xu X., Niu N.

Mapping of a Quantitative Trait Locus for the Bolting Trait in *Brassica rapa* under Vernalizing Conditions
Zhang L., Ji X.H., Wang Y.G. *, Feng H.

Overexpression of MYB29 Transcription Factor Increases the Beneficial Effects for Agriculture and Human Health of *Brassica oleracea* Plants
Zuluaga D.L., Prinzenberg A.E. *, Graham N., van Ommen Kloeke A.E.E., Klinder A., Wagstaff C., Verkerk R., Aarts M.A.E.

QTL Analysis of Bolting Time in Radish (*Raphanus sativus* L.) Based on SNP Markers
Yokoi S. *, Furuyama Y., Takahashi M., Aoki W., Takahashi Y., Ebina S., Mimida N., Takahata Y.

PCR-Based Identification of Cytoplasm Types in Male Sterile Accessions of *Brassicaceae*
Domblides E. *, Domblides A., Zayachkovskaya T., Bondareva L., Startsev V.

Creating a Source Material for Breeding Radish for Greenhouses
Yanayeva D.A. *, Khovrin A.N., Tereshonkova T.A.

Gene Expression Profiling of Developing Seeds in *Brassica napus* L. at Different Nitrogen Levels
Yan G. *, Chen B., Xu K., Gao G., Lu P., Wu X., Li F., Li J.

Useful Method for Distinguishing the Ploidy of Tube Plantlets Regenerated from Microspore Culture in *Brassica napus* l.
Wang C., Shi S. *, Wu J.

Development of Heterosis Hybrids of White Head Cabbage Through Self-Incompatibility and Cytoplasmic Male Sterility
Bondareva L. *, Startsev V.

Poster presentations

Molecular Characterization of Stress Responsive Family Genes in *Brassica rapa*
Ahmed N.U., Park J., Jung H., Thamilarasan S.K., Hur Y., Lim Y.P., Nou I. *

Identification and Characterization of Cold Responsive Genes in *Brassica*
Arasan S.K.T.K., Park J., Ahmed N.U., Jung H., Lim Y.P., Hur Y., Nou I. *

Nutritional Enhancement of Broccoli Sprouts Through Manipulation of Growth Conditions
Baima S. *, Maldini M., Natella F., Giusti A., Scaccini C., Morelli G.

Unravelling Molecular Mechanisms Involved during *Bacillus amyloliquefaciens* Mediated Induced Resistance in Plants

*Bejai S.**, *Johansson A.*, *Chen K.*, *Leelarasamee N.*, *Thoudal M.*, *Kjellin J.*, *Meijer J.*

Molecular Characterization of Brassicas Developed by EMS Mutagenesis with Tolerance to Sulfonylurea Herbicides

Braun R.H., *Christey M.C.**, *Conner A.J.*, *Gowers S.* and *Dumbleton A.*

On the Way to the Identification of a Downy Mildew Resistance Gene in *Brassica oleracea* L.

*Carlier J.**, *Alabaça C.*, *Rodrigues C.*, *Coelho P.*, *Monteiro A.*, *Leitão J.*

Characterization and Improvement of Sicilian Landraces of Violet Cauliflower

Chiarenza G. L., *Tribulato A.*, *Branca F.*

Organisation of Stem Canker Resistance QTL and Genome Duplications in *Brassica napus*

Fopa Fomeju B., *Falentin C.*, *Lassalle G.*, *Manzanares-Dauleux M.J.*, *Delourme R.**

Large scale genotyping in *Brassica napus*, *Brassica oleracea* and *Brassica rapa*

*Ganal M.W.**, *Durstewitz G.*, *Graner E.M.*, *Plieske J.*, *Polley A.*, *Wieseke R.*

Dependence of Pistils Stigmas Viability in Winter Rape on Its Genetical Origin

Gluhova N.A.

Genetic Modification of Oilseed Rape to Create New Quality and Flower Characteristics

*Hegelund J.N.**, *Lütken H.*, *Müller R.*, *Christensen B.*

Determination of Self-Incompatibility in Chinese Cabbage (*Brassica rapa*) by Multiplex PCR

*Lee H. E.**, *Chung H.*, *Park H.*, *Sang-Eun H.*, *Jong-Gyu W.*

The Usefulness of AFLP Markers for Determining Genetic Distance in Oilseed Rape (*Brassica napus* L.) Breeding Materials

*Liersch A.**, *Bartkowiak-Broda I.*, *Krotka K.*, *Wos H.*

An Efficient Regeneration System For Leaf of Heading Chinese Cabbge

Liu X., *Zhang L.**, *Ru L.*, *Li H.*

Change in the Expression of Anthocyanin Pathway Genes in Developing Brassica crops

Lo Piero A.R., *Lo Cicero L.*, *Ragusa L.*, *Branca F.*

Development of Highly Clubroot-Resistant Chinese Cabbage F1 Cultivar, 'Akimeki', Accumulating Three Resistance Genes, Crr1, Crr2 and CRb

*Matsumoto S.**, *Hatakeyama K.*, *Kato T.*, *Takashita S.*, *Miyazaki T.* and *Kondo T.*

Production of Doubled Haploid Plants of *Brassica rapa* Subspecies Using the Microspore Culture Technology for Core Collection Materials

Park M.Y., Bonnema G., Park S., Lim Y.P.*

Development of DH Inbred Lines Using Microspore Culture in Chinese Cabbage (Kimchi cabbage) (*Brassica rapa* L.)

Park S.*, Yoon MK., Lim Y.P.

Induction of Direct Somatic Embryogenesis from Immature Embryos of *Brassica oleracea* var. *sabauda* L.

Pavlović S.*, Milojević J., Vinterhalter B., Adžić S., Zdravković J., Cvikić D., Mitić N.

Evaluation of Intensity and Distance of Oilseed Rape Pollen Transfer

Poplawska W.*, Liersch A., Jedryczka M., Kaczmarek J., Wolko J., Ogrodowczyk M., Bartkowiak-Broda I.

Fingerprint Analysis of Selected Glutathione S-Transferase Superfamily Genes from Rape (*Brassica napus*)

Puglisi I., Lo Cicero L., Lo Piero A.R.*

Changes in Methylation Patterns in *Brassica Oleracea* L. during Time and in Response to Different Agroclimatic Conditions

Raggi L.*, Ciancaleoni S., Albertini E., Fé D., Torricelli R., Negri V.

Understanding the Root System in *Brassica napus*

Rahman M.

The Response Characteristics of Japanese Radish (Daikon) to Mechanical Wound Stress

Shiina T.*, Umehara H., Yoshida M., Soga A., Kaneta T., Nakamura N., Ito Y., Nakano K., Thammawong M.

Identification of *Brassica rapa* Homologue of *Fusarium* Wilt Resistance Gene, Foc-bo1 by Genome Synteny Analysis between *B. oleracea* and *B. rapa*

Shimizu M.*, Ebe Y., Kawanabe T., Pu Z., Fujimoto R., Kaji M., Zhang Y. and Okazaki K.

Quality Evaluation of Cultivars Types in *Brassica oleracea* L.

Solovyova A.E., Artemyeva A.M.*

Determination of Species, Varieties and Geographical Differences among Cabbage Genotypes Based on Electrophoresis of Storage Seed Proteins

Startsev V.*, Bondareva L., Dobrutskaya E., Gins V., Farber C., Antoshkina M.

Seed Quality Traits of DH Lines of Winter Oilseed Rape (*Brassica napus* l.)

Szala L.*, Cegielska-Taras T., Adamska E., Kaczmarek Z.

Creating Germplasm and New Tools to Investigate Post Harvest Senescence in *Brassica oleracea* L var. *italica*

Walley P.G., Carder J., Skipper E., Pink D., Buchanan-Wollaston V.

SESSION D - NUTRACEUTICAL AND PROCESSING

Oral presentations

Enhancement of Phenolic Compounds, Flavonoids and Isothiocyanate Content of Broccoli (*Brassica oleracea* var *italica*) in Response to Seaweed Applications

Lola-Luz T.*, Gaffney M., Hennequart F.

Nutriomic Approach on Chinese Cabbage Breeding

Lim Y.P.*, Park S.U., Kim S.Y., Park J.T., Chung S.O., Sung Y.J., Lee G.J., Hur Y.K., Jo M.H., Kim T.I., Lee E.M.

The influence of Temperature on the Efficiency of Moderate UV-B Treatment on the Flavonoid Profile in Kale (*Brassica oleracea* var. *sabellica*) and the Expression of Key Enzymes in Flavonoid Biosynthesis

Neugart S.*, Fiol M., Schreiner M., Rohn S., Zrenner R., Kroh L.W., Krumbein A.

Effects of Day Length and Temperature on Health Related Compounds in Broccoli (*Brassica oleracea* var. *italica*)

Steindal A.L.H.*, Mølmann J. B., Bengtsson G.B., Johansen T.J.

Genotypic Variation of the Glucosinolate Profile in Pak Choi (*Brassica rapa* ssp. *chinensis*)

Wiesner M.*, Zrenner R., Krumbein A., Glatt H., Schreiner M.

Variability in Antioxidant Compounds of Seeds and Sprouts of Broccoli (*Brassica oleracea* L. var. *italica*) and Rocket (*Eruca sativa*) in Relation to Temperature and Germination stage

Ragusa L.*, Tribulato A., Picchi V., Lo Scalzo R., Branca F.

Sensory Quality and Health-Related Substances of Broccoli Grown at Different Latitudes

Bengtsson G. B.*, Velasco Pazos P., Schreiner M., Mølmann J., Johansen T. J., Steinberg A. L. H., Cartea E., Krumbein A., Lea P., Skaret J., Hagen S., Seljåsen R.

Poster presentations

Brassicas and Their Glucosinolate Content for the Biological Control of Soilborn in Protected Cultivation

Argento S., Raccuia S.A., Branca F.

Measurement of Sulphur Compounds in *Brassica*

*Baty-Julien C.**, *Leroy G.*, *Gourrat K.*

Thermal Processing of Green and Red Cultivars of Curly Kale (*Brassica oleracea* L. convar. *acephala* var. *sabellica*) and Effects of Fresh and Processed Kale Extracts on Human Colon Cancer Cell Lines

*Borge G.I.**, *Olsen H.*, *Grimmer S.*, *Saha S.*, *Aaby K.*, *Wold A. B.*, *Schmidt G.*, *Skrede G.*

Glucosinolate profile in different Mediterranean Brassica species (n=9)

*Branca F.**, *Ragusa L.*, *Cartea E.*, *Tribulato A.*, *Velasco P.*

Phytochemical Profile of Kohlrabi (*Brassica oleracea* L. var. *gongylodes*) Cultivated in Italy

Branca F., *Ragusa L.*, *Lo Scalzo R.*, *Picchi V.*, *Argento S.*

Changes in Biochemical Qualitative Properties in Fresh-Cut Broccoli Genotypes during Storage

Conversa G., *Bonasia A.*, *Lazzizzera C.*, *Elia A.*

Chemical and Nutraceutical Profile in Typical Italian Typologies of Cauliflower

*Di Cesare L.F.**, *Migliori C.*, *Lo Scalzo R.*, *Campanelli G.*, *Ferrari V.*

Identification of Intermediary Breakdown Products Formed by the Thermally Induced Degradation of Aliphatic Glucosinolates

*Hanschen F. S.**, *Bauer A.*, *Rohn S.*, *Mewis I.*, *Schreiner M.*, *Kroh W. L.*

Characterization of the Main Glucosinolate Content and Fatty Acid Composition in Non Food *Brassicaceae* Seeds

Lazzeri L., *Bagatta M.**, *D'Avino L.*, *Ugolini L.*, *De Nicola G. R.*, *Casadei N.*, *Cinti S.*, *Malaguti S.*, *Iori R.*

Intestinal Fermentability of *Brassica*: Methods for Evaluating and Minimizing Digestive Discomfort

Leroy G., *Baty-Julien C.**, *Leguen V.*, *Mabeau S.*

Evaluation of Some Quality Parameters of Minimally Processed Violet and White-Type Cauliflowers

Licciardello F., *Muratore G.*, *Spagna G.**, *Branca F.*, *Ragusa L.*, *Caggia C.*, *Randazzo C.*, *Restuccia C.*

Evaluation of Phenolics, and Antioxidant Activity of Different Varieties of Cauliflower (*Brassica oleracea* var. *botrytis*) and their Changes during Conventional and Microwave Cooking

Lupidi G., *Acciarri N.**, *Achilli M.*, *Bramucci M.*, *Marchini C.*, *Amici A.*, *Ferrari V.*

Effect of Hot Air Treatment on Quality and Shelf Life of Minimally Processed Cauliflower

*Miceli A.**, *Vetrano F.*, *Romano C.*, *D'Anna F.*

Optimization of Methods of Myrosinase Activity Determination

*Piekarska A. *, Namieśnik J., Bartoszek A.*

Development of Modified Atmosphere Packages on the Quality of Sicilian Kale (*Brassica oleracea* var. *acephala*) Shoots

*Ragusa L. *, Melilli M.G., Branca F., Argento S., Raccuia A., Licciardello F., Restuccia C.*

Adapting Turnip-Tops and Turnip-Greens to Modern Consumer Habits

*Silva Dias J. *, Almeida A.B., Trindade P.B.*

Glucosinolates Composition in *Brassicaceae*

*Solovyova A.E., Artemyeva A.M. *, Schuetze W.*

Survey of Healthy Compounds in Plant Material from Different Species of *Brassicaceae*

*Tribulato A. * Branca F., Ragusa L., Lo Scalzo R., Picchi V.*

Brassica fruticulosa Cyr. and *B. incana* Ten. (*Brassicaceae*) as Mediterranean Traditional Wild Vegetables: a Valuable Source of Bioactive Compounds

*Tripodi G. *, Verzera A., Dima G, Condurso C., Ragusa S., Raccuia S.A.*

SESSION E - ISATIS WORKSHOP

Nutraceutical Values of Woad (*Isatis tinctoria* L.) Flower Buds of Ecotypes from Sicily, Italy

*Galletti S. *, Bagatta M., Ragusa L., Argento S., Branca F., Iori R.*

Woad (*Isatis tinctoria* L.): an Innovative Crop for the Mediterranean Agro-Industrial System

Guarnaccia P., Pinio M., Branca F., Testa G.

Capturing the Leaf Transcriptome of *Isatis tinctoria* L.

Parkin I., Kagale S., Robinson S.

Natural Indigo from *Isatis tinctoria* L. for the exploitation of old crops

Spagna G.¹, Todaro A.², Palmeri R.^{1}*

SESSION F - AGROMONY

Oral presentations

Agronomical Evaluation of Broccoli Raab Genotypes for Fresh Cut and Processing Products

*Calabrese N. *, Carito A.*

Effect of the Application of Gibberellic Acid at Different Stages of the Late Head Cabbage Rosette on Vernalization and Seed Yield

*Adzic S. *, Prodanovic S., Girek Z., Pavlovic N., Cvikic D., Pavlovic S., Zdravkovic J.*

Cultivars and Fertiliser Strategies Affect the Concentration of Glucosinolates in Curly Kale (*Brassica oleraceae* L. var. *sabellica* L.)

*Grønæk M. *, Kidmose U., Kristensen H.L.*

The *Brassicaceae* Biofumigation System for Plant Cultivation and Defense. An Italian Twenty Year Experience of Study and Application

*Lazzeri L. *, Malaguti L., Cinti S., De Nicola G. R., Ugolini L., Bagatta M., Matteo R., Casadei N., D'Avino L.*

Success and Failures of Biofumigation with Brassicas

*Gilardi G. *, Gullino M. L., Garibaldi A.*

Effect of Compost Application on Qualitative Traits in Cabbage

*Nicoletto C. *, Santagata S., Sambo P.*

Poster presentation

Biological and Productive Characterization of Different *Brassicaceae* Grown in the South of Italy

*Anastasi U. *, Cosentino S., Copani V., Sidella S.*

Strengths and Weaknesses Traits for Brassica Vegetable Crops Production of Turkey

Balkaya A.

Effects of Bio-Fertilizers on Broccoli Cultivars in Organic Farming

Boari F., Calabrese N., Schiattone M.I., Cantore V.

Quality Traits of Some Cauliflower Cultivars Grown in the “Valle dell’Ofanto” Area (Italy) as Affected by Post-Harvest Storage

*Candido V., Galgano F. *, Castronuovo D., Miccolis V., Favati F.*

Effects of Coverage with Nonwoven, and Fertilizer Level on the Wild Rocket in Organic Farming

Cantore V., Pace B., Schiattone M.I., Calabrese N., Boari F.

Characteristics and Seed Production of Sicilian Landraces of Violet Cauliflower

*Chiarenza G.L. *, Alonso Alos M., Argento S., Pinio M., Branca F.*

Seed Production and Plant Characterization of Sicilian Landraces of Broccoli

Chiarenza G.L., Pinio M., Branca F., Cavallaro C.

Growth and Nitrogen Uptake of Two Broccoli Cultivars

Conversa G., Bonasia A., La Rotonda P., Elia A.

Characterization of Different *Brassicaceae* Grown in Southern Italy for Glucosinolates Profile
Copani V., Anastasi U., Ruberto G., Cosentino S., Sidella S.

Phenology, Grain Yield and Quality of Ethiopian Mustard Grown in Southern Italy
Copani V., Cosentino S., Anastasi U., Sortino O., Lazzeri L.*

Effectiveness of Biofumigating *Brassica* Treatments in Greenhouse to Control Root-Knot Nematodes on Tomato and Melon
De Mastro G., D'Addabbo T., Ruta C., Verdini L.*

Morphological and Productive Characterization and Oil Yield of *Brassica napus* Grown in Different Areas in Italy
Del Gatto A., Raccuia S. A., Signor M., Argento S.*

Morphological and Productive Characterization of *Brassica carinata* Grown in Different Areas in Italy
Del Gatto A., Raccuia S.A., Signor M., Melilli M.G.*

Root Length Density and Yield Traits of Broccoli (*Brassica oleracea* L. var. *italica* Plenck) as Affected by Different Techniques of Seedling Growing and Transplanting
Gherbin P., Miccolis V., Candido V.*

Effects of NaCl Salinity on Yield, Quality and Mineral composition of Broccoli and Cauliflower
Giuffrida F., Giurato R., Leonardi C.*

Growth, Gas Exchange and Water Relations in Broccoli and Cauliflower as Affected by NaCl Salinity
Giuffrida F., Scuderi D., Giurato R., Leonardi C.*

Effects of Different Fertilization Levels on *Iberis semperflorens* Pot Culture
Iapichino G., Camerata Scovazzo G.*

Humic Acid Improved Growth and Yield Components of Hydroponic Greenhouse Grown Pak-Choi (*Brassica chinensis*)
Javanmardi J., Sehat M., Zahed M. J.*

Secondary Plant Metabolite Profile and Concentration in Leafy Asian Brassica Vegetable Affected by Organic and Mineral Nitrogen Supply
Krumbein A., Schreiner M., Ruehlmann J.*

Effect of a *Brassica juncea* Cover Crop on a Mono-Succession of Melon
Miceli A., Romano C., Vetrano F., D'Anna F.*

Quality comparison between Organic - and Conventional - Grown Cauliflowers: Results from the 3rd Year of Valorbio Project

Migliori C. *, Lo Scalzo R., Di Cesare L. F., Campanelli G., Ferrari V.

Effect of Anaerobic Digestates Application on Qualitative Traits in Early and Late Cauliflower

Nicoletto C. *, Santagata S., Sambo P.

Characteristics and Seed Production of Italian and Iberian Type of Kale (*Brassica oleracea* var. *acephala*) Grown in Sicily

Ragusa L. *, Chiarenza G.L., Pinio M., Tribulato A., Branca F.

Use of *Brassica napus* L. var. *oleifera* D.C. to Develop Sustainable Strategies in the Phytoextraction

Ruta C. *, Brunetti G., Cassano D., De Mastro G.

Effects of Harvest Time and plant density on Yield and Quality of Chinese Cabbage for Fresh-cut Production

Scuderi D. *, Giuffrida F., Leonardi C.

Response of *Matthiola incana* (L.) W. T. Aiton to Cultivation in Mediterranean Climate

Scuderi D. *, Toscano S., Romano D.

Characterization of the Economic system for Exploiting Sicilian Landraces of Cauliflower and Kohlrabi

Timpanaro G., Branca F., Foti V.T., Di Vita G.

Strong Heterosis in Yield is Seen in Chinese Cabbage F1 Hybrid Cultivars

Tomaru* Y., Kawanabe T., Abe H., Saeki N., Konno S., Shimizu M., Tomita H., Okazaki K., Kaji M., Dennis E.S., Fujimoto R.

Collard Green (*Brassica oleracea* var. *acephala*) Cultivation in Sicily

Vetrano F., Moncada A., Romano C., D'Anna F., Miceli A. *

SESSION G - PESTS AND DISEASES

Oral presentations

Fusarium oxysporum f. sp. and *Xanthomonas campestris* Patovars: Two Important Seed-Borne Pathogens of Wild Rocket Fourth Range Production in the Southern Italy

Sigillo L. *, Senape V., Serratore G., Bravi R.

Why to Study the Concentration of the Ascospores of *Leptosphaeria maculans* and *L. biglobosa*?

Kaczmarek J. *, Brachaczek A., Kasprzyk I., Sulborska A., Karolewski Z., Jędryczka M.

Antibiotic Effect of Glucosinolates and Derivatives on Different *Brassica* Diseases

Velasco P. *, Lema M., Alonso I., Cartea M.E.

Molecular Characterization of Portuguese Isolates of *Leptosphaeria maculans* Using PCR-ISSR and RAPD Markers

Godinho J.S. *, Balesdent M.H., Pereira E. M., Dias J.S.

Poster presentations

Evaluation of Sicilian Wild and Cultivated *Brassica* Genotypes for Resistance to *Xanthomonas campestris* pv. *campestris*

Bella P., Ragusa L., Strano C.P., Tribulato A., Branca F., Catara V.

Feeding Preference and Development of the Cabbage Moth, *Mamestra brassicae* (Lepidoptera: Noctuidae), on Cabbage Varieties

Cartea M.E. *, Soengas P., Lema M., Velasco P.

Study of Induced Systemic Resistance in Cabbage Against *Xanthomonas campestris* pv. *campestris* Following Biological and Chemical Seed Treatment

Desai D., Braun R., Visnovsky S., Pitman A., Christey M. *

The Inheritance of Brown Heart Susceptibility in Swede (*Brassica napobrassica*)

Fadhel F. *, Fuller M., Burchett F., Jellings A.

New Disease of Wild and Cultivated Rocket in Italy

Gilardi G. *, Gullino M.L., Garibaldi A.

Optimal Time of Fungicide Application of Oilseed Rape Against *Sclerotinia* Stem Rot

Kaczmarek J. *, Brachaczek A., Jedryczka M.

Receptor Kinase-Mediated Regulation of Seed Size in *Arabidopsis thaliana*

Gacek K., Marcos J.G.

Occurrence of Blackleg on Rapeseed in China

Li Q., Hu B. *, Rong S., Hou S., Fei W., Jiang Y., Chen F., Wu X., Fan Z., Lei W.

Investigations on the Resistance of Some Processing White Head Cabbage (*Brassica oleracea* L. var. *capitata* subvar. *alba*), Varieties to Root-Knot Nematodes [*Meloidogyne incognita* (Kofoid & White) Chitwood]

Özbakır M., Balkaya A. *, Mennan S.

Effect of Flooding on the Survival of *Leptosphaeria* spp. in Canola Stubble

Peluola C., Fernando W.G.D., Huvenaars C., Kutcher H.R., Peng G. *

Fine Mapping of Race-Specific Blackleg Resistance Genes in *Brassica napus* L.

Tang S.*, Zhao J., Gore S., Ripley V., Wiggins M., Preuss R., Rider K., Ubayasena L., Powers C., Meyer D., Thompson S., Channabasavaradhya C.

SESSION H - CLUBROOT

Oral presentations

Molecular Cloning of Crr1a, a Gene for Resistance to Clubroot Disease (*Plasmodiophora brassicae* Woronin) in *Brassica rapa* L.

Hatakeyama K.*, Suwabe K., Tomita R.N, Kato T., Nunome T., Fukuoka H., Matsumoto S.

Deciphering the Functional Typology of Resistance QTL Through Metabolomics

Gravot A., Wagner G., Lariagon C., Delourme R.*, Manzanares-Dauleux M.J.

Long-term Soil Data Sets Reveal Shifts in Populations of *Plasmodiophora brassicae*

Jonsson A.*, Wallenhammar AC., Dixelius C.

A Molecular Based Seedling Assay System to Study the Germination and Primary Infection by *Plasmodiophora brassicae*

Lewis M.*, Wakeham A., Kennedy R.

Crop Rotation and Cultivar Resistance Are Effective for Control of Clubroot on Canola While *Bacillus subtilis* Biofungicides Are Not

Peng G.*, Lahlali R., Pageau D., Hwang S.F., Hynes R.K., Anderson K., McDonald M.R., Gossen B.D., Strelkov S.E., Turkington T.K., Yu F.Q., Falk K.C., Boyetchko S.M., McGregor L., Hupka D., Geissler J.

Use of Genetics and Genomics Approaches to Fine Map the Clubroot Resistance “CRb” Locus in *Brassica rapa*

Ramchiary N., Im S.B., Piao Z., Lim Y.P.*

QTL Mapping of Isolate-Specific Resistance to *Plasmodiophora brassicae* in *Brassica rapa*

Jing J., Chen J., Zhang T., Piao Z.*

First Glance at the Genome of the Clubroot Pathogen *Plasmodiophora brassicae*

Schwelm A.*, Fogelqvist J., Dixelius C.

Poster presentations

Biological Control of Clubroot (*Plasmodiophora brassicae*) by an Endophytic Fungus

Auer S.

Identification and Mapping of Clubroot Resistance Genes

*Chu M., Yu F., Falk K.C. and Peng G.**

Clubroot Resistance Breeding in Oilseed Rape

Diederichsen E., Frauen M.*

Search for Genetic Resistance to Clubroot in *Brassica* Collection, Interspecific Hybrids and Mutants of *Brassica napus*

Jedryczka M., Wojciechowski A., Olejniczak J., Niemann J., Kaczmarek J.*

Infectious Potential for Clubroot in French Oilseed Rape Fields

Jestin C., Leflon M., Waller F., Pinochet X.*

Induced Resistance is Involved in Suppressing Clubroot on Canola by Several Biocontrol Agents

Lahlali R., Peng G., McGregor L., McDonald M.R., Yu F., Gossen B.D., Hwang, S.F., Hynes R.K., Boyetchko S.M., Geissler J.*

The Effect of Micronutrients on the Germination and Infection of *Plasmodiophora brassicae*

Lewis M., Wakeham A., Kennedy R.*

Occurrence of *Plasmodiophora brassicae* in Finnish Turnip Rape and Oilseed Rape Fields

Rastas M., Latvala S., Hannukkala H.*

Characterization of the RPb1 Gene, Mediating Resistance to *Plasmodiophora brassicae* in *Arabidopsis thaliana*

*Rehn F., Stolz A., Galfe N, Reinhardt S., Mukherjee O., Diederichsen E., Siemens J.**

The New Approach of Clubroot Control (*Plasmodiophora brassicae*) Using Plants Extracts and Catch Crops

Robak J., Gidelska A.*

Testing of Resistance to Clubroot of Interspecific Hybrids Within the Genus *Brassica* Using Hydroponic Cultures

Starzycki M., Jedryczka M., Starzycka E., Kaczmarek J.*

Genetic Variability Among *Plasmodiophora brassicae* Collections From Different Regions in Germany

Strehlow B., Struck C.*

In-Field Distribution of *Plasmodiophora brassicae* Measured Using Quantitative Real-Time PCR and the Influence of Soil Physiochemical Parameters on Disease Development

Wallenhammar AC., Almquist C., Söderström M., Jonsson A.*



BRASSICA2012

Session A

CRUCIFER GENETIC WORKSHOP

Oral presentation

Session A-1

CHARACTERISING DIVERSITY IN THE BRASSICA GENOMES

Keynote lecture

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The genome sequence of an organism provides the basis for gene discovery, the analysis of genetic variation and the association of genomic variation with heritable traits. Genome sequence variation can vary from single nucleotide polymorphisms to presence/absence of large regions or rearrangements. Second generation sequencing technologies and applied bioinformatics tools can provide an unprecedented insight into genome structure and variation, with applications for understanding the evolution of Brassica species and advancing crop breeding strategies.

Advances in data production and bioinformatics capability now make the re-sequencing of complex polyploid genomes routine. This provides the opportunity to expand genomics from gene and molecular genetic marker discovery to developing a broader understanding of the role of diversity in adaptation and selection. This, in turn, enables comparative genomic approaches to truly comprehend the effect of diversity on genome structure and how this impacts on the form and function of organisms, their growth and development, and response to environment, pests and diseases.

The sequencing and re-sequencing of different Brassica varieties has identified genome wide variation. Bioinformatics tools have been produced and applied to interrogate and annotate this abundant data, and genome wide variation has been integrated with genetic maps and phenotypic information. The Brassica genomes, when combined with genome diversity information provide an insight into the evolution of these important crop plants and their wild relatives together with tools to advance breeding of improved varieties with enhanced agronomic traits

The characterisation of diversity across *Brassica* genomes will provide an understanding of recent selection pressures on plant genomes, the genomic components that contribute to adaptation, and the genes that have been the target of selection. This may lead to significant increases in crop production.

Session A-1

BREEDING A DYNAMIC POPULATION OF NEW TYPE BRASSICA NAPUS CONTAINING UNIQUE ELITE GENES FROM OILSEED BRASSICA SUBGENOMES VIA GENOME SELECTION

Keynote lecture

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Various evolutionary driving forces, such as different chromosomal events followed by natural selection in various environments, sexual and geographic isolation, caused the divergence of each of the *Brassica* basic genome (A, B and C) to subgenomes composing different *Brassica* species in which unique genes embodied. Intersubgenomic hybrid oilseed, which were obtained from conventional rapeseeds (*B. napus*, AⁿAⁿCⁿCⁿ, superscripts representing the name of species were introduced to define each subgenome. and new type *B. napus* obtained by intensive introgression of subgenome A^r from *B. rapa* (A^rA^r) and C^c from *B. carinata* (B^cB^cC^cC^c) shows great heterosis potential for oilseed production. A genepool of a new type *B. napus* has been developed and its complexity of genetic structure has been revealed by molecular markers. Hundreds of elite plants have been selected from the pool to cross with more than 100 selected elite lines or commercial hybrids of *B. napus* with various subgenomic introgressions of either A^r, C^c or A^j (from *B. juncea*). SNP-chip/GBS will be applied for genomic selection against the offspring from the crosses, and the genetic effect for key SNP markers has been evaluated with several training populations, both diparental and natural of *B. napus*. A route for breeding a dynamic population of new type Brassica napus containing unique elite genes from all of subgenomes of oilseed Brassicas via genomic selection will be discussed.

Session A-1

THE WILD AND THE GROWN – REMARKS ON *BRASSICA*

Keynote lecture

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Brassica is a genus of the Cruciferae (Brassicaceae). The wild races are concentrated in the Mediterranean area with one species in CE Africa (*Brassica somalensis* Hedge et A. Miller) and several weedy races reaching E Asia. Amphidiploid evolution is characteristic for the genus. The diploid species *Brassica nigra* (L.) Koch (n = 8), *Brassica rapa* L. emend. Metzg. (n = 10, syn.: *B. campestris* L.) and *Brassica oleracea* L. (n = 9) all show a rich variation under domestication. From the naturally occurring amphidiploids *Brassica juncea* (L.) Czern. (n = 18), *Brassica napus* L. emend. Metzg. (n = 19) and the rare *Brassica carinata* A. Braun (n = 17) also some vegetable races have developed. The man-made *Brassica* × *harmsiana* O.E. Schulz (*Brassica oleracea* × *Brassica rapa*, n = 29, n = 39) or similar hybrids serve also for the development of new vegetables. *Brassica tournefortii* Gouan (n = 10) from another *Brassica*-cytodeme, different from the *Brassica rapa* group, is occasionally grown as a vegetable in India. *Brassica* has developed two hotspots under cultivation, in the Mediterranean area and in E Asia.

Cultivation by man has changed the different *Brassica* species in a characteristic way. The large amount of morphologic variation, which exceeded in many cases variations occurring in distinct wild species, has been observed by the classical botanists by adding these variations to their natural species by using Greek letters. Later taxonomists used the category botanical variety (var.). In this way impressive systems have been established, e.g. for *Brassica oleracea*. Later on, the other species followed. The variation from E Asia, particularly in the species *Brassica rapa* and *Brassica juncea*, was much later included into the investigations, simply because of lacking information. But also this material was included, in the last one hundred years, in classifications according to the International Code of Botanical Nomenclature, ICNBN (McNeill et al. 2006). An overview is provided of the infraspecific taxa in *Brassica* vegetable species. This is one possibility to demonstrate the rich variation of the cultivated races. Included here are our experiences with field studies in the Mediterranean area (especially Italy) and E Asia (Korea, China). A short discussion is devoted to the classification of material according to the International Code of Nomenclature for Cultivated Plants, ICNCP (Brickell et al. 2009). Reducing the possibilities for classification largely to cultivars and cultivar groups, this Code is certainly adapted to modern agri- and horticulture, including the seed business. Whereas cultivated plants experience a fast evolution, depending on the speed of breeding progress in different periods of the last less than 10.000 years, starting from the so called Neolithic revolution, wild plants are following usually slower evolutionary pathways over a much longer time span. It is difficult to classify wild and cultivated plants under the same system. But *Brassica* provides material, also showing a complex reticulate evolution difficult to classify, which can help developing ways for a general framework.

Session A-1

RECONSTRUCTING ANCESTRAL GENOME(S) OF THE MESOHEXAPLOID ANCESTOR OF *BRASSICA RAPA* AND TRIBE BRASSICEAE

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Together with 46 other genera is *Brassica* forming a monophyletic tribe Brassiceae comprising altogether about 238 species. Recent genetic, cytogenetic and genomics studies provided compelling evidence for an ancient whole-genome triplication (WGT) event shared by all members of the Brassiceae. The WGT event occurred 5 to 14 million years ago, post-dating the Brassicaceae-specific Alpha whole-genome duplication but pre-dating more recent origins of *Brassica* allotetraploids (e.g., *B. carinata*, *B. juncea*, *B. napus*). Several molecular studies placed the Brassiceae together with three other tribes into lineage II, one of the three well-supported lineages resolved in the Brassicaceae phylogeny. However, genome evolution as well as phylogenetic relationships in lineage II and 18 other tribes treated as expanded lineage II remain far from settled. Recent analysis of the draft genome sequence of *B. rapa* (Wang et al. 2011, Nat. Genet.) revealed three differentially fractioned subgenomes reflecting the mesohexaploid origin of *Brassica*. The available *B. rapa* sequence data and multi-species genome comparisons allowed us to get insight into the structure of the three ancestral subgenomes and subsequently to reconstruct “diploid” ancestral karyotype(s) which participated in the origin of the Brassiceae mesohexaploid progenitor. A general overview of the genome evolution in *Brassica*, the Brassiceae and closely related tribes will be given.

Session A-2

SNP-BASED HIGH DENSITY MAP OF *BRASSICA NAPUS* L.

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Brassica napus L., an allotetraploid (AACC, $2n = 4x = 38$) formed from the diploid *B. rapa* (AA, $2n = 2x = 20$) and *B. oleracea* (CC, $2n = 2x = 18$), is the most important vegetable oilseed crop in the world. Single nucleotide polymorphism (SNP) is the most useful marker for genetic diversity study, genetic mapping, association mapping and molecular breeding because of its abundance in the genome and amenability for high throughput genotyping. Research has indicated that 18,000-30,000 SNPs with even genome distribution are needed in order to efficiently identify the genes/loci for important traits through precision mapping and association mapping in major crops like *B. napus*. In this poster, we describe the construction of a high density consensus map for *B. napus* based on the SNPs identified at Dow AgroSciences (DAS) using different genome complexity reduction and next-generation sequencing technologies. Due to the low polymorphisms (10-25%) among modern canola lines, we developed and obtained eight di-haploid (DH) populations, which can map more than 90% of the validated SNP markers at DAS, for consensus map construction. By now, a total of 6,300+ SNP markers have been mapped in four of the eight DH populations, and a consensus map of 2,559.2 cM has been constructed for trait mapping, gene isolation and molecular breeding at DAS.

Session A-2

MEIOTIC SEGREGATION IS INITIATED BY RECENT AND ANCIENT HOMOELOGOUS REGIONS IN THE HAPLOID GENOMES OF *BRASSICA* INTERSPECIFIC HYBRIDS

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The *Brassica* A, B and C genomes diverged from a common hexaploid ancestor, as evidenced by whole genome triplication in these diploidised genomes that have subsequently been reshaped through chromosome rearrangements. Relatively recent polyploidy events have also brought the three genomes together in pairwise combinations: *B. juncea* ($2n = AABB = 36$), *B. napus* ($2n = AACC = 38$) and *B. carinata* ($2n = BBCC = 34$). Therefore, these allotetraploid species possess genomes containing regions of both ancient and recent homoeology. In previous cytogenetic research, it was observed that meiotic chromosome pairing occurred between regions of recent A, B and C genome homoeology and within each of the A, B and C genomes (presumably between regions of ancient genome triplication). However, little direct evidence has been obtained for genetic recombination between mapped regions of recent or ancient homoeology. We used two experimental populations derived from the crosses (*B. juncea* × *B. napus* ($F_1: AABC$)) × *B. carinata* ($BBCC$) and (*B. juncea* × *B. carinata* ($F_1: BBAC$)) × *B. napus* ($AACC$) to investigate meiotic segregation and recombination between and within the *Brassica* A, B and C genomes. Essentially normal homologous pairing and recombination occurred between the A genomes of *B. juncea* and *B. napus* in the $AABC$ × *B. carinata* population, with few non-homologous associations observed. However, molecular karyotyping of the $BBAC$ × *B. napus* population revealed very high frequencies of A-C association in regions of known recent homoeology, as well as A-A and C-C associations between regions of ancient homoeology. These results indicate that regions of homology, recent homoeology and ancient homoeology in the A, B and C *Brassica* genomes all retain sufficient similarity to allow meiotic pairing and segregation in interspecific *Brassica* hybrids.

Session A-3

EXPLORING THE BRASSICA B GENOME AT THE WHOLE GENOME SEQUENCE LEVEL

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Progenitors of the three Brassica diploid species, *B. rapa* (A genome), *B. oleracea* (C genome and *B. nigra* (B genome) form the apices of U's triangle, and together with the amphidiploid species formed from their pairwise hybridization, encompass all economically important Crucifereae crops. *Brassica nigra* although perhaps the oldest recorded Brassica species, with references from 3000 BC, has been neglected, especially with respect to breeding improvements, largely due to its use as a mustard crop being usurped by *B. juncea*, since *B. nigra* could not be easily mechanically harvested. As well as representing effectively half the genome of the important mustard crop *B. juncea*, the B genome harbours favourable alleles for a number of important agronomic traits, specifically resistance to abiotic and biotic stresses. Although closely related at the gene sequence level there has been limited success in transferring useful traits from the B genome into the A or C, the reasons for which are still debated. We have developed a whole genome sequence for *B. nigra*, combining the high sequence coverage of Illumina next generation sequencing (NGS) with the improved scaffolding offered by large insert Roche 454 mate-pair libraries. The current assembly covers 452 Mb, an estimated 71% of the predicted 632 Mb genome and has been linked to the genetic map with high density sequence tag mapping. We will present the results of comparative genome analysis between the three Brassica diploid genomes, which offers insights into the unique nature of the Brassica B genome and its potential for furthering our knowledge of Brassica genome evolution.

Session A-3

IDENTIFICATION OF RESISTANCE LOCI TO *VERTICILLIUM LONGISPORUM* IN ARABIDOPSIS AND BRASSICA

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Verticillium longisporum is a soil-borne fungus infecting cruciferous hosts. It penetrates host roots, grows towards the xylem and spreads within the xylem vessels to colonise the whole plant. Infected plants show a complex pattern of disease symptoms and developmental reactions, incl. premature seed ripening, chlorosis or stunting. *Brassica* species as well as *Arabidopsis thaliana* are hosts of *Verticillium longisporum*. This study aims at the identification of genes controlling disease parameters and implications host development.

We identified host accessions in *Arabidopsis* and *Brassica alboglabra* that differed strikingly in their susceptibility to *V. longisporum* and used these as parents for mapping experiments. In *B. alboglabra*, a closely related species of *B. oleracea*, we could identify 2 major QTL that explained significant proportions of the variation for the degree of fungal colonisation and AUDPC in F3 families. QTL for the control of different stunting parameters showed some overlap with QTL for AUDPC or colonisation. Two major QTL controlling differences in developmental velocity were identified that did not show interdependence to disease resistance. As for *B. alboglabra*, fungal shoot colonisation was the most reliable resistance parameter in repeated experiments with *Arabidopsis*. Resistance to *Verticillium*-induced stunting was inherited more independent from resistance to shoot colonisation than in *B. alboglabra*. QTL for colonisation resistance did show linkage with QTL that controlled development or with the morphological marker *erecta*. Fine-mapping of different disease parameters in the *erecta* region in near-isogenic lines revealed a close linkage of loci controlling either stunting or colonisation resistance.

QTL and candidate genes from *Arabidopsis* will be used to identify homologs/ orthologs in *B. alboglabra* and to check for co-localization with QTL that were identified in this species. Cloning of genes conferring either colonisation resistance or stunting resistance from *Arabidopsis* will allow the functional analysis of both types of resistance.

Session A-3

HOW HOMOLOGOUS RECOMBINATION IS BOOSTED IN OILSEED RAPE

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Selection and population bottlenecks have reduced the genetic variability within oilseed rape (*Brassica napus*, AACC, 2n=38) varieties whereas a large genetic diversity is available among its progenitors (*B.rapa*, AA, 2n=20 and *B.oleracea*, CC, 2n=18). We previously showed that it is possible to tap into the genetic diversity of *B.rapa* by boosting the homologous recombination between A genomes in AAC hybrids. This boost may be driven either by the number of C chromosomes remaining as univalents in meiosis or by the nature of these chromosomes. To test these alternative hypotheses, we produced AA hybrids carrying in addition 0, 1, 3, 6 or 9 univalents. The nature of C chromosomes was determined by molecular markers and cytogenetic controls which showed a meiotic behaviour close to the one expected. The genetic map lengths for each hybrid were established and then compared to that of the control AA without C chromosomes in addition. These comparisons revealed that the recombination rates were 1.4 and 2.7 times higher in the hybrids carrying C6 or C9 alone than in the control. Furthermore, this enhancement factor reached 3.1 and 4.1 in hybrids carrying 6 and 9 C chromosomes. Overall, the presence of C9 chromosome was the main factor affecting the recombination rate. Interestingly, we found that its presence was also the main factor reducing crossover interference. The consequences of such results for breeding programmes and gene cloning will be presented.

Session A-3

GENETIC ARCHITECTURE OF FLOWERING TIME VARIATION IN BRASSICA RAPA; A GENETICAL GENOMICS APPROACH

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The present study aims to understand and to identify gene networks involved in flowering time and identifying genes underlying flowering time QTL by a genetical genomics approach. We constructed a genetic map of DH68 (Yellow Sarson x Pak Choi) based on *B. rapa* homologues of *A. thaliana* flowering time genes. These markers connect the physical and genetic *B. rapa* maps which is a prerequisite for genetical genomic studies. The DH68 population was used to identify flowering time QTL (fQTL) under different environments and in different years. The same population was profiled for the whole genome transcript variation. A genome wide eQTL analysis was performed and gene networks in flowering time were constructed. The combined data allowed us to prioritize a small number of flowering time candidate genes for further study on functional roles. A major regulatory role for BrFLC2 is illustrated.

Session A-3

QUANTITATIVE TRAIT LOCI MAPPING IN *BRASSICA RAPA* REVEALED THE STRUCTURAL AND FUNCTIONAL CONSERVATION OF GENETIC LOCI GOVERNING MORPHOLOGICAL AND YIELD COMPONENT TRAITS IN THE A, B, AND C SUB-GENOMES OF *BRASSICA* SPECIES

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Brassica rapa is an important crop species that produces vegetables, oilseed, and fodder. Although many studies reported quantitative trait loci (QTL) mapping, the genes governing most of its economically important traits are still unknown. In this study, we report QTL mapping for morphological and yield component traits in *B. rapa* and comparative map alignment between *B. rapa*, *B. napus*, *B. juncea*, and *Arabidopsis thaliana* to identify candidate genes and conserved QTL blocks between them. A total of 91 QTL were identified in different crucifer blocks of the *B. rapa* genome. A few blocks contained more QTL than other duplicated paralogous blocks. Through synteny analysis with *A. thaliana*, *B. rapa* candidate genes and intronic and exonic single nucleotide polymorphisms in the parental lines were detected from whole genome re-sequenced data, a few of which were validated by mapping them to the QTL regions. Comparative mapping identified 5 key major evolutionarily conserved crucifer blocks (R, J, F, E, and W) harboring QTL for morphological and yield components traits between the A, B, and C sub-genomes of *B. rapa*, *B. juncea*, and *B. napus*. The information for these candidate genes could be used for breeding *B. rapa* and other related species.

Session A-3

IDENTIFICATION AND CHARACTERISATION OF CANDIDATE BLACKLEG RESISTANCE GENES IN *BRASSICA NAPUS* USING NEXT GENERATION SEQUENCING

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A thorough understanding of the relationships between plants and pathogens is essential if we are to continue to meet the agricultural needs of the world's growing population. The identification of genes underlying important quantitative trait loci (QTL) is extremely challenging in complex genomes such as *Brassica napus* (canola, oilseed rape or rapeseed). However, recent advances in next generation sequencing (NGS) enable much quicker identification of candidate genes for traits of interest. Here we demonstrate this with the identification of candidate disease resistance genes from *Brassica napus* for its most devastating fungal pathogen, *Leptosphaeria maculans* (blackleg fungus). These two species are locked in an evolutionary arms-race whereby a gene for gene interaction confers either resistance or susceptibility in the plant depending on the genotype of the plant and pathogen. Preliminary analysis of the complete genome sequence of the diploid progenitor of *B. napus*: *B. rapa*, identified numerous candidate CC-NBS-LRR and TIR-NBS-LRR domain protein encoding genes, several of which were clustered around a region syntenic with major loci for blackleg resistance in *B. napus*. Molecular analyses of the candidate genes using *B. napus* NGS data are presented and the difficulties associated with identifying functional gene copies within the highly duplicated *Brassica* genome are discussed.

Session A-3

GENOME SEQUENCING AND ANALYSIS OF THE EMERGING OILSEED CROP *CAMELINA SATIVA*

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Camelina sativa (false flax), a novel oilseed crop with several desirable agronomic and oil-quality attributes, is being developed as an industrial oil platform crop. Camelina seed oil holds great potential to be a viable and sustainable feedstock for biofuel and bio-lubricant production, thus having significant economic impact. However, currently limited genomic or genetic resources are available for this emerging oilseed crop. To enable genomics-assisted breeding for further improvement of Camelina, we have assembled a draft genome sequence of a doubled haploid line (DH55). To achieve this, we generated 170 Gb of raw sequence using Illumina HiSeq2000 and Roche pyrosequencing platforms. A total of 80 Gb high quality sequence, equivalent to ~100-fold genome coverage, was assembled resulting in 41,000 scaffolds with an N50 size of 946 Kb spanning 620 Mb, representing 78% of the estimated 790 Mb genome of Camelina. High density sequence tag mapping in a segregating population is being utilized to assist with aligning the genome sequence to the genetic map and to construct chromosome scale pseudomolecules. Whole plant transcriptome based on RNAseq data derived from multiple tissues collected from various stages throughout the life cycle of Camelina is being characterized to help assist in the genome annotation process. Comparative bioinformatics analysis of the Camelina draft genome with other sequenced crucifer genomes (*Brassica rapa* and *Arabidopsis*) has revealed striking conservation of gene synteny and provided evidence for a whole genome triplication event. It is evident from preliminary analyses that the proportion of genes retained in each of the subgenomes within Camelina is higher than that observed in *Brassica rapa*, suggesting that Camelina may have undergone a relatively recent polyploidization event. The genome sequence of Camelina is expected to enrich the sequence resources of Brassica genomes and provide novel insights into the evolution of polyploid genomes within Brassicaceae.

Session A-3

IDENTIFICATION OF QTLS DETERMINED BLACK ROT RESISTANCE AND MORPHOLOGICAL CHARACTERS IN *BRASSICA RAPA* L.

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Here we report on quantitative trait loci (QTL) analysis of 43 morphological and phenological traits (flowering time, growth-related traits, leaf, seed, flower and fruit traits), and black rot resistance traits in *B. rapa* using two DH mapping populations (DH38: PC175 x YS143 and DH30: VT115 x YS143) resulted from crosses between Yellow sarson, Pak choi, and Vegetable turnip accessions, obtained from Wageningen University. Plants of DH populations have been grown in the field and greenhouse conditions in St.-Petersburg area, Russia, during three years. For flowering time 3 QTLs for DH30 and 7 for DH38 were detected, 4-5 QTLs for growth-related traits, 12-15 for leaf traits, 12 QTLs for flower, seed, fruit traits. Principal component analysis and co-localization of QTL indicated that some components of the genetic control of leaf and growth-related traits and of flowering time might be the same. Evaluation of resistance of *B. rapa* DH lines to 6 races of black rot, caused by *Xanthomonas campestris* and *X. arboricola*, determined large differences between lines. QTL analysis allowed obtained for DH30 five SSR markers linked to resistance for five races of pathogen in linkage groups 3, 7, and 9. For DH38 it is obtained 16 markers linked to resistance for six races in R01, R02, R03, R04, R05, R06, R08, and R09. The most important loci of resistance in two populations are located in R03 and R06. For the first time in Russia genetic components (QTLs) determined valuable characters in *B. rapa* were established.

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BRASSICA2012

Session A

CRUCIFER GENETIC WORKSHOP

Poster presentation

Poster A-1

AGROBACTERIUM MEDIATED TRANSFORMATION AND INTEGRATION OF STRESS GENES INTO CAULIFLOWER (BRASSICA OLERACEA VAR. BOTRYTIS L.)

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Cauliflower is member of the cruciferaceae family and cultivated all over the world as in important culinary vegetable with associated health promoting properties. It is however, easily damage by abiotic stresses (salinity, drought and frost) during its growth. Ascorbate peroxidase (APX) is the enzyme implicated in the resistance of plant to free radicals and overexpression of these can confer resistance abiotic stresses such as salinity, drought and frost. In the last 2 decades, dramatic progress has been made in the application of recombinant DNA technology, including direct gene transfer to plants. The Agrobacterium mediated transformation system has proven to be the most commonly used protocol for the introduction of new genes and expressed in Brassica plants. This study is efforts to transfer constitutively expressed APX gene into cauliflower by Agrobacterium mediated method.

Poster A-2

STRUCTURAL AND FUNCTIONAL DIVERGENCE OF THE ABI1 HOMEOLOGOUS GENES IN *BRASSICA NAPUS*

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Whole-genome duplication as result of the paleopolyploidization process and the extensive chromosomal rearrangements have been confirmed by numerous studies in the Brassica species. A consequence of this process is duplication of individual genes and hence their large proportion are members of multi-gene families. Despite the sequence homology, these genes often exhibit significant differences in the expression patterns in effect of their sub- or neofunctionalization process. Understanding of the evolution and functional divergence of duplicated genes is significant for genetic improvement of crops and formation of new phenotypes. In this study we intended to determine mechanisms of structural and functional divergence of the detected ABI1 homeologous genes arising from polyploidy in *Brassica napus*. The ABI1 gene encodes protein phosphatase 2C and belong to PP2C family, group A. It is a key component and repressor of the abscisic acid (ABA) signaling pathway involved in the regulation of multiple signaling pathways through reversible phosphorylation of proteins. In Arabidopsis ABI1-encoding gene was identified as unique gene, but is represented by 6 copies in the allotetraploid *B. napus* genome. We identified and initially characterized 5 out of 6 ABI1 homeologs in *B. napus*. Based on sequence analysis, we determined their structure and genome origin (A or C genome). Although, a high sequence and structure similarity level among these ABI1 homeologs is observed, they exhibit changes in the expression profiles in different tissues, stages of development and in response to the abiotic and biotic stresses. One of major mechanism regulating the expression of duplicated genes (except changes in DNA sequence) are cis-acting effects. Therefore, we initiated the detailed analysis of gene expression regulation by using promoter-GUS and -GFP fused constructs. The promoter activity of 2 homeologs with GUS and GFP assays is analyzed in transgenic *B. napus* and *A. thaliana*. Additionally, in preliminary analysis, *in silico* identification of potential cis-regulatory elements in promoter regions of all ABI1 homeologs was carried out. The selected aspects of this studies will be discussed.

Poster A-3

BRASSICA RAPA GENOME ASSEMBLY V2.0: AN IMPROVED GENOME OF BRASSICA RAPA

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We updated the *B. rapa* genome from V1.0 to V2.0. In the new assembly V2.0, 150bp paired-end reads were generated to assemble the contigs by using a Kmer size of 91. To improve scaffolding, we further sequenced 2.6Gb and 3.2Gb libraries with the insert size of 40kb and 20kb, respectively. The *B. rapa* genome v2.0 reached a scaffold N50 ~1.2Mb, which was much improved from the version 1.0 (scaffold N50 ~348Kb). The assembled size of version 2.0 was ~370Mb, about 76% of the predicted 485Mb *B. rapa* genome size and 85Mb more than the version 1.0. We used also mRNA-seq data from different organs and different developmental stages of *B. rapa* to improve the prediction of gene models. In totally, we annotated 48,266 genes in this updated genome, which was 7,092 more than that in the version 1.0.

Poster A-4

QTL ANALYSIS OF MORPHOLOGICAL TRAITS USING DIFFERENT POPULATION IN CHINESE CABBAGE (*BRASSICA RAPA* SSP. *PEKINENSIS*)

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Plant morphology is one of the main characters in breeding of leafy vegetable. A QTL analysis was performed to identify the genetic regions for responsible plant morphological trait. A total of 32 traits such as heading habit and leaf related trait were investigated in two different populations, DH/RIL population, with year replication. Transgressive segregations were observed for most of the traits in two populations. A total of 215 QTLs having the LOD thresholds decided by applying permutation (1000 repetitions) test were detected in DH population and 63 QTL regions affecting 28 morphological traits represent at least over two times. Twenty two common traits which were measured in DH population were investigated in RIL population. A total of 98 QTLs affecting 19 traits were distributed in 10 linkage groups. A total of 13 QTLs belongs to 8 traits were represented by repetition experiment at least over two times. In comparison of the results, QTLs by different population type and seasons, we identified 26 consensus QTLs and candidate genes corresponding to traits. These robust QTL results provide useful information for further research and breeding.

Poster A-5

CROSSES OF RAPESEED-RADISH DISOMIC ADDITION LINE *D* WITH *BRASSICA RAPA* L. TO EVALUATE THE EFFECT OF THE RADISH CHROMOSOME IN PAKCHOI BACKGROUND

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A complete set (*a-i*) of disomic rapeseed-radish chromosome addition lines ($2n=38+2$) was described by Budahn et al. (2008). Some radish traits which may be interesting for *Brassica rapa* should be transferred using the set of rapeseed-radish addition lines as a bridge between radish and *B. rapa*. Gene *HsI^{Rph}* providing resistance against beet cyst nematode, *Heterodera schachtii* (Schmidt), was mapped on radish chromosome *d*.

In this study, the rapeseed-radish addition line *d* was used, as female parent, for crosses with *Brassica rapa*. Pakchoi was used as pollen donor in the crosses/backcrosses after emasculation of the female parent. F₁ hybrids and the progenies from backcrosses were identified by morphological investigation, cytological and molecular marker techniques. The presence of alien radish chromosome *d* was tested by chromosome specific SSR markers developed by Hashida et al. (poster this conference). Only individuals with the alien radish chromosome were used for the further backcrosses. Finally 15 individuals which contained radish chromosome *d* were obtained from BC₃ generation. Further cytological studies should be carried out to confirm elimination of the C-genome chromosomes before stabilization of Pakchoi-radish chromosome addition by self pollination. Transfer of the radish chromosome *d* should be a model system for the transfer of all other radish chromosomes with traits of great importance for *B. rapa*.

Poster A-6

TRANSCRIPT ANALYSIS OF GENES RELATING TO SEED OIL CONTENT IN *BRASSICA NAPUS* L.

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Rapeseed (*Brassica napus* L.) is one of the most important oil crops in the world. To improve the quantity of oil in seeds, basic and applied studies have been attempted. In oil content of seeds, a large genetic variation has been known in rapeseed. We performed microarray analysis in developing seeds between different oil content lines (high and low oil content) to identify the genes contributing to differences of oil contents. Consequently, 220 genes showed more than 3-fold higher expression in common to the two high oil lines (144, CN71) compared with low oil line (96). And also, it was revealed that many genes relating to the acyl-lipid metabolism of *Arabidopsis thaliana* showed higher expression in the high oil content lines. In the present study, we examined transcript expression levels of identified genes in developing seeds of different oil content lines in rapeseed.

For expression analysis, genes, which showed higher expression in high and/or low oil content lines and related to lipid metabolism, were selected. Transcript expression of these genes was analyzed in the developing seeds collected at 20, 30, 40, 50, 60 and 70 DAP.

Ten highly expressed genes in high and/or low oil content lines including 2 genes of unknown function were tested. Of these genes, 3 genes showed higher expression in high oil content lines during early stages of seed development (30-40 DAP). Ten highly expressed genes relating to lipid metabolism, which consist of 6 genes in plastid and 4 genes in endoplasmic reticulum, were also tested. Four genes showed higher expression in high oil content lines during seed development. These results suggested that genes selected from microarray analysis might be related to the difference of oil contents in seeds.

Poster A-7

MAPPING ROOT AND FLOWERING TIME QTL IN *BRASSICA NAPUS*: EVIDENCE FOR PLEIOTROPY

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Despite their obvious role in sensing and responding to the soil environment, the biology of plant roots remains a neglected topic of research. Clearly, an understanding of the variation that exists at phenotypic and genotypic levels benefits basic understanding and breeding efforts aimed at improving crop production. Here, we sought to understand the genetic architecture of the quantitative trait, root pulling force (RPF; the vertical force required to remove a plant from the soil), and its relationship to other agronomically relevant traits. Our field experiments in 2009 and 2010 were grown under irrigated and non-irrigated treatments utilizing a doubled haploid (DH) population derived from a cross between an annual and a biennial inbred variety. We measured: days to flower (DTF), wet shoot biomass, root diameter, seed yield and RPF and performed QTL analyses of these traits in the R/qtl software environment using our genetic linkage map constructed from 397 markers. We found a strong genetic correlation between DTF and RPF under both irrigated and non-irrigated conditions ($r_g = 0.66$ ($p < 0.0001$) and $r_g = 0.49$ ($p < 0.0001$), respectively). We also found that 2 major QTL for each trait co-localized on chromosomes N10 and N12, suggesting pleiotropy. Each QTL covers a genomic interval in which a paralog of the major flowering time gene *FLC* is expected to be located. Subsequent analyses of RPF using QTL models which condition for DTF found that the QTL on N10 remained significant and may suggest that the transcription factor *FLC* is acting directly on some aspect of root morphology affecting RPF rather than solely through its effect on DTF. These results provide us with a greater understanding of the complexity of plant development and evidence for the involvement of flowering time genes beyond simple induction of anthesis.

Poster A-8

ISOLATION AND EXPRESSION ANALYSIS OF AN *OGUCMS*-RELATED AT-HOOK DNA BINDING PROTEIN, BOMF2, IN CABBAGE FLOWERS

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AT-hook DNA binding proteins play an important role in regulating cell-specific gene expression. One of the AT-hook DNA binding protein, BoMF2 is a novel type of transcription factor that regulated gene expression through chromatin modification in stamen development and critical for OguCMS in *Brassica oleracea*. It was cloned by using a up-regulated expression EST sequence in OguCMS cabbage stamens as a querying probe. The full length cDNA of *BoMF2* was deduced as 1025bp with a 5'-UTR of 71 bp and 3' UTR of 171bp. The open reading frame (ORF) comprises 783bp, encoding a putative protein of 260 amino acids (molecular weight of 27.20KD and an isoelectric point (pI) value of 9.550). Phylogenetic analysis revealed that the *BoMF2* was closely related to At2g42940(AHL16) in *Arabidopsis*. Detailed real time RT-PCR illuminated it was preferentially expressed in cabbage anther, had an expression peak in tetrad to early microspore stages of anther development and up-regulated 12.4 times in OguCMS cabbage flower buds. Based on onion epidermis cells transient expression assays with plasmids pA7-BoMF2-GFP, We confirmed that BoMF2 appeared to be localized in the nucleolus. In general, these results indicated that BoMF2 is a novel AT-hook DNA binding protein that may be involved in anther development and play an important role in the anther abortion of *OguCMS*.

Poster A-9

CHROMOSOMAL ASSIGNMENT OF RADISH LINKAGE GROUPS USING A COMPLETE SET OF DISOMIC RADISH-RAPESEED CHROMOSOME ADDITION LINES

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Radish, *Raphanus sativus* L. ($2n = 18$), is a vegetable with high economic importance, especially in Asia. It is used also as fodder and oil crop. Some linkage maps of radish were published, but never assignment of linkage groups to radish chromosomes was possible. Budahn et al. (2008) described a complete set of disomic radish-rapeseed chromosome addition lines (*a-i*) offering the chance to anchor genetic maps. Nakatsuji et al. (2011) developed a radish map with 336 markers on nine linkage groups with a total length of 672 cM.

19 SSR- and 1 STS marker (at least two for each linkage group) were assigned to the radish chromosomes. No incongruence between the linkage groups and the assigned chromosomes was detected. Additionally three of the nine radish chromosomes (*c*, *d* and *h*) were characterized cytogenetically by fluorescence in situ hybridization (FISH) using 5S- and 18S/25S-rDNA probes. These tools have also been used to assign the linkage groups of two other radish maps. The anchored linkage map should be used further for QTL analysis of agronomically important traits.

Poster A-10

MORPHOLOGICAL VARIATION OF RESYNTHESED *BRASSICA NAPUS* AFTER INTERSPECIFIC HYBRIDIZATION

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Brassica napus (AACC, 2n=38) originated from interspecific hybridization of *B. rapa* (AA, 2n=20) and *B. oleracea* (CC, 2n=18) followed by the amphidiploidization (AC, 2n=19) of the F₁ hybrid through spontaneous chromosome doubling. Generally *B. napus* is more productive than *B. rapa* but its duration is much longer compared to *B. rapa*. In Bangladesh, short duration rapeseed varieties are advantageous to grow as a catch crop between two major rice growing seasons (Aman and Boro rice). To develop short duration *B. napus* varieties that fit in Bangladesh's cropping system, short duration varieties of *B. oleracea* (*B. alboglabra* and CrGc) and *B. rapa* (Tori-7 and BARI Sarisha-14) were crossed to produce resynthesized *B. napus* plants. Chromosome doubling was confirmed by root-tip chromosome counting. Out of 1681 crosses, 17 hybrids have been developed through ovary culture where cross-ability [(No. of plants obtained/ No. of flowers pollinated) × 100] ranged from 0.0 to 1.4 % among five different cross combinations. Using *B. oleracea* as a female parent produced more F₁ hybrids compared to the reciprocal crosses. Different leaf types (*B. rapa*, *B. oleracea* and mixed types) and flower colors (yellow and white) were found in the resynthesized *B. napus* individuals. Plant heights varied from 34 cm to 93 cm and pollen fertility ranged from 34 to 96%. The F₂ generation showed distinguishable variation in days to flowering (44 to 61 days). Between the F₁ and the F₂ generations, pollen fertility was found similar in the same line and variation in plant heights was higher in the F₂. Further research is to investigate characteristics of the F₃ generation.

Poster A-11

RADISH GENETIC MAP CONSTRUCTED BY HIGH-DENSITY EST-BASED MARKERS WITH SCAFFOLD SEQUENCES

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Radish (*Raphanus sativus* L. n=9) is one of the major vegetative crops in Asia. Radish called 'Daikon' has some unique morphological or physiological traits. To construct a genetic linkage map, we designed mainly expressed sequence tag (EST)-based single nucleotide polymorphism (SNP) markers, and genotyped F₂ plants obtained by crossing of inbred line 'Aokubi' as a root thickening type and inbred line 'Sayatori' as a non-thickening type by dot-blot analysis. Nine hundred and sixty-five dot-blot SNP markers and 46 SSR markers were assigned to nine linkage groups with a total length of 844.5 cM. By BLASTN, most sequences of the EST-SNP markers were found to have homologous genes in *Arabidopsis thaliana*. Since Shirasawa et al. (2011) constructed a radish genetic map with 843 DNA markers including EST-SSR and RAPD, the current map and the previous one were integrated to form a high density genetic linkage map with about 1,600 markers using 120 consensus EST-SSR markers. Additionally, genomic DNA of inbred line 'Aokubi' was sequenced by a Next Generation Sequencer, Illumina GAIIX. One hundred thirty-nine Gb data were assembled into 265,257 scaffolds, in which the longest size was 147 kb and N50 was 13.9 kb. Assembled sequences, 309 Mb, covered half of expected genomic size of radish. More than 1,500 (97%) DNA markers in the integrated map were contained in the scaffolds. Comparison of the genome structure of *R. sativus* and the A, B, and C genomes of *Brassica* species is in progress and will be discussed.

Poster A-12

GENOME-WIDE IDENTIFICATION, CLASSIFICATION AND EXPRESSION ANALYSIS OF GENES ENCODING PUTATIVE FASCICLIN-LIKE ARABINOGALACTAN PROTEINS IN CHINESE CABBAGE (*BRASSICA RAPA* L.)

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Fasciclin-like arabinogalactan proteins (FLAs), a subclass of arabinogalactan proteins (AGPs), have both predicted AGP-like glycosylated regions and putative fasciclin (FAS) domains, which are suggested to function in cell adhesion and communication. Previous studies have identified 21, 27, and 34 FLAs in *Arabidopsis* (*Arabidopsis thaliana*), rice (*Oryza sativa*), and wheat (*Triticum aestivum*), respectively. In this study, we identified 33 FLAs in the annotated genome of Chinese cabbage (*B. rapa* ssp. *pekinensis* line Chiifu-401-42). Sequence analysis indicated that FAS domains each contain two highly conserved regions, named H1 and H2, and that 17 FLAs from *B. rapa* (BrFLAs) possess both of these regions. Prediction of glycosylphatidylinositol (GPI) modification sites suggested that 15 BrFLAs were GPI-anchored to the plasma membrane. Additionally, 25 BrFLAs may have been duplicated during the processes that shaped the triplicated genome of the mesopolyploid *B. rapa*. Expression analyses indicated that *BrFLA1*, *BrFLA11*, *BrFLA13*, *BrFLA28* and *BrFLA32* were specifically expressed in inflorescence. Meanwhile, *BrFLA9* (homologous to *AtFLA12*) is specifically expressed in stem, and *BrFLA6/22* (homologous to *AtFLA11*) is also highly expressed in stem, suggesting *BrFLA6/9/22* may have the same functions as *AtFLA11/12* in *A. thaliana*. The identification and bioinformatic analysis of FLAs in *B. rapa* lays a foundation for studying their biological functions in plant growth and development as well as evolutionary history of this gene family in *A. thaliana* and *B. rapa*.

Poster A-13

THE MESOPOLYPLOID HISTORY OF AUSTRALIAN AND NEW ZEALAND CRUCIFERS

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Whole-genome duplication (WGD) events, cyclically increasing the genetic diversity of angiosperm plants, are at different rates followed by genome fractionation toward diploid-like genomes. During recent years, several *bona fide* diploid species were shown to be ancient polyploids. The incidence and age of WGD events, as well as the extent of subsequent genome diploidization vary widely between angiosperm phyla.

Due to the feasibility of comparative chromosome painting (CCP) in the Brassicaceae, we could reconstruct the fate of a mesopolyploid WGD event in “diploid” Australian and New Zealand crucifer species. CCP analysis showed that genomes of endemic Australian species (*Stenopetalum nutans*, *S. lineare*, and *Ballantinia antipoda* with $n=4$, 5, and 6, respectively) descended from the eight ancestral chromosomes (AK1-AK8) through an allopolyploid WGD. Nuclear and maternal gene phylogenies corroborated the hybrid origin of the mesotetraploid ancestor and suggest that the WGD occurred - 6 to 9 million years ago (mya). Similarly, the ancestral genome of ten New Zealand *Pachycladon* species (all with $n=10$) originated through a mesopolyploid event involving two genomes with the eight ancestral chromosomes, 1 to 2 mya. Both, presumably independent, WGD events were followed by taxon-specific reductions of chromosome number, with less extensive inter-genomic reshuffling in the evolutionary younger *Pachycladon* ancestor. Inter-genomic rearrangements and chromosome “fusions” were mediated by inversions, whole-arm translocations, and by end-to-end translocations associated with centromere inactivation and/or loss. Our data underline the importance of multiple whole-genome duplication events in the angiosperm genome evolution, elucidate diploidization mechanisms transforming polyploid genomes into “diploid” ones, and demonstrate that chromosome number per se is not a reliable indicator of ploidy level.

Poster A-14

WHOLE-GENOME TRIPLICATION AND SPECIES RADIATION IN THE SOUTHERN AFRICAN TRIBE HELIOPHILEAE (BRASSICACEAE)

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The unigeneric tribe Heliophileae includes 90 *Heliophila* species, all endemic to southern Africa. The tribe is morphologically the most diversified Brassicaceae lineage in every aspect of habit, foliage, flower, and fruit morphology. Despite this diversity, virtually nothing is known about its origin and genome evolution. Here we present the first in-depth information on chromosome numbers, rDNA *in situ* localization, genome structure, and phylogenetic relationship within the Heliophileae. Chromosome numbers in 27 *Heliophila* species range from 2n=16 to 2n=88, but 2n=20 and 22 prevail in 77% of species. Chromosome-number variation largely follows three major lineages (A, B, and C) resolved in the ITS phylogeny. The B-clade species possess mostly 2n=20, while 2n=22 is the dominating number in the C-clade ones. The numbers and positions of 5S and 45S rDNA loci vary between species and cannot be employed as phylogenetically informative characters. Seven species with different chromosome numbers and from the three ITS clades were analyzed by comparative chromosome painting. In all species analyzed, 90% of painting probes unveiled three homeologous chromosome regions in *Heliophila* haploid chromosome complements. These results suggest that all *Heliophila* species, and probably the entire tribe Heliophileae, experienced a whole-genome triplication (WGT) event. We hypothesize that the mesohexaploid ancestor arose through hybridization between genomes resembling the Ancestral Crucifer Karyotype with n=8. The WGT has been followed by species-specific chromosome rearrangements (diploidization) resulting in descending dysploidy towards extant quasi-diploid genomes. More recent neopolyploidization events are reflected by higher chromosome numbers (2n=32-88). The WGT might have contributed to diversification and species radiation in the Heliophileae.

Poster A-15

GENE EXPRESSION DIVERSITY IN *BRASSICA* C GENOME CROPS AND WILD RELATIVES ASSESSED USING RNA-SEQ

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Diversity in wild and cultivated plants can be partly explained by allelic diversity, and partly by differences in gene expression. We wished to survey gene expression diversity in leaf and root tissues among accessions in our *Brassica* C genome and *oleracea* Diversity Fixed Foundation Set collections.

Using the Illumina high-throughput sequencing platform we analysed mRNA expression in leaf and root tissue from 130 diverse accessions of wild and cultivated C genome *Brassic*as from our collections. By aligning the sequence reads from expressed genes to a draft annotated *B.oleracea* genome sequence, we were able to identify genes whose expression varied significantly between wild C genome accessions, between crop morphotypes and between leaf and root tissues in individual morphotypes. By clustering expression across accessions we were able to develop a view of the diversity of the collections, complementary to the view provided by analysis of genetic variation. This data set also gives us window into identifying cases of differential splicing of individual genes between accessions and tissues, the use of different paralogous genes by different lineages, and potentially allows us to associate gene expression with phenotypic variation.

Poster A-16

USE OF NATURAL VARIABILITY TO IDENTIFY AND VALIDATE THE GENETIC DETERMINANTS OF OIL CONTENT IN RAPESEED (*BRASSICA NAPUS* L.)

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The increased production of rapeseed oil is a major objective and can go through the increase of seed yield and / or oil content of the seed. This latter trait is under complex genetic determinism: 14 QTLs were identified in the 'Darmor-bzh x Yudal' doubled haploid population (DY-DH). Genetic studies conducted in other populations have revealed additional genomic regions. However, conventional approaches for QTL detection have the major limitation to restrict genetic diversity study. Association mapping approaches explore a range of genetic variability larger and thus appear as a solution.

An initial characterization of genetic diversity for the oil content of rapeseed has been initiated and will help to bring results on the validation of QTLs. For this, we focused on a QTL on chromosome A01 (QTL-DYA01), which is stable under different environments and accounts for about 13% of the phenotypic variability for oil content. A collection of 95 genotypes maximizing the genetic diversity of winter oilseed rape (hereafter referred to as the WOSR-95 population) was investigated and the following steps were performed: 1) analysis of diversity using neutral molecular markers (SSR) allowed structuring the WOSR-95 population, 2) genotyping of the WOSR-95 population for markers linked to QTL-DYA01 was performed, 3) the phenotypic data (oil content) were acquired during two years of field testing. Data analysis was to combine the genetic diversity observed in the different markers with the phenotypic variability observed in the field.

The expected results are: 1) validation of QTL-DYA01 on a collection of winter rape and 2) initiation of a process of association genetics to identify the determinants of oil content in oilseed rape.

Poster A-17

INTERTRIBAL HYBRIDIZATION BETWEEN *BRASSICA OLERACEA* L. AND *ERUCA SATIVA* L.

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Cauliflower cv. Korso was pollinated by a rucola breeding line to transfer CMS Ogura cytoplasm from *Brassica oleracea* to *Eruca sativa*. The five F₁ plants showed an intermediate phenotype, an almost normal growth habit and expressed male sterility. But no seed set was obtained after pollination with the *Eruca* parent. Therefore allotetraploid hybrids generated by colchicine treatment were backcrossed with *Eruca*. BC₁ plants were transferred to soil after embryo rescue. The BC₁ plants showed a more or less normal seed set and the seeds were sown directly into soil. All BC₂ plants expressed male sterility and showed full seed set compared to rucola parent. Under glasshouse conditions, BC₃ progenies were investigated for important agronomical traits such as plant vigour, biomass production, flowering, male sterility and seed set.

Hybrid status and elimination of *Brassica* genomic DNA in backcross generations were checked using RAPD markers and GISH analysis. Mitochondrial as well as plastid DNA was tested by Southern hybridization. The ploidy level was determined using flow cytometry. Flower-biological and flower-histological investigations were carried out for each backcross progeny. Usefulness of male-sterile *Eruca sativa* material for hybrid breeding will be discussed.

Poster A-18

IDENTIFICATION OF METABOLIC QTLS FOR GLUCOSINOLATE CONTENT IN *BRASSICA OLERACEA*

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Brassica oleracea include vegetables and forages of economical importance like, cabbage, broccoli or kale. Glucosinolates are secondary metabolites present in *B. oleracea* and other Brassicaceae. These compounds and their breakdown products have been shown to confer pest and disease resistance. Certain glucosinolates have anticarcinogenic activities in mammals. To identify loci controlling glucosinolate quantitative variation in *B. oleracea* we carried on an mQTL mapping experiment using the population BolTBDH, derived by crossing a DH of broccoli and a DH rapid cycling. One hundred and fifty five individuals were sowed in a greenhouse in a randomized design with two replications. Seed, leaf, and flower buds samples were taken from each individual and the content of glucosinolates was analyzed by UHPLC. Individual analyses were carried on by glucosinolate and sample type with PLABQTL. Significant mQTLs were spread all over the 9 linkage groups of *B. oleacea* and they explained a percentage of phenotypic variability ranging from 10-50% in seeds, from 10-53% in leaves and from 12-50% in flower buds. mQTL for individual glucosinolates were integrated using a meta-analysis with BioMercator 2.1. Twenty-two consensus mQTLs were detected in total, from these, 13 were present in seed, 15 in leaves and 15 in flower buds. Three mQTLs were exclusively found in flower buds, while one mQTL was exclusively found in seeds or leaves, indicating a metabolic regulation of glucosinolate synthesis which is mostly independent of the organ. Eight mQTLs determine the content of aliphatic glucosinolates and two mQTLs the content of indolic glucosinolates. The rest of consensus mQTLs determined the content of combinations of the three types of glucosinolates. Currently, a syntenic analysis comparing the *B. oleracea* genome with that of *Arabidopsis* is carried on to find candidate genes responsible for the variability of the consensus mQTLs detected.

Poster A-19

GENETICS OF EPIDERMAL CELL CHARACTERS IN *BRASSICA RAPA* L.

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Genetic analysis of epidermal cell characters, the presence or absence of seed coat mucilage and leaf hairs, were performed in a large number of accessions of *Brassica rapa* L. While the accessions with non-mucilaginous seed coat and hairy leaf are found in Europe and the Eastern Japan, the accessions with mucilaginous seed coat and hairless leaf are found in South Asia and the Western Japan. In order to understand the reason why such geographic differentiation occurred, the phylogenetic analysis based on 21 simple sequence repeats (SSRs) loci for 189 accessions was carried out. And a linkage analysis of epidermal cell characters in F2 population derived from crossing between DH lines of European and Asian turnip was demonstrated.

Phylogenetic analysis showed that the accessions could be categorized into two groups, the West and the East in Eurasia. The population structure was also detected between the Eastern Japan and the Western Japan. The results of a linkage analysis exhibited that one and two genes control the presence of seed coat mucilage and leaf hairs, respectively. These two characters are controlled by different genes. The candidate gene, *BrGL2* (an ortholog of *GLABRA 2*) and *BrGL1* (an ortholog of *GLABRA 1*), single copy genes in *B. rapa* was linked the seed coat mucilage and leaf hairs, respectively.

It is reported that the epidermal cell characters are involved in biological functions. Seed coat mucilage relates to retain seed germination rate in high osmotic pressure and seed longevity, and leaf hairs relate to protect plants against insect and to increase freezing tolerance. We will discuss the artificial and natural selection for epidermal cell characters.

Poster A-20

PRODUCTION AND CHARACTERIZATION OF THE CHROMOSOME ADDITION LINES OF AUTO- AND ALLOPLASMIC *BRASSICA NAPUS* WITH SINGLE B GENOME CHROMOSOME OF *BRASSICA JUNCEA*

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It is one of the important breeding object to investigate B genome chromosome in *Brassica*. Therefore, we have tried to product B genome chromosome addition lines and to study about each B genome chromosomes.

Auto- and alloplasmic *Brassica napus* with single B genome chromosome (MALs) (AACC+1b, 2n=39) were bred from backcrossing progenies of the interspecific F₁ hybrids between *B. napus* (AACC) and *Brassica juncea* (AABB). The alien chromosomes of the B genome were identified using the B genome specific microsatellite markers, the chromosome configuration of pollen mother cells (PMCs) at metaphase I (MI) and morphological characteristics. Therefore, 7 types of alloplasmic and autoplasmic MALs were totally identified. Alloplasmic MAL were 6 types, a-h except for the e- and the f type, in autoplasmic ones were also 6 types, except for the a- and f type. These 7 types of MAL plants showed predominantly the chromosome configuration of 19_{II}+1_I in MI. In morphological traits, it is suggested that B genome chromosome were affected the external form tending to appear in the form of a flower in particular. Transmission rate of B genome chromosome from MAL were revealed from 1.9 to 36.4%. It is possible that added B genome chromosome can be maintained the next generations.

The *B. napus* with single B genome chromosome (MALs) may provide the useful material for studying the relationships of B genome chromosomes between A or C genome chromosomes in genera *Brassica*.

Poster A-21

HIGH-THROUGHPUT SINGLE NUCLEOTIDE POLYMORPHISM (SNP) DISCOVERY AND VALIDATION IN *BRASSICA NAPUS* L.

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Single Nucleotide Polymorphism (SNP) is the most useful marker for genetic diversity study, fine mapping, association mapping and molecular breeding because of its abundance in the genome and amenability for high throughput genotyping. Development of SNP markers for canola molecular breeding is very challenging because of the polyploidy nature of *Brassica napus* L. as well as lack of reference genome sequence availability. However, combination of next-generation sequencing with genome complexity reduction technologies provided a rapid, efficient and cost-effective approach for SNP discovery in model and non-model organisms, including polyploid species such as *B. napus* L. Here, we describe a high-throughput SNP discovery in *B. napus* by sequencing of reduced representation libraries generated using Restriction-Site Associated DNA (RAD), Complexity Reduction of Polymorphic Sequence (CRoPS®) and in-house genome complexity reduction technologies or through shotgun resequencing. After sequencing, all reads were aligned to assembled contigs and an in-house SNP discovery pipeline was used for SNP discovery. The SNP discovery pipeline used sequencing depth at SNP position and other parameters, thus enabled us to distinguish allelic (intragenomic) variants (true SNPs) from non-allelic (intergenomic) variants (nucleotide polymorphisms between paralogs, false SNPs). More than 95,000 putatively intragenomic SNPs were identified by sequencing 18 *B. napus* lines, which captured more than 80% diversity of 397 *B. napus* lines used at Dow AgroSciences. After high throughput validation with Illumina Infinium Bead Chips, 65-90% of the putative SNPs identified from different technologies were polymorphic among the 397 *B. napus* lines.

Poster A-22

DEVELOPING A SET OF SNP MARKERS TO STUDY GENETIC DIVERSITY IN A BRASSICA A GENOME DIVERSITY COLLECTION

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A collection of *Brassica rapa* accessions was gathered mostly from genebanks. The accessions originated from different countries and represented mostly oilseed types. The aim was to study genetic diversity in the collection using SNP markers developed in the project. For discovering SNPs, resequencing with amplicon sequencing was used applying the recently released *B.rapa* genome sequence. Amplicons were designed to include one intron, to be 350-400 bp long and to amplify only from one site in the genome. Primers were developed for 384 amplicons, including 143 genes of specific interest, and the rest to cover the whole genome of *B.rapa* evenly. The functioning of primer pairs was tested in one genotype: PCR products were run on the gel to verify that only one clear product was amplified. Over 80 % (=315) of the primer pairs met this criterium, and were used for amplicon amplification from 8 selected genotypes. PCR products were normalized to the same concentration, and pooled from each genotype. Adapters with MID sequences were ligated for identification of the different genotypes, and all the amplicons were finally sequenced in one 454 sequencing run on Junior GS. SNP or indel differences between genotypes in the amplicons were visually scored in Tablet software. A total of 384 suitable SNP/indel differences were selected for SNP analysis with Illumina Golden Gate genotyping assay, which is based on Illumina BeadArray technology. For some amplicons, more than one SNP/indel was selected, so 262 different genes were included in the BeadArray. Results of the diversity analysis will be discussed in the poster.

Poster A-23

EFFECTS OF SUPPRESSION OF *MSH1* ON MITOCHONDRIAL GENOME IN *BRASSICA NAPUS*

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Msh1 gene is a nuclear gene that contributes stability of mitochondrial genome through control of the DNA recombination between short repeats distributed in the genome. To understand the effects of *Msh1* on the stability of mitochondrial genome in cytoplasmic male sterile (CMS) *Brassica napus*, which produced by cell fusion between oilseed rape and radish CMS line. We constructed an RNAi vector with a part of *Msh1* gene of *Brassica rapa* cv. 'Sunshine' and introduced in *B.napus* cv. 'Westar' by *Agrobacterium* mediated transformation. The transgenic m rapeseeds showed some abnormalities of the morphology such as male semi-sterility and bushy-type shoot in the T₀ generation, and leaf variegation was observed in the next generation. We will show the results of the *Msh1* suppression on the mitochondrial genome in the T₀ and T₁ generations.

Poster A-24

CHANGES IN GENE EXPRESSION OF HARVESTED CABBAGE IN RESPONSE TO MECHANICAL WOUND STRESS

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Mechanical stress, imposed on plant cells by a variety of physical stimuli during harvesting and handling of fresh horticultural products, induces a wide range of cellular responses through cellular receptor, signal transduction, and gene activation. To understand response behavior of cabbage to mechanical stresses (wounding and dropping), the characteristics of gene expression related to stress signal transduction and cellular response of cabbage were investigated during 300 min since the application of the stress. Quantitative real-time PCR data showed that candidate stress-responsive genes respond differently to specific stress application. The wounding treatment (punching the cabbage leaf by a cork borer) significantly induced the transient expression of calmodulin (CaM)-encoding genes, *BoCam1* and *BoCam2*, within an hour after the treatment. These genes are considered to be involved in stress signal cascades in plant cells, including cabbage. The increased expression of phospholipase D (PLD)-encoding genes, *BoPLD1* and *BoPLD2*, was also detected after the treatment. In response to the wound stress, the expression of *BoCam1* and *BoPLD2* appeared to be highly induced than that of *BoCam2* and *BoPLD1*, respectively. The dropping treatment (letting the cabbage head dropped from a height of 40 cm against a cork) predominantly induced the *BoCam1*, *BoCam2*, *BoPLD1*, and *BoPLD2* expression. Additionally, the stable increased expression characteristics of genes were observed in wounded cabbage caused by dropping treatment. In conclusion, CaM-encoding genes and PLD-encoding genes are likely wound-responsive genes that are induced to high expression levels after encountering stress. Moreover, because of their predominant response to the mechanical stress, CaM-encoding genes and PLD-encoding genes should be taken into consideration in further study of postharvest mechanical stress as it may be associated with the signaling cascade and cellular stress response in cabbage.

Poster A-25

COMPARATIVE GENOME ANALYSIS, IDENTIFICATION OF FLOWERING PATHWAY AND DEVELOPMENT OF GENOME-WIDE MOLECULAR MARKERS BASED ON GENOME SEQUENCE IN *BRASSICA JUNCEA*

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Brassica juncea (n=18) was theoretically derived from interspecific hybridization of *B. rapa* (n=10) and *B. nigra* (n=8) according to the ‘U-triangle’ and evolutionally possessed unique traits so far. We employed high-throughput sequencing approach to analyze the survey of genome of *B. juncea*. Totally, 28.35 Gb data was retained to estimate the genome size of *B. juncea* as 978 M and the genome contained high proportion of repeat because of a fat tail in the distribution displays. After the initial assembly of genome, we got 1537 and 12982 N50 of contig and scaffold in size. Comparative analysis of GSS of *B. juncea* and genome of *B. rapa* revealed that 42.35% reads were mapped to the *B. rapa* genome and little difference on mapped pari-end reads and single-end reads suggesting more structure variation in *B. juncea* genome than *B. rapa* genome. The mapped reads were 98.15 % identity to *B. rapa* genome suggesting close phylogenetic relationship between *B. juncea* and *B. rapa*. We detected 2816069 SNP sites with high heterozygosity and 155210 structure variation (SV) between *B. juncea* and *B. rapa*. Most SNP and SV were located in exon, intron, transposon and intergenic region of genome. Furthermore, the 1-3 bp Indel polymorphisms were observed to be dominant in genome and 3 bp-sized Indel was most abundant in CDS. These SNP, SV and Indel polymorphisms caused encoding different protein, changed gene function and lost function of gene. We identified vernalization-dependent and photoperiod-dependent flowering pathways coexist in allopolyploid *B. juncea* suggesting better adaptation for survival during evolution. Genome-wide molecular markers were developed including 1171640 SNP, 49941 indel, SSR and 538 structure variation (SV) markers totally after re-sequencing another inbred line in *B. juncea*. Taken together, high-throughput sequencing approach even low genome coverage is pushing forward the molecular genetics especially in non-model plant.

Keywords: *Brassica juncea*, comparative genome analysis, flowering pathway, high-throughput sequencing, molecular marker.

Poster A-26

GENETIC DISSECTION AND FINE MAPPING OF DOWNY MILDEW RESISTANCE AT VARIOUS DEVELOPMENTAL STAGES IN *BRASSICA RAPA*

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Downy mildew, caused by *Hyaloperonospora parasitica*, is a destructive disease of the Chinese cabbage (*Brassica rapa* ssp. *pekinensis*). Previously, a doubled-haploid (DH) population derived from a cross between 91-112 and T12-19 via microspore culture was used to localize the genetic factors affecting downy mildew resistance, and a major QTL *BraDM* was detected on linkage group A08. In this study, a rapid leaf disk test for resistance/susceptibility of downy mildew was established and used to investigate the resistance in this DH population at three developmental stages of adult plants (rosette, heading and flower stages). In a detailed linkage map, four QTLs were identified involving in downy mildew resistance. Of these, the major QTL *BraDM* identified previously at seedling stage was found to be effective at every developmental stage; the others only effective at certain developmental stages. *BraDMa1* on linkage group A06 was effective at rosette stage; *BraDMa2* on linkage group A04 and *BraDMa3* on linkage group A08 showed effective at heading stage. To fine mapping *BraDM*, we constructed a BC1 population, including 650 individuals, which was derived from two DH lines, DH-88 (highly resistant to downy mildew) and DH-60 (highly susceptible to downy mildew) with the similar genetic background but heterozygous at *BraDM* loci. Five InDel markers and one CAPS marker were mapped flanking *BraDM*, limiting *BraDM* to a interval of 1.4 cM with the flanking marker of BVIDT0801 and kbrb058m10-1. By Blast analysis, the physical distance between BVIDT0801 and kbrb058m10-1 is 194 kb with 42 open reading frames in this interval. This information will be helpful for future identification and isolation of the candidate gene. The tightly linked markers that we have developed for *BraDM* will be a useful tool in the marker-assisted selection of this gene in *Brassica rapa* improvement programs.

Poster A-27

HIGH-THROUGHPUT DISCOVERY OF CHLOROPLAST AND MITOCHONDRIAL DNA POLYMORPHISMS IN BRASSICACEAE SPECIES BY ORG-ECOTILLING

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Information on polymorphic DNA in organelle genomes is essential for evolutionary and ecological studies. However, it is challenging to perform high-throughput investigations of chloroplast and mitochondrial DNA polymorphisms. In recent years, EcoTILLING stands out as one of the most universal, low-cost, and high-throughput reverse genetic method, and the identification of natural genetic variants can provide much information about gene function, association mapping and linkage disequilibrium analysis and species evolution. Up to now, no report exists on whether this method is applicable to organelle genomes and to what extent it can be used.

To address this problem, we adapted the CEL I-based heteroduplex cleavage strategy used in Targeting Induced Local Lesions in Genomes (TILLING) for the discovery of nucleotide polymorphisms in organelle genomes. To assess the applicability and accuracy of this technology, designated ORG-EcoTILLING, at different taxonomic levels, we sampled two sets of taxa representing specimens from Brassicaceae with 3 chloroplast genes (*accD*, *matK* and *rbcL*) and 1 mitochondrial gene (*atp6*). The method successfully detected nine, six and one mutation sites in the *accD*, *matK* and *rbcL* genes, respectively, in 96 *Brassica* accessions. These mutations were confirmed by DNA sequencing, with 100% accuracy at both inter- and intraspecific levels. We also detected 44 putative mutations in *accD* in 91 accessions from 45 species and 29 genera of 7 tribes. Compared with DNA sequencing, the false negative rate was 36%. However, 17 mutations detected in *atp6* were completely identical to the sequencing results.

These results suggest that ORG-EcoTILLING is a powerful and cost-effective alternative method for high-throughput genome-wide assessment of inter- and intraspecific chloroplast and mitochondrial DNA polymorphisms. It will play important roles in evolutionary and ecological biology studies, excavating related genes associated with agronomically important traits of high yield and fine cytoplasmic quality, and identifying point mutations of mitochondrial genes leading to diseases in humans and animals.

Poster A-28

COMBINED QTL MAPPING AND TRANSCRIPTOMIC APPROACH TO UNRAVEL THE GENETICS OF TURNIP FORMATION IN *BRASSICA RAPA* SSP. *CAMPESTRIS*

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Brassica rapa displays enormous morphological diversity in the organs used for consumption and is an excellent model to study genetics and mechanisms underlying the morphological characteristics and their inter-relationships. In this research we aim to understand the genetic basis and physiological mechanisms that underlie turnip formation. To achieve this goal, several strategies have been applied. Two RIL (recombinant inbred lines) populations have been developed in which a Japanese vegetable turnip VT-115 is used as the common mother, and the father genotypes for the crosses are an oil type RC-144 and a pakchoi type PC-105. Genetic linkage maps of both populations were constructed and several QTL underlying turnip formation and other morphological traits were mapped. Gene expression studies using a customized *B. rapa* oligo-microarray, RNA-seq and qRT-PCR techniques identified a group of candidate genes that were differentially expressed during turnip development of a homozygous Japanese vegetable turnip line DH-VT-117. Knowledge of the genes that reside in the QTL region that showed different expression levels during turnip formation will unravel the genetic mechanism that define storage organ formation.



BRASSICA2012

Session B

GENETIC DIVERSITY, CONSERVATION AND USE

Oral presentation

Session B-1

IS THERE A NEED FOR THE SVALBARD GLOBAL SEED VAULT AND ARE OUR GENETIC RESOURCES SAFE FOR THE FUTURE? EXAMPLES IN *BRASSICA*

Keynote lecture

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Climate change, escalating environmental problems and need for an increased but sustainable food production puts a high pressure on an efficient use of the world's plant genetic resources (PGR). There are today several immediate threats for conservation. We are not only losing material in nature and in cultivation but even worse - in our gene banks! Disasters like earthquakes, tsunamis and flooding as well as technical, economical and political problems may cause severe losses of valuable material in gene banks. These damages are due to the fact that there is an ineffective or in many cases non-existing back-up system for many of the gene banks in the world. With these perspectives the Global Seed Vault was established in 2008. The Vault is situated in the arctic area at 78° N – in the northernmost larger settlement on earth. Here permafrost is prevailing keeping a constant temperature in the rock of -5°. The Vault has an extra cooling device for keeping the temperature down to - 18°, standard for gene bank material. Svalbard is a geologically and politically stable area, which makes it an ideal place for the international efforts for an effective and safe back up security system for all unique gene bank accessions of the world. Full capacity of the Vault is ca 3 million accessions, which is well above the estimated number of unique accessions. *Brassica* species will be taken as examples of material stored in the Svalbard Global Seed Vault.

Session B-1

AS EASY AS ABC: APPLYING THE CIRCADIAN CLOCK MODEL DEVELOPED IN ARABIDOPSIS TO BRASSICA CROPS

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Arabidopsis thaliana has served as a model species for the study of the architecture and function of the circadian clock. Experimental disruption of the clock via large-effect mutations reduces fitness. Therefore, it is of both academic and practical interest to explore the extent to which the circadian clock model developed in *Arabidopsis* can be generalized to other plants, especially crop plants, and to ask whether manipulation of the circadian clock can enhance agricultural yield. Accordingly, we have extended our studies to *Brassica rapa*, an agricultural crop closely related to *Arabidopsis*. Using a recombinant inbred population derived from cultivated oil seed and rapid cycling varieties we have mapped Quantitative Trait Loci for circadian period, water use efficiency (WUE), shade avoidance, and other morphometric traits. There is striking colocalization of QTL for circadian period with QTL for shade avoidance and WUE. Current efforts emphasize the exploration of the possible mechanistic relationship underlying this colocalization. With regard to crop accessions, history of domestication appears to affect the evolution of net carbon assimilation and stomatal conductance among replicates grown in either controlled or field settings. Specifically, oil seed varieties have higher values of both traits than do cabbage and turnip vegetable crops, and take longer to recover from drought. *Brassica rapa* has undergone triplication through two whole genome duplication events since its separation from *Arabidopsis*. This triplication has been followed by considerable fractionation (gene loss). Fractionation following whole genome duplication is not random in that dosage sensitive genes, such as those that are highly networked or those whose products contribute to protein complexes, are preferentially retained. We tested the hypothesis that circadian clock genes, which are dosage sensitive, should be preferentially retained in *B. rapa* and found that this is indeed the case.

Session B-1

GENETIC DIVERSITY IN *BRASSICA* CROPS AND WILD RELATIVES

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Allelic variation is the basic raw resource for crop improvement. However, elite breeding germplasm generally has a lower genetic diversity than the wider species gene pool. In addition, the wild species relatives are a potentially rich source of alleles for sustainable crop improvement that is largely untapped.

We are addressing these issues by generating *Brassica* diversity collections composed of homozygous lines which we refer to as Diversity Fixed Foundation Sets (DFFSs). We have produced DFFSs for *B. napus* (BnaDFFS), *B. oleracea* (BoLDFFS) and the wild C genome species (BCgDFFS). Homozygosity simplifies the genetic analysis of the lines and greatly improves the reproducibility of phenotypic analysis. One of the key purposes of these diversity sets is to identify novel sources of beneficial traits and we will present results for recent screens.

We have used two approaches to determine the allelic variation in the BoLDFFS and BCgDFFS. In the first we identified a panel of single nucleotide polymorphisms (SNPs) from whole genome resequencing of the parents of three *B. oleracea* mapping populations. 1560 of these SNPs were selected on the basis of wide genome coverage and polymorphism in each population and converted to KASPar SNP markers. In addition to using the KASPar markers for genetic analysis of the diversity sets, we also used them to update and integrate the genetic maps for the three mapping populations to greatly improve our ability to link quantitative trait loci to genome sequence data for candidate gene identification. In the second approach we performed transcriptome sequencing of standardised leaf and root samples of these lines and will present this data in a separate paper. For *B. napus* we are using the new Illumina *B. napus* 60K Infinium array to provide a very detailed analysis of the allelic variation.

Session B-1

ASSOCIATION MAPPING OF AGRONOMICALLY IMPORTANT TRAITS IN *BRASSICA RAPA* L.

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Association mapping in plants uses linkage disequilibrium (LD) that is present in natural and breeding populations including collections of plant genetic resources in genebanks. Using this tool, the genetic diversity expressed as allelic polymorphisms can be related to the observed phenotypic variation. We have used VIR core collection of *Brassica rapa* L. for association mapping of agronomical valuable characters. Phenotypic variation of bolting time, growth related traits (plant and leaf sizes), leaf morphological traits connected with quality, biochemical and immunological traits (resistance to 6 races of black rot and leaf spot) was evaluated and genetic diversity was measured using a set of 135 SSR- and 123 S-SAP (CACTA) markers. Cluster analysis revealed accurate positions of *B. rapa* accessions in two major clusters: East-Asian vegetables include subclusters of Chinese cabbage divided into four groups, pak-choi and Japanese greens; Indo-European-Asian cluster is divided on oilseeds according to their geographical origin and separate group of turnips in Nepal-Indian subcluster. Genetic distance was calculated by NTSYSpc, cluster analysis by Neighbor joining method. The phenotypic traits were integrated into the tree and closely related molecular markers were checked for influence on these traits. Significance levels of associations were checked by parametric and nonparametric tests. We have found two markers for each of the following traits: bolting time, plant diameter and weight, leaf type, lamina length, -width, -edge, -color, -hairs and -surface, petiole presence, -length, -width, -thickness, -color and -border. Our results should be useful for screening of collection and breeding material.

Session B-1

POSSIBILITY OF EXPLOITATION OF SERBIAN LOCAL VARIETIES AND LANDRACES OF CABBAGES: CASE OF "FUTOSKI CABBAGE" FROM FUTOG REGION.

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Many varieties of cabbage are grown in Serbia, but two local varieties, Futoški i Srpski melez, predominate the cabbage production in Central Serbia and the Vojvodina Province. Their advantage over the introduced varieties is that they are better suited to the local taste and methods of use (pickling of whole heads or sauerkraut, preparation of traditional local dishes and fresh consumption). It should be noted that local cabbage populations are widely grown in remote parts of Serbia. A recently completed SEED-Net Project was aimed at collecting local cabbage populations on the territory of Serbia. Populations were collected in the locations of Boljevac, Čurug, Deronje, Donji Katun (Varvarin), Futog, Taras (Elemir), Leskovac. The sources of collected materials were mostly local markets, home gardens and home stores in isolated villages, where old farmers still maintain land races of various brassicas in small quantities and for domestic use. These cabbages have juicier and thinner leaves, which predisposes their heads for fine grating and also makes them pliable and easy to roll up during food preparation. It is characteristics like these that give the local populations an advantage over hybrids. The climatic and soil characteristics in the typical and traditional production area favor the production of late cabbage varieties. Fresh cabbage from Vojvodina production area is a good raw material for fresh use and biological fermentation. The winter cabbage is suitable for biofermentation and long storage in the form of sauerkraut. The fermented Futoski cabbage has been certified at one point as a product with geographic origin.

Session B-1

ANALYSIS OF PHENOTYPIC DIVERSITY IN CHINESE COLLECTION OF *BRASSICA RAPA* L.

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To investigate phenotypic variation of 1 962 *Brassica rapa* germplasm in oilcrops middle-term genebank of China, we identified and evaluated their 13 phenotypic traits (leaf shape, seed color, resistance to *Sclerotinia sclerotiorum*, resistance to turnip mosaic virus, and resistance to *Peronospora parasitica*, growth period, plant height, branch height, plant branch, pods per plant, seeds per pod, thousand seeds weight, and theoretical yield per plant). The variation degree and distribution of 13 phenotypic traits were obtained. Results indicated that winter-type accessions from Shanxi province had longer growth periods, while spring-types from Qinghai, Gansu and Inner-Mongolia provinces had shorter growth periods. Semi-winter-types from Sichuan and Yunnan provinces had more pods per plant, and those from Sichuan province had higher plant height also. Spring-types from Tibet had larger seed size.

According to their geographic regions, phenotypic traits group and proportion of sample, a collection of 244 *B.rapa* landraces was selected from 1 962 *B.rapa* germplasm. Genetic diversity results showed that Chinese *B.rapa* landraces possessed abundant morphological polymorphism with higher average diversity index(as 1.709) than that of alien germplasm(as 1.250). Genetic diversity of different ecotypes showed that semi-winter-type had the richest diversity, and then followed by the spring-type and the winter accessions. UPMGA(unweighted pair group method with arithmetic mean) results showed that 244 representative *B.rapa* landraces could be classified into 4 groups(I,II,III and IV) with a genetic similarity coefficient as 0.204. The 4 groups involved 117,38,34, and 55 landraces, respectively. The group I,II,III and IV were mainly consisted of semi-winter ecotype, spring ecotype, spring ecotype and winter ecotype, respectively. Based on principal coordinates analyses, 244 landraces were separated into 3 gene pools including spring, semi-winter and winter resources, which were consistent with UPGMA result.

Session B-2

HISTORY AND PRELIMINARY EVALUATION OF GALEGA KALE LANDRACES

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Galega kale is usually a headless leafy cole with long petioles and large midribbed leaves inserted on an elongated stem, reaching over one meter at maturity, before bolting. Galega kale is the key ingredient of the traditional Portuguese soup called “Caldo Verde”, meaning “Green Broth”. In the past Galega kale was seen in most home gardens and surrounding most vegetables patches, producing tender fresh leaves thus, always available, when the “Caldo Verde” was to be prepared. Nowadays, due to the concentration of people in urban areas and the lack of home gardens, the Galega kale leaves are now mainly mass produced by specialized growers, and transformed by processors where they are finely chopped, packed and delivered to local supermarkets in Portugal and to other European countries with large Portuguese communities. Traditionally, growers carefully select their own landraces for non-bolting and paid particular attention to specific morphological traits. The rudimentary process of seed production used by the growers and the lack of isolation, allowing intercrossing between kales and surrounding cabbages, contributed to a great morphological heterogeneity. Besides Galega kales landraces are being increasingly lost due to absence of selection and seed production by young generation of growers. As there are no breeding cultivars of Galega kale available in the market, kale cultivars, mainly of marrow stem kale, are imported from France but they lack market quality and are susceptible to diseases. Some landraces of Galega kales were collected in the past but there has been no comprehensive programme for characterising these genetic resources. A breeding programme was introduced to produce cultivars adapted to large scale growing and processing of finely chopped greens for “Caldo Verde”. In a preliminary evaluation, some landraces were eliminated while others selected by yield parameters, leaf characteristics and shelf life.

Session B-2

GENETIC DIVERSITY IN BRUSSELS SPROUTS AND CONSERVATION OF F1 HYBRIDS IN GENE BANK COLLECTIONS

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Genebanks aim to conserve and manage the genepool diversity of crop species and ensure its continuing availability for research and plant breeding. In addition to the obvious sources of crop genetic diversity seen in crop wild relatives and landraces, useful variation may also be found in early F1 hybrid varieties, particularly where these were derived from regional breeding programmes. Such F1s represent potentially regionally adapted genetic variation in a form which is relatively derived and may be easier to incorporate into crop improvement programmes than unimproved landraces. A good example is that of Brussels sprouts in the UK, where the first F1 hybrids were developed in the 1980s. Seed from early F1 hybrids is conserved in Warwick Genetic Resources Unit, but as the parental lines are not, regeneration following standard protocols is problematic, and although the seed is kept under ideal conditions for long-term storage, inevitably viability will reduce.

We used microsatellite markers to assess genetic diversity in a range of open-pollinated and F1 hybrid Brussels sprouts, and to follow the allelic diversity in four accessions as they underwent a series of regeneration treatments. None of the treatments would recreate the F1 hybrid itself, but as the core aim of the genebank is to conserve allelic variation, a regeneration which offered the best compromise between resources required and alleles conserved would be extremely useful.

EVALUATION OF SICILIAN WILD BRASSICA SPECIES (N=9) FOR GLUCOSINOLATE PROFILE AND ANTIOXIDANT COMPOUNDS

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Mediterranean wild *Brassicaceae* species (n=9) are of great interest for their use to improve *Brassica oleracea* crops as well as other brassicas. Among the tasks of the Brassica Working Group (BWG) of the European Cooperative Program for Genetic Resources (ECPGR), managed by Bioversity International, priority was given to the safeguard of these species and the related gene pool. DISPA in the frame of the EU GEN RES “AEGRO” project (<http://aegro.jki.bund.de/aegro>) coordinated the case of study of the Sicilian wild population mainly related to *B. incana*, *B. macrocarpa*, *B. rupestris* and *B. villosa*, which were compared with a kale landrace and a population of *B. montana* collected in central Italy.

The activities of AEGRO project were committed mainly to individuate wild Brassica populations in Sicily, to collect and conserve seed samples, to characterize and evaluate them for several traits such as antioxidant compounds.

A lot of wild population seeds was used for sowing and set up, by the obtained plantlets, an evaluation field at the experimental farm of Catania University. After one year from transplanting, leaves samples were collected for each plant in view to have three bulk replicates for each population.

The phytochemical content was analyzed for the total glucosinolates and their profile, ascorbic acid, total polyphenols and carotenoid content, and the antioxidant capacity by DPPH and crocin bleaching assay (CBA).

The highest values of glucosinolates was detected in a population in *B. rupestris*, the ascorbic acid in *B. montana*, total polyphenols in *B. montana*, β -caroten and anthocyanin showed highest values in *B. rupestris*. The data of antioxidant capacity gave interesting results for *B. Montana* for DPPH and *B. incana* for CBA. Interestingly, a significant reciprocal correlation was found between the two methods of antioxidant capacity measurement ($r_{xy} = 0.705$).

A positive significant correlation by simple regression analysis has been found between total polyphenols and antioxidant capacity, with the highest index ($r_{xy} = 0.811$, $p < 0.01$) found for CBA and total carotenoid content. As expected, no relationship was found between total glucosinolate content and antioxidant capacity.

Session B-2

TAXONOMIC IMPLICATIONS OF SEED MORPHOLOGY AND SEED COAT SCULPTURES IN *BRASSICA* SECT. *BRASSICA*

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The seed coat is the direct interface between embryo and external environment, acting as main modulator in the plant life cycle with key functions of regulation and protection. The taxonomic value of the macro- and micro-morphological characters of seeds and outer coats has been clearly demonstrated in angiosperms systematics, being very conservative and stable features. Several reports were published for *Brassica* species (Stork *et al.* 1980; Koul *et al.* 2000, Zeng *et al.* 2004; Karcz *et al.* 2005) which showed highly diverse morphology and complex coat patterns providing valuable characters for the analysis of taxonomic treatment and evolutionary relationships.

Brassica Sect. *Brassica* is a rather controversial group including several wild taxa and cultivated varieties and landraces of *B. oleracea*. This study aims to characterize the seed morphology and seed coat patterns in all wild species of this Section, defining their systematic and phylogenetic implications.

Eighteen exomorphic parameters, including shape, size, color, surface texture, were detected from 50 seeds for each accession. SEM investigations were carried out on 5-10 seeds from each sample, considering the arrangement and shape of epidermal cells, the architecture of anticlinal and periclinal cell walls (primary sculptures), their fine cuticular ornamentation (secondary sculptures). Seed coat sculptures at low magnification (20X) have a reticulate model. Higher magnifications (200-500X), however, provide more complex patterns, classified in 4 basic types: simple reticulate, micro-reticulate, reticulate-foveate, reticulate-rugose, on account of fine network profile. Significant differences are found in the overall cell shape (\pm polygonal or irregular) and the size, height and alignment of the meshes, which may be lax to compact, sharply angled to smooth, regular or irregular. Great variation among different taxa and populations is also observed in the anticlinal and periclinal cell walls and cell lumen, which can be straight to \pm undulate, depressed, concave, flat or convex, smooth to \pm markedly wrinkled, foveolate and / or papillose.

Based on these data, information from seed morphology and microsculpturing clearly provide taxonomic features useful to define distinct species. Combined with other morphological, genetic and biochemical data, they can help to clarify and better define the taxonomic position and phylogenetic relationships amongst the critical taxa of the genus *Brassica*.



BRASSICA2012

Session B

GENETIC DIVERSITY, CONSERVATION AND USE

Poster presentation

Poster B-1

**STUDIES ON ENRICHMENT OF VARIETIES ASSORTMENT AT AUTUMN CABBAGE
BRASSICA OLERACEA L. VAR. *CAPITATA***

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Improving the assortment of varieties at vegetables from cabbage group needs a permanent attention and is motivated by the fact that the variety remains one of the main drivers of growth of production. In Romania is all the more so, whereas it creation of varieties in vegetables from the cabbage group was given a limited space, compared to other vegetable species. The local assortment can be improved by creating open-pollinated varieties and F1 hybrids. Difficulties in creating open-pollinated varieties of these species are due to allogamy involving depreciation of original material (population), by consanguinization, migration and genetic drift. Our breeding objectives considered at creating of a new variety were: productivity, quality, crack resistance, age of maturity, storage resistance, resistance to diseases and pests. Silvana variety, a new variety of autumn cabbage, open pollinated with superior characteristics and qualities comparing the existing range of cultures, represents the result of our breeding activity. This variety was obtained through a complex of methods (individual selection on families, selecting half "SIB"), in a biological material already existing in culture. Genotype was easily separated from the existing population mass, because the selection was made on elite quantitative attributes, especially the qualitative features of vegetative edible part, so before bloom. Silvana variety is superior to those existing in culture and is distinguished by: productivity, leaf softness - high demand of consumers for industrial use of autumn cabbage (pickling).

Poster B-2

EVALUATION OF VARIATION OF KALE (*BRASSICA OLERACEA* VAR. *ACEPHALA*) POPULATIONS COLLECTED FROM BLACK SEA REGION, TURKEY

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Kale (*Brassica oleracea* var. *acephala*) is one of the most important crops in the Black Sea Region of Turkey. In the first experiment year, 127 kale populations were collected from different geographical areas in this region. All populations of native kale genotypes were examined for morphological characteristics. Phenotypic diversity in their plant characters was assessed. Data were analyzed using principal component analysis (PCA). PCA revealed that the first five PC axes explained 67.0% of the total multivariate variation. Data were subjected to cluster analysis and several groups were identified with most of the populations. Clustered into ten groups, a dendrogram was prepared to evaluate morphological differences among populations. It revealed high variation. The results provide information on the diversity and it can assist geneticists and breeders to identify the populations with unique characteristics for inclusion in future breeding activities.

Poster B-3

IN PROGRESS ACTIVITIES OF THE BRASSICA WORKING GROUP OF THE EUROPEAN COOPERATIVE PROGRAMME FOR PLANT GENETIC RESOURCES (ECPGR)

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During Phase VIII (2009-2013) of the ECPGR Programme, its Brassica Working Group (BWG) decided to focus attention of all members to the priority actions represented by the development of AEGIS (A European Genebank Integrated System), and to characterise collections both of *B. rapa*, which could be defined as a multicrop species for its multiple utilisation, and of wild *Brassica* species (n=9) distributed in the Mediterranean basin,. For these species, there are a number of accessions held in the European collections that are poorly characterized.

It was agreed to identify about 100 'unknown' accessions of *Brassica rapa* and 20 accessions of wild *Brassica* species (n=9) with the aim of characterizing and evaluating them for several aspects such as bio-morphological traits, oils and nutraceutical compounds, DNA analysis, etc. The members of the Brassica WG are contributing to this initiative in relation to their main interest and competences. The activities are mainly cofinanced by specific projects which the members are carrying out and/or by the economical supports of breeders, research institutes/universities that are interested in regeneration, characterisation and evaluation activities. The results will also contribute to the implementation of AEGIS, since accessions will be proposed to be included as part of the European Collection. The characterization and evaluation activities will adopt common standards and descriptors.

The activities foreseen will be carried out by DISPA-University of Catania (UNICT), the Centre for Genetic Resources, the Netherlands(CGN), the Institute of Sustainable Agriculture of Cordoba (CSIC-Spain), and N.I.Vavilov Research Institute of Plant Industry (VIR). Brassica WG members discussed the minimum descriptors which will be similar to those previously used in the GENRES project CT99 108-112. Antioxidant compounds will be analysed with common protocols, whereas for DNA molecular analysis, standard AFLP and SSR primers will be utilised.

The characterisation fields will be set up in Sicily by UNICT where the climatic conditions are optimal for wild *Brassica* species (n=9) whereas *B. rapa* will be grown in St. Petersburg.

As far as the antioxidant and nutraceutical compounds are concerned, samples of roots, leaves and inflorescences of *B. rapa* accessions and of leaves of wild *Brassica* species, accessions will be freeze-dried by UNICT and VIR, whereas for the molecular analysis UNICT will extract the DNA from young leaf samples. At the end of the project, all data acquired will be available via the website of the Bras-EDB.

Poster B-4

BIO-MORPHOLOGICAL CHARACTERIZATION OF THE ECPGR BRASSICA WORKING GROUP COLLECTION

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A project planned by the Brassica WG involves the comparison of various populations of wild brassicas in the same environmental condition, with the aim to establish a collection of well characterized and diverse wild accessions. The accessions selected mainly on the basis of their availability and obtained from various European genebanks. We characterized 26 accessions of wild brassicas including *Brassica barrelieri*, *Brassica balearica*, *Brassica bourgeauii*, *Brassica cretica*, *Brassica desnottesii*, *Brassica drepanensis*, *Brassica fruticulosa*, *Brassica hilarionis*, *Brassica insularis*, *Brassica incana*, *Brassica macrocarpa*, *Brassica montana*, *Brassica oleracea wild type*, *Brassica rapa*, *Brassica rupestris* and *Brassica villosa*. The bio-morphological characterization was performed by the main IBPGR descriptors.

The data obtained were processed using the program SPSS by applying the hierarchical cluster analysis with the method of the complete linkage furthest neighbour. The cluster obtained classify the studied accessions in six main groups that have different characteristics. Group A is mainly different from the other groups for the pyramidal shape of the plants; group B is different for mainly to a less wavy leaf margin and leaves hairless; group C is different for higher transverse dimension of the plant, for the higher longitudinal and transverse dimension of the petiole and for the higher number of scars; Group D is different for the higher plant height, for the leaf blade length and width and for the main tomentosity of the leaves; Group E is mainly different for less height and diameter of the plant, for less length and width leaf blade and for less length and width petiole. It differs for less number of leaves per plant; Group F differs mainly for the presence of anthocyanin in the leaf, for the high number of leaves per plant and for plant branching.

Poster B-5

DIVERSITY OF KALES GROWING IN EUROPE AS BASIS FOR CROP IMPROVEMENT

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Kale (*Brassica oleracea* L. convar. *acephala* (DC.) Alef.) seems to be the most ancient crop belonging to *B. oleracea* cultigroup which is grown in European countries mainly in home gardens and peri-urban farms to satisfy the requirements of family and local communities. The produce is represented mainly by shoots which are utilized to prepare several traditional foods whereas the old leaves are utilized for feeding animals. Kale seems to be the ancestor of several *B. oleracea* vegetable crops because it is very similar to *B. oleracea* wild type, distributed along the European Atlantic coasts, and to several wild *Brassica* species (n=9), which are widespread around the Mediterranean basin. Kale was probably the first step of human selection for exploiting the large diversity present in the *Brassica* wild species (Snogerup *et al.* 1990). Recent linguistic and literary studies indicate the Mediterranean basin as the candidate center where domestication of vegetable brassicas took place and where kale is mainly grown and utilized. Kale, as a minor crop, is not well exploited and the traditional landraces are mainly propagated and maintained in cultivation by old growers in some European countries. The landraces are disappearing as a consequence of changed social and economic conditions and of introduction of new cultivars/crops providing higher yields. The kale collection studied was established in the frame of the Brassica Working Group (BWG) of the European Cooperative Programme for Plant Genetic Resources (ECPGR). We characterized 23 accessions, collected and/or conserved by eleven European genebanks and by some BWG members involved in kale utilization. Fifteen of these accessions were represented by landraces, five by commercial cultivars and three by *B. oleracea* wild types growing along European Atlantic coasts. We utilized several bio-morphological descriptors to detect kale diversity in Europe and to compare it with commercial cvs and *B. oleracea* wild type populations.

The data acquired ascertained the wide diversity of kales growing in Europe for several descriptors. The descriptors, which appeared more discriminating traits were plant branching and shape, as well as several leaf and petiole traits. Factorial and cluster analysis of this European kale collection grouped the accessions in relation to their geographic area. The data recorded show similar results obtained recently by Nordic European researchers on the basis of AFLP genetic analysis utilizing a similar kale collection Sweden (Christensen *et al.*, 2011).

Poster B-6

GENETIC RELATIONSHIP OF SICILIAN WILD AND CULTIVATED BRASSICACEAE USING FIVE SSR

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The Mediterranean basin have played an important role as centre of origin and diversification of several brassicas mainly related to *B. oleracea* which share an important primary gene pool with several wild *Brassica* species (n=9) of great interest for genetic improvement. In this frame we analyzed fifth-one accessions conserved by DISPA of Catania University of which represented mainly cauliflower and broccoli (eleven landraces, fourteen Sib-lines, twelve F1 hybrids set up by DISPA and six commercialized hybrids) and by several wild populations of *B. incana*, *B. macrocarpa*, *B. rupestris* and *B. villosa*. Five SSRs (microsatellite) markers (BoTHL1, PBCGSSRBo39, BoPLD1, BoAP1 and BoABI1) were used to assess genetic similarity among them. Among the markers utilized BoTHL1 showed eight alleles, PBCGSSRBo39 eleven alleles, BoPLD1 six alleles, BoAP1 showed twelve 12 alleles and BoABI1 nine alleles. Some alleles of some primers are present in commercial Hybrid F1 studied whereas the wild populations different alleles of both the broccoli and cauliflower F1 hybrids and landraces studied. A pairwise genetic distance (F_{ST} distance with sample size bias correction) was calculated for all genetic materials and a dendrogram of relationships was produced. All broccoli cultivars clustered with cauliflower cultivars as expected from the low genetic distance that exists between the two varieties ($F_{ST}=0.0261$). Major genetic differences were found within the wild species. The phylogenetic analysis grouped most of the wild species within the same cluster. The principal component analysis confirmed the previous results

Poster B-7

S-SAP MARKERS – A VALUABLE TOOL FOR GENETIC DISTANCE ANALYSIS IN *BRASSICA RAPA* L.

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Transposable Elements (TEs) contribute significantly to diversification between and within plant species. Therefore transposon based molecular markers could have special benefits for phylogenetic studies. Sequence-Specific Amplification Polymorphism (S-SAP) analysis was developed by Waugh et al. (1997). Among the Class II TEs of *Brassica* species, CACTA elements are of special interest, having highly conserved ends and highly variable internal sequences. *Bot1*-related sequences were found in different *Brassica* species. For S-SAP analysis fluorescence labeled *Bot1*-primers (Alix et al. 2008) have been combined with unlabeled Mse+3 or Eco+3 AFLP primers. Separation of amplification products on LI-COR 4300 DNA analyzer produced complex patterns of DNA fragments highly polymorphic in a core collection of 96 *Brassica rapa* accessions. Twelve primer combinations generated 123 polymorphic markers. Mean PIC value of the 123 S-SAP markers was 1.47-fold that of 149 SSR markers tested in the same core collection. The set of total 272 S-SAP and SSR markers was used for genetic distance analysis and association studies for morphological traits, metabolite content and resistance traits.

Poster B-8

EVALUATION OF A POSSIBLE BREEDING BETWEEN *BRASSICA NAPUS* CULTIVAR AND *SINAPIS ARVENSIS* IN THE FIELD

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Plant biodiversity studies have been performed in the Migliarino-San Rossore-Massaciuccoli Regional Park in Tuscany (Italy) within the frame of the European project LIFE08 NAT/IT/342 DEMETRA. This project aims at developing a quick monitoring index (QMI) to rapidly assess the potential risk generated by transgenic plants in characterized ecosystems or biotopes. For this reason test areas have been selected inside the protected area to evaluate plant (weeds and trees), animal, and soil microorganisms biodiversity. The proximity of the selected test area to cropped surfaces where Genetically Modified Plants (GMPs) might be cultivated has been taken into account. GMPs could spread pollen and hybridize with their wild relatives. To evaluate potential hybridization, *Brassica napus* L. var. *oleifera* Del. (oil seed-rape) were cropped. *B. napus* forms spontaneous hybrids with both of its wild progenitor species, *B. rapa* (wild turnip) and *B. oleracea* (wild cabbage) and there has been much interest in the extent of sexual compatibility between *Brassica napus* (oilseed rape) and wild *Brassica* species. Some wild plant species belonging to *Brassicaceae* have been selected in the protected areas, to evaluate possible hybridization and gene flow. The choice of *Brassicaceae* has been done considering the pollinating fauna and the flowering period. *Sinapis arvensis* was considered as one possible candidate to hybridize with oilseed rape. If GM oilseed rape is to be grown the possibility of his modified trait being transferred to *S. arvensis* needs serious consideration as the species are widespread.

Due to the close genomic relationship between these taxa, SSR primers designed for different *Brassica* species were tested to amplify both in *Brassica napus* and *Sinapis arvensis*. Therefore we looked for *S. arvensis* populations and oilseed *B. napus* cultivars which would hybridize in the field. Analysis are in progress.

Poster B-9

PRESERVATION OF VERZEGNIS TURNIP

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Turnip of Verzegnis is an ecotype of *Brassica rapa* subsp. *rapa*, grown by local inhabitants both for human and animal food and as source of income. Strange enough, in the local dialect, people of Verzegnis are called Gnaùs, that means “turnip”.

Actually Verzegnis' turnip is not well known and its cultivation is not frequent, not only in Carnia district, but in the entire Friuli Venezia Giulia.

Cultivation is quite complex due to the difficulties in finding seeds and the limited number of farmers (they are few and old).

Experimental activities about turnip are included in FuturBioErbe project; the main goal of the project is to recover and preserve turnip of Verzegnis increasing the growing area and promoting the diffusion in the region Friuli Venezia Giulia.

During the first two years several activities were performed. First of all was important to collect seeds among old farmers. Seeds were cultivated in different greenhouse (in pots or in land). Then plants were selected from a jury of farmers and experts on turnip.

In the second year plants were isolated and during blooming were manually fertilized to obtain a good number of seeds. Then seeds were grown in plateaux and new plants were transplanted in Verzegnis area during summer 2012 to be compared with Milan's turnip.

In the next and last year different agronomic techniques of cultivation will be tested and qualitative and quantitative analysis will be performed between Verzegnis and Milan turnips.

Poster B-10

KOREA BRASSICA RESOURCE BANK

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The genus *Brassica*, phylogenetically related to *Arabidopsis thaliana*, is one of economically important crops and a botanical model of plant polyploidization and rapid phenotypic evolution. We established the Korea *Brassica* Genome Resource Bank (KBGRB) in order to supply basic plant materials for structural/functional genomics and breeding of *Brassica*. Since the establishment of KBGRB in 2004, KBGRB has supported genomic materials for Multinational *Brassica* Genome Sequencing Project and collected over 10,000 accessions of *Brassic*as from different areas in the world. KBGRB has collected seeds including inbred lines and mapping population of various *Brassica* species, and DNA stocks including BAC libraries and cDNA libraries of *Brassica rapa*. Moreover, all germplasms of KBGRB have been propagated, maintained, and distributed to the scientists in the world. Currently, KBGRB has collected 11,041 accessions of *Brassica* species, 33,159 clones for cDNA libraries, and 222,336 clones for BAC libraries, and 1,398 genetic markers. KBGRB has distributed more than 621,345 clones, 280 genetic markers and 6,210 accessions of seed to researchers over 10 countries since 2004. Information and other requests for genomic resources of *Brassica* are accessible at <http://www.brassica-resource.org>.

Poster B-11

DEVELOPMENT AND APPLICATION OF AN SIMPLE SEQUENCE REPEAT PANEL FOR THE FINGERPRINTING OF CANOLA (*BRASSICA NAPUS*) CULTIVARS

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Canola (*Brassica napus*) is an economically important oil crop and many new cultivars have been developed and released every year to meet the market demand and environment challenges. For example, in China, the candidate cultivars in regional trial increased from 98 in 2004 to 201 in 2011. To maintain varietal purity and protect breeder and consumer rights, a reliable and definitive panel for cultivar fingerprinting becomes critical. In this study, 12 DH lines derived from distinctly different geographic regions were used to evaluate 97 SSR markers by a 3730XL DNA Analyzer. Considering PIC (polymorphism information content), repeatability, discrimination power and markers position in chromosome, 40 SSR markers were selected. Since the homozygote of the DH lines, we can differentiate the different loci and allele variations. In total, 52 loci with 136 alleles were detected. Using these markers, the genetic diversity and DNA fingerprinting of 201 candidate canola cultivars were investigated. As a result, all markers showed distinct polymorphism among the candidate cultivars, whilst, 52 loci with only 131 alleles were detected, which was lower than the DH lines, indicating that the selective effect in the breeding process narrowed the genetic background. PCA (principal components analysis) and population structure analysis revealed clear difference between CMS (Cytoplasmic male sterility) and GMS (Genic male sterility) hybrids, as well as among ecological regions in CMS. Three pairs of previously unknown identical candidate cultivars showed genetic similarity of 0.95 - 1.0, indicating the threshold of genetic similarity for variety identification should be under 0.95 at least. In addition, most of the similar varieties were bred by the same breeder and the most distinct ones came from different province or breeders, which was in accordance with the pedigree information. In conclusion, the SSR panel developed in this study is applicable not only for the fingerprinting of canola cultivars but also for analysis of population structure.

Poster B-12

ASSESSMENT OF GENETIC DIVERSITY IN IBERIAN LANDRACES OF *BRASSICA OLERACEA* BY MOLECULAR MARKERS.

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Brassica oleracea landraces are widely represented in European collections of plant genetic resources and those originating from the Iberian Peninsula are a significant source of genetic variation to conserve. In total, 542 landrace accessions are from Portugal (held at Banco Português de Germoplasma Vegetal) and 250 accessions are from Spain (Misión Biológica de Galicia).

The objective of this project was to evaluate a sub-set of landrace accessions with two molecular marker systems; ITS and SSR. A selection of the most diverse material was used for molecular evaluation, 100 accessions of *B. oleracea* from the Iberian collections were analysed. This subset consisted of three different morphotypes. Fifty accessions of kale (*B. oleracea acephala*), 18 accessions of cabbage (*B. oleracea capitata*) and 32 tronchuda (*B. oleracea costata*) were included in the study.

The study was performed on the ITS1 and ITS2 regions and 10 SSR markers. Based on ITS and SSR data, the results are similar: cabbage and tronchuda landraces form independent clusters whereas kale landraces are grouped in different clusters where Portuguese and Galician landraces clustered together. The relevance of these results to the proposed European Collection of plant genetic resources to be developed as part of the AEGIS concept (A European Genebank Integrated System) is discussed.

Poster B-13

SIGNALS OF INTER-CROSSING BETWEEN LEAFY KALE LANDRACES AND *BRASSICA RUPESTRIS* IN SOUTH ITALY

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In South Italy, landraces of leafy kales are grown in home gardens, sometimes in close proximity to populations of wild relatives. Inter-crossing between wild and cultivated populations might affect allele frequency patterns of the respective populations. This has implications for genetic resources conservation, understanding of domestication processes and overall variability of the brassica gene pool currently in use. AFLP studies on brassica germplasm collected in Calabria and Sicily show evidence that inter-crossing between leafy kale and *Brassica rupestris* is a common occurrence.

Poster B-14

USING CONTROLLED DETERIORATION TEST ON DETERMINING SEED VIGOUR PERFORMANCES OF CABBAGE (*BRASSICA OLERACEA* L. VAR. *CAPITATA*) SEED LOTS

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In this research, the effects of controlled deterioration test on cabbage seeds were investigated by using seed lots from four white head cabbage (*B. oleracea* L. var. *capitata*) varieties. Seed lots from Bafra, Yalova 1, Yalova Sarmalık and Bayraklı 85 varieties were used as experimental material in this work. This study have been conducted in seed germination and test laboratories of Food, Agriculture and Livestock, Variety Registration and Seed Certification Center, emergence tests have been conducted in a glass greenhouse of variety registration and seed certification center. In this study, emergence tests were conducted by sowing seed lots with % 77- 98 germination in peat. Controlled deterioration test was conducted in 40-45°C with 20 % moisture content and within 24 - 48 hours applications. As a result, 45°C temperature 20% moisture content and 48 hours duration for controlled deterioration test. Results of this research provided us to classify seed lots according to their performance by using controlled deterioration test.

Poster B-15

GENETICS OF LOW TEMPERATURE RESPONSE OF PHOTOSYNTHESIS AND GROWTH IN BRASSICACEAE

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Temperature is an important factor determining plant growth and performance. One of the first physiological parameters that is affected by low temperature is photosynthetic efficiency. However, very little is known about the mechanisms involved in the regulation of photosynthesis in cold and the underlying genetics.

We use photosynthetic efficiency of photosystem II (PSII), determined by chlorophyll fluorescence imaging, as an indicator for low temperature stress. An automated phenotyping system allows us high throughput and precise measurement of plant growth and chlorophyll fluorescence over the course of the day. Like this we obtain a unique dataset that provides information about the physiological status and growth of plants in response to low temperature at different time points during the stress and during recovery.

Screening a collection of different *Brassica oleracea* varieties we could show that the photosynthetic efficiency responded very quickly to the environmental changes and we could also establish correlations between photosynthetic and growth parameters in response to the low temperature conditions. These responses were predominantly genotype and not seed lot specific.

The detected natural variation and genetic determinism for cold responses is a promising start for a quantitative genetic analysis. We are currently analysing the data of a recombinant inbred lines population of *Brassica rapa* to determine genetic loci involved in low temperature performance.

Future experiments with a collection of *Arabidopsis thaliana* natural accessions will allow us to conduct an association mapping analysis and by homology search against the Brassica genome increase our power to determine candidate genes. Elucidating this genetic basis will provide useful information for future breeding programs and furthermore allow us to conclude on the corresponding physiological mechanisms for cold adaptation.

Poster B-16

ETHNOBOTANICAL USES OF *BRASSICACEAE* IN SICILY

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In the recent years, wild plants have increasingly become of relevant interest for several reasons: (1) the renewed interest in local traditional and neglected food plants; (2) the potential medicinal value of these species; (3) the intangible cultural heritage of their use. The erosion of traditional knowledge due to the depopulation of rural areas and the death of elderly people contrasts with the interest in local plant food and medicinal sources of the most cultured young and middle-aged of the urban classes. The *Brassicaceae* species are widely represented in Sicily: according to Pignatti (1982) in the island 116 species, belonging to 54 genera, are present among wild and cultivated ones. Some of these species have an ethnobotanical interest.

In this frame the aims of the work was to carry out an ethnobotanical survey, through interviews to representatives of small rural communities, on wild Brassica species in view to identify the traditional use and to compare the overall data on ethnobotanical literature related to Sicily.

The results showed that we have information about sixty species, sometimes linked only to the dialect name. The main use is linked to the food. For example, the bitter taste and the traditional culinary preparations increase the value of different species (*Brassica fruticulosa* Cyr., *B. nigra* (L.) Koch, *Bunias erucago* L., *Diplotaxis eruroides* DC., *D. tenuifolia* (L.) DC., *Nasturtium officinale* (R.) Br., *Raphanus raphanistrum* L., *Sinapis alba* L., *S. arvensis* L., etc.) for food. Some species, *Brassica rupestris* Raf., *B. oleracea* L., *Nasturtium officinale* L., *Raphanus raphanistrum* L.) were used in popular medicine. Particular is the use of *Matthiola incana* L. that is adopted in folklore manifestations related to St. Joseph's Day, when the flowers are used to completely decorate the horses, thus masking the unpleasant smell.

Poster B-17

**PROVENANCE-RELATED SEED HETEROGENEITY AND GERMINATION IN
*BRASSICA INSULARIS MORIS***

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Morphological seed heterogeneity, defined as the production of different types of seeds by a single individual, appears in many different species of angiosperms and it is also reported for the genus *Brassica*. *B. insularis* is a member of the *B. oleracea* L. group which occurs only in Corsica, Sardinia, Sicily (Pantelleria) and Tunisia (La Galite) and is inserted in the Annex II of Habitats Directive 92/43/EEC. Seeds of *B. insularis*, collected in 2010 from three Sardinian populations [Planu Sartu and Masua (SW Sardinia) and Isola dei Cavoli (SE Sardinia)], were analyzed through an image analysis system. In addition, germination tests were conducted on seeds from each population, in the light (12/12), at a range of constant temperatures (5-25°C) and at an alternating temperature regime (25/10°C). The morphocolorimetric analysis, clearly identified seeds from different populations (in 97.6% of the cases) and discriminated three chromatic categories (black, orange and brown) for seeds belonging to the “Isola dei Cavoli” population, with a percentage of correct identification of 90%, while seeds of the two other populations were morphologically homogenous. Germination ranged from 45% to 60% for “Planu Sartu” and “Isola dei Cavoli” populations, respectively and temperature had not a significant effect. Seed moisture content (%) did not vary significantly among chromatic categories, highlighting as heterogeneity was not related to different stages of seed maturation. Germination was highly influenced both from colour and temperature, and high germination percentages (ranging from 50% to 80% for black and orange seeds, respectively) were detected at 5°C. These results showed the significant variability in morphology and germination among *B. insularis* seeds from different provenances, indicating the presence of morphological and physiological seed heterogeneity in this species.

Poster B-18

THE USE OF *BRASSICACEAE* BY THE ANCIENT ROMANS

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There are two main sources of information on plants used by the Romans: archaeological evidence and classical literature. The Latin texts describing plants and their uses are several. Probably the most extensive information is available from Plinius the Elder work but generally there are difficulties to identify plants from the author's descriptions. This may be because there are problems in translation, or simply that the writer did not describe the plant accurately, or in sufficient detail. Some plants have changed due to cultivation or cross-fertilisation, thus genotypes described may no longer resemble to their modern counterparts. Among the plants known by the Romans, the *Brassicaceae* played an important role, partly because many of these species are native to the Mediterranean basin.

With the aim to achieve as much information as possible on the Romans botanical knowledge on *Brassicaceae* species, the operas of nine Latin authors were studied and each species was listed in a specific data base. For every identified species we listed the Latin name, family, origin and uses. The latter have been classified into 5 groups: food, medicinal, utilitarian, magical and ornamental. Specifically, 41 taxa (both species and subspecies) belonging to 21 genera were identified. Romans devoted particular attention to the *Brassica* genera. Marcus Porcius Cato, for instance, narrated the therapeutic applications of some species in the CLXV chapter of 'De Agricultura'. Plinius the Elder in the Naturalis Historia (chapter XIX) described different typologies, cultivation cycles, in order to extend the production time, of some *Brassica*, as well as grafting methods adopted for propagation of some species. Some plants of this important family have been mentioned by Plinius the Elder for the sweet scent of their flowers like *Matthiola fruticulosa* (L.) Maire in Jahandiez & Maire and *M. incana* (L.) R. Br. in Aiton.



BRASSICA2012

Session C

BIOTECHNOLOGY AND BREEDING

Oral presentation

Session C-1

GENETIC VARIATION AND INHERITANCE OF SECONDARY SEED DORMANCY IN WINTER OILSEED RAPE

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Secondary seed dormancy in oilseed rape is a phenomenon that allows seeds to survive for many years in the soil without germination. Following soil cultivation, dormant seeds may germinate in subsequent years and they are the main reason for the occurrence of volunteer oilseed rape plants in successive crops. Genetic reduction of secondary dormancy in oilseed rape could provide a mean to reduce the frequency of volunteer plants and especially the dispersal of transgenic oilseed rape. However, little is known about the genetic variation, the inheritance and the genotype x environment interactions on the secondary seed dormancy of winter oilseed rape.

The objective of the present study is to analyze the genetic variation, the genotype x environment interactions and the inheritance of secondary seed dormancy, and to estimate correlations to other seed traits in a set of 28 current winter oilseed rape cultivars and in the winter oilseed rape DH population Express 617 x R53 tested in multilocal field experiments. Among the cultivars, secondary seed dormancy ranged from 8% to 56% and the heritability was high (0.97). A large and significant variation for secondary seed dormancy was also detected among the DH lines which ranged from 5 to 96%; the heritability was with $h^2=0.85$ again high. Two major and three minor QTL for secondary seed dormancy were detected which together explained 42% of the phenotypic variance. Two of the QTL for secondary seed dormancy on N13 and N18 showed overlapping confidence intervals with QTL for germination rate and the additive effects had opposite signs, indicating that secondary dormancy is related to a low seed germination rate. Results showed that secondary seed dormancy is a heritable trait and that selection for low secondary seed dormancy is possible.

Session C-1

ENVIRONMENTAL AND GENETIC FACTORS CONTROLLING SHOOT APICAL MERISTEM ARREST IN *BRASSICA OLERACEA*

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Genetic and environmental factors influence shoot apical meristem (SAM) development in plants. The interplay between these factors can lead to abnormalities, such as SAM arrest, resulting in a stop of further organ development. This phenomenon is also called blindness and develops mostly in the early phase of plant development. Prior to the differentiation of the SAM the young plants can develop no, one or two normal leaves. *Brassica oleracea* suffer from this arrest that can result in significant production losses, for plant raisers and breeding companies.

Our aim is to study environmental and genetic factors influencing the occurrence of blindness. We developed an assay to induce blind plants in susceptible genotypes of *Brassica oleracea*, by incubating seeds at 0-1 °C for several days. After this cold treatment, most of the seeds germinate and produce a high number of blind plants. This percentage of blind plants varies per genotype pointing towards a genetic component controlling blindness. A Quantitative Trait Loci (QTL) analysis, using AGDH mapping population, revealed one locus with a significant contribution to the sensitivity for blindness. This QTL was reproducible for three sequential seed productions. At the moment we are determining the transcriptome of blind and normal lines of the population, aiming to identify the differentially expressed gene or genes in the QTL location. Expression of three meristem related genes (BoSTM, BoCLV1, BoCLV3) was analysed with two contrasting lines from the population to determine the optimal sampling moment for the genome-wide expression studies. All three genes were down regulated early during seedling development in plants of a blindness sensitive line compared to a resistant one.

Additionally we are quantifying cell proliferation by studying DNA synthesis in intact tissue of normal and blind plants to proof that the SAM is arrested and that during the development of blindness SAM cells of blind plants show lower rates of DNA synthesis until the complete arrest.

Session C-1

EXPRESSION OF TRANSCRIPTIONAL FACTORS AND STRUCTURAL GENES OF ANTHOCYANIN BIOSYNTHESIS IN PURPLE-HEADING CHINESE CABBAGE

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To investigate the mechanism of anthocyanin production in our new purple-heading Chinese cabbage, the expression of structural genes and regulatory genes involved in anthocyanin biosynthesis was examined between the purple line (11S96) and its non-purple parent (09S17) by the real-time fluorescent quantitative RT-PCR, as well as their leaves cross-section were observed and the anthocyanin content was measured. Leaf cross-section showed that the anthocyanin distribution is non-uniform in the purple heading leaf, specially enriched in the epidermis and its adjacent the mesophyll cells. Comparison with the negligible amount of anthocyanin in the non-purple parent, the anthocyanin in purple line is up to $0.80\text{mg}\cdot\text{g}^{-1}\cdot\text{FW}$ in purple inner heading leaves. Q-PCR analysis indicated that the EBGs (Early Biosynthesis Genes) *CHS*, *CHI*, *F3H*, *F3'H*, LBGs (Late Biosynthesis Genes) *UFGT* and *GST*, as well as the regulatory gene *MYB0* and *MYB1* were up-regulated in the inner leaves of purple heading; the other three LBGs, *DFR*, *ANS*, *LDOX* and the regulatory gene *MYB2*, *MYB4*, *MYB12* and *MYB111* were high up-regulated in all the leaves of the purple heading, which means that there is probably close relationship between these genes and the anthocyanin accumulation in this purple-heading Chinese cabbage, and *MYB2* and *MYB4* may play the dominant role in it.

Session C-1

INTERSPECIFIC HYBRIDIZATION AS A TOOL FOR BROADENING THE VARIABILITY OF USEFUL TRAITS IN RAPESEED (*BRASSICA NAPUS* L.)

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Amphidiploid rapeseed as a very important oil plant became widely cultivated crop in many countries in the world. The big increase of rapeseed acreage is causing some new problems connected with yielding and cultivation of this species. Moreover, searching of forms with improved traits is highly desirable. From that point of view interspecific crossing is a valuable tool for widening the variability of useful traits, e.g. seed quality and resistance to some diseases such as clubroot caused by *Plasmodiophora brassicae*. In this study, results of crossing of winter rape ($2n=4x=38$, AACCC) with five other species from the *Brassica* genus are being presented. Altogether from all cross combinations 140 siliquae containing in total 1623 ovules from which isolated embryos were incubated *in vitro*. In this way from all cross combinations 568 embryos were placed on the specified media. The highest effectiveness was received in the cross winter rape, MS-8 line \times *B. campestris* ssp. *pekinensis* (61.4 %). In two next combinations this effectiveness took out 53.1 % (MS-8 \times *B. fruticulosa*) and 51.1% (MS-8 \times *B. campestris* ssp. *trilocularis*). The lower effectiveness was being observed in combinations, where amphidiploid forms were taken as parental components, i.e. MS-8 line (maternal form) and *B. carinata* and *B. juncea*, where 20.5% and 27.4% of ovules contain embryos, respectively. Finally, 284 plants were rooted on Nitsh & Nitsh medium (H3). Furthermore, these studies are supplemented by a cytological characterization of individual, interspecific hybrids of *Brassica* showing their chromosome complement.

Session C-1

THE EFFECT OF MOLYBDENUM ON ARTIFICIAL SEED PRODUCTION, COLD TOLERANCE AND UP-REGULATION OF *CBF/DREB1* OF BRASSICA OLERACEA VAR. BOTRYTIS

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The production of cauliflower clones multiplied by tissue culture and distributed as artificial seeds could be useful alternative to F1 hybrid varieties and could also be useful in the context of the maintenance of elite clonal germplasm in cauliflower breeding programmes. A full protocol for the production of cauliflower artificial seeds was optimised by the encapsulation of cauliflower microshoots derived from the meristematic layer of cauliflower curd. The effect of different concentrations of Molybdenum (Mo) used with cauliflower microshoots liquid culture media ranged between 0 to 240 ppm on both number and average weight of the microshoots produced was investigated. The use of concentrations higher than 30 ppm Mo completely killed the explants. While the number of developing microshoots was not significantly affected by Mo concentration up to 30 ppm, the use of 30 ppm Mo significantly reduced the growth of microshoots assessed as average fresh weight. The use of 15 ppm concentration of Mo improved the cold tolerance of artificial seeds both with and without acclimation by low temperature (4 °C). The expression of *CBF/DREB1* the cold/drought transcription factor was also affected by presence of Mo especially in conjunction with low temperature acclimation. Moreover, the use of 15 ppm Mo had the capacity to up-regulate *CBF/DREB1* without any cold treatment. The results obtained in this study could have an important application for the use of an exogenous application of Mo to raise the efficiency of cauliflower cold hardiness even in the absence of cold acclimation.

Session C-2

INHERITANCE OF CYTOPLASM MALE STERILITY AND SCAR MARKERS LINKED TO FERTILE GENE IN RADISH

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To identify the inheritance of Cytoplasm Male Sterility and molecular markers closely linked to the CMS restorer gene in radish (*Raphanus sativus* L.), RAPD and SCAR technology combined with bulked segregation analysis were used to analyze 360 plants of four F₂ and three BC₁ populations. The results showed that the fertile gene and sterility gene were a pair of independent genetical alleles, and the fertile gene completely dominated the expression of sterility gene in the genetic process. Two RAPD markers S88-1526 and S1344-415 linked to fertile gene locus were identified from 500 RAPD primers. These two molecular markers were located on two sides of the sterility gene with a distance of 4.2cM and 6.9cM respectively. One RAPD molecular marker S88-1526 was successfully converted to SCAR marker as scar-88, whose recombination frequency was 4.28% with a genetic distance 4.24 cM to the fertile gene locus in population BC₁.

Session C-2

MAPPING OF A QUANTITATIVE TRAIT LOCUS FOR THE BOLTING TRAIT IN *BRASSICA RAPA* UNDER VERNALIZING CONDITIONS

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Premature bolting can occur during spring cultivation of heading Chinese cabbage in East Asia when the plants encounter low temperatures (vernalization), which leads to economic loss. The aim of this study was to identify genes responsible for varietal differences in the bolting trait in *Brassica rapa* under environmental conditions that promote vernalization. To achieve this goal we constructed a linkage map with 107 simple sequence repeat (SSR) and 54 insertion/deletion (InDel) markers based on a segregating population of 186 F₂ individuals. The resulting map consisted of 10 linkage groups and covered a total length of 947.1 cM, with an average genetic distance of 5.84 cM between adjacent markers. QTL analysis of the bolting trait was performed by two phenotypic evaluations (bolting index and flowering time) based on the scores in an F₂ population in the spring of 2010, and scores in F_{2:3} families in autumn 2010 and spring 2011, respectively. A total of 26 QTLs that controlled bolting were detected, accounting for 2.6% to 31.2% of the phenotypic variance. The detected QTLs with large effects clustered mainly on linkage groups A02, A06, and A07. These QTLs will be useful for marker-assisted selection (MAS) in a breeding program for late bolting or bolting-resistant cultivars in *Brassica rapa* crops.

Session C-2

OVEREXPRESSION OF *MYB29* TRANSCRIPTION FACTOR INCREASES THE BENEFICIAL EFFECTS FOR AGRICULTURE AND HUMAN HEALTH OF *BRASSICA OLERACEA* PLANTS

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Glucosinolates (GSLs) are secondary metabolites naturally produced by cruciferous plants. Their active enzymatic hydrolysis products, isothiocyanates (ITCs), play important roles in human health and agriculture. GSLs, and especially ITCs, are considered the main responsible for the cancer preventative properties of Brassica plants. For instance Sulforaphane, the ITC generated from the 4-Methylsulphinylbutyl GSL (glucoraphanin), is released when several Brassica crops, especially *B. oleracea* varieties, are consumed. Conversely, different kinds of ITCs, derived from aliphatic and aromatic glucosinolates, are highly toxic to several plant pests and pathogens. These properties make them useful as a more sustainable alternative to chemical fumigants. For example, Allyl ITC, the hydrolysis product of the 2-propenyl aliphatic GSL (sinigrin), is demonstrated to be toxic to Nematodes, Fungi and some herbivores. DH AG1012 genome has a high similarity to the Broccoli genome, thus being a potential *B. oleracea* model for studying and improving Broccoli. On the other hand, Winspit, a wild *B. oleracea* genotype which has an *AOP2* functional allele, produces the Alkenyl GSLs sinigrin and gluconapin in high quantities compared to other *B. oleracea* wild populations and cultivars. Therefore, the glucosinolate gene set present in Winspit makes it an interesting genotype for soil biofumigation in agriculture. In this work, we isolated the Winspit *MYB29* gene and used it for overexpression and expression of it in the DH AG1012 genotype. A complete expression analysis of the genes controlling the Aliphatic GSL pathway was done for the transgenic and control plants. Additionally, the antigenotoxic activity of the DH AG1012 expressing *WinMYB29* was evaluated. In this research, the difference in the functionality of *AOP2* gene between wild and cultivated genotypes was crucial for studying the aliphatic GSL pathway and the role that *MYB29* plays in the production of glucoraphanin and sinigrin in *B. oleracea*.

Session C-3

THE INNOVATIVE EXPLOITATION OF BRASSICA VEGETABLES IN THE HEALTH QUALITY FOOD PRODUCTION CHAIN. FROM FIELD TO FORK

Keynote lecture

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The specific chemical composition of vegetables belonging to *Brassicaceae*, as regards both nutrients and non-nutrients, renders them particularly suitable for the health food production chain. The ability of these plants to accumulate heavy metals may be utilised for phytoremediation of arable soils, but also as a vehicle to fortify foods in micronutrients such as selenium. The bioactive organosulfur compounds: glucosinolates and their degradation products, glutathione or S-methylmethionine owing to their chemical properties can target a number of biological processes implicated in human organism homeostasis. Consequently, rationally exploited brassica vegetables may be a promising ingredient for research based production of foods addressing both general public, as well as specific groups of consumers. Moreover, isothiocyanates, released upon myrosinase catalysed hydrolysis of glucosinolates, due to their reactivity towards amino groups, may play a role of “chemical detoxification system” neutralising carcinogenic amines formed during heat processing of meat foods. The agricultural waste of brassica crops containing glucosinolates and their degradation products represents a valuable raw material for crop protection in the process of biofumigation, at the same time constituting green manure. The multidirectional opportunities and examples of innovative exploitation of chemical and biological properties of brassica vegetables will be discussed.

Session C-3

QTL ANALYSIS OF BOLTING TIME IN RADISH (*RAPHANUS SATIVUS* L.) BASED ON SNP MARKERS.

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Radish (*Raphanus sativus* L.) is an important vegetable, especially in East Asia. Radish accounts for the largest cultivation area and annual production of all vegetable cultivated in Japan.

Bolting time is an important developmental/agronomic trait, and variation exists among radish cultivars. Their bolting habits are generally influenced by temperature, light intensity and/or day-length. Bolting time is one of a major determinant for root thickness in radish, because it has been known that the positive correlation exists between bolting time and root thickness, that is a phenomenon which root development of thickening was restricted after bolting. Because of this reason, late bolting cultivar has been selected in conventional breeding process. Recently, a number of molecular markers linked to important agronomic traits have been developed in many plants. Marker-assisted selection (MAS) has also been desired for the development of new cultivars and the introduction of bolting traits in radish breeding program.

Two Radish cultivars, Yumehomare and Taibyosobutori, (late flowering and early flowering phenotypes, respectively) were used in this study. In each parent, homozygosity was increased by 5 successive selfings. We crossed two genotypes and generated F₂ population. One hundred and eighty eight F₂ progenies and their parent plants were grown and 11 morphological traits such as plant height, leaf number, bolting time, root diameter were investigated at different growth stages. Using 103 SNP markers, 9 linkage groups (LGs) were constructed. QTL analysis revealed that among QTLs relating bolting time, a major QTL was detected on LG4. This QTL region is a comparative with the particular region of Arabidopsis chromosome 5 containing *FLC* (*FLOWERING LOCUS C*). We will present the locations of QTLs controlling the important agronomic traits including bolting time.

Session C-3

PCR-BASED IDENTIFICATION OF CYTOPLASM TYPES IN MALE STERILE ACCESSIONS OF *BRASSICACEAE*

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The plant collection being maintained at All-Russian Research Institute of Vegetable Breeding and Seed Production contains 43 accessions, including both fertile and sterile plants of white cabbage, savoy cabbage, Brussels sprouts, kohlrabi, daikon and radish.

Appropriate primers were selected out of summarized publication data to type the sterile cytoplasm among the accessions belonging to *Brassicaceae*.

Out of all primers 12 pairs were 138Xba and 138Eco, Orf138-F1 and Orf138-R, Orf138-F2 and OrfB-R1, ogu-5 'and ogu-3', orf138-5 'and orf138-3 ', Og-1 and Og-2, F * and R *, P11 and P12, orfBF and orfBR, orfBF' and orfBR2, P6 + (orfB) and P6-(orfB), Me6 and Em7 to identify *Ogura* cytoplasm. Three primer pairs pol-5 'and pol-3', Orf224f and Orf224r, P21 and P32 were used to find *polima* cytoplasm. The *napus* cytoplasm can be determined with two pairs of primers nap-nap-5 and 3, P21 and P22. Such primers as rad-5 'and rad-3' were also taken to detect normal radish cytoplasm.

As a result, the *Ogura* type cytoplasm was clearly defined in cabbage, daikon and radish according to specified PCR products observed.

On the contrary, no locus associated with cytoplasm sterility of *napus*, *polima* and *Ogura* was found in supposed sterile samples of Savoy cabbage, Brussels sprouts and kohlrabi.

The *orf138* locus is known as a marker of *Ogura* type cytoplasm and used to characterize the CMS. The aligned sequence of the products obtained with primers Orf138-F2 and OrfB-R1 in accessions of radish, daikon and cabbage showed their similarity to the mitochondrial locus Orf138 corresponded to type A *Ogura* sterility.

It is worth mentioning that all samples had the deletion of two nucleotides AA at position 20-21. Moreover, an A/G base substitution at position 18 was observed in radish and cabbage sterile accessions. Additionally, a G/A base substitution was detected at position 410 in the samples of cabbage.

Session C-3

CREATING A SOURCE MATERIAL FOR BREEDING RADISH FOR GREENHOUSES.

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In recent years in Russia the production of radish in the greenhouse with the use of cassette technology, includingis of sliding hydroponic shelving, with periodic underflooding, supplementary lighting, and established the parameters of the microclimate.

The use of radish F₁ hybrids is the best solution to uniformity of products.

The purpose of our research is the creation of F₁ hybrids of radish with such features as: early ripening (18-20 days), high-yield (3.5 kg/m² or more), resistant to the shooting and the deficiency of light (winter cultivation), the ability to form roots at low temperatures, low erect rosette leaf, root crops dense rounded form.

In our work to create a of heterotic F₁ hybrids of radish, we used the line ogura-CMS and the phenomenon of self-incompatibility. As a result, four sterile lines and their fertile analogs have been obtained.. Were created four self-incompatibility inbred lines POK-2, KMN-3, KMN-8 and KRR-2. Of them KMN lines is characterized by high capacity combination on the feature "root mass" and the high value of the maternal effect. The line KMN is also valuable in the breeding to the shorten of leaf rosettes.

In the course of the study of population consisting of 90 radish varieties and F₁ hybrids of different origin and 17 inbred lines of second-and fourth-generation the following correlations was revealed: hypocotyl length with the length of the leaves ($r = 0,72$), root diameter ($r = 0,83$), the inverse length of the hypocotyl strong relationship with productivity ($r = -0,76$) and product yield of roots ($r = -0,72$).

Session C-3

GENE EXPRESSION PROFILING OF DEVELOPING SEEDS IN *BRASSICA NAPUS* L. AT DIFFERENT NITROGEN LEVELS

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Nitrogen is one of the major fertilizers to *Brassica napus* L.. To assess the influence of nitrogen fertilizer on protein and oil content, and identify nitrogen-response genes and their networks, we carried out 4 nitrogen gradients using *B. napus* cultivars Zhongshuang 11 and Parter (from Germany). Oil content and protein abundance in matured seeds were investigated. The gene expression profiles of seeds at 25 days after pollination (DAP) between nitrogen fertilizer-treated (180 kg ha⁻¹) and blank-control of both cultivars were conducted using microarray, and the expressions of *ACCase*, *DGAT2* and *PEPC* of seeds at different development stages between the treatment and control were analyzed. Our results indicate that (1) oil content was reduced but the decline was obviously higher in Parter than in Zhongshuang 11, whereas protein percentage was enhanced of both cultivars. (2) 827 and 3676 genes between treatment and control of Zhongshuang 11 and Parter were differentially expressed with 2-folds or even more, respectively. Among these differentially expressed genes (DEGs), 287 DEGs including 151/80 up-/down regulated and 4 10-fold or more change genes were shared between the two comparisons. About 50% DEGs could found functional hits and assigned biological roles, such as catalytic, binding and transcriptional regulation activities. The remaining genes have unknown hits at this moment. (3) Compared gene expression levels between treatment and control, the expression levels of *ACCase* and *DGAT2* had no obvious difference for the two cultivars during 7-25 DAPs; however, at 31 DAP, they were down regulated significantly in Zhongshuang 11, and both were up regulated 1 fold in Parter. The expression peaks of *PEPC* were brought forward in treatments of the two cultivars. The three genes responding to nitrogen fertilizer existed genotype differences. Our results shade light on exploring mechanism of nitrogen response and nitrogen-tolerance genotype identification in *B. napus*.

Session C-3

USEFUL METHOD FOR DISTINGUISHING THE PLOIDY OF TUBE PLANTLETS REGENERATED FROM MICROSPORE CULTURE IN *BRASSICA NAPUS* L.

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The microspore culture protocol in *B. napus* was established successfully in 1980s and has been used in breeding and other research areas. However, about 10-60% haploid plants appeared in some populations of plants regenerated from colchicine-treated microspores and could not be distinguished by eyes observing at seedling stage. Thus, the use of *B. napus* microspore culture system was decreased in breeding programs. For the ploidy identification of the plantlets, by using techniques of chromosome analysis and DNA content determination by flow cytometry is labor intensive, time-consuming and cost expensive, which cannot be used to identify a large numbers of plants. So, the ploidy level of the tube plantlets from *B. napus* microspores was studied through determining its chloroplast number in stomatal guard cells.

Methods: A piece of middle tissue was excised from the leaflet of the tube plantlets from lines 3163 etc. and put on slide to remove its mesophyll cells, then placed a drop of 1.2% I-IK on the epidermis with staining for two mins. Stained chloroplasts were counted under bright field microscope. A total of 10 stomata containing 20 guard cells per leaflet were tested for each plantlet each genotype. These plantlets determined were transplanted to field to check further their ploidy by the morphological characters at flowering and seed setting phases.

Results: The plantlet having 6-8, 10-13 or 15-24 chloroplasts per stomata in more than 7 out of 10 stomata per leaflet is haploid, doubled haploid and tetraploid, respectively, which is consistent with traits of the flowers and seed set in plants tested grown in field. This chloroplast counting procedure is an effective and reliable method for rapid and easy distinguishing the ploidy level of a large numbers of plantlets from the microspore culture so as to eliminate or double haploids early, to increase the efficiency of doubled haploid system in *B. napus*.

Session C-3

DEVELOPMENT OF HETEROSIS HYBRIDS OF WHITE HEAD CABBAGE THROUGH SELF-INCOMPATIBILITY AND CYTOPLASMIC MALE STERILITY

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The main objective in breeding of white head cabbage in Russia is to increase productivity, quality and disease resistance through heterosis. The hybrid seed production of cabbage is based on self-incompatibility and utilization of cytoplasmic male sterility.

The horticultural characteristics of new inbred lines of cabbage developed in the All-Russian Research Institute of Vegetable Breeding and Seed Production are as follows:

- earliness of ripening (80-85 days from seeding stage till the stage of industrial ripeness of head);
- increased yield due to larger or more marketable heads (head weight averages 5 kg);
- resistance to *Fusarium* wilt and black root.

The inbred line with a marker trait correlated with ripeness was developed. Using saturating crossing, the lines with the Ogura cytoplasmic male sterility were developed. For each sterile line the maintainer lines of several groups of ripeness were selected. Using these lines, the new hybrid combinations of three groups of ripeness (early-, mid-, and late-ripening) were developed. Work on the identification of genes responsible for these traits is underway. The maintaining of the original male sterile lines is carried out through *in vitro* micropropagation.



BRASSICA2012

Session C

BIOTECHNOLOGY AND BREEDING

Poster presentation

Poster C-1

MOLECULAR CHARACTERIZATION OF STRESS RESPONSIVE FAMILY GENES IN *BRASSICA RAPA*

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Brassica is a very important vegetable group worldwide and for these crops biotic and abiotic stresses are major concern now. To address this concern, enhancement of resistance against stresses exploiting stress resistance related genes, offers the most efficient approach. Among the stress resistance related genes, chitinase and thaumatin-like genes are playing vital roles for enhancement of resistance in *Brassica*. In this study, 3,429 ESTs, obtained from the full-length cDNA library of *Brassica rapa* cv. Osome, were well annotated using bioinformatics tools and clustered into different functional categories including stress related genes. From these stress related genes, 3 chitinase and 2 thaumatin-like genes were identified and more 27 chitinase and 18 thaumatin-like genes were collected from the *Brassica* database. By sequences analysis and comparison study, 30 chitinase and 20 thaumatin-like genes were confirmed and these genes showed a high degree of homology with other published stress related genes. In addition, an organ specific expression of these genes was observed in chinese cabbage plants. Among the chitinase genes, seven genes showed responses after infection with *Fusarium oxysporum* f.sp. *conglutinans* in cabbage and ten genes showed responses after salt, drought and PEG stress treatments in chinese cabbage. On the other hand, seven thaumatin-like genes showed responses after cold, salt, drought and ABA stress treatments. All these data revealed that these genes may be involved in resistance against biotic and abiotic stresses for *Brassica*.

Poster C-2

IDENTIFICATION AND CHARACTERIZATION OF COLD RESPONSIVE GENES IN BRASSICA

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Brassica is a very important vegetable group worldwide. Cold stress is the major environment factor that limits the productivity of *Brassica*. Plants differ in their tolerance to chilling and freezing are major concerns and molecular biology techniques offer the most efficient approaches to address these concerns. In this study, we collected 903 ESTs of stress chilled tandem and consensus sequences from *B. rapa* tissue-specific EST database (*BrTED*) and analyzed cold treated *Br24K* microarray data. Gene ontology (GO) analysis of these ESTs revealed 94.2% of sequences well annotated towards reeds one dimensional concept and obtained 113 sequences responsive to abiotic stimulus under the category of biological process (BP). We then identified 20 highly cold responsive genes from abiotic stimulus group and *Br24k* microarray data and further analyzed expression after applying cold stresses (22 °C, 3 °C, -2 °C and -6 °C) in chinese cabbage. All genes responded to cold and freezing stress except *BrCRG4* and 15. *BrCRG* 1, 10, 11, 13, 17, 18 and 19 were highly expressed at 3°C and *BrCRG2*, 3, 5, 6, 7, 12, 14, 16, 17 and 20 were highly expressed at -2 and -6°C. These results revealed that the identified genes may be useful resources for developing cold and freezing tolerant *Brassica*.

Poster C-3

NUTRITIONAL ENHANCEMENT OF BROCCOLI SPROUTS THROUGH MANIPULATION OF GROWTH CONDITIONS

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The consume of *Brassicaceae*, widely diffused in the world, is highly recommended because of their recognised beneficial role. Young seedlings (sprouts), in particular, represent enriched sources of vitamins, minerals, and health promoting bioactive substances respect to adult plants. In addition, sprouts can be easily grown all year round at home representing a good alternative to increase vegetable consumption. Interestingly, many of the health promoting bioactive molecules are produced or vary their concentration in response to environmental growth conditions. In fact, to survive and grow under abiotic stress conditions, crops have evolved sophisticated adaptative response mechanisms based on a high metabolic plasticity.

In this work, we aim at studying the influence of different light and temperature regimes, as well as, of some chemical and hormonal treatments on bioactive molecules production in broccoli sprouts. We focused our attention mainly on glucosinolates, anthocyanins and phenolics compounds for their recognised health promoting role. We have developed a rapid and sensitive LC-MS/MS (MRM) method to analyse and quantify glucosinolates in broccoli sprouts. The results showed that exposure to light increase the glucosinolates and anthocyanins content in broccoli sprouts. Also, abiotic stresses (sucrose, mannitol, NaCl) and some stress responses hormones were able to increase accumulation of anthocyanins and glucosinolates, with sucrose being the most effective.

In addition *in vitro* studies to test the potential health promoting effect of extracts of broccoli sprouts grown under different conditions on different cell culture system are in progress.

Poster C-4

UNRAVELLING MOLECULAR MECHANISMS INVOLVED DURING *BACILLUS AMYLOLIQUEFACIENS* MEDIATED INDUCED RESISTANCE IN PLANTS

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Many bacteria are known to provide beneficial effects to plants. Colonization of plants by specific plant growth promoting bacteria can enhance plant growth and tolerance to a wide range of pathogens. The general mechanism through which bacteria induce systemic resistance (ISR) in plants has been reported to involve jasmonic acid (JA) and ethylene (ET) responses. The molecular mechanisms underlying priming from the time of recognition or beneficial bacterial to stress challenge of the plants are not fully elucidated.

In our laboratory, we study the mechanisms by which *Bacillus amyloliquefaciens* promotes plant growth and defense in *Brassica napus* and *Arabidopsis thaliana*. Previous transcriptomic, metabolomic and hormonal studies revealed a major resource allocation upon priming by *B. amyloliquefaciens*. Further studies using *A. thaliana* JA, SA and ET signaling mutants and GUS reporter lines revealed a complex strategy being employed by *B. amyloliquefaciens* to evade hemibiotrophic and necrotrophic pathogen onslaught. We also demonstrated the involvement of a novel transcription factor during priming and defense against pathogens.

Unraveling the underlying molecular machinery on the detection of bacteria mediated MAMPs and subsequent downstream signaling and response events have been in focus since the past decade. We show that some receptors have a parallel role in MAMP and PAMP perception systems. A higher degree of MAMP suppression was observed during the initial stages of colonization by *B. amyloliquefaciens*.

Poster C-5

MOLECULAR CHARACTERISATION OF BRASSICAS DEVELOPED BY EMS MUTAGENESIS WITH TOLERANCE TO SULFONYLUREA HERBICIDES

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The sulfonylureas are an extensively used class of herbicides that include commercial products such as chlorsulfuron. The mode of action of chlorsulfuron is to inhibit the chloroplast-localised enzyme AHAS (AcetoHydroxyAcid Synthase), a key enzyme in the formation of branched chain amino acids. This leads to a deficiency in these amino acids and the accumulation of the AHAS substrate, 2-ketobutyrate, which is toxic. Point mutations in the AHAS gene can result in herbicide-tolerant phenotypes by altering the herbicide binding site while retaining the catalytic activity of the enzyme. Five conserved domains are implicated in resistance to AHAS inhibitors across a range of naturally occurring and artificially generated resistant genotypes.

In previous work, *Brassica napus* (rape) rapid cycling lines with increased resistance to chlorsulfuron were developed through EMS mutagenesis of seeds. This material was then used to develop traditionally bred herbicide tolerant Cleancrop™ brassicas including HT Rape™. Molecular analyses of the conserved domains of the AHAS gene indicate a proline to leucine substitution in Domain A is responsible for the resistance.

Poster C-6

ON THE WAY TO THE IDENTIFICATION OF A DOWNY MILDEW RESISTANCE GENE IN *BRASSICA OLERACEA* L.

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We have recently mapped the dominant and monogenically inherited downy mildew resistance locus *Pp523* to the chromosome C8 of *Brassica oleracea* L. and constructed a physical-map (BAC contig) that encompasses this locus, using two BAC libraries available at the Plant Genome Mapping Laboratory, University of Georgia, USA. A minimal tiling path constituted by 13 BAC clones and spanning the resistance locus was also identified. Presently, STS markers derived from this physical map are being used to select clones from a genomic BAC library constructed in our laboratory for the downy mildew resistant line. This new BAC library contains 17280 clones with an average insert size of 125 Kbp, which represent approximately 3.5 equivalents of the broccoli genome. To date, 18 clones from this new library were selected and assembled in a contig (with two small gaps) that cover the major part of the previously mapped locus region. The assemblage of overlapping BACs has been done by PCR using STS markers derived from BAC-end sequences, followed by a confirmation of the sequences of the PCR products against the original BAC-end sequences. The BAC-end sequences of 12 of the newly identified clones were aligned with the *B. oleracea* genomic sequences available in the *Brassica* database (BRAD), allowing a more precise positioning in the genome.

Poster C-7

CHARACTERIZATION AND IMPROVEMENT OF SICILIAN LANDRACES OF VIOLET CAULIFLOWER

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In Italy several landraces of cauliflower are grown because of their popularity in different regions. During the last twenty years, at DISPA, Sicilian cauliflower landraces have been collected, characterized, evaluated and conserved with the aim to identify genotypes of interested. Sib lines and F1 hybrids, obtained by anther culture, have been obtained during breeding programs with the aim to enhance nutraceutical and agronomical traits in this crop. These activities allowed to supply qualified propagation materials to the local growers and to use the wide gene pool for F1 hybrids release. In the present work we characterized two local cultivars of Sicilian purple cauliflower, two selective lines of first-generation, four selective lines of second-generation, three selective lines of third-generation, one selective lines of fourth-generation, and four F1 hybrids obtained at DOFATA (former DISPA).

Morpho-biometric parameters such as fresh and dry weight, longitudinal and transverse dimension, core diameter, grain size, angle of curvature and chromatic parameters were collected in order to define qualitative traits of the curds and to highlight differences among the genotypes.

A sample of lyophilized curd was used to determine, for every genotype, the total content of glucosinolate compounds (ISO 9167-1 1992), particular attention was paid to glucorafanina and glucobrassicina; moreover ascorbic acid, total polyphenols, anthocyanins and β -carotene were determined as well.

Molecular analysis were performed using microsatellite known in the literature for this crop. The study of the genetic profile allowed us to detect the traits of agronomic and/or technological value of the characterized genotypes.

The morphological characterization highlighted different traits of the different genotypes; the analysis of antioxidant compounds allowed to identify high values of Sicilian genetic materials compared to commercial F1 hybrids. Molecular analysis provided an accurate classification of materials by dividing them into cultivars, selective lines, DISPA hybrids and commercial hybrids.

Poster C-8

ORGANISATION OF STEM CANKER RESISTANCE QTL AND GENOME DUPLICATIONS IN *BRASSICA NAPUS*

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Taking into account the structural organisation of Brassica genomes will allow to understand the impact of genome duplications and rearrangements on the organisation, the effects and the diversity of genetic factors involved in complex traits. For quantitative resistance, fine comparative analysis of the genomic regions carrying the QTL would provide data on the potential diversity of the underlying factors, according to the number of QTL in duplicated regions and to the similarity between these regions. With the assumption that the combination of QTL with different mechanisms or different effects on the pathogen can increase the sustainability of the resistance trait, the diversity of the involved QTL should be assessed to be able to rationalize their use in plant breeding. Previous studies led in our laboratory identified genetic factors for quantitative resistance of oilseed rape to stem canker through linkage analysis and association mapping approaches. The recent development of high throughput genotyping has opened new perspectives of analysis.

The aims of this study are *i*) to take advantage of dense SNP genotyping data to make a more detailed association analysis between markers and resistance trait and *ii*) to study the structural organisation of QTL for resistance in relation to the duplications occurring in *B. napus* genome.

To identify with more precision the marker-resistance trait associations, we conducted a genome-wide association analysis on a panel of 117 oilseed rape varieties using 3228 SNP markers and phenotyping data collected on one year disease trial. By a synteny approach with *Arabidopsis thaliana* genome, we put in relation the position of the QTL and the duplications occurring in the oilseed rape genome.

Knowledge of the structural organisation of the genetic factors involved in quantitative resistance will provide evidences on the potential diversity usable for plant improvement.

Poster C-9

LARGE SCALE GENOTYPING IN *BRASSICA NAPUS*, *BRASSICA OLERACEA* AND *BRASSICA RAPA*

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While most SNP development projects in the Brassicas have focused on the allopolyploid *Brassica napus* (AC), SNPs in the diploid ancestors *Brassica oleracea* (C) and *Brassica rapa* (A) are also of interest since these two species are important vegetables. We have investigated the use of a combined panel of *Brassica napus*, *Brassica oleracea* and *Brassica rapa* lines to identify SNPs that are polymorphic not only in *Brassica napus* but also in one of the two ancestral species. Furthermore, we have used the data from the two ancestral species to discriminate intergenomic SNPs, i.e. those between the A and C genome of *Brassica napus* from useful or intragenomic SNPs, i.e. those that are located in one of the two genomes and only present between different lines. Using this approach, it was possible to obtain a very high success rate for developed SNP markers both with respect to polymorphism and functionality in the different *Brassica* species. We will also report on data concerning the development of specific allele calling procedures for the diploid *Brassica oleracea* and *Brassica rapa* species as well as for *Brassica napus*. The respective success rates and accuracy is exemplified based on the analysis of germplasm from the three species as well as from mapping populations.

Poster C-10

DEPENDENCE OF PISTILS' STIGMAS VIABILITY IN WINTER RAPE ON ITS GENETICAL ORIGIN

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Peculiarity of stigmas of pistils' viability, dynamics of seed setting in winter rape varieties and forms differing in erucic acid and glucosinolates content and, as well as, forms with sporophytic self-incompatibility and the original mutant sterile form are studied. The researches have been carried out in 2011-2012 ys. in the experimental fields of Plant Production Institute nd. a. V. Ya. Yuryev, Kharkov, Ukraine. Varieties and original forms of winter rape differing in the content of erucic acid and glucosinolates were used as the initial material. The forms of "00" type (a low content of erucic acid and glucosinolates) were studied and self-pollinated lines – 110M-10, 127M-10, 132M-10; the forms of "0+" type – selfed lines 124-10, 221-10, 305-10; the forms of "+0" type – cultivars Victor, Janus, Emerald; forms of "++" type – cultivars Fyedorovskiy, Marens, Uspikh, as well as, self-incompatible forms 5C, 15C-10, 32C-10 and the original sterile form of mutant origin were used. In the cluster 7-8 buds were left and castrated. As an insulating material Agrofiber P-17 of the Polish trademark was used. The viability of pistils' stigmas was studied by means of artificial pollination of castrated flowers with the three-day interval. The number of seed setting pods was counted on 3 insulators at an average. Viability in stigmas of the pistil lasted 3-7 days, in the forms of "+0", "++" type, 7-15 days in the cultivars and forms of "0+", "00" type, 14-21 days in the self-incompatible and sterile forms. A fast loss of sensitivity to pollen by pistil's stigma was observed in the forms of "+0" and "++" types. On the contrary, in self-incompatible and sterile forms a gradual increase of sensitivity of pistil's stigma to pollination and its gradual fall. It has been noted that in comparison with 2011 pistil viability period in self-incompatible and original sterile forms decreased by 19 % approximately, in the rape forms of "00" and "0+" types – by 12 % roughly. The forms of rape, of "+0" and "++" types responded less to temperature changes, duration of viability undergone the least changes, decreased by 9 %. The rape forms of "+0" and "++" types are defined by a greater number of seed setting pods at flowers' pollination on the date of castration (on the average 4,8-5,0 seedpods) and a sharp reduction in seed setting during flowering (flower's life). A maximum of sensitivity of a pistil's stigma to pollen in "00" and "0+" forms was on the 3rd – 6th day after castration. The pollination of self-incompatible and sterile forms can be carried out quite long, starting from the 4th day after castration up to the 16th day, but to obtain hybrids of winter rape on the basis of self-incompatibility and CMS it is necessary to have sources with a prolonged period of pollen-forming ability.

Poster C-11

GENETIC MODIFICATION OF OILSEED RAPE TO CREATE NEW QUALITY AND FLOWER CHARACTERISTICS

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Oilseed rape (*Brassica napus* L.) is among the most important oilseed crops in the world. Genetically, oilseed rape is a close relative to the model plant *Arabidopsis thaliana*, both found in the *Brassicaceae* family. A translational science approach, where molecular knowledge from *Arabidopsis* within floral development and hormonal signalling is combined with practical know-how from ornamental breeding, flower and senescence physiology, holds great prospective for crop improvement. Potentially, new plant cultivars with changed characteristics affecting crop yield and performance could be generated. In the present study, we transform oilseed rape with a wild type strain of *Agrobacterium rhizogenes*. During transformation, *A. rhizogenes* root-loci-genes (*rol*-genes) are integrated into the rape genome resulting in the generation of hairy roots. After selection of putative *rol*-transformed roots followed by regeneration of plants *in vitro*, plants with a changed morphology can be generated. The rape transformation method comprises expression optimization of bacterial virulence-genes in *A. rhizogenes*, explant type and regeneration experiments. Positive transformants in rape will be validated on DNA and RNA levels using polymerase chain reaction (PCR) and quantitative real time PCR. In ornamentals, the presence of *rol*-genes results in plant compactness, increased rooting and changed flower characteristics, however, on the molecular level *rol*-gene induced changes are not well characterised. Via the hormonal signalling pathways characterised in *Arabidopsis* and the insertion of *rol*-genes into oilseed rape, we aim to broaden the understanding of the functions of *rol*-genes on phenotypes related to flowering and crop yield.

Poster C-12

DETERMINATION OF SELF-INCOMPATIBILITY IN CHINESE CABBAGE (*BRASSICA RAPA*) BY MULTIPLEX PCR

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Self-incompatibility (SI) is one of several mechanisms plants, which evolved to prevent inbreeding and to promote outcrossing. SI is genetically controlled by a single locus that has been designated as S. Generally, the S haplotypes of *Brassicaceae* crops, such as cabbage, broccoli, radish, and Chinese cabbage, has been investigated by conventional test-cross. However, it takes several months to determine the S haplotypes using traditional breeding methods, and the results obtained are sometimes uncertain because of environmental effects and physiological factors. To overcome these difficulties, simple and reliable methods for crossing have been developed in many crops. However, effective method to detect S haplotypes has not been reported due to their highly polymorphic sequences. In this study, we developed newly multiplex PCR primer based on sequences of S-locus receptor kinase (SRKs) and S-locus glycoprotein (SLG) of *Brassica rapa*. The designed primers gave different sizes of amplification from genomic DNA of five S haplotypes (S22, S25, S46, S54, and S55) on a 2% agarose gel. The multiplex PCR is a simple and fast method for marker assisted selection (MAS). The results of this study suggest that the multiplex PCR methods will effectively be utilized to identify the S haplotypes of *Brassicaceae* breeding lines at the early seedling stage.

Poster C-13

THE USEFULNESS OF AFLP MARKERS FOR DETERMINING GENETIC DISTANCE IN OILSEED RAPE (*BRASSICA NAPUS* L.) BREEDING MATERIALS

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Potential benefit of heterosis in practical hybrid breeding depends on specific combining ability and genetic distance of parental lines. The level of genetic diversity between parents has been proposed as a predictor of F₁ performance and heterosis for biomass and seed yield.

The objectives of this study was to estimate the genetic distance between breeding materials which can be utilized in the breeding of Polish winter oilseed rape hybrids. The collection of 78 genotypes included 25 CMS *ogura* and 36 restorer lines. The set of genotypes was completed with winter *B. napus* cultivars and genotypes of European and Asian origin.

In order to determine genetic distance in oilseed rape genotypes the analyses of AFLP markers were performed. 11 primer-enzyme combinations (*Eco*RI and *Mse*I) were used. Genetic distance among genotypes were estimated in accordance with Nei and Li (1979) coefficient of dissimilarity. The genotypes were grouped using the unweighted pair group method using arithmetic average.

A total of 262 reproducible, polymorphic markers were obtained. Genetic distance ranged from 0.1 within CMS *ogura* lines (MS 2 and MS 3) to 0.79 within CMS *ogura* and restorer line. UPGMA clustering based on Nei and Li coefficient of dissimilarity separated the 78 genotypes into five distinct clusters: CMS *ogura* (2); restorer lines (2) and one cluster representing European cultivars and Polish breeding lines.

AFLP dendrogram grouped genotypes closely related by pedigree with respect to breeder and/or country of origin.

Poster C-14

AN EFFICIENT REGENERATION SYSTEM FOR LEAF OF HEADING CHINESE CABBAGE

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The effects of different combination of hormones, cutting treatments and inoculation methods on regeneration rate of adventitious bud were studied in four heading Chinese cabbage with leaf as explants. The results showed that the best regeneration rate and regeneration coefficient reached as high as 87.63% and 2.62 respectively in MS+4.0 mg.L⁻¹ 6-BA+ 0.28 mg.L⁻¹ NAA. The leaf was cut off close to the hypocotyl, and the whole leaf with petiole were kept as explants. The explants were then made to stand on its leaf stub. Comparing the four genotypes, the highest regeneration rate reached 87.63% in 06J28, while the highest regeneration coefficient reached 3.76 in 92S105. The best result was also got in the medium supplemented with 0.3 mg.L⁻¹ TDZ and 0.28 mg.L⁻¹ NAA in 06J28, the best regeneration rate and regeneration coefficient were 80.73% and 1.89 respectively, and TDZ showed similar effect with 6-BA.

Poster C-15

CHANGE IN THE EXPRESSION OF ANTHOCYANIN PATHWAY GENES IN DEVELOPING *BRASSICA* CROPS

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Plant-based foods contain significant amount of bioactive compounds which often provide both basic nutrition and desirable health benefits. In the last decades, special attention has been paid towards cruciferous edible plants due to their richness in secondary metabolites functioning as antioxidants. Among them, colored cauliflower and cabbage are very eye-catching, commercially available vegetables characterized by the presence of high levels of anthocyanin pigments fulfilling important protective role against biotic and abiotic stresses in plants, and, endowed of potent nutritional and healthy properties as component of the human diet. In this respect, the consumption of pigmented *Brassica* species has been strictly related to the reduction of the risk of certain chronic diseases including cardiovascular diseases and cancer. The anthocyanin biosynthesis pathway has been elucidated in many herbaceous and tree plants and most of the structural gene encoding the enzymes responsible for each steps have been isolated from *Brassica* genus. In this study, we monitored the expression patten of three structural genes involved in anthocyanin biosynthetic pathway: PAL (phenylalanine ammonia lyase), DFR (dihydroflavonol-4-reductase) and GST (glutathione S-transferase) during the development of different *Brassica* crops; the anthocyanin content was also measured in order to correlate gene expression with pigment accumulation. The results reveal that the induction of gene expression simultaneously occurs or slightly precedes the maximum levels of anthocyanin accumulation.

Poster C-16

DEVELOPMENT OF HIGHLY CLUBROOT-RESISTANT CHINESE CABBAGE F₁ CULTIVAR, 'AKIMEKI', ACCUMULATING THREE RESISTANCE GENES, *CRR1*, *CRR2* AND *CRB*

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Clubroot is a serious disease affecting Chinese cabbage cultivation. Cultivars with clubroot resistance (CR) play an important role in controlling the disease. However, this resistance has been broken down by genetic variability of the pathogen. Four pathotypes (group 1 to group 4) were identified in Japanese field isolates through the use of two commercial CR F₁ cultivars of Chinese cabbage. In this study we tried to accumulate CR genes by DNA marker-assisted selection for development of F₁ hybrid Chinese cabbage cultivar with high resistance and high quality. 'Parental line No.9' (PL9) carries two CR genes, *Crr1* and *Crr2*, which were introduced from genetic resource 'G004' derived from European fodder turnip 'Siloga' and shows high resistance to clubroot isolates from pathotype group 1 which is pathogenic to most of Japanese Chinese cabbage F₁ cultivars, group 2 and group 4, but not group 3. The parents of F₁ hybrid 'Akiriso', T-line and V-line, were crossed with PL9, respectively. T-line harboring another CR gene, *CRb*, confers resistance to pathotype group 3 and V-line is resistant to Verticillium yellows. The progenies were selected by DNA markers linked to *Crr1* and *Crr2*, and backcrossed with each parent. The DNA marker selection and backcrossing were repeated by 5 times and two introduced resistance genes became homozygous by self-pollination. Of new parental lines acquiring *Crr1* and *Crr2*, T-line pyramided with three CR genes showed resistance to all 4 pathotypes. Since *CRb* is a dominant resistant gene, F₁ hybrid between new parental lines also showed highly resistant in pot and field tests. Except of clubroot resistance, phenotypic characters of new F₁ hybrids were completely as same as those of 'Akiriso'. We dominated a new F₁ hybrid as 'Akimeki' which is the first F₁ cultivar with resistance to all isolates from pathotype group 1 to group 4 and Verticillium yellows.

Poster C-17

PRODUCTION OF DOUBLED HAPLOID PLANTS OF *BRASSICA RAPA* SUBSPECIES USING THE MICROSPORE CULTURE TECHNOLOGY FOR CORE COLLECTION MATERIALS

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For genetic mapping studies, biparental segregating populations are often useful; however, recombination is limited, giving rise to large genomic regions under QTL, and only alleles present in both parents can be studied. In Wageningen UR, a core collection is being developed representing all *Brassica rapa* morphotypes or subspecies and geographic origins. As most *B. rapa* accessions are heterozygous and heterogeneous, we started a project to fix the collection through microspore culture. The resulting Diversity Fixed Foundation Set will be an interesting resource for association mapping studies, which have as advantage that they present the allelic variation present in the collection and recombination is increased for mapping studies. Nineteen accessions of eight subspecies of *B. rapa* were used for microspore culture to develop doubled haploid lines. Eight morphotypes were represented: 3 Chinese cabbage, 2 Chinese turnip cabbage, 3 Pak choi, 5 Turnip, 3 Broccoletto, 1 Mizuna, 1 Komatsuna and 1 Turnip green from the 19 accessions examined. Embryos were obtained for 13 accessions, representing six subspecies (Komatsuna and Turnip green had no response). The embryo yields differed significantly among these 13 accessions. We regenerated normal plants from 10 accessions that survived more than 4 weeks in the soil using microspore culture. Nine accessions flowered after 4 weeks vernalization at 5 °C and seeds were harvested from 5 accessions. From Mizuna, we obtained 3,791 seeds from one plant and a total of 7,318 seeds were harvested from 5 accessions representing 4 subspecies (Chinese cabbage, Chinese turnip cabbage, Pak choi, and Mizuna). At present, we carry out experiment for obtain more seeds and induce embryos from the other plant materials.

Poster C-18

DEVELOPMENT OF DOUBLE HAPLOID INBRED LINES USING MICROSPORE CULTURE IN CHINESE CABBAGE (KIMCHI CABBAGE) (*BRASSICA RAPA* L.)

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Chinese cabbage (Kimchi cabbage) is one of the most important vegetables in Korea. In every meal times, adult korean enjoy Kimchi made of Chinese cabbage(Kimchi cabbage). To develop high quality varieties, breeders tried to make inbreds carrying functional materials in Chinese cabbage breeding programmes. To make genetically fixed inbred in short time, we tried to use microspore culture. Several varieties were used under different culture conditions to develop doubled haploid (DH) plants. Among 21 varieties collected from Korea and China, 18 varieties produced embryos and developed into plants. We started microspore cultivation from 9th in February and finished 14th in May 2012. The total number of plants developed from microspore was 1,244, 596, and 947 in Feb., Mar., and Apr., respectively. The effective condition of microspore cultivation was varied according to the variety. Using 11-FF197 accession, medium consisted of 'NLN (1X) + AgNO₃ (1mg/L) + Sucrose (13%) + NAA (0.05mg/L) + BAP (0.05mg/L)' produced 11 plants from 20 buds. For 11-FF302 accession, the medium consisted of 'NLN (1X) + AgNO₃ (1mg/L) + Sucrose (13%)' produced 265 plants from 10 buds.

Thirteen combinations of medium were used to find the most appropriate conditions for microspore cultivation in Chinese cabbage. However, the effect of plant hormone, micro nutrients, and even NLN concentration was varied following to variety. Even though, we developed 2,813 plants using microspore cultivation of 18 selected varieties showing yellow inner leaf color in Chinese cabbage.

Poster C-19

INDUCTION OF DIRECT SOMATIC EMBRYOGENESIS FROM IMMATURE EMBRYOS OF *BRASSICA OLERACEA* VAR. *SABAUDA* L.

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An efficient plant propagation system through somatic embryogenesis by using immature zygotic embryos was established in Savoy cabbage (*Brassica oleracea* var. *sabauda* L.). The effects of zygotic embryo age, presence of 1 mg/L 2,4-dichlorophenoxy acetic acid (2,4-D) (B5-D) in the culture medium, and the pH (5.0 and 5.8) of the B5 plant growth regulator (PGR)-free (B5-0) induction media were investigated. It appeared that the developmental stage of the immature zygotic embryos used as explants was the most important factor for somatic embryogenesis. Highest frequency of somatic embryogenesis (86.67%) was achieved directly on zygotic embryos at cotyledonary stage (1.8 mm long) of development that cultured on B5-0 medium. Optimal pH of the medium was 5.0. Mean number of primary somatic embryos per explant on B5-0 pH 5.0 medium was 7.2. The inclusion of 2,4-D into the induction medium significantly reduced both frequency of somatic embryogenesis (to 53.34 %) and mean number of embryos per explant (to 2.62) in cotyledonary zygotic embryos. After transferring to PGR-free Murashige and Skoog (MS-0) medium 69.69% of the primary somatic embryos of the Savoy cabbage produced secondary somatic embryos (SSEs). Effective recurrent somatic embryogenesis may be an appropriate practical solution for clonal propagation and genetic modifications of Savoy cabbage.

Poster C-19

EVALUATION OF INTENSITY AND DISTANCE OF OILSEED RAPE POLLEN TRANSFER

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Oilseed rape (*Brassica napus* L. ssp. *oleifera* Metzg.) is both self- and cross-pollinated. The frequency of outcrossing is dependent on the genotype of the cultivar and environmental conditions. Cross pollination is estimated to be between 0 and 90% depending on cultivar. Outcrossing mainly occurs between fields in the neighborhood but also pollen dispersal is observed in long distance. The extent of gene flow of genetically different types is largely dependent on the scale of pollen production and dispersal and on distance between fields in a particular season and place.

The objective of this study was to investigate the frequency and distance of pollen dispersal in Polish environmental conditions to determine the size of the buffer zone between the crops of different types of oilseed rape.

The experimental fields were isolated by at least 5 km from other oilseed rape plantations. The monitoring of intensity and distance of pollen oilseed rape transfer was done in season 2011 and 2012 using passive and active pollen traps. Passive traps were placed at five compass directions around the field at the distance of 140 m in increments of 5 m. Hirst-type volumetric pollen traps (Burkard Manufacturing, UK) were positioned on a linear transect in the direction of the prevailing wind (S-E) at the distance of 10, 90 and 180 m from the edge of the field. Calculation of pollen concentrations was based on pollen number visualized on Vaseline-covered and stained Melinex tapes that were mounted on microscope slides and quantified with light microscopic techniques.

The results show that the highest intensity of pollen was generally found in the first 40 m from the edge of field, at each compass direction, a decrease in intensity with increasing distance was observed. However, in the peak of flowering pollen grains flow up to 180 m of the investigated distance.

Poster C-20

FINGERPRINT ANALYSIS OF SELECTED GLUTATHIONE S-TRANSFERASE SUPERFAMILY GENES FROM RAPE (*BRASSICA NAPUS*)

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The glutathione S-transferases (GSTs; EC 2.5.1.18) are a widely distributed family of detoxifying dimeric enzymes found in both invertebrates and vertebrates, plants, yeast and bacteria. GSTs catalyze the nucleophilic addition of glutathione (GSH) to the electrophilic groups of a large variety of hydrophobic toxic chemicals. Following their uptake, the conjugation of such molecules to GSH increases their solubility and facilitates their further metabolic processing. In plants, the conjugated products are either sequestered in the vacuole or transferred to the apoplast thus potentially contributing to reclaim cells from unwanted compounds. It is well documented that plant GSTs show major transcriptional regulation as the levels of their transcripts markedly increase in response to a wide variety of stressful conditions, such as herbicides, chilling, hypoxic stress, dehydration, wounding and pathogen attack. However, the regulation of existing GSTs (post-translational regulation) by reversible covalent modification is still poorly investigated. This *in silico* study was designed to identify the putative correlation between structural and functional characteristics of plant GST classes belonging to *Brassica napus*. Consequently, the protein sequences of the expressed GSTs have been retrieved from UniGene, classified and then analyzed in order to assess the evolutionary trend. Moreover, the fingerprint analysis was performed with SCAN Prosite in the attempt to correlate meaningful signature profile and biological information. The fingerprint analysis revealed that specific signature profiles related mainly to protein phosphorylation are in the GST classes thus suggesting that they might be subjected to reversible activation by phosphorylation-mediated regulation. This approach provides the knowledge of the relationship between the presence of conserved signature profile and biological function in the view of future selection of GSTs which might be employed in either mutagenesis or genetic transformation studies.

Poster C-21

CHANGES IN METHYLATION PATTERNS IN BRASSICA OLERACEA L. DURING TIME AND IN RESPONSE TO DIFFERENT AGROCLIMATIC CONDITIONS

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Among the *Strategies for Organic and Low-input Integrated Breeding and Management* (SOLIBAM) project aims, there is the identification of epigenetic modifications caused by farming under different agronomic and/or pedoclimatic conditions. SOLIBAM project is funded by the European Commission under the Seventh Framework Programme (GA 245058, coord. Véronique Chable, INRA).

Plants of two hybrid varieties of broccoli (*Brassica oleracea* L. var. *italica* Plenck), Iron Man (IM) and Santee (SN) were grown in three experimental fields in 2010-2011 and in four experimental fields in 2011-2012 under different climatic and management conditions: low input (LI) and organic agriculture (OA). Young and healthy leaves were collected from both varieties and from each experimental field at three different dates during winter time. Differences in methylation state of samples collected in 2010-2011 were identified applying the *Methylation-Sensitive Amplified Polymorphism* technique (M-SAP) using 5 different primer combinations on genomic DNA extracted from 36 samples (2 plants for each of the 2 hybrids, grown in 3/4 different experimental fields and collected at 3 different times).

M-SAP analysis of materials collected in 2010-2011 revealed the presence of 210 amplicons, 25 specific of IM, 13 of SN and 172 that were found in both hybrids. Among the amplicons from methylated regions, 83 in IM and 64 in SN underlined changes in methylation patterns in relationship to different sampling dates, while 17 in Iron Man and 19 in Santee are putatively related to the different agro climatic growing conditions. Intriguing enough, 9 of these amplicons are common between the two analyzed hybrids.

The best M-SAP primer combinations will be used to analyze samples collected in 2011-2012 to eventually confirm the presence/absence of amplicons putatively related to response to abiotic stress and different climatic and management conditions. Dynamics of methylation patterns modification in *Brassica oleracea* are reported and discussed.

Poster C-22

UNDERSTANDING THE ROOT SYSTEM IN *BRASSICA NAPUS*

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Rapeseed/canola (*Brassica napus* L.) has both winter and spring form available. Scarce information on genetic control of root system in *B. napus* is available. This work investigated the roots morphology and its possible contribution to seed yield in *B. napus*. The work was conducted at a greenhouse of North Dakota State University, Fargo, USA. Root establishment in pots was observed visually for the parents, F₁, F'₁, and F₂ progenies. The winter type has vigorous root system whereas the spring type has weakly developed root system. The root system was scored on a scale of 1 to 5 on the basis of root development in the pots. Plants with weak bottom and surface roots, such as those observed in spring type parents were scored 1, followed by 2 which has more bottom and surface roots, 3 has intermediate bottom and surface roots, 4 has strong bottom and surface roots, and 5 has the strongest bottom and surface roots resembling the root system of the winter parents. The F₂ root system data was divided into two groups for inheritance study. The plants scored as a 1 formed the first group, and all other root types formed second group. The F₁ and F'₁ of winter and spring crosses showed a vigorous root system, indicating the dominant nature of vigorous root system in *B. napus*. Vigorous root system was found to be controlled by three genes. Root length showed positive and significant correlation with seed yield/plant for both cross and reciprocal cross, whereas dry root weight was not significantly correlated with seed yield. Significant and positive correlation was also observed between root length and late flowering, and dry root weight and late flowering. This is the first report on genetics of root system and its contribution to seed yield in *B. napus*.

Poster C-23

THE RESPONSE CHARACTERISTICS OF JAPANESE RADISH (DAIKON) TO MECHANICAL WOUND STRESS

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The postharvest damage caused by physical stresses (compression, shock, vibration) during packing, storage, and transportation in bulk influences the quality and the yield of fresh produce. We found three predominant types of physical damage characteristics (abrasion, peeling, and bruising) of Japanese radish (Daikon, *Raphanus sativus* var. *longipinnatus*) under the bulk transport simulation. Additionally, the types of damage including abrasion, peeling and bruising of Daikon were observed differently, depending on the specific layers in the container. In order to understand the basic changes in molecular mechanisms in relation to mechanical/physical stress of Daikon, we have utilized mechanical wounding as the stress stimulus to study the expression of candidate stress-responsive genes in Daikon. The predominant increases in *RsCHI* (chalcone isomerase-encoding gene) and *RsPAL* (phenylalanine ammonia lyase-encoding gene) expression levels were observed at 4 h and 6 h after the wounding treatment. In contrast, the expression level of *RsSOD* (superoxide dismutase-encoding gene) was detected at low level, and the significant increase in expression of *RsSOD* was found at 10 h and 12 h after the treatment. An increase in expression of candidate stress-responsive genes of Daikon demonstrates the change in cellular response to the mechanical wound stress. Overall, it appears that the physical damage of Daikon was likely affected by transport under bulk condition. Moreover, applying a mechanical wounding stimulus clearly affected the expression of stress-responsive genes (*RsCHI* and *RsPAL*). The rapid expression of these genes may consequently affect the cellular metabolism and quality changes of Daikon. The further study in order to find acceptable level of gene expression to avoid the development of severe damage of Daikon should be taken into consideration. Additionally, the appropriate design of packaging conditions for the actual transport condition will enable to distribute Japanese radishes by newly developed bulk container without the unacceptable level of damages.

Poster C-24

IDENTIFICATION OF *BRASSICA RAPA* HOMOLOGUE OF FUSARIUM WILT RESISTANCE GENE, *FOC-BO1* BY GENOME SYNTENY ANALYSIS BETWEEN *B. OLERACEA* AND *B. RAPA*.

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Brassica crops are severely infected by the soil-borne pathogen *Fusarium oxysporum* f. sp. *conglutinans*. Fusarium wilt disease leads to leaf yellowing, wilting, defoliation, stunted growth, and plant death. As a result, plants suffer severe losses in both yield and quality. In our previous study, the fusarium wilt disease resistance gene, *Foc-Bo1*, was identified by QTL analysis in *Brassica oleracea*. *Foc-Bo1* was located on the linkage group 7. The analysis of fine-mapping revealed that *Foc-Bo1* was located in 0.7 cM intervals between the two molecular markers.

In this study, the comparative analysis between *Foc-Bo1* region and the *B. rapa* genome sequence (Brassica DB) showed 22 genes in the syntenic region of *B. rapa* genome. The *B. oleracea* BAC and cosmid clones harboring the *Foc-Bo1* region were sequenced. We found putative disease resistance genes, which are named as *FocBo1^A* and *FocBo1^B*. Similarly, *B. rapa* homologues were named as *FocBr1^A* and *FocBr1^B*. To characterize the two candidate genes in *B. rapa*, we carried out RNA sequence analysis using the resistant (R09) and susceptible (S11) lines. Comparison of the number of copies of the two genes (*FocBr1^A* and *FocBr1^B*) revealed high level of expression of *FocBr1^A* and *FocBr1^B* in the resistant line, but no expression in the susceptible line. For MAS with *FocBr1*-specific markers, simultaneous phenotyping and genotyping in the F₂ population derived from F₁ hybrid between R09 and S11 revealed an almost perfect match between phenotyping and genotyping. These results indicate that *FocBr1^A* and *FocBr1^B* are candidate genes that confer resistance against fusarium wilt. *FocBr1* and *FocBo1* are probably originated from a common ancestor gene. Our study indicates that genome synteny analysis between *Brassica* species is very useful for identifying disease resistance genes.

Poster C-24

QUALITY EVALUATION OF CULTIVARS TYPES IN *BRASSICA OLERACEA* L.

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Crops belonging to the *Brassica* genus are widely grown in Russia. Russian world-wide *Brassica oleracea* L. collection kept in N.I.Vavilov Institute of Plant Industry (VIR) includes 2724 accessions. For an intraspecies differentiation of *Brassica* collections morphological and ecologo-geographical approaches were used by T.V. Lizgunova (1984). For instance the collection of white cabbage was divided into 7 eco-geographical groups and 33 cultivars types, distinguished on morphological, biological and agronomic traits; Savoy – into 5 groups and 16 types; cauliflower – into 6 groups and 18 types, etc.

Biochemical composition of all *B.oleracea* crops has been investigated. 50-90% accessions depending of crops from VIR collection, belonging to all cultivars types, were evaluated for dry matter, sugars, vitamin C, organic acids, protein, carotene, chlorophylls, total glucosinolates and nitrates content. The cultivars types of all crops evaluated in this study display a wide diversity of characters for selection and combination of interesting genotypes for important characters to obtain improved varieties. Among the cultivar types the sources of nutritive and bioactive substances were revealed.

Poster C-25

DETERMINATION OF SPECIES, VARIETIES AND GEOGRAPHICAL DIFFERENCES AMONG CABBAGE GENOTYPES BASED ON ELECTROPHORESIS OF STORAGE SEED PROTEINS

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Each breeding line can be identified by polypeptide components. Cruciferins belong to the class of globulins, one of the groups of *Brassica* seed storage proteins. Specific patterns of polypeptide components within a genome may help to identify inter-genomic hybrids.

Differences in A-genomic species have been found, to which all East-Asian *Brassica* species belong and which are characterized by the high molecular components 26-28. Components 23 and 28 are relatively consistent in these species. Species that represent this genome lack the molecular components 8 and 22.

The specific components (positions 4, 8, 12, 18, 22, and 25) found in white head cabbage can be considered as markers. At the same time they can differ in their manifestation. Although the differences in cruciferin polypeptide composition among studied varieties were found they were too scant and cannot be used as molecular markers for reliable cultivar identification.

The same genotypes of white head cabbage growing in geographically distant regions (Turkey, Uzbekistan, Central, North-West and South Regions of Russia) showed differences in their cruciferin polypeptide compositions concerning the combination of basic components of different intensity. Among three studied cauliflower varieties growing in Russia and Uzbekistan one specimen cultivated in Russia showed differences in its cruciferin spectrum. The cruciferin spectrum of 20% of genotypes was characterized by scant component at position 12. The manifestation of other components was similar.

Poster C-26

SEED QUALITY TRAITS OF DH LINES OF WINTER OILSEED RAPE (*BRASSICA NAPUS* L.)

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The objective of the study was to estimate winter oilseed rape DH lines in terms of fat, protein, fiber (NDF and ADF) and glucosinolate content, as well as the seed colour and to specify the effect of the influence of environment on these traits .

44 doubled haploid lines obtained from F₁ hybrids of the reciprocal crosses between yellow-seeded DH Z-114 and black-seeded DH H₂-26 were studied in two years. Analysis of variance indicated that both sources of variation tested in the trial, years and genotypes were significant for all traits, with the exception of NDF content. In this case only genotypes were significantly different. . A significant interaction years ×DH lines was found for fat, protein, ADF and glucosinolate content, as well as for seed colour.

Double haploid lines were also studied in terms of the positive effects of transgression for fat and protein content and negative for NDF, ADF and glucosinolates content. Among 44 examined genotypes, 10 DH lines revealed positive effects of transgression for fat content and two DH lines for protein content. Three lines with negative transgression were found for NDF content and one for ADF content. The results from both years of research have revealed significant negative correlation between seed colour and fat, NDF, ADF content in the direction of the lighter colour, the lower fat and fiber content. Significant positive correlation was noticed between NDF, ADF and fat content. Protein content was negatively correlated with NDF and ADF content but significant correlation between protein and fat content was not observed.

Poster C-27

CREATING GERMPLASM AND NEW TOOLS TO INVESTIGATE POST HARVEST SENESCENCE IN *BRASSICA OLERACEA* L VAR. *ITALICA*

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Natural plant senescence is a complex trait that marks the end of a photosynthetically active period in a plants life cycle. This may end with the death of the plant or the beginning of a dormant period. Crop plants that are harvested during their vegetative phase are placed into artificial stress induced senescence resulting from the removal of a water supply. Physiological signs of stress include wilting and rapid yellowing. Broccoli is particularly prone to post harvest yellowing; this is a major factor that contributes to the short shelf life of this crop. To explore the polygenic nature of this trait, we trialed lines from a unique 'broccoli x broccoli' doubled haploid (DH) population and mapped significant QTL for yellowing and broccoli morphological traits onto a new *B. oleracea* L var. *italica* linkage map.

We have mapped two 'days to yellowing' (dty) QTL, these have no significant correlations to QTL for morphological traits (head diameter, butt diameter, water loss) therefore the effect was not due to pleiotropy. Using markers linked to the QTL, lines that contained beneficial alleles were selected for our backcross (BC) program. We have generated a further set of DH lines from the BC₁. These lines have been genotyped, and useful recombinants were trialed in 2010. We are currently at BC₃ in the resource development program. Selected recombinants that help to delineate QTL will act as a further resource to map candidate genes. This poster outlines the development of the population, linked resources and their utility.

Poster C-28

BRASSICA RAPA SSP. CHINENSIS (PAK CHOI) AS MODEL PLANT TO IDENTIFY GENES INVOLVED IN INDOLE GLUCOSINOLATE BIOSYNTHESIS

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Glucosinolates are secondary plant metabolites of the order Brassicales and are contributing to the plant defense systems. Epidemiological studies generated considerable pharmacological interest in the breakdown products of glucosinolates due to their beneficial biological activities in mammalian consumers. However recently, *Brassica* extracts were found to be mutagenic, especially 1-methoxyindol-3-ylmethyl glucosinolate (1MOI3M-GS) produces DNA adducts in mammalian cells. Therefore identification of genes involved in formation of specific secondary metabolite profiles are important for breeding activities to produce vegetables enriched in healthy compounds and depleted in others (1MOI3M-GS).

A secondary metabolite profiling studies identified Pak Choi as interesting *Brassica* model. Elicitor treatments revealed that methyl jasmonate application specifically increased the 1MOI3M-GS content up to 30-fold in Pak Choi while induction in *Arabidopsis thaliana* was low. Transcriptome analysis with the 2×104k *Brassica* Array identified genes putatively involved in 1MOI3M-GS biosynthesis. In planta functional analysis was performed using *Arabidopsis* T-DNA insertion mutants in the respective homologs, and putative candidates were verified by heterologous expression of the Pak Choi genes in *Arabidopsis*. Using this strategy we identified *Brassica rapa* oxygenases involved in 1MOI3M-GS biosynthesis.



BRASSICA2012

Session D

NUTRACEUTICAL AND PROCESSING

Oral presentation

Session D-1

**HIGH GLUCORAPHANIN BROCCOLI – THE DEVELOPMENT OF BENEFORTE™
BROCCOLI AND EVIDENCE OF HEALTH BENEFITS**

Keynote lecture

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I shall briefly review the evidence from epidemiological studies and cell and animal models for health benefits of sulforaphane, and then describe the development of high glucoraphanin broccoli, and current human intervention studies that seek to obtain evidence of health benefits. I will describe the genetic basis to the high glucoraphanin trait and its effect on sulphur metabolism and partitioning, and evidence from human studies that consuming high glucoraphanin broccoli perturbs lipid metabolism.

Session D-1

ENHANCEMENT OF PHENOLIC COMPOUNDS, FLAVONOIDS AND ISOTHIOCYANATE CONTENT OF BROCCOLI (*BRASSICA OLERACEAE* VAR *ITALICA*) IN RESPONSE TO SEAWEED APPLICATIONS

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Broccoli is one of the main vegetable crops grown in Ireland, these vegetables are rich in vitamins as well as phytochemicals like phenolic compounds, flavonoids, and isothiocyanates. A diet rich in brassicas has been associated with the inhibition of chemically induced carcinogenesis in humans. A series of field experiments was carried out to evaluate the effect of pure novel cold process commercial seaweed extract on yield and quality of two broccoli cultivars (Ironman and Red Admiral) over a two year period. Treatments included water control, dry and calcified seaweed, liquid seaweed application of 3L/ha, 30L/ha and 300L/ha. Liquid seaweed extracts were applied once a month. Cold process seaweed extracts increased all bioactive compounds significantly in both cultivars consistently for the duration of the trial. Total phenolic (TPC), total flavonoid (TFC), and total isothiocyanate content (TIC) were higher in all seaweed treatments compared to the control. In the green broccoli trial (Ironman) TPC ranged from 40-87 mg/ 100g FW, TFC ranged from 5.7-8.4 mg/L and TIC ranged from 1.6- 4.2 µM/L. In the purple broccoli trial (Red Admiral), TPC ranged from 49-154 mg/100 g FW, TFC from 7.7-20 mg/L and TIC from 5-11 µM/L. Seaweed treated plants had significantly higher phytochemical content. Application rate was not a statistically significant factor. In both crops, seaweed application increased yield but only at the highest rate. In Ironman, the yield increase reached 5.5% and in Red Admiral, 24.6%. Results indicate that seaweed extract has an effect on the yield of both broccoli cultivars but only at the highest application rate. There was a consistent effect on the increase of the phytochemical content of treated plants, even at the lowest application rate. The response of broccoli cultivars in terms of increase in phytochemicals suggests the potential utilisation of cold processed seaweed extracts in the production of high value vegetables with increased health benefits.

Session D-1

NUTRIOMIC APPROACH ON CHINESE CABBAGE BREEDING

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Development of nutriomics in relation to functional foods is becoming an imperative issue for human health. *Brassica* species have a lot of attention as important vegetables containing very high amounts of functional compounds such as glucosinolates, β -carotene, lutein, phenolic acids, flavonoids, etc. Since the draft sequencing of the Chinese cabbage genome was completed, functional genomics research has been rapidly expanded to understand metabolic pathways and regulatory mechanisms of related genes. Since the establishment of Korea *Brassica* Genome Resource Bank in 2004, our research group has supported genomic materials for Multinational *Brassica* Genome Sequencing Project and collected over 10,000 accessions of *Brassic*as from different areas in the world. Meantime, Collected germplasms were analyzed for their functional compounds and selected the accessions which have high level of functional compounds, such as glucosinolates, vitamins, β -carotene, lutein, flavonol, Fe, Ca, etc. With the accessions and microspore culture, we have developed doubled haploid lines and finally selected inbred lines containing high concentration of specific compounds. Our research group also analyzed the metabolic pathways and functions of genes related to the production of secondary metabolites; i.e., identification of 39 full length CDs for carotenoids from *Brassica*. Further study of the genetically fixed lines with specific amount of functional compounds under controlled environment cultivation system would produce more significant data for the nutriomics research.

Session D-1

THE INFLUENCE OF TEMPERATURE ON THE EFFICIENCY OF MODERATE UV-B TREATMENT ON THE FLAVONOID PROFILE IN KALE (*BRASSICA OLERACEA* VAR. *SABELLICA*) AND THE EXPRESSION OF KEY ENZYMES IN FLAVONOID BIOSYNTHESIS

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Kale (*Brassica oleracea* var. *sabellica*) has a wide spectrum of structurally different flavonol glycosides. The 71 flavonol glycosides identified, can be non-acylated or acylated with different hydroxycinnamic acids.^[1] The aim of this study was to investigate if subsequent doses of moderate UV-B radiation can enhance flavonol glycosides and how temperature influences the efficiency of moderate UV-B radiation.

The cultivar 'Winterbor' was grown to a 4-5 leaf stage in the greenhouse at ambient conditions. Plants were exposed to multiple UV-B doses of 0.5 KJ m⁻² d⁻¹ applied as 5 subsequent treatments (total dose 2.5 KJ m⁻²) with 24 h acclimatization in between. The experiments were conducted either at 5°C or at 15°C and ambient PAR.

Generally, the efficiency of subsequent doses of moderate UV-B radiation on flavonol glycoside concentration was higher at 15°C than at 5°C, and the response of flavonol glycosides was structure-specific as well. E.g. at 15°C the monoacylated quercetin tri- and tetraglycosides were increased after treatment with UV-B doses of 1.5 KJ m⁻² up to 2.5 KJ m⁻² while the corresponding kaempferol glycosides decreased. Molecular investigations using semi-quantitative real-time RT-PCR showed concomitantly enhanced mRNA expression of *flavonol 3'-hydroxylase*. A consequence of these results could be a new crop management for *Brassica* species to enhance the health promoting secondary metabolite profile of vegetables.

Session D-1

EFFECTS OF DAY LENGTH AND TEMPERATURE ON HEALTH RELATED COMPOUNDS IN BROCCOLI (*BRASSICA OLERACEA* VAR. *ITALICA*)

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Cultivation conditions in Norway generally have lower temperatures during the summer and much longer days (24 h photoperiod in the high north) compared to more southerly latitudes. It has been claimed that fruits and vegetables grown at northern latitudes can be healthier and tastier than those grown further south. Previous studies, including on carrot, strawberry and blackcurrant, indicate that such northern produce can have special qualities, such as higher content of vitamin C and "better" flavor. The content of health related chemicals in northern broccoli, have not been studied before, and this was the main subject for our study. We have specifically looked at the content of vitamin C, glucosinolates, the flavonols quercetin and kaempferol, and soluble sugars. In our experiments, broccoli plants were grown in climate chambers under controlled light and temperature regimes. Long and short days were combined with high and low temperatures. Our preliminary results showed a higher content of vitamin C at low growth temperatures (15/9 °C day / night) compared to higher temperatures (18/12 °C day / night), while the flavonols had the highest content at high temperature and short day treatment. These results, as well as other findings, will be presented and discussed.

Session D-2

GENOTYPIC VARIATION OF THE GLUCOSINOLATE PROFILE IN PAK CHOI (BRASSICA RAPA SSP. CHINENSIS)

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Glucosinolates are secondary metabolites of *Brassica* crops. Glucosinolates are β -D-thioglucoside-N-hydroxysulfates containing amino acid-derived variable side chains. Upon tissue damage glucosinolates are hydrolyzed by plant derived myrosinase and breakdown products, like isothiocyanates, nitriles, and oxazolidinethiones are renowned for biological activity and toxicity. *Brassica rapa* var. *chinensis* is one of the most consumed *Brassica* vegetable in Asian countries. Here we present the classification of diverse pak choi cultivars regarding their glucosinolate profile and relative mRNA expression level of involved genes to further establish pak choi as *Brassica* model plant for further studies.

From 12 days old sprouts and mature plants of 13 pak choi cultivars glucosinolates were determined using an established extraction procedure followed by HPLC-MS. Data of identified glucosinolates were subjected to principle component analysis and three groups of pak choi sprouts could be distinguished. The group differences were marked by variations in the aliphatic glucosinolate profile such as the differing levels of 3-butenyl and 2-hydroxy-3-butenyl as well as by their varying proportional ratios. Additionally, the three groups of pak choi sprouts varied by the presence or absence of 2-hydroxy-4-pentenyl. This classification is also reflected by the relative mRNA expression level of 2-oxoacid-dependent dioxygenase. *Due to the ontogenetic modifications during plant development in mature leaves, the relative amounts of aliphatic and indole glucosinolates show only small variations between the different cultivars. Thus, it is not possible to use the glucosinolate profiles of mature pak choi plants for differentiation in cultivar groups.*

The distinct differences in the glucosinolate profile of sprouts will be used in future studies for *in vitro* and *in vivo* analyses. E.g. in feeding studies the additive, synergistic or suppressive effects of various glucosinolate compositions and their corresponding breakdown products within the same *Brassica* plant tissue might be linked to health related functional effects.

Session D-2

VARIABILITY IN ANTIOXIDANT COMPOUNDS OF SEEDS AND SPROUTS OF BROCCOLI (*BRASSICA OLERACEA* L. VAR. *ITALICA*) AND ROCKET (*ERUCA SATIVA*) IN RELATION TO TEMPERATURE AND GERMINATION STAGE.

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Brassicaceae family is well known to be rich in bioactive compounds such as sulphur containing glycosides called glucosinolates and antioxidants. The glucosinolates are naturally hydrolyzed by the myrosinase enzyme or enteric microflora into isothiocyanates that are known to efficiently reduce risks of degenerative diseases.

Antioxidant compounds are generally represented by ascorbic acid, polyphenols and carotenoids. Recently the request of vegetables sprouts on the market increased as consequence of their richness in vitamins, minerals, proteins and enzymes, especially for what regards those belonging to the *Brassicaceae* family.

We investigated the variation of glucosinolate profile and the antioxidant compounds (total phenols, ascorbic acid and carotenoids) content during germination process at 10 °C, 20 °C and 30 °C of broccoli (*Brassica oleracea* var. *italica*) and rocket (*Eruca sativa*).

Germination rate differed in relation to the species and temperature, being detected higher in the rocket and at 20 °C.

The glucosinolate profile differed among the two species and in relation to the temperatures; in broccoli six components have been identified while two were present in rocket.

The phenols well correlated within the experiment showing different amount in relation to the two species. In broccoli the highest amount of antioxidant was detected at 10 °C and 20 °C, while in Rocket only at 10 °C.

Seeds extracts showed the lowest response. The same trend was found for total phenols and ascorbic acid content. A simple linear regression test was performed, with the best response between the two antioxidant assays, followed by the significance between antioxidant assays and phenols content. No regression was found between glucosinolates content and antioxidant indexes.

Session D-2

SENSORY QUALITY AND HEALTH-RELATED SUBSTANCES OF BROCCOLI GROWN AT DIFFERENT LATITUDES

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A semi-field experiment was conducted 2009 to 2011 to investigate possible differences in effects of primarily ambient temperature and solar radiation on broccoli (*Brassica oleracea* var. *italica* cv. Lord) grown at four northern latitudes: Spain (Galicia, 43°), Germany (Brandenburg, 52°) and Norway (Grimstad, 58°; Tromsø, 69°). All other growing conditions were kept identical at the locations, i.e. soil, seeds, fertilization, precipitation shelter, insect netting, and use of specially designed pots with water supply according to the plant's need. Florets prepared from broccoli heads of commercial maturity were randomized and used for analysis of intensity of sensory attributes and contents of glucosinolates, flavonol aglycons and vitamin C.

The development of broccoli heads was normal during three summer seasons with the exception of Spain for two summer seasons, probably due to too high temperature. Therefore, the experiment was repeated two autumn seasons in Spain and those samples were used for sensory analysis. Most of the sensory attributes measured were significantly different between the locations. Preliminary results for flavonol contents and profile also indicate large differences between the locations.

(All authors have contributed with large and important parts of the work.)



BRASSICA2012

Session D

NUTRACEUTICAL AND PROCESSING

Poster presentation

Poster D-1

BRASSICAS AND THEIR GLUCOSINOLATE CONTENT FOR THE BIOLOGICAL CONTROL OF SOILBORN IN PROTECTED CULTIVATION

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Soil-borne diseases is a widespread problem in protected cultivation in Sicily especially after the prohibition of methyl bromide use. Secondary metabolites of some *Brassica* species are biofumigant able to contrast the main soil-borne agents in a view to establish health and environmental friendly farming.

In order to assess the effectiveness in nematodes control (*Meloidogyne* spp.) on tomatoes was carried out, adopting factorial design, a field trial in an representative farm of protected cultivation (Ragusa) using the flour of dry plants of *Brassica juncea*, *Eruca sativa*, *Raphanus sativus* and *Brassica macrocarpa*. The first three species were previously studied by some Authors as biofumigant, while *B. macrocarpa* is an endemic Sicilian wild species appears interesting since recent scientific evidence showed high glucosinolate content in leaves, 90% represented by sinigrin, and the effectiveness of its dry leaves inserted into the soil to control root-knot nematodes in tomato crops (*Meloydogine* spp.). Sinigrin content in the tissue of the Brassica species studied varied between 3.1 $\mu\text{M g}^{-1}$ d.m. of *B. juncea* and 7.3 $\mu\text{M g}^{-1}$ d.m. of *E. sativa*, while for *R. sativus* and *B. macrocarpa* were ascertained about 4.7 $\mu\text{M g}^{-1}$ d.m.

Flour was distributed underground (0, 60 and 90 g m⁻²), the mean dose corresponded to the active principle contained in the commercial formulate (Nematorin) applied in 3 g m⁻² as reported on the instructions. Disease index detected on the tomato roots at the end of the growing cycle (Lamberti score, 1971), although low in general, resulted in all thesis lower than the control (0.30) and the commercial formulated (0.20), whereas it was and between 0 (*E. sativa* 60 g m⁻² and *R. sativus* 90 g m⁻²) and 0.13 (*B. juncea* 90 g m⁻²).

Poster D-2

MEASUREMENT OF SULPHUR COMPOUNDS IN *BRASSICA*

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Sulphur compounds are involved in sensory quality of Brassica vegetables. Vegenov has developed tools to measure the volatile sulphur compounds and their precursors: S-methyl cysteine sulfoxide (methiin) and glucosinolates. This work was realised on cauliflower (*Brassica oleracea* L. var. *botrytis*). The aim was to analyse volatile sulphur compounds which contribute to the flavour and odour of cooked cauliflower and relate them to individual glucosinolates and methiin contents in raw cauliflowers.

For analysis of the volatile sulphur compounds, extractions were taken by Solid Phase MicroExtraction (SPME) from headspace on cooked cauliflower samples. Qualitative analysis and semi-quantitative analysis were performed by gas chromatography coupled with mass spectrometry (GC/MS). More than one hundred compounds were identified by this method. Gas chromatography coupled with olfactometry on two very differently cooked cauliflowers allowed us to identify key odorant compounds amongst the molecules identified by GC/MS.

Individual glucosinolate contents were determined after extraction and desulfatation by HPLC-DAD. Our method allowed us to identify 21 glucosinolates. In cauliflower, we identified 13 of them: Glucoiberin, Epiprogoitrin, Progoitrin, Glucoraphanin, Sinigrin, Gluconapin, 4-OH-glucobrassicin, Glucoerucin, Glucoiberin, Glucobrassicin, Gluconasturtiin, 4-methoxyglucobrassicin and Néoglucobrassicin. S-methyl cysteine sulfoxide was also determined by HPLC-DAD after an ethanol extraction and a derivatization step with dansyl chloride.

These analyses were performed to explain different sensory characteristics measured by sensory analysis. Qualified panellists were trained to identify and score the perceived intensity of 22 characteristics describing odour, flavour and texture of raw and cooked cauliflowers. These tools can be useful to help “Brassica vegetable companies” (breeders, producers, industry etc.) evaluate and improve the quality of their products.

Poster D-3

THERMAL PROCESSING OF GREEN AND RED CULTIVARS OF CURLY KALE (*BRASSICA OLERACEA* L. CONVAR. *ACEPHALA* VAR. *SABELLICA*) AND EFFECTS OF FRESH AND PROCESSED KALE EXTRACTS ON HUMAN COLON CANCER CELL LINES

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Curly kale (*Brassica oleracea* L. convar. *acephala* var. *sabellica*) is a leafy green or red/purple colored vegetable belonging to the Brassicaceae family. The plant is robust and tolerates a broad range of agricultural and climatic conditions. Brassica vegetables can be subjected to various forms of processing before storage and to make them more suitable for human consumption. Common processing steps include blanching, freezing, thermal treatments and, occasionally, warm-holding. Processing affects the plant tissue and the phytochemicals present, and may lead to alteration in health-related qualities. Processing may influence phytochemicals positively by releasing compounds and increasing their bioavailability, or negatively by physical loss and chemical degradation of the phytochemicals. Thus, it is crucial to gain more knowledge on the fate and final concentration of the phytochemicals before and after vegetable processing, in order to estimate the availability of the phytochemicals in the human diet.

Green and red cultivars of curly kale, grown at an experimental field in Ås, Norway, were evaluated for phytochemicals before and after processing involving blanching, freeze storage, and different heat treatments; boil-in-water, boil-in-bag and sous vide, as well as warm holding of the processed kale. The levels of flavonoids, hydroxycinnamic acids, vitamin C, glucosinolates, carotenoids and antioxidant capacity were followed during the different processing steps. Heat treatments significantly reduced the concentration of health-related phytochemicals, and the results on the different processing methods will be presented. The processing affected the two kale cultivars differently, with a greater loss of phytochemicals in the green cultivar compared to the red kale cultivar. Extracts of the kale cultivars were used in cell proliferation studies with three human colon cancer cell lines (Caco-2, HT-29 and HCT 116), and the fresh plant material had a stronger antiproliferative effect than extracts from processed plant material.

Poster D-4

GLUCOSINOLATE PROFILE IN DIFFERENT MEDITERRANEAN BRASSICA SPECIES (N=9)

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Mediterranean basin represent an important centre of origin and diversification of *Brassicaceae* and above all of *Brassica oleracea* because are widespread several Brassica wild species (n=9) which seem support an intense genetic flux among them. In fact, the genetic self-incompatibility system characterize some populations and landraces favors the genetic recombination and the set-up of several forms and types within the same crop, such as in broccoli, cauliflower, kale, etc. That is observed mainly for bio-morphological traits but great variance has ascertained for antioxidant profile.

We compare Glucosinolate (GLS) profile of wild and cultivated *Brassica* species (n=9) analyzed by DISPA (Catania, Italy) and MBG (Pontevedra, Spain) in view to define variation occurs among crops and origin. In particular, DISPA grew and analyzed twelve wild species from Italy, thirty-eight kale grown in four European countries, nine cauliflower landraces and four commercialized cultivars, four broccoli landraces and three commercialized cultivars. MGB grew and analyzed eleven kale, five cauliflower, ten cabbage, three Brussels sprouts, three kohlrabi and three broccoli among landraces and commercialized cultivars .

Leaves were immediately transferred to the laboratory and frozen at -80 °C. All samples from laboratory and field experiments were lyophilized. The dried material was powdered and utilized for GLS analysis. The extraction protocols were similar for both laboratories in Italy and Spain. The data acquired were utilized to prepare the numerical matrix used to analyze any correlation among GLS compounds and for principal component analysis and cluster analysis. Variation of glucosinolate compounds showed several positive and negative correlations between and within the aliphatic, indolic and aromatic type.

The accessions studied were classified in seven groups different for GLS profile characterized by main GLS compounds such as the group were the main are represented by Neo-Gluco Brassicin and Gluconasturtiin, or by Gluconapin, or by Progoitrin and other minor GLSs, or by Glucoraphanin, 4-OH Gluco Brassicin and Gluco Brassicin, or by Glucoiberin and Sinalbin, or by Glucoerucin, or finally by Sinigrin and Glucoibervirin.

The wild Brassica populations showed the highest variability among them and the other Brassica crops, but the major part of the genetic materials analyzed clustered in the same truss. The commercial cultivars seem to be similar to some landraces and clustered in the biggest truss. Of course we registered great variability for GLS profile among and within the crops analyzed according to the recent scientific evidence about the genetic base of metabolic pathways for GLS synthesis.

Poster D-5

PHYTOCHEMICAL PROFILE OF KOLHRABI (*BRASSICA OLERACEA* L. VAR. *GONGYLODES*) CULTIVATED IN ITALY

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Cruciferous vegetables provide important nutrient such as vitamins A, folic acid, calcium, and dietary fiber and are low in calories and fat. *Brassicaceae* family have significant amounts of bioactive compounds such as sulphur containing glycosides called glucosinolates, but also antioxidants compounds such as total polyphenols, anthocyanins and carotenoids.

Among them, Kohlrabi (*Brassica oleracea* L. var. *gongylodes*) is a native plant from northern Europe. Stems of kohlrabi may be white, purple or green depending on the cultivar. This plant can be eaten raw or steamed or in another way, they are a source of vitamin C and potassium. The present work provides a phytochemical survey on five Kohlrabi genotypes (CR13, CR17, CR22, CR25 and CR29) cultivated in southern Italy by measuring the content in glucosinolates (GLS), total phenols (TPC), ascorbic acid (AsA), total anthocyanins (ATH), total carotenoids (CAR) and antioxidant capacity on different part of the plant (skins, trunks and leaves) and putting into relationship these parameters.

In the leaves have been found four glucosinolates, such as glucoraphanin, glucobrassicin, gluconasturtiin and neo-glucobrassicin but the main has been glucoraphanin. The glucosinolates found in the trunk have been same of the leaves more glucoerucin, but in the trunk the glucosinolates value was generally lower than in leaves. It has to be pointed out an elevated variability of glucosinolates presence in trunks, as it can be inferred from the deviation standard values.

Total polyphenols amount has been found slightly higher in leaves than trunks and the same trend was found for ascorbic acid. For anthocyanins the high values has been found for leaves in comparison with skins, except for CR 17 skin, that showed the highest amount. For total carotenoids the value have been similar for all accessions.

The data of antioxidants capacity differed in accordance with the used method: they have been generally established higher in leaves for DPPH quenching, with the exception of CR22 and CR29 in trunks, with a very strong variability, while the peroxy radical (ROO[·]) scavenging had high values in leaves and in skins, with the highest one in skins of CR17, due to the presence of anthocyanins, enforced by the highest simple regression value ($r_{xy}=0.81$).

As expected, significant simple regression values have been found between DPPH quenching and AsA content, and between ROO[·] and CAR, but, interestingly, an unusual r_{xy} value of 0.50 has been found between GLS and DPPH, suspecting a role of glucosinolates in the antioxidant capacity.

Poster D-6

CHANGES IN BIOCHEMICAL AND QUALITATIVE PROPERTIES IN FRESH-CUT BROCCOLI GENOTYPES DURING STORAGE

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Cruciferous vegetables crops are rich source of phytochemicals which are known to possess antioxidant activity. In particular, broccoli [*Brassica oleracea* L. conv. *botrytis* (L.) Alef. var. *cymosa* Duch.] is recognised as health-promoting vegetable. It is one of the most important vegetables produced in Southern Italy, frequently consumed by people. The content of bioactive compounds varies with the genotype, processing, and post-harvest. Therefore, a study was planned to characterize and evaluate changes in fresh (FW) and dry weight (DW), in total phenolics content and antioxidant activity in fresh-cut processed florets of seven broccoli cultivars (TSX-0788 F1 - cv₁, TRI-8006 F1 cv₂, SRX 1340 F1 - cv₃, SRX 1350 F1 - cv₄, SRX 3090 F1 - cv₅, SRX 1332 F1 - cv₆, Parthenon - cv₇) after 7, 14, and 21 days of fresh storage at 5 °C in OPP packaging.

Corymbs were harvested in spring from a commercial farm located in Foggia (Southern of Italy) province. Post-harvest storage was stopped on day 14th for cv₁, cv₂ cv₃ cv₄ because of off-odours and yellow discoloration occurred. Results indicate that at harvest cultivars cv₁, cv₂, cv₃, cv₄, cv₅, and cv₆ showed the highest corymb DW concentration (13.9 g 100 g⁻¹ FW, on average) followed by cv₇ (12.2 g 100 g⁻¹ FW). After 14 d of storage corimb DW concentration decreased from 1.9 (cv₄) to 0.1 (cv₇) g 100 g⁻¹ FW. The reduction in FW ranged from 0.11 (cv₅) to 0.23% (cv₆) and from 0.36 (cv₅) to 0.69% (cv₄), respectively after 7 and 14 d of storage. On average, at harvest and after 7 d of storage total polyphenolic content was 1.25 mg of gallic acid equivalent (GAE) g⁻¹ FW and it increased up to 1.46 mg GAE g⁻¹ FW after 14 d of storage. At harvest and after 14 d of cold storage antioxidant activity was, on average, 8.4 and 10.2 μmol eq trolox g⁻¹ FW.

Poster D-7

CHEMICAL AND NUTRACEUTICAL PROFILE IN TYPICAL ITALIAN TYPOLOGIES OF CAULIFLOWER

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A comparison between four different typologies of cauliflower (*Brassica oleracea* L, var. botrytis) characterized by the colour of the head (Verde di Macerata, Violetto di Catania, Bianco Jesino, Verde Romanesco) was made.

Samples were conventionally cultivated in one growing season in the field of CRA-ORA, Monsampolo del Tronto (AP) and subjected to a comparative quality characterization: sulphur volatile substances, glucosinolates, ascorbic acid, total polyphenols, total carotenoids and antioxidant capacity.

As regards the amount of sulphur volatiles, fundamental for the organoleptic impact after cooking, the Bianco Jesino and Violetto di Catania showed the highest content.

The glucosinolates, recognized as healthy compounds by their potential anticancer activity, were characterized for the single compounds, glucoraphanin, glucobrassicines and sinigrin with different amounts in each typology, with the lowest presence in Bianco Jesino as total amount.

The nutraceutical profile, evaluated by the ascorbic acid, total polyphenols and total carotenoids dosage, revealed the presence of interesting amounts in Violetto di Catania and Verde Romanesco, confirmed by the measurements of antioxidant capacity.

These indexes can be used as important tools and markers for establishing the typical cauliflower within a determined territory. In this particular case, Violetto di Catania and Verde Romanesco resulted the best cv according to their nutraceutical potential.

Poster D-8

IDENTIFICATION OF INTERMEDIARY BREAKDOWN PRODUCTS FORMED BY THE THERMALLY INDUCED DEGRADATION OF ALIPHATIC GLUCOSINOLATES

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Brassica plants such as cabbage, broccoli or mustard contain glucosinolates (GSL), sulfur containing secondary plant metabolites.⁽¹⁾ Especially their enzymatically formed hydrolysis products – e.g. isothiocyanates (ITC) and nitriles – are of particular interest in food research because of their alleged anticarcinogenic effects. Thermal processes such as cooking and canning degrade GSL and ITCs and nitriles will be formed.⁽²⁾

The aim of the current study was the investigation of thermal degradation pathways of aliphatic GSL, such as sinigrin (allyl GSL), in model systems. The influence of various parameters on the GSL-breakdown, e.g. the pH value, temperature, iron ions, and water content, were studied to identify factors influencing the breakdown and the degradation pathways of the GSL.

GSL-breakdown as well as formation of the volatile breakdown products (nitrile and ITC) and non-volatile products, e.g. D-thioglucose and D-glucose, were investigated using high-performance liquid chromatography-diode array detection (HPLC-DAD) and gas chromatography-flame ionization detection (GC-FID).

The thermally induced degradation of GSL and the resulting breakdown products were found to be dependent on the water content of the sample, on the concentration of iron(II), and on the pH value of the medium. Under aqueous conditions (pH 5.3) the aliphatic GSL sinigrin degraded to D-glucose and the corresponding ITC. Basic medium reduces the thermal stability of the GSL as well as of the ITC, as reported previously.^(3, 4) Iron(II) reduces, especially in presence of additional vitamin C, the GSL concentration and the corresponding nitrile and D-thioglucose was formed. Under dry conditions the desulfo-GSL was identified for the first time as an intermediary breakdown product in the thermal degradation of aliphatic GSL.

Poster D-9

CHARACTERIZATION OF THE MAIN GLUCOSINOLATE CONTENT AND FATTY ACID COMPOSITION IN NON FOOD BRASSICACEAE SEEDS

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Plants of the Brassicales order, and, in particular, the species belonging to the *Brassicaceae* family, are characterised by the presence of the myrosinase (MYR)-glucosinolate (GLs) system, an efficient internal defensive system that plays a role in the control of several types of pathogens. When plants are damaged by wounding or by a pathogen attack GLs are hydrolyzed by MYR and release isothiocyanates (ITCs), bio-active degradation products harmful to pathogens or pests. The exploitation of this system has led to the definition of the “Biofumigation” technique arising from the use of the biocidal properties of ITCs released from GL containing plants and materials. In addition, *Brassicaceae* seeds are characterized by an oil content ranging from 10 to 45 % of their dry matter. *Brassicaceae* oils contain different fatty acid compositions that confer important tribological properties for the production of bioenergy, lubricants and molecules for lipochemistry. For a better exploitation and knowledge of the *Brassicaceae* biodiversity, our research group has been collecting seeds from several wild and cultivated non food species. They have been analyzed for their GL and fatty acid composition. Furthermore, plants from the most interesting species selected for their GL and fatty acid profile were tested for their adaptability to the full-field cultivation. In the present work, a collection of seeds of about sixty *Brassicaceae* species, preserved and available at CRA-CIN, has been characterized. The results confirm a wide variation in their profile bringing out interesting perspectives for their application in non food fields.

Poster D-10

INTESTINAL FERMENTABILITY OF BRASSICA: METHODS FOR EVALUATING AND MINIMIZING DIGESTIVE DISCOMFORT

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Brassica vegetables are spontaneously cited by consumers as causing digestive discomfort after their consumption. The sensory and nutritional laboratory of Vegenov has applied a method first developed on artichoke to identify a solution to limit the disturbance after cauliflower consumption. Bloating and flatulence were the main symptoms caused by cauliflower. First, the fibre compounds suspected to cause inconvenience was identified with bibliographic research and we optimized a dosage method to determine fibre content. The compounds suspected were cellulose and pectin. Secondly, we evaluated the different factors altering the amount of pectin and cellulose e.g. agronomic factors (varieties, size) and technological factors (storage, cooking treatment etc.). Thus, thanks to a better understanding of fibre content changes, it was possible to identify solutions to limit fibre content and therefore digestive discomfort. Biochemical analyses showed a high variation of fibre content with a drop by 35 % in the case of a particular association of preservation and cooking treatment. An *in vivo* study was performed in order to understand better the effectiveness of these potential solutions on digestive discomfort perception. We developed methods for assessing intestinal discomfort by measuring gas production (using a breath hydrogen monitor) and registering the perceptions of the panel during the study with a health questionnaire. We observed that the “digestion friendly” option significantly reduced gastrointestinal symptoms like bloating, abdominal pain and stool emissions. With our method, we were able to find a solution to reduce digestive discomfort. This approach can also be used for other vegetables that cause digestive discomfort.

Poster D-11

EVALUATION OF SOME QUALITY PARAMETERS OF MINIMALLY PROCESSED VIOLET AND WHITE-TYPE CAULIFLOWERS

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The impact of minimal processing technology on the shelf-life of Violet and White-type cauliflowers packaged in two different films was evaluated during 21 days of storage at 4°C. White cauliflower was characterized by a weight loss by 8% after 5 days of storage in the perforated film, as a result of moisture loss, while packaging in the permeable film was effective at reducing the weight loss to about 0.3%. For what concerns the Violet-type cauliflower, the weight loss in the perforated film exceeded 22% after 5 days, while it was limited to 0.3% in the permeable film. The colorimetric analysis on racemes did not reveal, for each cultivar, significant differences during storage, while important changes were observed on the cut surfaces of the White cultivar. The use of a perforated film allowed the headspace gas composition to remain at environmental levels throughout storage, while with the use of a permeable film, both cauliflower varieties reached a steady-state O₂ level ranging from 11 to 13% and a CO₂ level of 8.5% after 72 hours. Although the initial mesophilic bacteria counts in both cultivars were high, all the analyzed samples were in compliance with the recommended microbial limits of total plate counts (8 log cfu/g). Count values of moulds and yeasts reached the limit values (5 log cfu/g) after a few days of storage, especially for the White-type packaged in the perforated film, while a different behaviour was shown by *Enterobacteriaceae*, which evidenced a higher load throughout the considered storage period in samples packaged in the permeable film.

The use of a non-perforated, permeable packaging determines a suitable atmosphere, limiting moisture loss. Violet cauliflower is more suitable for minimal processing due to the color stability on the cut surface area, however microbial quality of raw materials represents an essential condition for the shelf life extension.

Poster D-12

EVALUATION OF PHENOLICS, AND ANTIOXIDANT ACTIVITY OF DIFFERENT VARIETIES OF CAULIFLOWER (*BRASSICA OLERACEA* VAR. *BOTRYTIS*) AND THEIR CHANGES DURING CONVENTIONAL AND MICROWAVE COOKING

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In this work the antioxidant activity of different types (white, green, violet and orange) of cauliflower florets and stems, and their changes during conventional and microwave cooking, were investigated. The different samples of cauliflower florets and stem were cooked by conventional boiling or by microwave over up to 300 seconds. Total phenolics retained after the different cooking methods in the florets and stems were determined as the total retained antioxidant activity evaluated with ABTS, DPPH, FRAP and ORAC assay.

The results showed that most of the antioxidant components and antioxidant activity in cauliflower were lost during cooking on all cauliflower varieties tested, with lower losses when microwave was used under particular conditions. Phenolics content and total antioxidant activity of the samples correlate very well with all used methods. The loss of activity resulted lower for orange and violet cauliflower with respect to white or green varieties, when we used microwave method in the presence of a small amount of water and dedicated commercial cooking bags. The decrease in concentration of different phenolic components was also evaluated by HPLC-MS, as a function of cooking methods. The results obtained suggest that some common cooking treatments can be used on the different varieties of cauliflower to enhance the nutritional value of these vegetables, increasing bioavailability of potential health-promoting constituents.

Poster D-13

EFFECT OF HOT AIR TREATMENT ON QUALITY AND SHELF LIFE OF MINIMALLY PROCESSED CAULIFLOWER

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Cauliflower, as other cabbages, has a high nutritional value due to its content of vitamins, antioxidants and anti-carcinogenic compounds. Cauliflower inflorescences are harvested when they are still totally immature. The request of minimally processed vegetable is continuously increasing, and there is a growing interest for new fresh cut products. Cauliflower is suitable to be used as a minimally processed vegetable, but harvesting and the following processing can cause a severe stress determining the appearance of accelerated senescence symptoms. The effect of hot air treatment on minimally processed cauliflower was investigated. Florets were put in PE sealed bags before treating at 48°C for 180 min and then stored at 4°C for 21 days. During storage period, weight loss, color, firmness, SSC and pH were evaluated. Cauliflower showed a good shelf life and overall quality maintenance. Hot air treatment reduced its susceptibility to browning.

Poster D-14

OPTIMIZATION OF METHODS OF MYROSINASE ACTIVITY DETERMINATION

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The enzyme myrosinase (β -thioglucosidase glucohydrolase, EC 3.2.3.1) is found mostly in *Brassicaceae* plants that also contain glucosinolates (GLS), a sulfur-containing secondary metabolites. Myrosinase catalyzes the hydrolysis of the thioglucosidic bond in GLS releasing a range of products of different structure depending on the parent GLS, hydrolysis conditions, presence of ferrous ions and additional protein factors. The final compounds, mainly isothiocyanates (ITC), are known to trigger a number of health promoting effects, most importantly they inhibit tumorigenesis and play the role in the prevention of heart diseases. ITC are also a major component of plant natural protection system against phytophages and therefore they have found application in the ecological method of crop protection (biofumigation).

However, to fully exploit the potential of Brassicas the indepth understanding of enzymatic mechanism behind their biological activity is required. This implies that the reliable and reproducible methods of determination of myrosinase activity are needed. In literature, different protocols are described and accordingly the published values of myrosinase activity vary substancially. Therefore, we decided to optimize the procedures of two direct methods of determination of myrosinase activity: spectrophotometric and pH-stat assay. The optimization was carried out for a wide range of concentrations for two substrates (sinigrin, glucotropaeolin) and commercial myrosinase prep. Also the influence of sample preparation method and temperature conditions were investigated. The optimized protocols were then verified with the use of plant samples including edible parts of Brassica vegetables and seeds.

Poster D-15

DEVELOPMENT OF MODIFIED ATMOSPHERE PACKAGES ON THE QUALITY OF SICILIAN KALE (*BRASSICA OLERACEA* VAR. *ACEPHALA*) SHOOTS

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Since some decades, in the present global economic crisis the request for fresh-cut products is showing an increasing trend, and it is one of the most remunerative sectors of the agriculture.

In this frame we pointed our attention to diversity fresh-cut vegetable items by several minor and/or underutilized crops and in some case by wild species gathered and utilized as vegetables in Sicily.

Kale (*Brassica oleracea* var. *acephala*) seems to be of great interest for fresh-cut production both for its environmental friendly growing techniques and nutraceutical properties, as it is rich in antioxidants compounds as ascorbic acid, total polyphenols, carotenoids and glucosinolates.

The use of low oxygen (O₂) and high carbon dioxide (CO₂) atmosphere appeared useful for maintain quality and extend shelf-life for several vegetables. In this condition the plant reduces respiration process that involves a complex biochemical reactions, delayed ripening and senescence and also reduces fungal development. Besides refrigeration is important for elongate the shelf-life but modified atmosphere packaging (MAP) is an important complementary technique.

We studied a kale Sicilian type tested with three modified atmosphere (air, 70%N₂:30CO₂; 100%CO₂), three temperature levels (0, 4 and 8°C) and three time storage (0, 3 and 7 days).

Every day and at any temperature were monitored percentage of oxygen and carbon dioxide, chromatic parameters (L*, a* and b*) and dry weight to observe the variation.

Results showed the best method was utilization of 70% N₂:30% CO₂ at temperature of 4°C, the product is kept in good condition for all seven days. This study has allowed of knowledge the respiration process and variation of color of the leave in relation at modified atmosphere, time and temperature of storage.

Poster D-16

ADAPTING TURNIP-TOPS AND TURNIP-GREENS TO MODERN CONSUMER HABITS

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Turnips are very common in Portugal and produce three distinct vegetables: turnip-roots, turnip-greens and turnip-tops. Turnip-tops and turnip-greens are unique and very popular vegetables in Portugal used in traditional dishes. In fact, there are food festivals in Portugal featuring only turnip-tops dishes.

Turnip-tops are the fructiferous stems with the flower buds and the surrounding leaves. Turnip-greens are the young leaves, grown following a spinach like growing technique with a high density population of plants, and harvested when reaching about 20 cm. There are special turnip landraces used by growers for each of these specialized productions. Although in recent years there was a decline in consumption of both vegetables, associated with the loss in competitiveness towards other vegetables and consumer requirements.

The production of frozen turnip-tops and turnip-greens is an interesting way for adapting a traditional vegetable to the modern consumer habits. A summary of the results of two agronomic trials: i) influence of population and harvest date in the yield, morphology and quality of turnip-greens; and ii) influence of cultivars and harvest dates in the yield and quality of turnip-greens, will be presented.

Poster D-17

GLUCOSINOLATES COMPOSITION IN *BRASSICACEAE*

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Glucosinolates (GSL) are important secondary metabolites in plants, which are responsible for taste, flavour, provide anticarcinogenic, antioxidative, immune stimulated and antithrombotic effects for human health and from other side they are ranked among natural pesticides against diseases and pests and can be used as natural biofumigants in soil after ploughing in their biomass as green fertilizing. GSL give rise to a wide range of biologically active breakdown products isothiocyanates, indoles, and oxazolidinethiones upon enzymatic degradation by the endogenous enzyme myrosinase. In our studies it was carried out analysis of total and component content of glucosinolates in five genera of *Brassicaceae*: *Sinapis alba*, *Lepidium sativum*, *Eruca sativa*, *Diplotaxis muralis*, *Brassica juncea*, *Brassica rapa* by HPLC method. 21 component of glucosinolates profile were determined, among them 15 aliphatic GSL, four indol GSL and two benzoyl GSL. Although the new glucosinolates were not found, significant differences in total amounts and glucosinolate profiles between crops and cultivars were determined. All studied accessions of white mustard and six accessions of Indian mustard with high level – more than более 40 µmol/g of sinalbin and sinigrin respectively – are convenient for biofumigation. 17-compound GSL content of *Brassica rapa* was revealed. Some leafy forms with high level of nutritious value are recommended for breeding to improve quality of food, for medicinal purposes, and to enlarge an assortment of cruciferous vegetables. Large potential of variability of glucosinolates component content permits to recommend the use concrete forms and cultivars of cruciferous plants in different directions: for valuable food products and for soil fumigation.

Poster D-18

SURVEY OF HEALTHY COMPOUNDS IN PLANT MATERIAL FROM DIFFERENT SPECIES OF *BRASSICACEAE*

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The content in healthy compounds is becoming a strong instrument for evaluating the quality of plant material. For this purpose, seeds, initial and 7-days sprouts of Brassica and other species have been analyzed and compared for their phytochemical content (ascorbic acid, total polyphenols, total carotenoids, anthocyanin and glucosinolates) and antioxidant capacity (DPPH method).

In the seeds the ascorbic acid resulted more highest, with 7,6 mg g⁻¹s.s., for broccoli “Ramoso calabrese medio precoce”, the total polyphenols content showed more highest amount for white mustard, the total anthocyanins resulted present only in rocket “coltivata a foglia lobata” and the total carotenoids content showed more highest amount for leek “S.Giovanni 90”, broccoli “Ramoso calabrese medio precoce”, Bruxelles sprouts “Mezzo nano” and wild mustrad.

In the sprouts the contents in antioxidant compounds generally has been increasing of initial sprouts at 7 days for more highest species.

The contents in glucosinolates in the seeds showed value more higher than to 0 and 7 days, but among the different days the highest value has been for spouts at 0 day.

A strong variability has been obviously found in the glucosinolates profile, but the trend in their production resulted in a descending trend from seeds to 7-days sprouts.

On the other hand, the main hydrosoluble phytochemicals assayed (total phenols and ascorbic acid) resulted in an ascending trend from seeds to 7-days sprouts. The antioxidant capacity generally resulted higher in 7-days sprouts, with some exception in seeds. Non Brassica species had a low amount in phytochemicals, while a low correlation by simple regression has been found only between total polyphenols and antioxidant capacity.

Poster D-19

BRASSICA FRUTICULOSA CYR. AND B. INCANA TEN. (BRASSICACEAE) AS MEDITERRANEAN TRADITIONAL WILD VEGETABLES: A VALUABLE SOURCE OF BIOACTIVE COMPOUNDS

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Two species of Brassicaceae (Crucifer) family, used and appreciated as traditional wild vegetables, including *Brassica fruticulosa* Cyr. and *Brassica incana* Ten., were examined as potential source of bioactive volatile compounds. The volatile constituents released by the chopped leaves and roots were extracted and analysed by solid-phase microextraction - gas chromatography - mass spectrometry (SPME-GC-MS). A large number of volatile constituents were identified: alcohols, aldehydes, esters, acids, ketones, terpenes, C₁₃-norisoprenoides and sulfur compounds. Volatiles included isothiocyanates with a well known anticancer activity; the largest amount resulted in the roots with 3-butenyl isothiocyanate the most represented in both species, of great interest also the good amount revealed in the leaves of *Brassica fruticulosa* Cyr. The revaluation of these plants, a vegetable source of high antioxidant power, will be interesting for consumer health by the production of new commercial herbal products and/or dietary supplements of high quality and low cost.



BRASSICA2012

Session E

WORKSHOP ISATIS

Oral presentation

Session E

NUTRACEUTICAL VALUES OF WOAD (*ISATIS TINCTORIA* L.) FLOWER BUDS OF ECOTYPES FROM SICILY, ITALY

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Woad (*Isatis tinctoria* L.) is a wild plant widely diffused throughout Europe, Italy included. In ancient times it was exploited as a dye-plant, to obtain the indigo colour, until the appearance of the synthetic dye on the markets at the beginning of 1900. Recently this plant was rediscovered also for its cosmetic and anti-inflammatory properties. Belonging to *Brassicaceae* family, woad contains glucosinolates, bioactive compounds possessing nutraceutical properties. Although woad is not considered as an edible vegetable worldwide, rural people living around Mount Etna in Sicily, Italy, are accustomed to collect the flower buds from the extensively diffused wild plants in the late winter, to be consumed after boiling as ingredients for salads or omelets.

In order to investigate the nutraceutical value of woad, fresh flower buds were collected on Mount Etna slopes in Sicily over two years and analysed for their bioactive compound content. Woad samples contained considerable amounts of glucosinolates, ranging from 80 up to over 100 $\mu\text{mol g}^{-1}$ dw, which are values higher than those found in other Brassica vegetables. Only gluconapin and the most interesting glucobrassicin, which is the natural precursor of indole-3-carbinol, a multiple antitumorigenic compound, were found. High mean values were also recorded for total polyphenols and flavonoids (25 GAE mg g^{-1} dw and 7 CE mg g^{-1} dw, respectively). Consequently, also the antioxidant activity (ORAC) of the woad flower buds resulted among the highest reported for other Brassica vegetables, being about 300 $\mu\text{mol TE g}^{-1}$ dw.

Since processing can partly reduce the glucosinolate content of vegetables, different cooking ways, such as boiling and microwaving, were compared. Boiling for 10 min caused losses of 34% of total glucosinolates, while microwaving (10 min, 800W) only reduced the glucosinolate amount by 3%.

The results obtained suggest that woad flower buds could be considered as a functional food with possible antitumoral properties.

Session E

WOAD (ISATIS TINCTORIA L.): AN INNOVATIVE CROP FOR THE MEDITERRANEAN AGRO-INDUSTRIAL SYSTEM

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The genus *Isatis* (*Brassicaceae*) encompasses about 30 species, most of them producing in their leaves the blue pigment indigo (indigotin), one of the oldest natural dyes known to man. *Isatis tinctoria* L., the earliest known source of indigo in Europe where it was cultivated since the Middle Ages, is a biennial and mainly outbreeding species not yet subjected to any formal breeding programme and expressing a variety of phenotypes which differ for morphological and phytochemical characteristics.

Indigo is not found as a native compound in plants, but as a product of a secondary metabolite named indoxyl. Since the biosynthetic pathway of indigo production has not yet completely understood, the purity and quality of the natural product could not be controlled, being it one of the main reasons for their replacement by synthetic indigo at the end of the 19th century. New technologies to process indigo products have recently been developed encouraging the importance of the reintroduction of indigo-producing plants into European farming in order to make naturally dyed products available to a much wider market to fulfill the increasing interest amongst consumers for ecological standards and natural products made from renewable sources.

As a result of the historical disappearance of plant indigo-producing farming, there is a considerable lack of knowledge regarding the basic cultivation of these plants and its indigo production. The “Impronte” research project (www.impronte.org) funded by Sicilian Region (PSR 2007-13, Mis. 124) started in 2011 and will run until 2013. It aims to give Sicilian farmers a way to make a high quality indigo product to satisfy present and future demands from industry. There are eight partners representing all links in the production chain: growing the crops, ensuring seed supplies, improving yields, extracting the indigo, purifying the product and testing it for dye quality.

First results show a wide genetic variability of *Isatis tinctoria* L., with good adaptation to the Mediterranean climate conditions, giving large biomass production and high indigo yields during the first vegetative year. This low technology-demanding crop could represent an interesting alternative to the traditionally cultivated vegetables in this region thanks to the positive influence of the moderate temperatures, that allow successive harvests during an extended growing season, and of the intense light regimes, that increase indigo precursor content

Session E

CAPTURING THE LEAF TRANSCRIPTOME OF *ISATIS TINCTORIA* L.

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Woad or *Isatis tinctoria* is a valuable member of the Brassicaceae that has been utilized in traditional medicine since antiquity but it is most commonly known for its previous use in the production of blue dye for the textile industry. In our lab *I. tinctoria* has proved a compelling species for studying pathways producing health-promoting compounds but also as a control species for the study of the plants response to low temperatures. There are few genomics or genetic resources available for *I. tinctoria*, in order to assist in our research we have developed a snapshot of the woad transcriptome using Roche 454 next generation sequencing. Young leaves were used to develop a cDNA library, for which 374,719 good quality, filtered reads were generated (average length, 325 bp). These reads were assembled into 32,533 Isotigs using Newbler v2.6, 80% of which showed strong sequence similarity to 12,852 and 30 annotated nuclear and plastid genes of *Arabidopsis thaliana*, respectively. A summary of the developed transcriptome and its use in a larger study comparing 15 Brassicaceae species with adaptive phenotypes of interest will be discussed.

Session E

NATURAL INDIGO FROM *ISATIS TINCTORIA* L. FOR THE VALORISATION OF SICILIAN CROPS

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Isatis tinctoria L. or woad (Brassicaceae) is an upright herbaceous biennial species up to 120 cm in height. It is in Mediterranean countries, a common plant cultivated through out centuries to produce the blue dye indigo. With increasing concern for sustainability and a demand from consumers for naturally sourced products, there is a revival of interest in natural indigo as an agricultural crop product. Indigo is formed after the extraction of indigo precursors in the leaves of these plants: mainly isatans in woad. These compounds are extracted by steeping leaves in warm water. With woad, the addition of alkali to the steep water releases free indoxyl, which forms indigo after a vigorous aeration. Indigo is hydrophobic and insoluble in water, so that it sediments readily, and the solid indigo can be readily washed and dried. Indigo is synthesized from two precursor molecules of indoxyl, deriving from plant secondary metabolism. Recently clarified the nature of indigo precursors in woad (*Isatis tinctoria* L.), by identifying the major indoxyl glycoside as isatan A (indoxyl-3-O-(6-O-malonyl-b-D-ribohexo-3-ulopyranoside)), and by correcting the structure of the related isatan B (indoxyl-3-O-b-D-ribohexo-3-ulopyranoside). The seasonal variation of indoxyl glycosides in woad leaves was investigated and the influence of various post-harvest treatments was studied and Isatans A and B disappeared completely when the leaf material was submitted to a conventional drying process. In contrast, the indican content in leaves increased significantly, and the extent of this increase was temperature-dependent. These precursor can be identified and quantified by HPLC method since their quantity is dependent on the species and the harvest period. The modern extraction method of indigo from woad uses the water solubility of the indigo precursors in steeping the leaves in hot water. The precursors are broken down to indoxyl and sugar moieties by endogenous enzymes in plant (glycosidases), but in the extraction method this is done by alkali with aeration. The purity of plant-derived indigo even with the modern extraction method is somewhat low when compared to the synthetic indigo. Natural indigo contains besides indigo, impurities such as indirubin, indigo-brown, indigo gluten and mineral matter. Several authors indicate that indigo can be extracted from woad with a purity of 90% if three conditions are met: the leaves contain a sufficiently high yield of indigo precursors; the leaves are rinsed free of soil; and the indigo is sedimented in an acid medium.



BRASSICA2012

Session F

AGRONOMY

Oral presentation

Session F

AGRONOMICAL EVALUATION OF BROCCOLI RAAB GENOTYPES FOR FRESH CUT AND PROCESSING PRODUCTS

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Broccoli raab (*Brassica rapa* L) also called turnip top or rapini, is widely cultivated in central and southern Italy; the edible portion is composed of the green-immature inflorescences (heads) and the tender stems with its most young leaves. In the last years the interest of the consumers to this vegetable is rising; moreover, a substantial increase of consumption could come from processed (frozen) or fresh-cut products (FCP), that offer to consumers convenience, high nutritional value and flavour while still maintaining their freshness.

This paper provides information on the suitability of different genotypes of broccoli raab to obtain frozen and FCP. In addition growing techniques to improve yield and quality were investigated.

Two year open field trials were carried out on five genotypes of broccoli raab. Sowing time and plant density were also evaluated in order to enlarge the harvest season of vegetables. Total yield, biometric traits and colour measurements were determined on broccoli raab plants at each harvest. To prepare FCP, young stems, leaves and inflorescences were selected, trimmed, washed and drained. Panel tests were arranged on the FCP to evaluate organoleptic attributes; the results were graphically represented by sensorial profiles.

‘Quarantina’ and ‘Fasanese’ genotypes resulted best suitable for fresh cut production; total yield was higher than others, whereas the refuse percentage of products was very low. The number of axillary heads was highest in ‘Mezzo tempo di Fasano’. Plant density affected total yield and morphological and qualitative traits of all broccoli raab genotypes.

Session F

EFFECT OF THE APPLICATION OF GIBBERELIC ACID AT DIFFERENT STAGES OF THE LATE HEAD CABBAGE ROSSETE ON VERNALIZATION AND SEED YIELD

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Seed production of head cabbage (*Brassica oleracea* var. *capitata* L.) in conditions of continental climate is carried out by introducing plant in a specific developmental stage of vegetative rosettes, in the meteorological conditions under which the vernalization process occur. Vernalization is a physiological process of transformation vegetative meristemic tissue into generative tissue, and in this way avoiding one stage of organogenesis – cabbage head. This process takes place in conditions of low positive temperatures, with a specified duration, which is possible in our country in the period of autumn and winter. Only a limited number of plants during this cold period experience this transformation. Experiment on one late head cabbage genotype, was set up in Institute for Vegetable Crops, in four replications with three sowing dates (15th August, 1st September, and 15th September). The seedling was planted, in field conditions at 20th October 2010, in the experimental field size 70x50 cm (28 500 plants per ha). Plants were treated with gibberellic acid (GA₃), concentration of 300 ppm. Application of GA₃ was conducted in two periods: before the beginning and at the end of the period with optimum temperature for vernalization. The results of this experiment showed statistically significant differences of sowing dates and seed treatment on yield ($p > 0.05$). Also, results showed statistically significant differences of interaction between sowing date and the percentage of blossom treated plants at both levels of significance ($p > 0.01$, $p > 0.05$). A minimum percentage of blossom plant was 40 and a maximum 80, and average yield varied from 539 kg to 1621 kg.

Session F

CULTIVARS AND FERTILISER STRATEGIES AFFECT THE CONCENTRATION OF GLUCOSINOLATES IN CURLY KALE (*BRASSICA OLERACEAE* L VAR. *SABELLICA* L)

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Curly kale contains glucosinolates which have been of great interest since they were shown to have some health beneficial effects such as a preventive effect against cancer. Choice of cultivar and application of nitrogen and sulphur fertilisers during production are known to have an influence on glucosinolate concentration in other Brassica species. The aim of this study was to investigate the interactions between choice of cultivars, nitrogen and sulphur fertiliser on glucosinolate concentration in a crop of curly kale. Modern and traditional cultivars from the NordGen germplasm collection were used in this study.

In a first experiment the traditional 'Tiara' and modern 'Reflex' cultivars were grown at 90, 135, 185 or 230 kg N ha⁻¹ in a field experiment during the season of 2011. In a second experiment the 'Reflex' cultivar was grown at 90, 135 or 185 kg N ha⁻¹ combined with 0 or 30 kg S ha⁻¹ during the same period. Glucosinolates were extracted from freeze dried leaves and analysed by high-performance liquid chromatography.

The results showed that the traditional cultivar 'Tiara' had a higher total concentration of glucosinolates but a lower yield of edible leaves compared to the modern cultivar 'Reflex'. The ratio of aliphatic to indole glucosinolates was highest in 'Reflex'. The highest concentration of glucosinolates was obtained at different levels of nitrogen in the two experiments, whereas application of sulphur always increased the glucosinolate concentration irrespective of nitrogen application in 'Reflex'.

These findings underline the importance of the choice of cultivar and fertiliser strategy for nitrogen and sulphur when we aim for bio-enrichment seen in a future health perspective.

Session F

THE *BRASSICACEAE* BIOFUMIGATION SYSTEM FOR PLANT CULTIVATION AND DEFENSE AN ITALIAN TWENTY YEAR EXPERIENCE OF STUDY AND APPLICATION

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Among non-chemical alternatives to pesticides, the use of natural compounds with a high biological activity presents several environmental benefits, mainly due to their renewability and biodegradability, that generally allows a reduction in greenhouse gas emissions in comparison to the use of conventional compounds. In particular, in these last twenty years, interesting perspectives were provided by the study and the exploitation of the typical glucosinolate (GL) – myrosinase (MYR) defensive system of the *Brassicaceae* family. The high biocidal activity of the GL-MYR hydrolysis derived products has been observed *in vitro* on several soil-borne fungi, nematodes and insects and provides a natural and practical alternative to chemical fumigants for controlling some soil borne pathogens and pests. Over these years, plants from several *Brassicaceae* species were selected as biofumigant green manures. In addition, some plants materials based on residual defatted seed meals containing high GL content were characterized, formulated, patented and tested in full-field application. Since several years, they have been marketed in different countries as dry pellets or as a component of liquid formulation for a distribution to epigeal or hypogeal plant apparatus. A review about the biofumigant system, reporting the results of its efficacy in application fields as horticulture, floriculture, fruit cultivation and post-harvest treatment, will be presented. Finally, environmental aspects of the application of biofumigant system in alternative to conventional pesticides will be discussed, taking into account also the secondary benefits of biobased products such as the fertilizing and biostimulant properties. The synergic application of these natural compounds, year after year, can maximize the biofumigation containing effect and can offer the farmer a new option in plant cultivation and management.

Session F

SUCCESS AND FAILURES OF BIOFUMIGATION WITH BRASSICAS

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The use of *Brassica* species as green manure is a type of biofumigation involving the release of isothiocyanates, thiocyanates, nitriles or oxazolidinethiones that control multiple soil-borne problems. Biofumigation can be achieved by incorporating fresh plant material, seed meals, or dried plant material treated to preserve isothiocyanate activity or by using brassica intercrops. Thus, brassica residues can provide varying levels of pathogen control, either alone or when combined with other disinfestation methods. However, some recent studies indicate that the effectiveness of organic amendments including brassica residues is variable and, in some cases, soil amendments with *Brassica* products can enhance disease severity. The negative effect of these amendments may result from increased pathogen inoculum potential when the substrate serves to sustain saprophytic growth of plant pathogens or from the increase of inoculum potential of pathogens carried out by using the brassica amendments. Some examples of success and failure of the use of biofumigation against soil-borne pathogens of vegetable crops will be reported.

Session F

EFFECT OF COMPOST APPLICATION ON QUALITATIVE TRAITS IN CABBAGE

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The use of alternative fertilizers plays nowadays an increasing importance because of increase of price of mineral fertilizers and organic matter decrease in cultivated soils. Veneto Region plays an active role in waste recycling and composting activities that allows to use new organic matter sources in order to improve soil properties. In this study we evaluated the effect of compost use on the agronomic and nutritional qualitative traits of cabbage.

The experiment was conducted at the University of Padova considering the following theses: control unfertilized (F0), treatment with mineral fertilizers (FMIN) in which the 100% of the nitrogen requirement was supplied in mineral form, treatment where 50% of the nitrogen requirement was made in mineral form and the remaining demand with compost (F50), 100 % of nitrogen requirements necessary for the culture was made from compost (F100) and 200% of demand in nitrogen was supplied through compost (F200). Dry matter content, antioxidant capacity (AOA), total phenols (TP), ascorbic acid (AA), phenolic acids, organic nitrogen and nitrate (NO₃) were evaluated as qualitative traits. It was also determined the quantity of Al, Ca, Cu, K, Mg, P, Zn and heavy metals (Cd, Cr, Hg, Ni, Pb).

Results showed that AOA and TP were higher in plants grown with F0 and F200. About AA values the most interesting result was observed in F200 too. The main phenolic acids detected were chlorogenic and p-coumaric acid on which fertilizer treatment did not significantly influenced. However, for both acids, the highest values were measured by plants cultivated with F200. In assessing the content of NO₃, plants of the thesis that during the crop cycle were held to a higher proportion of available nitrogen (F50 and FMIN), showed the highest concentrations of nitrogen compounds. Concerning heavy metals Cd, Hg, Pb were below the detectable limit.



BRASSICA2012

Session F

AGRONOMY

Poster presentation

Poster F-1

BIOLOGICAL AND PRODUCTIVE CHARACTERIZATION OF DIFFERENT BRASSICACEAE GROWN IN THE SOUTH OF ITALY

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A number of *Brassicaceae* such as Rapeseed (*B. napus* L. var. *oleifera* D.C.), Ethiopian mustard (*Brassica carinata* A. Braun), Indian mustard (*Brassica juncea* (L.) Czern), White mustard (*Sinapis alba* L.) and Oil radish (*Raphanus sativus* L. var. *oleiformis* Pers.) can provide raw materials for food and non-food sectors, since their biomass is rich in a spectrum of valuable constituents distributed in the seeds (mainly oil and fatty acids, protein) as well as in other tissues (above all glucosinolates) of the plant. Glucosinolates can be useful as bioactive ingredients (e.g. in reducing the risk of cancer in humans) and as biocide agents (e.g. in controlling different biotic constraints of the crops). Therefore, these species are considered interesting as cash and catch crops to promote the transition towards the sustainability of cereal based farming systems prevalent in semiarid environment of southern Italy. For these reasons, an experiment was carried out, in a plain area in the province of Catania (eastern Sicily). A set of nineteen cultivars (variety and hybrids) belonging at the above-mentioned species were compared in large plots replicated three times arranged in a randomised-blocks. The sowing was performed in winter period. Weather data were recorded, and the main phenological stages of the plants were measured during the cropping cycle. At harvest, grain yield and its components were determined. Seed samples were analysed in order to characterise the lipid and protein components. The data were processed using analysis of variance. The time course of temperature and the amount of rainfall throughout the growing seasons were in accordance to that of the long-term period.

The results highlighted an appreciable variability among the genotypes in terms of duration of the biological cycle and its phases. The genotypes reached the flowering stage from the end of March to the start of May, and ended the cycle (seed ripening) between mid-May and mid-June. In particular, the earliest genotypes were found within *B. carinata*, *B. juncea*, *Sinapis* and *Raphanus*. On the other hand, two cultivars of *B. carinata* reached the better performance in terms of grain yield, near to 2 t ha⁻¹. Significant differences between the tested genotypes were observed for oil and protein content of the grain.

The experiment revealed that overall, the studied cultivars evidenced a good adaptability and some of them ensured an acceptable production level under the semiarid environmental conditions of Sicily.

Poster F-2

STRENGTHS AND WEAKNESSES TRAITS FOR BRASSICA VEGETABLE CROPS PRODUCTION OF TURKEY

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Brassicas are widespread as wild, weedy and cultivated forms throughout Turkey. Vegetable brassica crops are an important and highly diverse group of crops grown world-wide that belong mainly to the species *Brassica oleracea* and *Brassica campestris*. Turkey has a significant place in world vegetable production. Turkey is fourth after China, India and the United States in world vegetable production. The total production of vegetable species in Turkey is 26.700.000 ton. In Turkey, cabbage is the most economically important member of the genus *Brassica*. The type of vegetables grown in Turkey has been diversified in the recent years. New crops include Brussels sprouts, Chinese cabbage, kohlrabi, Pak choi and aromatic vegetables. Among many factors that make Turkey a strong vegetable sector, appropriate ecological conditions, proximity to foreign markets, and implement new technologies quickly transferred into the country, include the fact that labor costs are still low compared to developed countries. In this study, it was given as comprehensive strengths and weaknesses traits for *Brassicas* vegetable production of Turkey.

Poster F-3

EFFECTS OF BIO-FERTILIZERS ON BROCCOLI CULTIVARS IN ORGANIC FARMING

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Results of open field trial to study the effects of bio-fertilizers, applied by fertigation, on growth traits, morphological, yield and qualitative characteristics of different cultivars of broccoli (*Brassica oleracea* var. *italica* Plenck), are reported. Gas exchange parameters as net assimilation, transpiration, stomatal conductance and water use efficiency, were also evaluated during the cropping cycle.

In addition, the results of a trial to compare the effects of conventional and organic grown techniques on the growth and yield traits of seedlings (cv. Marathon) are showed.

The trials has been carried out in winter period in Valenzano (Bari, Southern Italy) on a clay-loam soil. Three levels of organic fertilization (0 - 60 and 120 kg ha⁻¹ of NewFert based on fleshing hydrolyzed, by Chimica Dr. Fr. D'Agostino s.p.a., Bari-IT) on 4 cultivar of broccoli ('Marathon', 'Lord', 'Chevalier', 'Iron'), were compared.

The different fertilizer treatments affected significantly the diameter of the inflorescence, which increased with the increase of the dose of bio-fertilizer and ascorbic acid content that, instead, was reduced. The cultivars showed good qualitative characteristics especially nitrate content that did not exceed 350 mg kg⁻¹ f.w. The most productive cultivar was 'Chevalier' (11.8 t ha⁻¹). Nitrate content was low, particularly in 'Lord' and 'Marathon' (161 mg kg⁻¹ f.w), while a high level of ascorbic acid was recorded for 'Chevalier' and 'Iron' (72 mg 100 g⁻¹ f.w.). 'Chevalier' and 'Iron' presented the best characteristics for what concern colour, more green and shiny.

The type of seedlings did not result in significant effects on biometric and production parameters.

Poster F-4

QUALITY TRAITS OF SOME CAULIFLOWER CULTIVARS GROWN IN THE “VALLE DELL’OFANTO” AREA (ITALY) AS AFFECTED BY POST-HARVEST STORAGE

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In this research, carried out at Gaudio (41°03' N; 15°42' E, Southern Italy - Basilicata Region), some quality characteristics of the “Valle dell’Ofanto” cauliflower, that has been recently appointed the certification of collective brand, were investigated. In particular, 6 white head cauliflower cultivars, characterized by a different length of the cultivation cycle (2 early, 2 medium and 2 late cultivars), were studied.

The above cultivars were transplanted in open field at the middle of August 2004 and harvested from the middle of October 2004 to the end of March 2005. At harvest time yield traits and head sizes of cauliflowers were measured. Among the qualitative traits, color, dry matter, nitrate and vitamin C content were assessed on the fresh florets and after a storage period lasting 15 days at 0 °C and 95% R.H. The “Valle dell’Ofanto” cauliflower, showed to be characterized by a high content in sugars and vitamin C, even if significant differences in some qualitative parameters (e.g. vitamin C and nitrate content) were affected by cultivars and crop cycle length. On the other hand, the storage at controlled temperature and R.H. did not substantially change the qualitative characteristics of the heads, but caused only a slight increase of dry matter and soluble solids levels, especially in those cultivars more susceptible to weight loss.

Poster F-5

EFFECTS OF COVERAGE WITH NONWOVEN, AND FERTILIZER LEVEL ON THE WILD ROCKET IN ORGANIC FARMING

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The results of a study conducted to assess the effects of coverage with non-woven fabric (NW) and fertilization level on yield and quality of wild rocket in organic farming, are reported. The work is focused also to assess the variations of soil and air temperature near the canopy, caused by the cover with nonwoven.

The research was carried out during autumn-winter period in southern Italy. Two cropping techniques (crop covered with NW and not covered, indicated respectively NWC and NC) and 3 levels of organic fertilization (0 – 1 and 2 t ha⁻¹ of guano) on wild rocket (*Diplotaxis tenuifolia* L. DC.), were compared.

The covering with NWC has influenced the microclimate near the plants. In particular, air temperature at the surface of the soil and that of soil of 5 cm in depth, as average of the crop cycle, were respectively about 0.8 and 1.8 °C higher than those in the crop not covered. Moreover, the coverage with NWC has resulted in a lower thermal excursion, reducing the risk of frost damage. NWC has enhanced the weight of the plants and the total yield, but has increased also the percentage of waste. Yield and ascorbic acid content increased by increasing the fertilizer level in the 2nd picking. In NWC, the nitrate content of the second picking leaves has remained low and did not change increasing the level of fertilizer, at the contrary of the NC crop in which it increased with the increase of fertilizer.

Poster F-6

CHARACTERISTICS AND SEED PRODUCTION OF SICILIAN LANDRACES OF VIOLET CAULIFLOWER

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In Italy there are several landraces of cauliflower very popular for local communities of different regions. These landraces are endangered by introduction of new F1 hybrids which risk to contaminate traditional gene pool with a serious genetic drift. In this frame DISPA since twenty years have collected, characterized, evaluate and conserve these landraces identified genotypes of interested and have set up sib lines and F1 hybrids by DHs of interest for their nutraceutical traits. These activities are supplying for qualified propagation materials both to satisfy local market demand and GDOs and to support functional foods chains. In this context Sicilian violet cauliflower landraces, characterised by high rusticity and adaptability to Mediterranean climatic condition could allow friendly environmental crops and contain the use of pesticides and fertilizers with a reduced environmental impact in agriculture.

We were characterised and evaluated fourteen landraces of cauliflower growing in several areas of Sicily, utilising the principal IBPGR descriptors used for this crop. Six curds for each landraces, harvest at commercial ripening time were collected to detect some characters such as fresh weight, longitudinal and transverse dimensions, core diameter, grain size, colour, angle of curvature and the chromatic parameters ($L^* a^* b^*$) of the curd.

With regard to seed production we registered seed yield collecting six plants for landraces at seed physiological maturity.

The landraces characterized showed significant differences especially for some characters that seemed more variables such as the plant height, number of leaves for main stem, longitudinal dimension and shape of the leaf and colour of the margin.

The plant biometric parameters showed high variance mainly for fresh weight, height, diameter, angle of curvature, chromatic parameters and dry weight of the curd. Seed production has presented a significant variance among the landraces.

The range of seed production varied from 0,8 to 12,27 q/ha.

Poster F-7

SEED PRODUCTION AND PLANT CHARACTERIZATION OF SICILIAN LANDRACES OF BROCCOLI

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Broccoli seems to have been domesticated in the eastern Mediterranean where it began the process of diversification that has given rise to current types and cultivar of which many are today grown in south Italy and in Sicily. The denomination of the landraces occurred often in relation to the colour, harvest period, locality.

We were characterised and evaluated 10 different local cultivars of broccoli growing in several areas of Sicily utilising the principal IBPGR descriptor used for this crop.

Six curds of each landraces harvest at commercial ripening were collected to detect some characters such as the fresh weight, longitudinal and transverse dimension, diameter core, grain size, colour, angle of curvature and the chromatic parameters of the system (L* a* b*) of the curd.

With regard to seed production we registered seed yield collecting six plants for landraces at seed physiological maturity.

The landraces characterized showed significant differences especially for some characters that seemed more variables such the plant height, number of leaves for main stem, longitudinal dimension of the leaf, section of the petiole and the margin of the leaf.

The plant biometric parameters showed high variance mainly for fresh weight, height, maximum diameter, angle of curvature, chromatic parameters a* and b*.

Seed production has presented a significant variation among the landraces in the study probably this is related to the biological materials and environmental conditions in Sicily. The range of seed production varied from 2,5 to 21,75 q/ha.

Poster F-8

GROWTH AND NITROGEN UPTAKE OF TWO CULTIVAR OF BROCCOLI *BRASSICA OLERACEA* L. CONV. *BOTRYTIS* L. ALEF. VAR. *CYMOSA* DUCH.

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The dry weight (DW) accumulation and partitioning, and the N uptake and allocation for two broccoli cultivars ('Parthenon' - *Sakata seeds*- and 'Montecarlo' - Syngenta seeds) grown in a Mediterranean climate. Experiment was arranged in a randomized block design with three replications. Transplanting was performed on September 25st 2008 (plant density 3.5 m⁻²). Plants were fertilized with 130 kg·ha⁻¹ of N and 120 kg·ha⁻¹ of P₂O₅. Starting from 21 days after transplant up to commercial maturity of the corymbs, 8 destructive samplings were done. At each sampling, fresh and the dry weight of leaves, stalks and corymbs, leaf area and leaf number, was recorded. Kjeldahl-N concentration was also determined on dried plant parts material. DW data for each cultivar were used for modelling dry mass with three non linear functions (logistic, Gompertz and Hill), each one fitted using time (days after transplanting) and thermal time (day-degrees - °CD) as independent variables. The best fit was obtained with the logistic function using °CD as independent variable. Nitrogen uptake data for each cultivar were also used for modelling the decrease in N concentration in the DW as affected by plant DW increase, by using the exponential curve: %N = aDW^{-b} . Cultivar 'Parthenon' had higher leaf number and area than 'Montecarlo', but leaf DW was not different between the two cultivars. 'Montecarlo' showed higher stalk DW than 'Parthenon'. No differences were observed in the harvest index (0.26) and in the corymbs fresh yield (11.4 t ha⁻¹, on average). Total N uptake was higher in 'Montecarlo' (194.2 kg ha⁻¹) than in Parthenon (183.4 kg ha⁻¹), while corymb N uptake was 74 and 71 kg ha⁻¹, respectively.

Poster F-9

CHARACTERIZATION OF DIFFERENT *BRASSICACEAE* GROWN IN SOUTHERN ITALY FOR GLUCOSINOLATES PROFILE

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Glucosinolates (GSLs) are secondary metabolites synthesized by the *Brassicaceae*. The quantity and quality of such constituents in the tissues of a given Brassica species changes as far as the developmental stage and the part of the plant are concerned and also as a result of the influence of the genotype, growing environment and agronomic management. These phytochemicals are known for a range of biological properties (anti-nutritive, anti-carcinogenic, anti-microbial, anti-pest, anti-disease) and therefore are taken into consideration for possible medical, food/feed and agricultural uses. Currently, within the sustainable and organic farming systems, there is interest for plants synthesizing GLSs, which can be used for crop protection applying different biofumigation strategies including break-crops, green manures and derived biobased formulations (pellets and liquid), substantially based on the activation of glucosinolates-myrosinase system, which occurs after the damage of the plant tissue as a result of the enzymatic (Myrosinase, Myr) hydrolysis of GLSs in iso-thio-cyanates (biocidal substances). In southern Italy where a number of crops, especially some vegetables widespread both in the field and greenhouse (potato, tomato, etc.), are susceptible to a range of soil-borne pathogens and pests, the use of GLSs-Myr system is considered an effective control tool alternative to conventional fumigation and synthetic agrochemicals. In this view, an experiment was carried out to assess the content and composition of GSLs in different *Brassicaceae* such as *Brassica carinata* A. Braun, *Brassica juncea* (L.) Czern, *Sinapis alba* L. and *Raphanus sativus* L. var. *oleiformis* Pers. grown in eastern Sicily. A set of nineteen cultivars were sowing in winter period in large plots replicated three time arranged in a randomised-blocks. Representative plant samples were collected intact at two significant stages of biological cycle (flowering and seed maturity). Total aboveground biomass was weighted to determine the dry matter yield, and representative sub-samples of whole plant were crushed and freeze-dried for GLSs extraction and determination. In addition, the defatted meal of harvested seed samples of each genotype was analyzed for GLSs. The data were subjected to ANOVA.

The comparison of the tested genotypes revealed appreciable variation in GLSs profile, and differences between the sampling during the growing cycle were found highlighting the possibility to establish an optimal combination between dry matter accumulation and GLSs concentration in the plant tissues to maximize the biofumigation effect. This result seems to be easier to reach with some of the studied species, mainly *B. juncea* and *R. sativus*.

Poster F-10

PHENOLOGY, GRAIN YIELD AND QUALITY OF ETHIOPIAN MUSTARD GROWN IN SOUTHERN ITALY

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The knowledge of the behaviour of Ethiopian mustard (*Brassica carinata* A. Braun) genotypes on different sites and seasons is a key step of the research to assess the opportunity of spreading this multifunctional crop (food and non-food) in farming systems of southern Italy, which are based almost always on cereals. For these reasons, a two-year experiment (2007-2008 and 2008-2009) was carried out in central-eastern Sicily, in a hilly and plain representative area. Three cultivars of Ethiopian mustard selected from the Italian germplasm source (ISCI7, CT 207, CT 204) were sown in autumn-winter period in large plots replicated three times arranged in a randomised-blocks. During the trials, weather data were recorded. The main phenological stages of the plants were measured throughout the cropping cycle and grain yield was determined at harvest. Grain samples were analysed for oil, protein and glucosinolate content.

The data were statistically analysed using appropriate 3-way ANOVA. In each experimental site, the time course of weather conditions during the growing seasons conformed to that of the long-term period.

The results indicated that, although the differences between the genotypes, the duration of the growing cycle was appreciably conditioned by the site. The earlier genotypes, CT 204 and to a minor extent ISCI 7, reached better performance in terms of grain yield, and oil, protein and glucosinolate yields. Therefore, the choice of genotype represents a crucial aspect for Ethiopian mustard cultivation in southern Italy, but owing to the significant interactive effect of this factor with the other ones (year and site), the biological and productive behaviour of the studied cultivars was markedly affected by the different weather conditions occurred during the trials.

Overall, it was possible to establish that the tested cultivars have a good adaptation to the semiarid environmental conditions of Sicily, and that the agronomic management of the crop does not require significant changes of the mechanization and farm structure.

Poster F-11

EFFECTIVENESS OF BIOFUMIGATING BRASSICA TREATMENTS IN GREENHOUSE TO CONTROL ROOT-KNOT NEMATODES ON TOMATO AND MELON

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Intensive vegetable cropping systems enhanced infestations of root-knot nematodes (*Meloidogyne* spp.) and related damages in Salento region, southern Italy. Sustainable alternatives to chemicals for the control of these pests may be represented by nematicidal green manures or plant-derived formulations. Biofumigating green manures of Brassica plants may be limited by the need to insert a brassica crop within intensive rotations with short intervals among the crops. Technical feasibility and effectiveness of brassica green manure biofumigation for the control of root-knot nematodes were investigated in a greenhouse experiments on a tomato - melon succession. Green manures of *Brassica juncea* and *Eruca sativa* were compared with a brassica seed meal commercial pellet, alone or combined with *E. sativa* manure, two commercial formulations of quillay or tagetes, chemical nematicide cadusaphos and an untreated soil.

In the tomato crop, green manure of *E. sativa* resulted in the lowest *M. incognita* population densities and gall formation, as its results were not significantly different from chemical but lower than *B. juncea* green manure, the pellet and quillay formulation. This last treatment was statistically similar to the untreated control. *E. sativa* green manure, either alone or combined with the pellet, provided also a tomato yield two-three times higher than control, whereas *B. juncea* and cadusaphos almost doubled the yield of untreated soil.

In the following melon crop the lowest nematode densities were recorded in soil treated with cadusaphos or the commercial pellet. Green manure of *E. sativa*, alone or combined with the pellet, resulted in a higher suppressivity than *B. juncea*, whereas a limited nematicidal activity emerged for the quillay formulation. The highest melon yield were provided by the seed meal pellet, followed by the combination of *E. sativa* green manure and pellet and by both single green manures.

Poster F-12

MORPHOLOGICAL AND PRODUCTIVE CHARACTERIZATION AND OIL YIELD OF BRASSICA NAPUS GROWN IN DIFFERENT AREAS IN ITALY

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Despite the potential offered by the rape cultivation as biofuels crop, in Italy its cultivation is still almost absent. A further motivation to the spread this crop might arise from the exploitation of secondary products (flour and cake) useful in application fields.

In 2010-2011, thanks to the Project EXTRAVALORE the agronomic evaluations were carried out on 38 varieties of rape, the most spread in the Italian market, purchased by 16 seed companies. The genetic screening was performed three different localities of Italy: in the North (Palazzolo - UD), Centre (Osimo - AN) and South Italy (Cassibile - SR).

The environments significantly influenced the morpho-phenological traits and the production of the studied varieties. The best performances were obtained in Central Italy, where the the rape, on average of genotypes, flowered before than in North and South Italy. Moreover the plant height resulted higher, these results confirm the good adaptability to this environment for this crop.

Among the cultivars, “Zoom” and “Albatross”, showed yields close to 4.8 t ha⁻¹; other fifteen varieties have exceeded the average value. As regards the seed oil content, two cultivars (“Katabatic” and “Adriana”) showed an average value of 480 g kg⁻¹.

In the northern Italy it has been obtained intermediate yields, with a more marked delay in flowering. The differences among genotypes resulted more marked than that recorded in the other environments; “DK Expower” was the most productive, with “Hornet”, “Hybriswing” and “Fregat”, with values higher than 3.0 t ha⁻¹. The highest oil content was recorded in “Albatross” and “Primus”, with values higher than 480 g kg⁻¹. In Sicily, because of the the weather condition some cultivars (“Kutiba”, “Ilia”, “Tassilo”, “Adriana” and “Anaconda”) have not been able to flowering, probably for the lack of vernalization. The seed yields and the seed oil content, lower than 450 gkg⁻¹, resulted the lowest as compared to the other environments.

Poster F-13

MORPHOLOGICAL AND PRODUCTIVE CHARACTERIZATION OF *BRASSICA CARINATA* GROWN IN DIFFERENT AREAS IN ITALY

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In recent years the cultivation of *Brassica carinata* has been attracting the interest of the research, even in countries characterized also by unfavorable climatic conditions for its cultivation thanks to its features such as, the more vigorous and productive potential, the greater resistance to biotic and abiotic stresses than rapeseed for oil in winter season and the absence of dehiscence of the siliques. Recent evaluations of the Italian territory, however, have highlighted the lower productivity, especially with regard to oil yield, due to oil content in the seeds, 10% lower, as compared to rapeseed. A further motivation to the spread this crop might arise from the exploitation of secondary products (flour and cake) useful in application fields. In 2010-2011, under the Project EXTRAVALORE agronomic evaluations were performed on four commercial varieties of *B. carinata*, in different areas of Italy: the North (Palazzolo - UD), Centre (Osimo - AN) and South Italy (Cassibile - SR).

The environments significantly influenced the morpho-phenological traits and the production of the studied varieties. The best performances were obtained in Central Italy, where the *B. carinata* reached the highest grain yields. On average of the three environments the two varieties "CT 180" and "CT 204" have exceeded the production of 2 t ha⁻¹ of grain, reaching the yield of 1 t ha⁻¹ of oil only in the locality of Central Italy. The differences among genotypes for oil content suggests the necessity of breeding programs for the creation of varieties with higher oil content to fill the gap, due mainly to the relatively recent interest in this crop and the consequent lack of progress in genetic improvement obtained.

Poster F-14

ROOT LENGTH DENSITY AND YIELD TRAITS OF BROCCOLI (*BRASSICA OLERACEA* L. VAR. *ITALICA* PLENCK) AS AFFECTED BY DIFFERENT TECHNIQUES OF SEEDLING GROWING AND TRANSPLANTING

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The research was carried out in Lavello plain (South of Italy, Basilicata region, 41°03' N; 15°42' E, 180 m asl), in order to study the response of broccoli (cv Marathon F1) both root length density (RLD) and corymbs yield as affected by: 1) different techniques of production of seedlings (containers of volume equal to 13, 46, 90 and 180 cm³), 2) different periods of transplant (24/08/2005 and 26/09/2005) and 3) different procedures of transplantation (with or without biodegradable container). A double rows lay out of planting (40 cm between rows, 30 cm between plants on the row, 170 cm between the doubled rows), for a crop density of 2.94 plants m⁻², was performed. The harvest started on 02/11/2005 and 12/12/2005, and ended after 10 and 20 days respectively for the two transplantation times. Along with harvests, soil samples were taken at different positions (close to the plant, between the rows, between the doubled rows) and at different depths (0-20, 20-40, 40-60 cm) in order to measure RLD according to Newman's method (1966). The results showed that the RLD and the corymbs yield (as so as all the main yield components) were significantly influenced by: 1) the volume of containers, from 1.9 to 2.4 cm cm⁻³ and from 9.7 to 13.5 t ha⁻¹ respectively for the smallest and the largest container; 2) the time of transplant, with values of 2.4, 1.9 cm cm⁻³ and 14.6, 10.9 t ha⁻¹ respectively for the first and the second time; 3) the presence of the container at transplant, with values of 2.0, 2.3 cm cm⁻³ and 12.9, 13.5 t ha⁻¹ for the transplant with and without container, respectively. Moreover, with reference to the RLD, were observed, as expected, a reduction with: 1) increasing distance from the plant (values from 3.3 to 1.3 cm cm⁻³ respectively for samples collected near the plant and between the doubled rows) and 2) with increasing depth (values from 3.7 to 0.8 cm cm⁻³ respectively for the 0-20 and the 40-60 cm layer).

Poster F-15

EFFECTS OF NaCl SALINITY ON YIELD, QUALITY AND MINERAL COMPOSITION OF BROCCOLI AND CAULIFLOWER

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A greenhouse experiment was conducted to study the effects of NaCl salinity on yield, quality and mineral composition of Broccoli and Cauliflower. *Brassica oleracea* L. var. *italica* and *Brassica oleracea* L. var. *botrytis* plants were grown in a substrate culture (sand) using three nutrient solutions containing 0 (control), 20 or 40 mM NaCl. The electrical conductivity of control nutrient solution was 1.9 dS m⁻¹. The increase of NaCl caused a decrease in the marketable yield from 9.9 (control) to 6.2 t ha⁻¹ (40mM NaCl) and from 27.9 (control) to 19.9 t ha⁻¹ (40 mM NaCl) in broccoli and cauliflower, respectively. The dry matter percentage of heads increased as consequence of salt stress more in cauliflower than broccoli, whereas the rises of soluble solids (+25%) and titratable acidity (+40%) were similar in both species. In both crops, head concentrations of Na⁺, Cl⁻, N, P and S increased with the rise of NaCl in the nutrient solution, whereas Ca²⁺ and K⁺ decreased. Salinity influenced also the quality characteristics of broccoli and cauliflower after 10 days of storage at 4°C. Dry matter, titratable acidity and total soluble solids increased as consequence of storage more in salinized treatments than unstressed control.

Poster F-16

GROWTH, GAS EXCHANGE AND WATER RELATIONS IN BROCCOLI AND CAULIFLOWER AS AFFECTED BY NaCl SALINITY

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In the present investigation we studied the effects of NaCl on the growth and physiological response of broccoli and cauliflower. A control nutrient solution and two salinized treatments with different NaCl concentrations (20 and 40 mM) were assayed under greenhouse conditions. Plants were cultivated by using an open soilless system and the electrical conductivity of the basic nutrient solution was equal to 1.9 dS m⁻¹. The rise of the NaCl concentration in the nutrient solution caused a similar decrease in plant dry weight of cauliflower and broccoli that varied, respectively, from 226.9 to 201.1 g plant⁻¹ and from 236.5 to 208.2 g plant⁻¹. The reductions of plant dry weight were not related to the assimilation rate of the plants being the net photosynthesis not influenced by NaCl salt stress. The total water potential of leaves decreased significantly at the increase of NaCl concentration with a reduction of about 80% in cauliflower and 160% in broccoli. The important effect of NaCl salinity on the water status of both crops was confirmed by the results of leaf relative water content that decreased with increasing salinity from 90.5% (0 mM NaCl) to 82.4% (40 mM NaCl) and from 92.4% (0 mM NaCl) to 81.4% (40 mM NaCl) in cauliflower and broccoli, respectively. The exposure to NaCl salinity determined an increase of Na⁺ and Cl⁻ concentrations on plant tissues and a nutrient (K⁺, Ca²⁺) imbalances due to ion antagonism effects. Moreover, the old leaves showed the highest concentrations of Na⁺ and Cl⁻ to preserve the integrity of the young leaves and reproductive organs. The results obtained on plant water relations, gas exchange and ion content in the tissues demonstrated that the negative response of broccoli and cauliflower to salt stress depends on both osmotic and ion specific effects.

Poster F-16

EFFECTS OF DIFFERENT FERTILIZATION LEVELS ON *IBERIS SEMPERFLORENS* POT CULTURE

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The genus *Iberis* (family Brassicaceae) includes 30 species of annual and perennial herbs and subshrubs that grow in a wide area from western and central Europe to the Mediterranean Basin. *Iberis semperflorens* L. is a perennial evergreen subshrub (30-60 cm high) endemic to southern Italy and Sicily. Due to its abundance of white flowers and extensive flowering period occurring during the winter months, the species has good potential for pot cultivation and outdoor ornamental use in Mediterranean-type climates. Reliable and efficient propagation methods for *I. semperflorens* nursery production have been determined. However, there are limited information on cultural techniques of this winter flowering species. The aim of this research was to compile fertilizer pot culture recommendations for this unexploited Mediterranean species. Plug plants bearing 8-10 leaves were transplanted into plastic pots (16 cm in diameter) containing a 3 peat:1 perlite growing medium fertilized with various levels of Osmocote 16 N – 8 P – 10 K (0.75, 1.5, 3.5 g l⁻¹). Plant height, number of leaves per plant and number of inflorescences per plant were monitored during the growing cycle. Our results suggest a good adaptation of *I. semperflorens* to pot cultivation and significant relation between fertilization levels and flowering response.

Poster F-17

HUMIC ACID IMPROVED GROWTH AND YIELD COMPONENTS OF HYDROPONIC GREENHOUSE GROWN PAK-CHOI (*BRASSICA CHINENSIS*)

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Effects of humic acid supplementation into a hydroponic greenhouse production system of Pak Choi (*Brassica chinensis*) were studied. Fifty mL of different levels of humic acid (HA) from 1000 to 11000 mg/L were applied four times in a weekly manner. Growth parameters plant height and diameter, leaf number and chlorophyll content, fresh and dry weight, dry matter percent, number of deformed leaves and flowering percent were studied. All plants treated with 11000 mg/L HA showed malformed leaves and 30% of plants were aborted. There was no significant difference among treatments for leaf chlorophyll content. The highest leaf number, leaf fresh weight and dry matter percent were observed at 5000 mg/L HA. A 12% increase in plant fresh weight was observed in 5000 mg/L HA comparing to control plants. The highest flowering percent in pak choi plants was observed at 6000 mg/L HA. According to results, application of 5000 mg/L HA is recommended for the highest yield of pak choi in hydroponic system.

Poster F-18

SECONDARY PLANT METABOLITE PROFILE AND CONCENTRATION IN LEAFY ASIAN *BRASSICA* VEGETABLE AFFECTED BY ORGANIC AND MINERAL NITROGEN SUPPLY

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Leafy Asian *Brassica* vegetables are gaining increasing attention by European consumers since they contain rich sources of health promoting secondary plant metabolites such as glucosinolates, flavonoids, carotenoids, and chlorophylls (Krumbein et al. 2005). In this study, we test our hypothesis that low N supply dependent on organic or mineral N supply via various N uptake will increase the concentrations of flavonoids and glucosinolates but decrease the concentrations of carotenoids and chlorophylls in leafy *Brassica* vegetables.

The leafy Asian *Brassica* specie *Brassica juncea* was grown in the field with four different levels of farmyard manure (0, 30, 60, 90 t ha⁻¹) corresponding to the N supply of 0, 15, 30, 45 kg ha⁻¹, respectively. In addition, four treatments with mineral N supply (0, 30, 60, 90 kg ha⁻¹) were tested. Secondary plant metabolites were determined by HPLC-DAD-ESI-MS² (1, 2).

Increasing mineral N supply resulted in increasing N uptake of the plant up to 107 kg ha⁻¹ while the N uptake of organic fertilised plants was independent of the level supplied with amounts between 13 and 20 kg ha⁻¹. Various relationships were calculated between N uptake and secondary plant compounds investigated. Generally, N depletion is known to stimulate activation of the flavonoid and glucosinolate pathway via a number of genes and enzymes (3, 4). Interestingly, N supply of 30 kg ha⁻¹ led to a plant N uptake of 20 kg ha⁻¹ for organic fertilised plants in comparison to 56 kg ha⁻¹ for mineral fertilised plants resulting in lower flavonoid and glucosinolate concentrations by 30 % and 22 %, respectively in mineral N fertilised plants. Our result highlights the importance of N availability for formation of secondary plant metabolites dependent on N supply form used.

Poster F-19

EFFECT OF A *BRASSICA JUNCEA* COVER CROP ON A MONO-SUCCESSION OF MELON

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The ban of methyl bromide and the need for low environmental impact of agriculture has recently increased the interest in the biofumigant effects of Brassica species on soil-borne pathogens. Many researches have tested the toxic effects of Brassica green manures on several soil-borne pathogens in vitro, but field studies have not assessed definitely the efficiency of biofumigation at the field scale and the effect on crop development and production quality. The aim of this research was to evaluate the effect of a winter-spring cover crop of *Brassica juncea* compared to bare soil on a mono-succession of melon. The biomass of the *B. juncea* plants or of the weeds eventually developed in bare soil, used as control, were managed as follows: (i) crushed and left on the soil as organic mulching; (ii) crushed and incorporated into the soil; (iii) crushed and incorporated into the soil under PE mulching along melon crop rows. Soil changes during the two years trial were monitored by evaluating: organic matter content, availability and content of mineral elements and Cation Exchange Capacity. Marketable and unmarketable production, fruit weight and fruit quality (thickness of the epicarp and pulp, percentage of edible part, soluble solid content, firmness, etc.) were evaluated on melon. The use of *Brassica juncea* as a cover crop influenced soil characteristics and was effective on melon compared to bare soil when the biomass was incorporated to the soil, while no difference was found on melon production and quality when PE mulching was used.

Poster F-20

QUALITY COMPARISON BETWEEN ORGANIC- AND CONVENTIONAL-GROWN CAULIFLOWERS: RESULTS FROM THE 3RD YEAR OF VALORBIO PROJECT

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The aim of VALORBIO research project, funded by Italian Ministry of Agriculture, is the valorization of typical horticulture products by the extended use of organic farming. Specifically, the presence of healthy chemicals was monitored in different genotypes of cauliflower (*Brassica oleracea* L, var. botrytis) by comparing conventional and organic agrosystems.

Four cauliflower genotypes were cultivated in 12-years certified organic fields at CRA-ORA, Monsampolo del Tronto (AP), and the conventional ones according to consolidated practice of conventional agriculture. The considered cultivars were Emeraude (green), early and late Velox (green) and Jesino (white).

As nutraceutical quality traits, the amount of sulphur volatiles and glucosinolates were measured as well as ascorbic acid, total polyphenols, total carotenoids and antioxidant capacity.

The differences between the two systems of cultivation were discussed by comparing what happened in the previous years, such as a general increase of volatiles and glucosinolates in organic samples and a general equilibrium of antioxidant compounds in organic and conventional systems.

Poster F-21

EFFECT OF ANAEROBIC DIGESTATES APPLICATION ON QUALITATIVE TRAITS IN EARLY AND LATE CAULIFLOWER

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The use of anaerobic digestates coming from organic matrices as fertilizers, in reference to qualitative and nutritional traits in cauliflower was evaluated. These matrices plays nowadays an increasing importance since they could be employed as a source of organic matter and nutrients to the soil. The sludge used derived from the anaerobic digestion of plant material as by product obtained from fruit distillation.

The experiment was conducted at the University of Padova considering two varieties of cauliflower (early and late). The following treatments were considered: control unfertilized (F0), treatment with mineral fertilizers (FMIN) in which the 100% of the nitrogen requirement was supplied in mineral form, 50% of the nitrogen requirement was made in mineral form and the remaining demand with digestate (F50), 75 % of nitrogen requirements was supplied with digestate (F75) and 100% of demand in nitrogen was supplied through digestate (F100). Dry matter content, antioxidant capacity (AOA), total phenols (TP), ascorbic acid (AA), phenolic acids, organic nitrogen and nitrate (NO₃) were evaluated as qualitative traits in corymb. It was also determined the quantity of Al, Ca, Cu, K, Mg, P, Zn and heavy metals (Cd, Cr, Hg, Ni, Pb).

Concerning the dry matter percentage, F0 plants and the early variety showed higher values. About AOA, TP, AA and phenolic acids all theses showed high values and none of them was influenced by fertilization. Antioxidant compounds content was affected also by genotype and the early variety showed the highest values. In assessing the percentage of nitrogen and NO₃ values, F50 and FMIN plants showed the highest concentrations of nitrogen compounds whereas, increasing digestate percentage, a decrease of these values was observed. Heavy metals content was very low or absent in the edible part. High levels of mineral compounds were found and they were not influenced by digestate application.

Poster F-22

CHARACTERISTICS AND SEED PRODUCTION OF ITALIAN AND IBERIAN TYPE OF TALE (BRASSICA OLERACEA VAR. ACEPHALA) GROWN IN SICILY

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Kale is an underutilized crop which seems to be the first results of domestication process of Brassica oleracea wild type complex. This crop is still now grown in all European countries in home gardens and periurban vegetable farms and it is appreciated for its rusticity and the high bioactive molecule. Kale leaves, in fact, contain high amounts of total polyphenols, ascorbic acid and glucosinolates.

Seeds are important for a wide range of horticultural crops, their development depends by many factors such as genetic, physiological and environmental. An important prerequisite for the successful production of seeds are the optimal timing of harvest.

In view to enhance kale production and provide to the growers qualified genotypes attention was paid to the evaluation of twenty-three types of kale grown in Italy and the Iberian peninsula for seed production; to the landraces four commercial cultivars were compared.

Besides seeds production in the experiment plants characterization, according to the main IBPGRI descriptors, was performed.

Seeds were sown in seedling trays and were transplanted when they had 4-5 expanded leaves. An experimental field with plant density of 2 plant m⁻² was realized with the aim to determine seed yield. The experimental design was a split-plot with three replicates. Siliquas were collected from two plants for each repetition when the seeds reached the physiological maturity.

Seeds production showed a significant differences among the compared genotypes; it varied from 0,59 q ha⁻¹ to 25,69 q ha⁻¹ respectively for one Italian and one Iberian type, in general the Italian landraces showed to be more productive. Plants characterization allowed to highlight that the descriptors which appeared more discriminating were plant shape, and as well as several leaf and petiole traits.

Poster F-23

USE OF *BRASSICA NAPUS* L. VAR. *OLEIFERA* D.C. TO DEVELOP SUSTAINABLE STRATEGIES IN THE PHYTOEXTRACTION

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Phytoextraction is an emerging green technology to extract heavy metals from polluted soils. *Brassica napus* L. var. *oleifera* D.C. is known for its metal tolerance and accumulator useful but also is cultivated also for energy purposes. To increase the advantages of phytoextraction, this species should also be evaluated for the ability to produce products with economic value that would certainly be an additional benefit. Therefore, a promising alternative could be thermochemical conversion process. The biomass produced by phytoremediation, combined with biomass generation and its commercial utilization as an energy source, can be turned into profit making operation and the remaining ash can be used as bio-ore.

This study evaluates the phytoextractive capacities and the biomass production of two commercial varieties, Betty (Apsob sementi) and PR46W31 (Pioneer Hi Breed), which could be used either for energy purposes.

Seeds of Betty (Apsob sementi) and PR46W31 (Pioneer Hi Breed) were sowed in pot on a multi-metal contaminated soil sampled in protected area of Alta Murgia (Southern Italy).

An uncontaminated agricultural soil collected nearby was used to fill control. At the end of the experiment, the morphological parameters were measured to define biomass yield. The phytoextraction potential of the heavy metal concentrations in the plant parts were determined with ICP-EOS spectrometer and the bioaccumulation coefficients calculated.

The results obtained are very interesting considering that we are restoring a protected area where other methods may prove impractical.

The morphological data obtained show that plant growth increases on the multi-metal contaminated soil compared to control, producing considerable biomass. In our conditions, although both varieties are tolerant to heavy metals, *B. napus* PR46W31 demonstrated the best capacity of multi-metal absorption. In particular, in this experiment, 'PR46W31' behaves as Cr hyperaccumulator.

An evaluation of the potential biomass obtainable in a phytoremediation process expressed in energetic terms showed a positive balance, adding energy production to the environmental benefits of phytoremediation.

Poster F-24

EFFECTS OF HARVEST TIME AND PLANT DENSITY ON YIELD AND QUALITY OF CHINESE CABBAGE FOR FRESH-CUT PRODUCTION

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The introduction of new products for fresh-cut needs the screening of suitable cultivars and the definition of the appropriate cultivation techniques to improve the quality and reduce the negative effects of the processing on shelf-life. The aim of this study was to evaluate the effects of harvest time (40, 50 and 60 days after the transplant) and plant density (7, 10 and 16 plants m⁻²) on the yield and quality characteristics of two cultivars of Chinese cabbage ('Bilko' and 'Manoko') for fresh-cut production. The increase of harvest time and plant density affected differently the yield of the cultivars; in particular 'Bilko' showed a relative yield increase greater than 'Manoko' as the harvest time was delayed from 40 to 60 days after the transplant. Similarly the relative increase of the yield at the increase of plant density from 7 to 16 plants m⁻² was higher in 'Bilko' (+44%) than 'Manoko' (+17%). Also the quality characteristics of fresh-cut product were differently affected by the harvest time and plant density in two cultivars considered. 'Manoko' showed lower dry matter and higher nitrate content than 'Bilko'; however these differences were more evident at the highest plant density. 'Bilko' showed higher CO₂ concentration in the packages than 'Manoko' at the harvest of 60 days due to higher respiration activity. The results of this experiment showed that the plant density and harvest time affected the yield and quality characteristics of Chinese cabbage. However the interaction effects between these factors and the cultivars on some characteristics (e.g. dry matter and nitrate content) should be considered to obtain a product suitable for the fresh-cut markets.

Poster F-24

RESPONSE OF *MATTHIOLA INCANA* (L.) W. T. AITON TO CULTIVATION IN THE MEDITERRANEAN CLIMATE

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In relation to the favorable winter conditions in the Mediterranean areas, it could be possible to expand the season production of the stock (*Matthiola incana* L.), a very interesting specialty cut flower, through the use of unheated greenhouses. In this frame the aim of this study was to evaluate the response of cultivar of stock to the cultivation in unheated greenhouse. Two different experimental trials were carried out. In the first trial were evaluated two cultivar ('Prousse Pink' and 'Maria') and five sowing times (February, April, June, August and November); in the second experiment was evaluated the response of four cultivar ('Prousse Pink', 'Maria', 'Prousse White' e 'Prousse Red') sowed in November. In the first trial only 'Maria' showed a profitable production in every sowing periods. In relation to the two cultivar considered the production time ranged from 100 days in April to 187 days in sowing period of August. However the production time affected the stem characteristics: in particular the fresh weight ranged from 71.1 to 308.1 respectively for the sowing periods of April and August, while the strength index was higher in the stems sowing in August and November. In the second trial the genotype affected stem characteristics and production time. The flowering time for 'Prousse White' and 'Prousse Pink' was at the beginning of January while for 'Maria' and 'Prousse Red' in March. The cultivar affected the fresh weight (from 141.3 g in 'Prousse Red' to 227.9 g in 'Maria') and length of the stem (from 85.2 cm in 'Prousse Pink' to 96.7 cm in 'Maria'). The results showed that the unheated greenhouse is profitable to expand the production season of the stock but it should be considered the choice of most suitable cultivar to improve the yield and the quality characteristics of the stems.

Poster F-25

CHARACTERIZATION OF TRADITIONAL BRASSICA VEGETABLE PRODUCTION SYSTEMS IN SICILIAN PERI-URBAN HORTICULTURE

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Among the vegetable species widespread in the Sicilian peri-urban vegetable farms and in the inland areas of the Island it is possible to observe different forms and types of Brassica vegetable crops distinguished by several traits of quality, which play a significant role from agronomic, economic and social point of view. In these contexts several diversified economic activities, integrated and/or environmental friendly, of interest also in term of landscaping are supported by several actors (including the so-called "farmers keepers") that trigger a logic of endogenous development typical of local production systems and rural development, for full exploitation of natural resources and local historical traditions. These are local systems of production, not so much for the specific spatial configuration of the aggregate observed at local level (with micro, small and medium-sized enterprises "concentrated" with reduced "density"), as well as for the inter-business and inter-social (the so-called "thickening" of relations between enterprises, public institutions and local society). In this frame, various activities of SMEs (agricultural, tourist and trade) support articulated production systems and play an important role of the local economy; they have a strong social content for the presence of life and culture forms historically connected to the local communities and finally because they are developed in differentiated contexts from the environmental point of view, for the presence of ecosystems and landscapes very different from urban ones. In this contexts, the vegetable Brassica crops represent the nucleus around have started or are starting several production chains by managing traditional activities, originated to make it possible to overcome the limits relating to seasonality, perishability and amplitude of the market, to realize the economies of scale. That perspective could increase the economies of scale, in view to diversify the production also by particular quality traits, particularly useful in the challenge of the competition between local and global that characterizes the modern agro-food systems. Although it is widely-accepted the socio-economic-environmental valence of these productions often they suffer from a low level of protection which greatly limit the possibilities of development and, in some cases, their survival. Our work focuses on the "Cavolfiore Violetto Catanese" and on the " Cavolo Rapa di Acireale", actively grown in some areas of the Eastern Sicily and selected by growers on the basis of some criterias such as the recognition of the product and the genetic specificity. We utilized both of them for assessing within these local production systems the presence of relationship systems defined between stakeholders (local governance) and their degree of interrelation. The results show the central role of "guardian farmers" who, thanks to their work for the conservation of cauliflower and kohlrabi landraces and passing on and jealously guarding the "seeds of knowledge 'generation to generation within small enterprises, usually located in suburban areas, with limited relations systems whose strengthening can become a strategic factor for the success and competitiveness of the entire local system.

Poster F-26

STRONG HETEROSIS IN YIELD IS SEEN IN CHINESE CABBAGE F1 HYBRID CULTIVARS

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Heterosis (or hybrid vigor) is a phenomenon in which hybrid progeny exhibit superior performance over their parents. It has been observed in many plant and animal species. Heterosis is widely used in many crops and vegetables to make high-yielding cultivars. However the molecular mechanism of heterosis is still to be elucidated and there is no consensus model. As most crucifer vegetable cultivars use F₁ hybrid seed production systems, these species should show heterosis with particular parental combinations. However there are few reports analyzing heterosis at either early- or late-developmental stages in crucifer vegetables.

In this study, we characterized the heterosis phenotype using two commercial F₁ hybrid cultivars and their parental lines in Chinese cabbages (*Brassica rapa*) at early- and late-developmental stages. Heterosis is already observed in both F₁ hybrid cultivars at early developmental stages, from germination to 14 days after sowing: F₁ hybrids showed larger leaf area and greater fresh weight. At later development stages, we examined the final yield under field conditions. The two F₁ hybrid cultivars showed 36% and 77% increase in harvest compared to the average of the parental lines. Previously we observed that heterosis phenotypes were clearly observed in early stages of shoot development in *Arabidopsis thaliana* (Fujimoto *et al.* 2012 Proc. Natl. Acad. Sci. USA 109: 7109-). We consider this early developmental advantage in F₁ hybrids of both Chinese cabbage and *A. thaliana* might be important for increased biomass at later stages or for yield.

Poster F-27

COLLARD GREEN (*BRASSICA OLERACEA* VAR. *ACEPHALA*) CULTIVATION IN SICILY

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Collard greens (*Brassica oleracea* var. *acephala*) are a group of plants cultivated for its thick, slightly bitter, edible leaves. As other Brassicaceae, have a good nutritional value as they are a good sources of vitamin C and soluble fiber, and contain various compounds with potent anticancer properties. In order to enhance collard cultivation in Sicily, different planting dates were tested.

The research was carried out during 2009-2010 in the experimental field of SAgA Department – University of Palermo. Plants of a Sicilian local cultivar, with high vigor, good emission of side shoots, large and moderately curly leaves and long and strong petiole, were planted in three different periods: end of November, end on January, end of March. The harvest of the main shoot was made when they had tender and non-fibrous leaves and petioles. Secondary shoots, eventually originated from axillary buds left on the plant, were also harvested. Data were recorded on: number of leaves/plant, stem diameter, fresh weight, dry weight, length and number of harvested shoots. The plants of the earliest transplant were harvested in March (main shoot), April and May (secondary shoots), while only the main shoot was harvested in the other plants. This determined an higher yield for the first transplant against the other planting dates. Early transplant influenced also the quality of the main shoots that had more leaves, higher percentage of edible part and less dry matter than those from the other transplant. Secondary shoots had worst quality than the main shoot.



BRASSICA2012

Session G

PESTS AND DISEASES

Oral presentation

Session G

FUSARIUM OXYSPORUM F. SP. AND XANTHOMONAS CAMPESTRIS PATOVAR: TWO IMPORTANT SEED-BORNE PATHOGENS OF WILD ROCKET FOURTH RANGE PRODUCTION IN THE SOUTHERN ITALY

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Wild rocket (*Diplotaxis tenuifolia*) is an important fourth range species; the most of its Italian production comes from the Southern Italy. *Fusarium oxysporum* (Fo) and *Xanthomonas campestris* (XC) are two dangerous pathogens that affect this species and are the cause of great yield losses. Fo is the causal agent of plant wilting while XC causes the bacterial leaf spot disease. Either Fo and XC are seed-borne pathogens and because of their aggressiveness many farmers check the seed health before sowing. In the last years, we frequently isolated these pathogens from infected plant samples; moreover we analysed 57 seed samples to assess the Fo and XC presence. Isolation on generic and semi-selective media were performed to detect the pathogens on symptomatic materials. To detect *Xanthomonas* sp. on seeds, a modified ISTA protocol (7-019) were used: the samples were separated into three 10.000 seed sub-samples, soaked in a buffer and the extract were plated on semi-selective media. The pathogenicity tests on xanthomonas-lyke colonies were performed by spraying a suspension on one month plants. The Fo detection on seeds were carried out by isolation on agar semi-selective medium and the suspected colonies were tested for pathogenicity using the dipping root inoculation method. A characterization of *X. campestris* colonies were performed by molecular and *in-vivo* tests. According to Vicente description (2006), the strains with vascular behavior were identified as *X. c. campestris* while the non vascular isolates were identified as *X. c. raphani*; only the first resulted positive to the amplification of *HrcC* gene (Zaccardelli *et al.*, 2007).The characterisation of xanthomonas strains is still in progress and it will be completed by further biochemical and molecular tests. Almost the 20% of seed samples tested resulted contaminated by XC. A fitosanitary control should be achieved to reduce the widespread of the pathogen in the cultivations.

Session G

WHY TO STUDY THE CONCENTRATION OF THE ASCOSPORES OF *LEPTOSPHAERIA MACULANS* AND *L. BIGLOBOSA*?

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The fungal species *Leptosphaeria maculans* and *L. biglobosa* contribute to one of the most damaging diseases of oilseed rape - stem canker of brassicas. This severe disease causes significant crop losses worldwide. The primary source of infection of oilseed rape by both species of the genus *Leptosphaeria* are ascospores that are formed in pseudothecia - fruiting bodies of the perfect stage of the fungus, located on crop residues from the previous growing season. Monitoring of the release of these spores allow to optimize the chemical protection and better combat the disease.

The concentration of ascospores in the air was determined using Burkard samplers. Seven-day operating traps were located in nine climatic regions in Poland and they operated from 1 September to 30 November in the years from 2009 to 2011. The field experiments were done in five locations with the use of winter oilseed rape cultivar PR46W10 F1. Protective treatments were done using a fungicide containing flusilazole, with weekly intervals starting from the end of September till mid-November.

In all locations the spores were found in air samples and their concentration and dates of the earliest detection differed in space and time. The earliest detection of the first ascospores was on 7 September 2010 in west part of Poland, the latest on 22 September 2011 in east Poland. The highest daily concentration of ascospores reaching up to 88 ascospores was observed on 20 September 2011 in south Poland. The ascospore release was also subjected to the diurnal cycle – the highest spore concentrations were observed during the day and the lowest at night. The results of field experiments demonstrated that the time of fungicidal treatments had a significant impact on the severity of stem canker. Sprays done in a period following the highest number of spores was the most effective.

Session G

ANTIBIOTIC EFFECT OF GLUCOSINOLATES AND DERIVATIVES ON DIFFERENT BRASSICA DISEASES

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Brassica crops are affected by several diseases worldwide. *Xanthomonas campestris*, *Pseudomonas syringae*, *Alternaria brassicicola* and *Sclerotinia sclerotinium* cause important damages in these crops. Glucosinolates are secondary metabolites derived from aminoacids with effect on the resistance to pests and diseases. The objective of this work was to evaluate the effect of 17 glucosinolates and their derivatives isothiocyanates on the development of abovementioned fungus and bacteria. Each pathogen isolate was grown in a Petri dish and replicated five times. On each dish, five discs were laid with different concentrations of each compound together with a blank disk and a gentamicin disk. The effect of the glucosinolate was measured as the diameter of growth inhibition. All compounds showed an inhibitory effect, which increased with higher concentrations. There was a high variation for inhibition among pathogen isolates and compounds. In general, glucobrassicin and gluconapin had an important effect on all pathogens, being benzyl isothiocyanate and gluconapin isothiocyanate the compounds which showed the weakest effect. Gluconapin was the most effective compound against *Pseudomonas* isolates. Glucorafanin was also very effective against one race of *Pseudomonas*. Related to *Sclerotinia*, differences between compounds were minor than with other pathogens, being glucobrassicin and allyl isothiocyanate, the compounds which showed the highest effect. The growth of *Alternaria* was inhibited mainly by indol-3-carbinol.

The inhibitory effect of glucosinolates on Xcc, was race-dependent (9 different races described). The most common races (1, 4 and 6) were together inhibited by glucoiberin and gluconapin. Sinalbin, glucoerucin and sinigrin had also an inhibitory effect on races 1, 4 and 6, respectively. The rest of the races were inhibited by different compounds.

The results showed that glucosinolates had an important effect on the development of two bacterial and two fungal brassica diseases and that there is a high variation of the glucosinolate effect among and within pathogens.

Session G

**MOLECULAR CHARACTERIZATION OF PORTUGUESE ISOLATES OF
LEPTOSPHAERIA MACULANS USING PCR-ISSR AND RAPD MARKERS**

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There are scarce information regarding *Leptosphaeria maculans* isolates from Portugal. This paper reports the first molecular study with Portuguese *L. maculans* isolates. PCR-ISSR and RAPD markers were used to study the genetic relationships of 18 Portuguese isolates of *L. maculans* from Beja region (south Portugal) to other 12 reference isolates representative of each group of the *L. maculans* species complex. Cluster and principal components analyses were conducted using PCR-ISSR and RAPD data from amplification with selected ISSR primers and RAPD markers, detecting 234 polymorphic fragments. The results showed that the 30 isolates clustered into two distinct groups (Tox⁺ and Tox⁰ isolates) and 4 subgroups: i) a large and compact subgroup containing all the Tox⁺ *L. maculans* “brassicae” isolates including all Portuguese isolates; ii) the unique “*Lepidium*” isolate; iii) a relatively heterogeneous subgroup with the Tox⁰ NA2 *L. biglobosa* “canadensis” isolates; and iv) a dispersed subgroup with the other Tox⁰ NA1 and NA3 *L. biglobosa* isolates and NA2 *L. biglobosa* “*erysimii*” isolate. There is low similarity between these three isolates. The Portuguese and foreign Tox⁺ *L. maculans* “brassicae” isolates could be further divided into phenetic groupings/clusters. These groupings did not corresponded to their pathogenicity groups revealed by plant differentials. The Portuguese *L. maculans* population do not seem to be different to that occurring in other countries (Australia, France, Germany, UK) which confirms that there is a large-scale mixing in *L. maculans* complex.



BRASSICA2012

Session G

PESTS AND DISEASES

Poster presentation

Poster G-1

EVALUATION OF SICILIAN WILD AND CULTIVATED BRASSICA GENOTYPES FOR RESISTANCE TO *XANTHOMONAS CAMPESTRIS* PV. *CAMPESTRIS*

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Disease resistance to races 1 and 4 of *Xanthomonas campestris* pv. *campestris* (*Xcc*) is very rare in *Brassica oleracea* whereas both race-specific and broad spectrum quantitative resistance have been found in other species of the genus *Brassica*. Observation of wild and cultivated *Brassica* genotypes in Sicily under natural occurring inoculum and previous screenings of local accessions suggested a high variability in *Xcc* resistance of the local germplasm. A subset of 38 *Brassica* genotypes of nine species in the collection of DISPA were therefore screened for resistance to *Xcc* using a race 4 bacterial isolate (the only race identified to date in Sicily). Leaves were inoculated by clipping secondary veins with a mouse-tooth forceps wrapped in cotton wool dipped in a 10^8 cfu ml⁻¹ bacterial suspension. Approximately 8 to 10 points of inoculations were made per leaf and the three youngest leaves in each plant were inoculated. The percentage of successful inoculation points and the severity of the symptoms assessed on a 0 to 3 scale based on the relative size of each lesion were recorded one and two weeks after inoculation. None of the species except for *B. napus* was resistant to *Xcc*. Four genotypes of other species showed a mean disease index <1, with symptoms developing from 48 to 69 % of the inoculation sites. Most of the remaining genotypes showed mean disease indexes between 1 and 2 and nine of them above 2 with up to 100% positive inoculations. The glucosinolate (GLS) content in inoculated and un-inoculated control plants of seven species was evaluated two weeks after inoculation. Specific GLS profiles were detected for the *Brassica* species and significant lower total GLs contents were detected in inoculated leaves in comparison to that un-inoculated. No correlation was observed between the GLS content and the disease resistance.

Poster G-2

FEEDING PREFERENCE AND DEVELOPMENT OF THE CABBAGE MOTH, *MAMESTRA BRASSICAE* (LEPIDOPTERA: NOCTUIDAE), ON CABBAGE VARIETIES

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The objective of this study was to investigate the plant defense mechanisms on cabbages, expressed as antixenosis or antibiosis, to the most abundant pest on Brassica crops in NW Spain: the cabbage moth (*Mamestra brassicae* [L.]). Six cabbage varieties were evaluated on the basis of differing resistance levels under previous artificial infestations with *M. brassicae*. No-choice-feeding and choice-feeding bioassays were performed by using leaf discs from plants at two different development stages, pre-heading (PH) and head formation (HF). Larval preference, leaf feeding, larval survival, larval development, pupal weight and sex ratio were recorded. At the same time, no-choice-feeding and choice-feeding tests were performed in field experiments to investigate the adult preference, leaf feeding and survival and larval development after plant infestations.

The plant development stage significantly affected the performance of varieties in both bioassays. In the choice-feeding bioassay, varieties BRS0409 and BRS0452 (both previously identified as susceptible) were the preferred by larvae at PH while BRS0525 and BRS0535 (both previously identified as resistant) were chosen at HF. This last variety was the most resistant in no-choice-feeding tests at PH, showing a high percentage of larval mortality (88%) and the slowest larval development. However, this high resistance decreased drastically as the plant develops and forms the head.

Antixenosis effect was not found in adult plants and under artificial infestation conditions, BRS0525 and BRS0535 confirmed their resistance in terms of percentage of mortality and larval development (larval weight and pupal time) as well as the percentage of leaf and head area consumed. Further studies are required to identify the mechanism of antibiotic resistance present in BRS0535 and the changes occurred in plant defense in this variety as it develops.

Poster G-3

STUDY OF INDUCED SYSTEMIC RESISTANCE IN CABBAGE AGAINST *XANTHOMONAS CAMPESTRIS* PV. *CAMPESTRIS* FOLLOWING BIOLOGICAL AND CHEMICAL SEED TREATMENT.

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Black rot caused by the seed borne pathogen *Xanthomonas campestris* pv. *campestris* (*Xcc*) is a devastating disease of Brassicas worldwide. The pathogen infects and multiplies in plant vascular tissues and, as the disease progresses, the veins of infected tissues turn black and characteristic V-shaped lesions appear along the margins of leaves. The control of black rot is difficult and usually attempted by growing disease-free seeds and adopting cultural practices that limit the dissemination of the pathogen. Chemical and bacterial/fungal seed priming have proven beneficial in fighting plant pathogens. In this study *Xcc*-infected cabbage seeds were treated with an in-house developed bacterial bio-control agent (BCA) and plant hormones. These seeds were grown in a growth chamber and the reduction of disease incidence and severity in the seedlings were recorded. Quantitative RT-PCR analysis of genes reported to be associated with induced systemic resistance (ISR) was carried out with the leaf samples. The seed treatment bioassays showed a reduction in disease incidence and severity. RT-qPCR results indicate a positive correlation of some genes to the bio-assay results. Our results show that treatment of cabbage seeds with priming agents enhances the resistance response of cabbage to *Xcc*.

Poster G-4

THE INHERTANCE OF BROWN HEART SUSCEPTIBILITY IN SWEDE (*BRASSICA NABOBRASSICA*)

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Five F1 hybrids were produced from inbred lines of Swede (*Brassica nabobrassica*) and assessed in field trial for susceptibility to Brown Heart disorder under different levels of Boron (0, 1400, 1800 and 2700) gh^{-1} . The trial was conducted on grower's fields in Cornwall / south western England.

All genotypes were significantly responded to Boron application and showed different ability to resist Brown Heart disorder. Increased level of boron completely overcame the problem in all genotypes except two of inbred lines and one F1 hybrid. Parent lines Ela and Ms.ela and the hybrid (Ms.ela \times Nor) were considered as very susceptible to brown heart disorder by showing the disorder symptoms at the higher level of boron where root discoloration gradate from sever browning at (0 gh^{-1}) Boron applied to slight discoloration at (2700 gh^{-1}) boron. Parent lines like (Res, Ms. res, Zzy and Nor) showed high ability to resist boron deficiency and showed no brown heart at all.

F1 hybrids appeared to be affected by their parent lines, wherever the genetic background of the brown heart resistance lines (Res, Ms. res, Zzy and Nor) were included in F1 genotypes high resistance to field brown heart was observed even though any of the susceptible parent lines was included in that genetic combination and wherever the susceptible lines appeared together in the genetic combination of F1 hybrids a high susceptibility to field brown heart was observed.

It is concluded that resistance to brown heart disorder is a dominant trait and the genotype for this trait is (BHBH or BHbh) while the susceptibility considered as a recessive trait (bhbh). Boron application may not help with high susceptible genotypes.

Poster G-5

NEW DISEASE OF WILD AND CULTIVATED ROCKET IN ITALY

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During the past years several diseases caused by soil-borne and foliar pathogens were observed for the first time in Italy on wild (*Diplotaxis tenuifolia*) and cultivated (*Eruca sativa*) rocket grown in intensive cropping system for fresh cut production. This sector is particularly exposed to the risk of the emergence of new diseases as a consequence of its dynamism, specialization and use of intensive cultivation techniques. Fusarium wilts have been observed in Italy on wild and cultivated rocket. Among soil-borne pathogens, attacks of *Rhizoctonia solani*, and *Pythium* spp. on rocket, were increasingly observed. Among foliar diseases, downy mildew of wild rocket and a leaf spot of wild and cultivated rocket caused by *Alternaria japonica* were recently observed as new foliar diseases on these crops. A new leaf spots caused by *Fusarium equiseti* was observed for the first time in the world on *Eruca sativa*. During spring 2012, symptoms of an unusual leaf spot disease caused by *Plectosphaerella cucumerina* were observed in several commercial greenhouses near Salerno (southern Italy) on plants of *D. tenuifolia*. Many of these diseases are observed for the first time in the world. These diseases emerged as major production problems in protected crops, where every year repeated cropping is carried out in the same soil. The biology, epidemiology, physiological characteristics of the causal agents are reported.

Poster G-6

OPTIMAL TIME OF FUNGICIDE APPLICATION OF OILSEED RAPE AGAINST SCLEROTINIA STEM ROT

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Sclerotinia stem rot caused by *Sclerotinia sclerotiorum* is a major pathogen of numerous crops, including oilseed rape, causing substantial decrease of seed yield. Airborne ascospores of this fungus usually infect plants via petals - good sources of nutrition, and are later deposited on leaves or stems. The aim of this study was to determine the effect of fungicide application in different times during flowering stage of oilseed rape and to compare the results of petal contamination with the subsequent infection of plants by sclerotinia stem rot.

The experiments were done in two seasons: 2010/2011 and 2011/2012 in Glubczyce (southern Poland). Fungicide treatments were done at weekly intervals from late April to mid-June, with Acanto 250 SC containing 250 g/L of picoxystrobin. Each season the petals were chosen randomly from an untreated variant, 6-7 times during the flowering stage. Scoring of fungal colonies was done based on media discoloration followed by sclerotia formation. The sclerotinia stem rot symptoms were evaluated before harvest. Each experiment variant was also evaluated according to the following characters: seed yield at 90% of dry matter content, mass of thousand seeds, the percentage of oil in seeds, the content of proteins, glucosinolates as well as acid and neutral detergent fibers.

The highest disease incidence was observed in the unsprayed variant. Application of fungicide reduced disease incidence. The percentage of infected plants depended on the time of fungicide application. The comparison of the availability of primary inoculum in the air and the subsequent plant infection have demonstrated that the infection of plants was much smaller than the infection of petals, however the correlation between these traits was highly significant. It suggests that the petal test supplied with weather forecast may serve as a good tool for predicting the incidence and severity of Sclerotinia stem rot.

Poster G-7

RECEPTOR KINASE-MEDIATED REGULATION OF SEED SIZE IN *ARABIDOPSIS THALIANA*

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Seed formation is the key event in the life cycle of flowering plants. Proper development of the seed is achieved through coordinated growth of its three components: embryo, endosperm and maternal integuments (seed coat). Recent genetic studies have revealed that the coordinated growth of these components is mediated by intercellular cell-cell communication. This mechanism is thought to involve leucine rich repeat receptor like kinases (LRR LRK) and small secreted peptides acting as ligands. However, the regulation of these processes remain largely unknown. In *A.thaliana*, *IKU2* encodes a LRR RLK and is a key regulator of endosperm development and seed size. Results of this study revealed that *IKU2* interacts genetically with *ERECTA* LRR RLK to regulate seed size. This finding has a particular relevance to understanding seed biology not only in *A.thaliana* but also in agronomically valuable *Brassica* crop species.

Poster G-8

OCCURRENCE OF BLACKLEG ON RAPESEED IN CHINA

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Blackleg is one of the most damaging diseases of rapeseed world-wide. The disease pathogen populations comprise two main species, aggressive *Leptosphaeria maculans*, and less aggressive *L. biglobosa*. *L. biglobosa* was first identified on both winter rapeseed in provinces of Anhui, Hubai and Guizhou, China in 2000. Does *L. maculans* appear in China?

Surveys for blackleg on rapeseed have been conducted since 2006 regionally in both winter rapeseed (*Brassica napus*) growing areas along the Yangtze River and in spring rapeseed (*Brassica napus*) growing areas in northern China. Rapeseed debris with blackleg symptoms was collected and the causal fungi were isolated from infected lesions. Cultural characteristics and PCR detection methods were used for identification of blackleg pathogen.

The results showed: Blackleg Occurrence: Blackleg infected plants were found at 41 cities and counties of 13 provinces (Anhui, Jiangsu, Jiangxi, Hunan, Hubai, Zhejiang, Shanghai, Henan, Sichan, Guizhou, Qinghai, Gansu and Inner Mongolia) in China Disease incidence were at low levels in most fields up to 95% in some fields.

Disease symptoms: Most plants were with upper stem lesions before harvest, few plants with leaf lesions were found.

Cultural characteristics: Isolates from germinating conidia tended to exhibit more rapid growth in vitro and germ tube extension, produce pigments in liquid culture. In contrast, isolates described as aggressive *L. maculans* are generally characterized by relatively slow germ tube extension, slow growth in vitro, the absence of water-soluble pigments in liquid culture.

PCR detection: Only *L. biglobosa* was detected from these samples and aggressive *L. maculans* was not found.

Poster G-9

INVESTIGATIONS ON THE RESISTANCE OF SOME PROCESSING WHITE HEAD CABBAGE (*BRASSICA OLERACEA* L. VAR. *CAPITATA* SUBVAR. *ALBA*), VARIETIES TO ROOT-KNOT NEMATODES [*MELOIDOGYNE INCOGNITA* (KOFOID & WHITE) CHITWOOD]

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This study was conducted during 2010–2011 as a field study in the field of Ondokuz Mayıs University, Agricultural Faculty, Horticulture Department and as a pot study in the acclimatized room under controlled conditions at Plant Protection Department. The main materials of experiment were *Meloidogyne incognita* (Kofoid & White) Chitwood infected soil and totally 27 white head cabbage (*Brassica oleracea* L. var. *capitata* subvar. *alba*), of which 4 were resistant and 23 was susceptible to root-knot nematodes. Roots gall were investigated according to 0–5 scale. In 2010 field experiment, 530-1 (2.2500), P33 (2.000), 173 (2.2500) and 183 (2,7500) cabbage lines have the lowest gall index. On the other hand, there was the highest observed in the roots of 195, 166, 542,115, 165, 148, 519, Yalova-1 and Bafra (5.000) lines. The results provide information on the resistant root-knot nematodes and breeding study of Turkish white head cabbage lines.

Poster G-10

EFFECT OF FLOODING ON THE SURVIVAL OF *LEPTOSPHAERIA* SPP. IN CANOLA STUBBLE

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Blackleg, caused by *Leptosphaeria maculans* (Desmaz.) Ces. & De Not, is an important disease of canola and rapeseed (*Brassica napus* L.) in many countries, including Canada. However, *L. maculans* has not been reported in China, where blackleg is caused by a less aggressive pathogen, *L. biglobosa* R.A. Shoemaker & H. Brun. Strategies that mitigate the risk of blackleg epidemics may help alleviate China's concern about accidental introductions of *L. maculans*. In China, winter rapeseed is the main oilseed crop followed often by a paddy rice or cotton. Paddy rice fields normally are flooded for weeks during late spring and summer. This study was conducted to assess the effect of flooding temperature (12–40°C) and duration (2–12 wks) on survival of *Leptosphaeria* spp. in canola stubble. Pieces of infested stubble were submerged in water in small glass jars containing 20 cc soil on a thermogradient plate capable of maintaining up to 96 independent temperature regimes simultaneously. Flooded stubble pieces were sampled every 2 wks, surface sterilized, and incubated on V8-juice agar for 10 d to recover the pathogen. Flooding for 2 wks reduced the pathogen recovery substantially relative to non-flooded controls, irrespective of temperature and no pathogen was recovered after 6 wks of flooding. The pathogen was eliminated more rapidly at flooding temperatures >20°C compared to 12–16°C. There was no difference between *L. maculans* and *L. biglobosa* in ability to survive flooding. Stem tissues degraded rapidly during the first 2 wks of flooding, corresponding to a rapid decline in pathogen survival in the same interval. These results indicate that a paddy rice crop following winter rapeseed should minimize the impact of blackleg by eradicating the inoculum of *Leptosphaeria* spp. in stubble.

Poster G-11

FINE MAPPING OF RACE-SPECIFIC BLACKLEG RESISTANCE GENES IN *BRASSICA NAPUS* L.

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Blackleg, caused by *Leptosphaeria maculans*, is one of the most devastating diseases in *B. napus* production in Australia, Canada and Europe. Development of Blackleg resistant canola varieties is the most effective and sustainable approach to control this disease. There are two types of resistance to *L. maculans* in *Brassica* species. The first is qualitative, single race-specific resistance, which depends on the presence of a resistance (*R*) gene in the canola and a corresponding avirulence (*Avr*) gene in the pathogen. Qualitative resistance provides complete resistance but is easy to break down. The second type of resistance is quantitative resistance, which is controlled by non race-specific genes of small phenotypic effect. Quantitative resistance is usually considered the most durable resistance, due to its effectiveness against a broad range of pathogenic isolates and lower selective pressure on the pathogen leading to a breakdown of resistance. However, quantitative resistance genes do not confer such effective protection as race-specific *R* genes. Pyramiding multiple race-specific resistance genes with non race-specific, quantitative resistance provides an effective and durable long-term approach to control the disease in *B. napus*. Although 14 race-specific genes and some quantitative trait loci (QTL) have been identified and mapped in different *Brassica* species in the past decades, high-throughput SNP markers tightly linked to the resistance genes have not been developed, and are not routinely used for marker-assisted selection (MAS) of the Blackleg resistance. Here, we leveraged a high-density consensus map developed at Dow AgroSciences, fine mapped a few race-specific resistance genes, and developed SNP markers for MAS of these resistance genes in *B. napus*.



BRASSICA2012

Session H

CLUBROOT

Oral presentation

Session H

MOLECULAR CLONING OF *CRR1A*, A GENE FOR RESISTANCE TO CLUBROOT DISEASE (*PLASMODIOPHORA BRASSICAE* WORONIN) IN *BRASSICA RAPA* L.

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Clubroot disease, caused by the obligate biotrophic protist *Plasmodiophora brassicae* Woronin, is one of the most economically important diseases of *Brassica* crops in the world. Although many clubroot resistance (CR) loci have been identified through genetic analysis and QTL mapping, none of these have been cloned in any *Brassica* species or *Arabidopsis*, and molecular mechanisms underlying *P. brassicae*–host interactions remain unknown. Here we report the map-based cloning of *Crr1a*, a gene conferring resistance to clubroot in *Brassica rapa*. *Crr1a*^{G004} encoded a Toll-Interleukin-1 receptor / nucleotide-binding site / leucine-rich repeat (TIR-NBS-LRR) protein expressed mainly in the stele or cortex of hypocotyl and roots, in leaves, and in apical meristem, but not in root hairs. Compared with the resistant allele *Crr1a*^{G004}, the susceptible allele *Crr1a*^{A9709} appeared to encode a truncated NBS-LRR protein lacking more than half of the TIR domain. Transgenic *Arabidopsis* harboring *Crr1a*^{G004} cDNA showed pathotype-specific resistance to *P. brassicae* isolates similar to that of untransformed *B. rapa* with a homozygous *Crr1* locus. This study provides a basis for further analysis of molecular mechanisms underlying *Brassica*–*P. brassicae* interactions and contributes significantly to breeding of highly resistant cultivars of *Brassica* vegetables.

Session H

DECIPHERING THE FUNCTIONAL TYPOLOGY OF RESISTANCE QTL THROUGH METABOLOMICS

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The use of quantitative partial resistance, alone or in combination with specific resistance genes, seems to be an effective way to get varieties with an improved level of durability. With the assumption that the combination of QTL with different mechanisms or different effects on the pathogen can increase the sustainability of the resistance trait, the mechanisms underlying the involved QTL should be deciphered in order to rationalize their use in plant breeding. To date there is an insufficient understanding of the cellular and molecular mechanisms associated to quantitative resistances, possibly due to the difficulty in cloning QTLs.

Clubroot is caused by the telluric obligate biotrophe *Plasmodiophora brassicae* and is one of the most damaging diseases of cultivated Brassicas worldwide. The use of genetic resistances, particularly quantitative partial resistance, is considered as one of the most efficient control and sustainable strategies. We report here a combination of biochemical and quantitative genetics approaches to elucidate the complexity of cellular responses involved in the quantitative resistance in the clubroot/*Brassicaceae* pathosystem.

A “without *a priori*” strategy was developed, using targeted and untargeted metabolomics to identify metabolic biomarkers associated to the cellular response to clubroot infection, in several genotypes of *Brassica napus* and *Arabidopsis thaliana* harbouring various levels of susceptibility/partial resistance. This highlighted large biochemical similarities between susceptibility and resistance responses, and suggested the role of amino-acid, glutathione, glucosinolate, jasmonate and other still unidentified compounds in the susceptibility and resistance processes.

This metabolomic approach was conducted in infected roots of segregating populations in *B. napus* and *A. thaliana*. Several colocalizations between QTL controlling symptoms and QTL controlling levels of metabolic biomarkers were identified. Resistance QTL were associated to different metabolic fingerprints, allowing i) to build a functional typology of several resistance QTL, and ii) to generate new hypotheses about the mechanisms underlying resistance/susceptibility to clubroot.

Session H

LONG-TERM SOIL DATA SETS REVEAL SHIFTS IN POPULATIONS OF *PLASMODIOPHORA BRASSICAE*

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BioSoM (Biological Soil Mapping) is the thematic research program at the NL-faculty at the Swedish University of Agricultural Sciences, that aims to provide scientific support to a new service for farmers enabling detection of soilborne pathogens. The BioSoM program will improve cropping reliability and will form an essential part to meet the challenge of optimizing crop production in Sweden in conditions that are affected by global processes and climate change. Soilborne plant pathogens are a major limitation in the production of marketable yields and are adapted to grow and survive in the bulk soil, causing root disease. Soilborne pathogens in general include many organism groups. In BioSoM we have an emphasis on fungi and protists (supergroup Rhizaria). Soilborne pathogens survive in the soil by e.g. resting spores, sclerotia or chlamdyspores. The lifetime of resting spores is often long, ranging from 10 to 20 years, and the time of survival depends on soil conditions, crop rotation etc. In one subproject we have analyzed the presence of *Plasmodiophora brassicae*, causing club root in Brassica crops, in archived soil samples from five long term field experiments using quantitative DNA-based detection methods previously developed. The field experiments were started in 1957 with a crop rotation allowing Oilseed Brassica crops every 4th year. Initially white mustard (*Sinapsis alba*) was grown, and was later replaced by summer oilseed rape (*Brassica napus*). The oilseed rape crops were devastated by clubroot at two experimental sites in 2006 and 2010 respectively. DNA has been extracted from soil samples collected in the field experiments, and the presence of *P. brassicae* has been quantified. The pathogen information will be compiled together with data from soil analyses and meteorological data in order to predict incited long-term changes.

Session H

A MOLECULAR BASED SEEDLING ASSAY SYSTEM TO STUDY THE GERMINATION AND PRIMARY INFECTION BY *PLASMODIOPHORA BRASSICAE*.

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The life cycle of *P. brassicae* consists (in part) of a single motile zoospore infecting a root hair cell, followed by replication within host cells before transposition to the root cortical cells where further spore formation occurs. Due to the complex nature of the lifecycle, it has been necessary to consider alternative methods to determine the viability of this organism. If DNA levels are considered at an appropriate stage of the life cycle (during the resting spore or primary zoospore stage) then the level of DNA is directly comparable to the number of spores present as these lifecycle stages have been shown to contain haploid nuclei.

The objectives of this study were to construct a seedling assay system which uses the sensitivity of molecular methods to allow the determination of the number of germinating spores as a measure of viability, so that factors affecting viability can be investigated. Employing quantitative PCR, a seedling assay has been developed that allows the germination of resting spores to be quantified thus giving an indicator of spore viability within two weeks. The potential for monitoring levels of infection resulting from resting spore germination was also investigated in relation to infection of *Brassica rapa* ssp. *Pekinensis* cv. Granaat (Chinese Cabbage). Infection of root tissues by zoospores produced from a resting spore suspension was demonstrated by quantifying the levels of *P. brassicae* DNA over the period corresponding to primary infection. It was found that significantly reduced levels of *P. brassicae* DNA were detectable across a range of resting spore suspension concentrations after incubation with the brassica seedlings, and that *P. brassicae* DNA could be isolated from root tissue after the incubation period which, along with microscopy, confirmed seedling infection.

Session H

CROP ROTATION AND HOST RESISTANCE ARE EFFECTIVE IN CONTROL OF CLUBROOT ON CANOLA WHILE *B. SUBTILIS* BIOFUNGICIDES ARE NOT UNDER FIELD CONDITIONS.

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Two field studies were conducted to assess the effect of crop rotation and cultivar resistance for management of clubroot on canola, together with potentially complementary biofungicide (*Bacillus subtilis*). In the first study, two granular formulations of *B. subtilis* were applied in-furrow at 50 kg/ha on resistant and susceptible commercial cultivars in three field trials near Leduc and Edmonton, Alberta and Normandin, Quebec. In a second study, *B. subtilis* was applied as seed dressing at 1×10^5 to 5×10^6 cfu/seed and evaluated on a susceptible cultivar in plots with a 1, 3, or 11-year break from the previous canola crop in Normandin. Clubroot disease pressure was high in each of the trials, with disease severity indices (DSI) ranging from 69% to 98% on the susceptible cultivar. None of the *B. subtilis* formulations reduced clubroot substantially in either study. In the first study, the resistant cultivar reduced DSI to below 15% and doubled the yield over that of the susceptible cultivar. In the second study, there was a higher level of pathogen inoculum in plots with a 1-yr break from canola than in the other two rotations, based on bioassay and qPCR assessment. Although DSI for all of the rotation treatments were high, approaching 100% in 1-year-break plots, longer crop rotations (3 and 11 years) reduced gall size slightly, showed less above-ground disease impact on canola plants, and yielded significantly more than the 1-year rotation. Even the 3-year break from canola alleviated the impact of clubroot substantially, with seed yield doubled when compared to the 1-year break.

Session H

USE OF GENETICS AND GENOMICS APPROACHES TO FINE MAP THE CLUBROOT RESISTANCE “*CRB*” LOCUS IN *BRASSICA RAPA*

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Clubroot disease, caused by the obligate plant pathogen *Plasmodiophora brassicae* Wor, is one of the most economically important diseases affecting *Brassica* crops worldwide including oilseed *Brassica napus*, vegetable brassicas such as *B. rapa* and *B. oleracea*. The genetic basis of clubroot resistance (CR) has been well studied and mapping of resistance loci have been reported in these species. We earlier reported mapping of clubroot resistance “*CRb*” locus in A3 chromosome of *B. rapa* (Chinese cabbage) using F_{2/3} mapping populations derived from resistance and susceptible parental cross and tightly linked sequence characterized amplified region (SCAR) markers were developed by converting amplified fragment length polymorphism (AFLP) markers. In this study, fine mapping of *CRb* locus was done using 1500 F₂ lines. Combination of genetic mapping using SNP and other gene specific markers, comparative mapping with *Arabidopsis thaliana* to identify potential resistance genes, and whole genome re-sequencing of resistance and susceptible parental lines were used to identify candidate gene causing resistance and susceptible phenotypes. We identified several candidate genes around the *CRb* region and parental nucleotide polymorphism were used to design SNP primers and validated by mapping. Of the 20 genes found to be putative candidate genes, one gene was found co-segregating with resistance phenotype. We developed few SNP markers (*CRbSNPs*) based on parental differences observed in that gene which could be applied for marker assisted breeding of clubroot resistance phenotype in *B. rapa*, and believed that this gene is responsible for causing clubroot resistance. The functional validation through transformation is underway.

Session H

QTL MAPPING OF ISOLATE-SPECIFIC RESISTANCE TO *PLASMODIOPHORA BRASSICAE* IN *BRASSICA RAPA*

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Quantitative trait loci (QTL) for resistance to clubroot caused by *Plasmodiophora brassicae* were studied using four field isolates of *P. brassicae* and 115 BC₁F₁ and their selfing progenies derived from a cross between Chinese cabbage (*B. rapa* spp. *pekenensis*) inbred line '59-1' as a susceptible recurrent parent and turnip (*B. rapa* spp. *rapifera*) 'ECD04' as a resistant donor parent. The BC₁F₂ families were assessed for resistance under controlled conditions with two replications. A genetic linkage map covering 1159.1 cM of *B. rapa* genome was constructed with BC₁F₁ population, and consisted of 254 markers including 100 unigene-derived microsatellite (UGMS), 132 simple sequence repeats (SSR) markers and 4 markers linked to published clubroot resistance (CR) genes. Composite interval mapping identified 8 QTLs which were contributed by ECD04. Among these QTL, 4 QTLs allocated in the same region of previously identified *Crr1*, *Crr2* (two regions) and *Crr3*. Four novel QTLs were mapped on linkage group A3 (*Pb2Ba3.1*, *Pb2Ba3.2*, and *Pb7Ba3.1*) and A7 (*Pb10Ba7.1*), respectively. The phenotypic variation explained by each QTL ranged from 8.3% to 25.2%. None of the QTL was effective to all isolates. Four QTLs were common to resistance for the two isolates of Pb2 and Pb7 on A1 and A7, respectively. Others showed isolate-specific resistance: one to isolate Pb2 on A3, one to Pb4 on A8, and two to Pb10 on A3 and A7. The QTLs resistance to two isolates in common explained 9.2~15.3% of phenotypic variation. The isolate-specific QTLs explained 8.3~25.2% of phenotypic variation. The results indicated a gene-for-gene relationship between *B. rapa* and *P. brassicae* for clubroot resistance.

Session H

FIRST GLANCE AT THE GENOME OF THE CLUB ROOT PATHOGEN *PLASMODIOPHORA BRASSICAE*

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Plasmodiophora brassicae is the casual agent of the clubroot disease of the *Brassicaceae*, one of the most damaging diseases within this plant family. Despite its agricultural importance, the biology of *P. brassicae* remains poorly understood. Due to its obligate biotrophic nature, *P. brassicae* remains impossible to grow in axenic culture and the typical experimental systems for working with *P. brassicae* are comparatively unsophisticated. Molecular studies are especially challenging with only approximately 100 known genes. We recently succeeded obtaining the whole genome sequence from a *P. brassicae* single spore isolate. The first assembly draft showed a total length of the genome sequence of 22.8 Mb, which broadly corresponded with the previously estimation of the genome size of 18–20.3 Mb. In addition to the genome data transcriptome analyses have been started. The exploitation of the genome will greatly advance the knowledge of this pathogen and will shed light into the evolutionary origin of *P. brassicae* and its biology, which will in the long run help to understand and control club root disease. Here we present some first results of the genome sequence analysis.



BRASSICA2012

Session H

CLUBROOT

Poster presentation

Poster H-1

BIOLOGICAL CONTROL OF CLUBROOT (*PLASMODIOPHORA BRASSICAE*) BY AN ENDOPHYTIC FUNGUS

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The clubroot disease caused by *Plasmodiophora brassicae* infects economically important crop species such as canola and causes high yield losses. The disease is difficult to control by chemical and cultural means.

In a previous study *Acremonium alternatum*, a soilborne endopyhtic fungus and known biological control agent in other countries, showed a promising antagonistic effect in clubroot infected plants. The means by which *Acremonium* controls pathogens is not known so far. Presumably the fungus induces resistance mechanisms in the host plant and thus delays the development of the pathogen. We want to test this theory in the model plant *Arabidopsis thaliana* and the economically important Canola (*Brassica napus*). To monitor the development of the infection within plants we use molecular methods as well as phytopathological techniques. The following questions are addressed: (A) How does *Acremonium* induce tolerance / resistance in *Arabidopsis*? (B) In which plant parts can the endophyte be found at different time points after inoculation? (C) Where does the interaction between *A. alternatum* and *P. brassicae* take place? (D) Which plant signals are involved? The long-term goal is to develop an inoculum from *Acremonium* spores or spore parts which can be integrated in pest control as environmentally friendly and lasting method for the reduction of clubroot infections.

Poster H-2

IDENTIFICATION AND MAPPING OF CLUBROOT RESISTANCE GENES

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Clubroot, caused by *Plasmodiophora brassicae* Woronin, is one of the most economically important diseases in *Brassica* species worldwide, and an emerging threat to canola production in western Canada. Cultivar resistance is the cornerstone for effective management of clubroot on canola. A total of more than 1,000 accessions of *Brassica* spp. were evaluated and 34 were identified highly resistant to the pathotype -3 race of *P. brassicae* in *B. rapa*, *B. oleracea*, *B. nigra* and *B. napus*. Selected resistant lines from different *Brassica* species were crossed with respective susceptible doubled haploid (DH) lines and segregating populations (test-cross, F₂ and BC₁) were produced for identification and mapping of clubroot resistance (CR) genes. Parents, F₁, F₂ and BC₁ were evaluated for resistance to *P. brassicae* pathotype 3, and selected parents were further tested against five *P. brassicae* pathotypes (2, 3, 5, 6, and 8, Williams) identified in Canada. DNA samples were analyzed with microsatellite markers. Single dominant resistance genes controlling clubroot disease were found in four of the lines, two in *B. rapa* (pak choy and Chinese cabbage) and two in *B. nigra*. Two resistance genes from the pak choy line FN and Chinese cabbage line JNC, herein designated *Rpb1* and *Rpb2*, respectively, were mapped to different genomic regions on *B. rapa* linkage group A3. Molecular markers closely linked to these CR genes were developed and verified to facilitate marker-assisted breeding. The *Rpb1* and *Rpb2* were highly resistant to all five *P. brassicae* pathotypes identified in Canada. Introgression of these CR genes into canola (*B. napus*, *B. juncea* and *B. rapa*) is in progress.

Poster H-3

CLUBROOT RESISTANCE BREEDING IN OILSEED RAPE

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Clubroot has become a serious threat to oilseed rape cultivation in oilseed rape crops worldwide. Control means are poor and mainly restricted to host resistance. *Plasmodiophora brassicae*, the causal agent of clubroot, forms physiologic races that differ for their virulence towards race-specific host resistance. Breeding for clubroot resistant cultivars requires knowledge of pathotypes present in the cropping area and the efficacy of resistance sources which are considered to be used for cultivar development.

The race-specificity of *Plasmodiophora brassicae* isolates from different areas was investigated in greenhouse tests using a *Brassica napus* differential set that is representing major sources for clubroot resistance. Opposite to expectations, virulent *P. brassicae* isolates towards major clubroot resistance genes originating from *B. rapa* were present in Europe, China and Canada. To monitor for the efficacy of the clubroot resistance from cv. ‘Mendel’ *P. brassicae* isolates from its cropping area were tested in the greenhouse. Besides a number of avirulent isolates certain areas were identified which seem to have a higher frequency of compatible isolates. Based on a limited number of *P. brassicae* populations it was indicated that compatible races were present in these areas already before the resistant cultivar was introduced. New breeding material exhibited a significantly higher level of clubroot resistance; however, highly virulent pathotypes were able to infect also the new breeding material.

Poster H-4

SEARCH FOR GENETIC RESISTANCE TO CLUBROOT IN BRASSICA COLLECTION, INTERSPECIFIC HYBRIDS AND MUTANTS OF *BRASSICA NAPUS*

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Clubroot is regarded as one of the most damaging diseases of oilseed rape worldwide. It is caused by a soil borne obligatory parasite – a protozoa *Plasmodiophora brassicae*. The pathogen is very persistent in soils, it becomes infective even after several years without the host plant. Agrotechnical methods, such as liming, are able to decrease the levels of plant infection, but unable to stop the disease. The most effective method to control clubroot is plant resistance. The aim of this work was to look for genetic resistance to clubroot in 360 accessions from the Polish Genebank Collection of the Institute of Plant Breeding and Acclimatization in Radzikow, including *Brassica oleracea*, *B. rapa*, *B. juncea* and other *Brassica* species. The studies also included interspecific hybrids between *B. napus* and *B. juncea* (20 lines), *B. napus* and *B. rapa* ssp. *pekinensis* (50 lines), and *B. napus* x *B. rapa* ssp. *trilocularis* (50 lines). The resistance to clubroot was also studied using 13 chemical mutants (MNUA) of *B. napus* with increased levels of tocopherols (400-500 ppm), 8 lines with decreased levels of indole glucosinolates (below 10 µM) and 5 semi dwarf lines. The resistance test concentrated on race 1 that prevails in the soils of Poland. Small plantlets (5-day-old) of all genotypes were planted to soils inoculated with single club isolates. The evaluation of resistance was done 8 weeks after planting, using 0-4 scale, where 0 was no disease symptom and 4 was a root transformed to a big club. The mean disease severity varied from 0.188 to 3.875. The mean infection of all interspecific hybrids was 2.287 and 1.998 for the mutants. The differentiation of plant response allowed to pick up the most resistant genotypes that are now being tested for resistance to other race 1 isolates and another races of *P. brassicae*.

Poster H-5

INFECTIOUS POTENTIAL FOR CLUBROOT IN FRENCH OILSEED RAPE FIELDS

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Clubroot caused by the protist, *Plasmodiophora brassicae*, is a disease that causes serious damage to Brassica crops, such as oilseed rape (*Brassica napus* L.). In France, clubroot was first detected in the 80's. It then spread in areas where the production of oilseed rape and cruciferous vegetables were important. Currently, it is feared that the pathogen might expand in the French soil, due to introduction of N-catch cruciferous crop, and especially in the new oilseed rape growing areas. Moreover, an overcoming of the genetic resistance varieties used in field seems to have appeared in recent years. Consequently, it has become necessary to evaluate the status and the progress of this disease in France. We have established a reporting system online to take inventory of the plots infested by the pathogen and to gather information (type of soil, choice of the varieties...) associated with these plots. From this survey, a sampling strategy of infested soil was developed, targeting different cultural and pedoclimatic contexts. The development of a quantitative PCR approach is in progress in our lab in order to determine the infectious potential of French soils. The results will give us a new vision on the clubroot problematic in France.

Poster H-6

INDUCED RESISTANCE IS INVOLVED IN SUPPRESSING CLUBROOT ON CANOLA BY SEVERAL BIOCONTROL AGENTS

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The commercial biofungicides Serenade[®] (*Bacillus subtilis*), Prestop[®] [*Clonostachys rosea* f. *catenulata* (Gilman & Abbott) Schroers, Samuels, Seifert & Gams], and the endophytic fungus *Heteroconium chaetospira* (Grove) M.B. Ellis reduced the severity of clubroot (*Plasmodiophora brassicae* Woronin) on canola (*Brassica napus* L.) by >85% when applied to the soil under controlled conditions. The treated plants were examined for gene expression related to jasmonic acid (JA), ethylene (Et), auxin (IAA), PR protein, and phenylpropanoid (Pp) pathways using qRT-PCR to determine whether induced host-defence responses were involved in biocontrol. In plants treated with Serenade[®] or Prestop[®], genes encoding JA (BnOPR2), Et (BnACO) and Pp (BnOPCL, BnCCR) were up-regulated while genes encoding IAA and PR proteins were down-regulated in roots relative to non-treated controls. The same pattern was also observed in leaves, but only the increase in Pp gene expression was significant. Plants treated with *H. chaetospira* showed higher transcript levels for Et, JA, IAA, PR-2 and Pp in roots, but the expression of Pp genes in leaves was insignificant based on a microarray study. These results indicate that induced host resistance is possibly involved in clubroot suppression by these biocontrol agents via the modulation of JA, Et and Pp pathways in root tissues. The up-regulation of Pp pathways in leaves by Serenade[®] or Prestop[®] implies that the acquired resistant response is systemic. The Pp pathways influence the production of several host-defense secondary metabolites including phenolics, salicylates and flavonoid phytoalexins.

Poster H-7

THE EFFECT OF MICRONUTRIENTS ON THE GERMINATION AND INFECTION OF *PLASMODIOPHORA BRASSICAE*.

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Within soil there are many factors such as nutrient levels that can interact, having a combined influence on the clubroot resting spores in the soil. This makes it difficult to determine the individual significance of each factor on the organism. In a controlled system such as that observed in a seedling assay, the detection of *P. brassicae* by molecular methods means that it is possible to assess the effect of factors both individually and in combination. There are limitations to seedling assays however the effects of Copper, Boron, Zinc, Molybdenum and Manganese on *P. brassicae* germination and primary infection were studied using this system.

The presence of copper decreased the level of root tissue infection and the concentration of copper was significant to the rate of decrease in infection. Similarly boron was also found to decrease the level of root infection, and again the concentration of boron was also significant. The presence of zinc was not found to have a significant effect at 0.5mEq L⁻¹ but at the higher concentration of 1mEq L⁻¹ there was a significant effect on the level of root tissue infection. The presence of all of the nutrients tested increased the rate of spore germination except for molybdenum at 0.5mEq L⁻¹. The level of treatment applied was only significant to root infection for boron and copper while the remaining micro nutrients applied at different levels were not significant on infection following resting spore germination.

Poster H-8

OCCURRENCE OF *PLASMODIOPHORA BRASSICAE* IN FINNISH TURNIP RAPE AND OILSEED RAPE FIELDS

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Clubroot, caused by *Plasmodiophora brassicae* Woronin, is a serious plant disease of cruciferous plants. A field survey of occurrence of clubroot in oilseed fields was conducted in Finland in 2007–2009 and in 1984–1989. At present, the disease is distributed throughout the oilseed cultivation area. Clubroot was found on average from 30% of fields, but its severity was low; fields with high numbers of infected plants and plants with severe symptoms were rare. According to the survey, cultivation frequency of cruciferous plants is the most important factor affecting clubroot occurrence and severity. Clubroot was found in soils with a wide range of pH-values (pH 5-7.6), but symptoms were most severe at low pH. According to the survey, and greenhouse and field trials, high temperature and moisture during the early growth period seem to favour disease development and can cause significant yield losses. In a survival trial, clubroot declined to close to zero after four years in the absence of host plants, but traces of the pathogen were still detectable after a 19-year trial period, making eradication of the pathogen very difficult. Due to long survival of the pathogen and its distribution to whole cultivation area, clubroot is an important limiting factor in increasing the cultivation area of oilseed crops in Finland.

Poster H-9

CHARACTERIZATION OF THE RPB1 GENE, MEDIATING RESISTANCE TO PLASMODIOPHORA BRASSICAE IN ARABIDOPSIS THALIANA

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Clubroot disease of *Arabidopsis thaliana* and other crucifers is caused by *Plasmodiophora brassicae*, a plant-pathogenic, obligate biotrophic protist. A hypersensitive response-like, isolate-specific resistance reaction can be observed in the *A. thaliana* ecotypes Tsu-0, RLD, Ze-0 and Ta-0. This resistance to *P. brassicae* is inherited monogenically and dominantly. It is suppressed in a *sgt1a* mutant background. We have identified and cloned *RPb1* gene (resistance to *Plasmodiophora brassicae*) and have shown expression by RACE. Transgenic expression of *RPb1* under the control of its native promoter in the susceptible ecotypes Col-0 or Cvi-0 confers resistance to *P. brassicae*. Two functional copies of *RPb1* (*RPb1a* and *RPb1b*), which encode identical amino acid sequences, have been found in the resistant ecotypes Tsu-0, Ze-0 and Ta-0, and only one copy (*RPb1a*) in accession RLD. The *RPb1* gene encodes a small protein with three predicted trans-membrane domains and no other known conserved domains. Transcriptome analysis of *P. brassicae* infected roots of the resistant ecotype Tsu-0 pinpoint to jasmonic acid dependent defense responses due to down-regulation of *JAZ* genes and induced expression of *PDF1.2* and *PDF1.2b*.

Poster H-10

THE NEW APPROACH OF CLUBROOT CONTROL (*PLASMODIOPHORA BRASSICAE*) USING PLANTS EXTRACTS AND CATCH CROPS

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Clubroot, caused by the obligate biotroph *Plasmodiophora brassicae*, is one of the economically most important diseases of *Brassica* crops in the world. In the last two years the most economic important is epidemically occurrence of clubroot on major oilseed rape growing areas in monoculture technology in Poland, similarly like in the Canadian canola crop. Presently at the Horticulture Institute at Skierniewice we are continuing the several experiments concerning of screening some natural products as plant extract from berry fruits (strawberry) SFET/08 AOD® and herbs such as: ST1 Ernia ® to compare with standard chemical product fluazynam (Altima 500 SC®).and calcium cyanamide. In the recent field trials the most effective in clubroot control indicated some plant extract SFET/08 AOD than standard chemical fungicide fluazynam. It was found that herbs plant extract Ernia induced natural resistance to *P. brassicae* in cabbage plants, but their effectiveness is very low and not repeatable from test to test. The new plants extract compounds creating the new possibility of clubroot control. Also many others agrrotechnical methods is developing for effective control of clubroot such as using brassicae catch crops (white mustard).Also the soil samples from infested by *P. brassicae* field from Poland are evaluated to check for the occurrence of a new pathotypes in infested oilseed rape crops following greenhouse biological test using differential ECD host set and P.H. Williams set to compare with PCR method. Finally we would like to summarize of map positions of infested fields and races specifications of *P. brassicae* in Poland using conventional and molecular technique.

In 2012, the study also included the "Catch crops" in order to reduce the degree of settlement and the threshold of soil by *P. brassicae*. The aim of this study is to develop realistic and effective methods of reducing the population of *P. brassicae* in infected soils mainly on plantations of large-scale rape in Poland. The study included, among others: Brassicas - host susceptible and resistant to *P. brassicae*: white mustard, winter rape resistant to *P. brassicae*, radish, cabbage and others. In preliminary studies it was found pot in the greenhouse cultivation of single gripper: resistant varieties of winter oilseed rape and white mustard for a period of 28 days in greenhouse conditions eliminated the presence of *P. brassicae* from previously contaminated soils. This was confirmed by biological tests. The satisfactory association of such strategies with reductions of resting spore populations in soil has not been achieved. Yamagishi et al (1986).

Poster H-11

TESTING OF RESISTANCE TO CLUBROOT OF INTERSPECIFIC HYBRIDS WITHIN THE GENUS *BRASSICA* USING HYDROPONIC CULTURES

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The genus *Brassica* is strongly attacked by clubroot, caused by *Plasmodiophora brassicae*, the pathogen belonging to protists. The disease symptoms are present on vegetable brassicas, as well as on agricultural crops, such as oilseed rape (*B. napus*). The disease is soil borne. The primary inoculum are flagellate spores transported by ground water. The spores penetrate plants through the root system, entering by injuries and root hairs. The test was done using 8 interspecific hybrids of the genus *Brassica* and 3 cultivars of *B. napus*: 1) *B. oleracea acephala* x *B. taurica*, 2) *B. napus* Yellow seed x *Eruca sativa*, 3) *B. taurica* x *B. cretica*, 4) *B. taurica* x *B. napus*, 5) *B. napus* (CMS ogura) x *B. campestris*, 6) *B. napus* x *B. juncea*) x *B. rapa*, 7) *B. napus* x *B. campestris*, 8) *B. oleracea acephala* x *B. napus*, 9) *B. napus* cv. Tosca, 10) *B. napus* cv. Mendel, 11) *B. napus* cv. Alister. Tests were done using two aggressive, single club isolates of *P. brassicae*, originating from Poland. The most resistant hybrids were *B. oleracea acephala* x *B. taurica* and *B. napus* Yellow seed x *Eruca sativa*. The most susceptible was the hybrid *B. taurica* x *B. cretica*. In case of limited plant material or the lack of seeds, hydroponic cultures can be successfully used for screening of plant resistance to clubroot.

Poster H-12

GENETIC VARIABILITY AMONG *PLASMODIOPHORA BRASSICAE* COLLECTIONS FROM DIFFERENT REGIONS IN GERMANY

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Clubroot disease, caused by the soil-borne, obligate plant pathogen *Plasmodiophora brassicae*, is an economically important disease of cruciferous crops including oilseed rape. Chemical control of the pathogen is not possible at present and cultural practices can only limit the infestation with *P. brassicae*. Therefore the development of resistant cultivars is considered the most economical and efficient method for clubroot control.

Different field isolates of *P. brassicae* could not be distinguished by phenotype except virulence pattern. Therefore pathogenicity-based classifications are used to differentiate field isolates. These bioassays are time and space consuming, and subject to varying environmental conditions. Molecular markers specific to isolates or pathotypes would be an efficient tool to identify *P. brassicae* field isolates.

The objectives of the current research were to develop a molecular approach to characterize *P. brassicae* populations concerning the genetic variability and genomic polymorphism directly related to pathotype classification. Amplified fragment length polymorphisms (AFLP) were detected within and between field isolates from regions in Germany with different oilseed rape history.

Breeding of clubroot-resistant plants requires an understanding of pathogen diversity and the variation of pathogenicity in *P. brassicae* populations. Molecular markers specific to *P. brassicae* isolates may be an important tool in breeding strategies to develop durable clubroot resistance in oilseed rape.

Poster H-13

IN-FIELD DISTRIBUTION OF *PLASMODIOPHORA BRASSICAE* MEASURED USING QUANTITATIVE REAL-TIME PCR AND THE INFLUENCE OF SOIL PHYSIOCHEMICAL PARAMETERS ON DISEASE DEVELOPMENT

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Clubroot in Brassica crops, caused by *Plasmodiophora brassicae* Woronin, is recognised as a serious soil-borne disease, associated with appreciable yield losses, and is considered one of the most economically important diseases of cruciferous crops. The disease is found world-wide throughout the growing areas of oilseed rape and vegetable Brassicas, with the reported outbreak of clubroot in parts of the rape-growing districts of western Canada of particular concern. In Sweden, outbreaks of clubroot in recent years have been reported to be more frequent in winter oilseed rape districts, and severe attacks have once again been reported from spring oilseed rape districts where severe outbreaks of the disease occurred 25 years ago. A protocol using real-time PCR for the direct detection and quantification of *P. brassicae* in soil samples previously developed, was used to determine the spatial distribution in a 25 ha field of spring oilseed rape (*Brassica napus*) sampled after harvest in central Sweden 2010.

The results show an overall spatial trend in the variation of the amount of *P. brassicae* DNA detected, with the lowest amounts of *P. brassicae* detected in the eastern part of the field, where also the pH values determined were on a higher level compared to that of the sampling points in the western part of the field. A correlation was found between pH value and amount of *P. Brassicae* DNA. The amount of *P. brassicae* DNA detected ranged from 225 to 33098 fg plasmid DNA g⁻¹ soil, that is of a considerably higher magnitude than previously determined in fields of winter oilseed rape. Analyses of soil type and other soil physiochemical parameters are underway. Continuous sampling in the following years will enable determining a measure of rate of decline of *P. brassicae* DNA.

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