



# 3<sup>rd</sup> Sulphyton Meeting

Campus of Conegliano

University of Padova



29 September – 1 October 2011

Organized by the Department of Agricultural Biotechnology





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Stanislav Kopriva, John Innes Centre, Norwich, U.K.  
Agnieszka Sirko, Institute of Biochemistry and Biophysics, Warsaw, Poland

**We would like to dedicate this meeting to**

**Our good friends from Japan whose valuable contribution to the plant sulphur research is unanimously recognized. Japan has been shocked by an immense tragedy, and we want to express to all our friends our sympathy**



## CONTENT

|                                | <b>Page</b> |
|--------------------------------|-------------|
| PROGRAMME:                     | 7           |
| INVITED LECTURE:               | II          |
| ABSTRACT ORAL PRESENTATIONS:   |             |
| SESSION I                      | 17          |
| SESSION II                     | 25          |
| SESSION III                    | 33          |
| INVITED LECTURE:               | 39          |
| ABSTRACT ORAL PRESENTATIONS:   |             |
| SESSION IV                     | 43          |
| SESSION V                      | 49          |
| SESSION VI                     | 55          |
| POSTER PRESENTATIONS:          | 61          |
| ABSTRACT POSTER PRESENTATIONS: | 65          |
| LIST OF PARTECIPANTS:          | 79          |



# PROGRAMME

## Thursday, September 29, 2011

- 12.00 – 15.00 Welcome and registration  
15.00 – 15.10 Opening of the 3<sup>rd</sup> Sulphyton meeting – *Mario Malagoli, Padova, Italy*  
15.10 – 16.00 **Invited lecture:** Thiols and the cell – a complex biochemical interplay.  
*Alfonso Pompella, Pisa, Italy*

### ***Session I - Sulfur compounds: crucial players in the plant stress tolerance.***

*(chair: Agnieszka Sirko, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland)*

- 16.00 – 16.30 The role of sulfur-containing secondary metabolites in resistance to pathogens and pests. *Felix Mauch, Fribourg, Switzerland*  
16.30 – 16.50 Compartment specific importance of ascorbate and glutathione during abiotic stress. *Bernd Zechmann, Graz, Austria*  
16.50 – 17.10 Coffee break  
17.10 – 17.30 Impact of drought stress on the sulfur assimilation pathway in *Zea mays*.  
*Nisar Ahmad, Heidelberg, Germany*  
17.30 – 17.50 S-containing metabolites might be important for the detoxification of mercury in plants. *Luis E. Hernandez, Madrid, Spain*  
17.50 – 18.10 Involvement of the gamma glutamyl cycle in the plant cell redox homeostasis.  
*Antonio Masi, Padova, Italy*  
18.30 – 23.00 Visit to the Collalto wine cellar and wine tasting.

## Friday, September 30, 2011

### ***Session II – Sulfur transport, assimilation and signalling in plants.***

*(chair: Malcom Hawkesford, Rothamsted Research, Harpenden, UK)*

- 9.00 – 9.20 The phylogeny of the plant sulfate transporter gene family: an update. *Peter Buchner, Harpenden, UK*
- 9.20 – 9.40 F-box protein, EBF2 is a component of the sulfur deficit signaling pathway in Arabidopsis. *Anna Wawrzynska, Warsaw, Poland*
- 9.40 – 10.00 Interplay of SLIM1 and miR395 in the regulation of sulfate assimilation in Arabidopsis. *Colette Matthewman, Norwich, UK*
- 10.00 – 10.20 A transcriptomic approach for the identification of genes involved in the response to chromium and sulphur in *Brassica juncea*. *Michela Schiavon, Padova, Italy*
- 10.20 – 10.40 Characterization of the chloroplastidic PAPS transporter in Arabidopsis thaliana. *Tamara Gigolashvili, Cologne, Germany*
- 10.40 – 11.00 Coffee break

### ***Session III – The sulphur metabolism regulation.***

*(chair: Stanislav Kopriva, Norwich Research Park, Norwich, UK)*

- 11.00 – 11.30 Adenosine 5'-phosphate reductase (APR2) mutation in Arabidopsis implicates glutathione depletion in selenate toxicity. *Doug Van Hoewyk, Conway, South Carolina, USA*
- 11.30 – 11.50 Adenosine 5'-phosphosulfate reductase, is regulated by HY5 in Arabidopsis. *Anna Koprivova, Norwich, UK*
- 11.50 – 12.10 Investigations of sulfite reductase in *Nicotiana tabacum*. *Hans Michael Hubberten, Potsdam-Golm, Germany*
- 12.10 – 12.30 Reprogramming of sulphur metabolism in *Lotus japonicus* during symbiotic nitrogen fixation. *Chrysanthi Kalloniati, Athens, Greece*
- 12.30 – 14.30 Lunch and Poster viewing

14.30 – 15.20 **Invited lecture:** Occurrence and impact of sulphur compounds in wine.  
*Doris Rauhut, Geisenheim, Germany*

***Session IV – The role of sulfite oxidase in plants.***

*(chair: Luit DeKok, Laboratory of Plant Physiology, University of Groningen, Groningen, The Netherlands)*

15.20 – 15.50 Sulfite oxidase is an essential component in the sulfite homeostasis network uncovered in tomato plants exposed to stress. *Moshe Sagi, Sede Boqer Campus, Israel*

15.50 – 16.10 Sulfite detoxification mechanisms in response to SO<sub>2</sub>-fumigation in *Arabidopsis thaliana*. *Domenica Hamisch, Braunschweig, Germany*

16.10 – 16.30 Coffee break

16.30 – 16.50 Sulfur metabolism at different concentrations of SO<sub>2</sub> in *Populus tremula* x *P. alba*. *Dörte Randewig, Freiburg, Germany*

17.00 – 18.00 Poster presentation and general discussion (*chair: Markus Wirtz, Centre for Organismal Studies, Plant Molecular Biology, University of Heidelberg, Germany*)

18.00 – 19.30 Relax and chattings

19.30 – late Conference Dinner

## Saturday, October 1, 2011

### ***Session V - Sulfur in environment and in agriculture.***

*(chair: Cornelia Herschbach, Institute of Forest Botany and Tree Physiology, Albert-Ludwigs-University, Freiburg, Germany)*

- 9.00 – 9.30 The long term variations of  $\text{SO}_4^{2-}$  availability contributed to the radiation and success of chlorophyll a+c algae in the oceans. *Mario Giordano, Ancona, Italy*
- 9.30 – 9.50 Perspectives for sulphur use efficiency in agriculture. *Jørgen Eriksen, Tjele, Denmark*
- 9.50 – 10.10 Impact of S fertilization and fungal pathogens on the release of gaseous sulfur compounds by oilseed rape. *Elke Bloem, Braunschweig, Germany*
- 10.10 – 10.30 Modelling the allocation of nutrients between the root and shoot of young S-deprived maize plants. *Dimitris Bouranis, Athens, Greece*
- 10.30 – 10.50 Coffee break

### ***Session VI - Serine acetyltransferase: a key actor on plant sulphur assimilation regulation.***

*(chair: Rainer Hofgen, Max Planck Institute of Molecular Plant Physiology, Potsdam-Golm, Germany)*

- 10.50 – 11.10 Two transcription factors are putative partners of Arabidopsis cytoplasmic serine acetyltransferase. *Monica Mierzwinska, Warsaw, Poland*
- 11.10 – 11.30 Characterization of the serine acetyltransferase family from *Vitis vinifera*. *Sara Amancio, Lisboa, Portugal*
- 11.30 – 11.50 Functional analysis of SERAT gene family in Arabidopsis. *M. Watanabe, Potsdam-Golm, Germany.*
- 11.50 – 12.10 The role of cyclophilin in activation of chloroplast serine acetyltransferase under oxidative stress. *Anna Speiser, Heidelberg, Germany*
- 12.10 – 12.30 Concluding remarks and Closing of the meeting
- 12.45 – 14.00 Lunch and goodbye

## INVITED LECTURE



## THIOLS AND THE CELL - A COMPLEX BIOCHEMICAL INTERPLAY

Alfonso Pompella

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Biological thiols are mainly present in aminoacids, of which cysteine is associated with a series of important functions. Cysteine residues are contained in proteins as well as in low molecular weight molecules. The most abundant and important intracellular low mol. wt. thiol is glutathione (GSH), a tripeptide present at mM concentrations. Thiols can defend the cell – either spontaneously or through the action of specific enzymes – by functioning as scavengers of potentially harmful compounds such as electrophiles, or reactive oxygen (ROS) and nitrogen species (RNS) that originate as by-products of various physiological reactions (mitochondrial respiration, cytochrome P-450 catalysis, phagocyte activation etc.). On the other hand, redox status of thiols can modulate the function of several crucial proteins in cell signalling, such as e.g. protein kinases, phosphatases and transcription factors. S-Glutathiolation – i.e. the formation of reversible disulfide bonds by GSH with cysteine residues in proteins – is a major reaction involved in these processes. Gamma-glutamyl transferase (GGT), recently studied in plant tissues as well, is the single enzyme activity capable of metabolizing GSH. Complexity of thiol biology is well represented by the functions of this enzyme, which originates thiol metabolites prone to interact with metal cations and generate ROS. Prooxidant phenomena are thus promoted at sites of GSH metabolism, a process apparently involved in several aspects of cellular pathophysiology including cell surface receptor function, DNA oxidation and resistance to cytotoxic drugs. S-Nitrosogluthathione, the main storage and transport form of nitric oxide (NO) within the body, is also metabolized by GGT leading to release of free NO, which suggests the possibility that the enzyme may serve as a means for site-specific delivery of NO in tissues.

*Studies supported by ITT-Istituto Toscano Tumori (Firenze) and FFC-Fondazione per lo studio della Fibrosi Cistica (Verona).*



## **ABSTRACT ORAL PRESENTATIONS**

(order of presentation)



## ABSTRACTS:

SESSION I - *Sulfur compounds: crucial players in the plant stress tolerance.*



## THE ROLE OF SULFUR-CONTAINING SECONDARY METABOLITES IN RESISTANCE TO PATHOGENS AND PESTS

Felix Mauch and Klaus Schlaeppi\*

*Department of Biology, University of Fribourg, Chemin du Musée 10, 1700 Fribourg, Switzerland*

The *Arabidopsis* mutant *pad2* shows strongly enhanced susceptibility to various pathogens. The identification of PAD2 as  $\gamma$ -glutamylcysteine synthetase suggested glutathione deficiency as the primary cause of enhanced disease susceptibility. The *pad2* mutant is known to have a defect in the synthesis of the S-containing phytoalexin camalexin. We recently found *pad2* to accumulate also less glucosinolates (GS) and, as a consequence, to be more susceptible to insect herbivores. The secondary deficiencies of *pad2* in S-containing metabolites suggests that 1) GSH and not cysteine serves as sulfur donor in the biosynthesis of both camalexin and GS and 2. the partial lack of these metabolites might be responsible for enhanced disease susceptibility. Disease resistance tests of various *Arabidopsis* mutants with single and combined defects in camalexin and/or GS biosynthesis led to the conclusion that enhanced disease susceptibility is caused by the combined deficiency in camalexin and indole-GSs. These compounds act sequentially in disease resistance with an early role for indole-GS in penetration resistance whereas camalexin is important at later infection stages. Interestingly, the early role of indole-GSs was independent of cellular destruction, thus suggesting a mode of action different to the classical 'mustard oil bomb'.

\* present address: Max Planck Institute for Plant Breeding Research, Department of Plant Microbe Interactions, Carl-von-Linné-Weg 10, Cologne, Germany

## COMPARTMENT SPECIFIC IMPORTANCE OF ASCORBATE AND GLUTATHIONE DURING ABIOTIC STRESS

Bernd Zechmann<sup>1,2</sup>, Maria Müller<sup>1</sup>, Graham Noctor<sup>3</sup> and Felix Mauch<sup>4</sup>

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Ascorbate and glutathione are important antioxidants and involved in the detoxification of reactive oxygen species, which are commonly formed during environmental stress situations. Changes in ascorbate and glutathione contents are therefore commonly used as stress markers in many fields of plant sciences. Inter- and intracellular ascorbate and glutathione contents and their ratio between certain cell compartments are important measurements of the plants ability to sense and fight oxidative stress and can give key information about the physiological condition of the plant.

Here a method will be presented that allows the quantification of ascorbate and glutathione in all cell compartments simultaneously at a high level of resolution. This method is based on immunogold cytochemistry with anti-ascorbate and anti-glutathione antisera and computer-supported transmission electron microscopy. By applying this method on different *Arabidopsis* mutants during environmental stress situations such as cadmium, light stress and high internal levels of reactive oxygen species it was possible to gain thorough knowledge about the subcellular distribution of ascorbate and glutathione in plants and on the importance of these antioxidants in certain cell compartments during abiotic stress.

Acknowledgement: This work was supported by the Austrian Science Fund (FWF-Project nr. P20619-B16 and P22988-B16).

## IMPACT OF DROUGHT STRESS ON THE SULFUR ASSIMILATION PATHWAY IN ZEA MAYS

Nisar Ahmad, Markus Wirtz and Rüdiger Hell

*Centre for Organismal Studies, University of Heidelberg, 69120 Heidelberg, Germany.*

Crop plants are exposed permanently to various biotic and abiotic stresses which cause a significant reduction in yield. Among abiotic stresses, drought is one of the most serious world-wide problems which alone cause 15% yield losses to crop plants. During stress conditions the normal homeostasis of the cell is disrupted which causes an increased production of reactive oxygen species (ROS). ROS play a dual role in response to stress. They function as important signaling molecules in stress response pathways and also cause damage to cells. Detoxification of the ROS is partially done by the glutathione-ascorbate cycle where  $\text{H}_2\text{O}_2$  is reduced to  $\text{H}_2\text{O}$  at the expense of reduced glutathione (GSH) in the final step, yielding oxidized glutathione (GSSG). Glutathione (GSH) is an important and abundant non protein thiol whose concentrations in cells can be increased in defense against various stresses. Maize seedlings show elevated levels of GSH and sulfur compounds of the assimilation pathway 12 days after onset of drought. However, high GSH is assumed to be a feedback inhibitor of the sulfate assimilation pathway, the primary source of cysteine synthesis. This apparent discrepancy will be presented with respect to the impact of drought stress on the sulfur assimilation pathway in maize.

## S-CONTAINING METABOLITES MIGHT BE IMPORTANT FOR THE DETOXIFICATION OF MERCURY IN PLANTS

Sandra Carrasco-Gil<sup>1</sup>, Ana Álvarez-Fernández<sup>3</sup>, Juan Sobrino-Plata<sup>1</sup>, Rocío Millán<sup>2</sup>, Danika L. LeDuc<sup>4</sup>, Joy C. Andrews<sup>5</sup>, Javier Abadía<sup>3</sup>, and Luís E. Hernández<sup>1\*</sup>

<sup>1</sup>Department of Biology, Universidad Autónoma de Madrid, Spain;

<sup>2</sup>CIEMAT, Madrid, Spain;

<sup>3</sup>Estación Experimental de Aula Dei-CSIC, Zaragoza, Spain;

<sup>4</sup>Department of Chemistry and Biochemistry, California State University, East Bay, CA USA;

<sup>5</sup>Stanford Synchrotron Radiation Lightsource, Menlo Park, CA USA.

Three-week-old alfalfa (*Medicago sativa*), barley (*Hordeum vulgare*) and maize (*Zea mays*) were exposed for 7 days to 30  $\mu$ M of mercury (HgCl<sub>2</sub>) to characterize the Hg speciation in root. Liquid chromatography coupled with electro-spray/time of flight mass spectrometry showed that Hg was bound to an array of phytochelatins (PCs) in root. Spatial localization of Hg in alfalfa roots by microprobe synchrotron X-ray fluorescence spectroscopy showed that most of the Hg co-localized with sulfur in the vascular cylinder. Extended X-ray Absorption Fine Structure (EXAFS) fingerprint fitting revealed that more than 79% of Hg was bound *in vivo* to organic-S compounds, i.e. biomolecules containing cysteine. To evaluate the relevance of Hg-PC complex formation for Hg detoxification and tolerance, the biothiol profile was studied in *Arabidopsis thaliana* mutants *cad2-1* (with low glutathione content) and *cad1-3* (unable to synthesize PCs) in comparison with wild type plants. These mutants were more sensitive to Hg, and the HPLC-ESI-TOFMS analysis showed that none of them accumulated Hg-biothiol complexes, highlighting the importance of S-containing metabolites in Hg detoxification.

## INVOLVEMENT OF THE GAMMA GLUTAMYL CYCLE IN THE PLANT CELL REDOX HOMEOSTASIS

Serena Tolin<sup>1</sup>, Giorgio Arrigoni<sup>2</sup>, Bernd Zechmann<sup>3</sup>, Annarita Trentin<sup>1</sup>, Sonja Veljović Jovanović<sup>4</sup>, Antonio Masi<sup>1</sup>

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<sup>2</sup> Dept. Biological Chemistry, University of Padua, and VIMM (Venetian Institute of Molecular Medicine), Italy

<sup>3</sup> Institute of Plant Sciences, University of Graz, Austria

<sup>4</sup> Institute for Multidisciplinary research, Belgrade, Serbia

The existence of a gamma-glutamyl cycle in plants that is functional to the retrieval of extracellular glutathione (GSH) has been demonstrated in recent literature. In this cycle, glutathione is extruded to the apoplast, and sequentially degraded to its constituent aminoacids by gamma-glutamyl transferase (GGT) and cys-gly dipeptidase (CD) activity. Aminoacids are then taken up and glutathione is reassembled inside the cell. While increased GGT activity has been reported to be associated with oxidative stress conditions, the exact significance of the gamma-glutamyl cycle in plant physiology remains obscure.

*Arabidopsis thaliana* GGT1- knockout mutants exhibit no clear phenotype but a shorter life cycle and premature senescence; extracellular washing fluid analysis and immunodetection in these mutants also indicates increased apoplastic glutathione and ascorbate concentration.

We carried out a comparative proteomic analysis on tissues from wildtype and GGT1- knockout *Arabidopsis thaliana* plants, by means of protein labelling by iTRAQ and LC-MS/MS for simultaneous identification and relative quantification. Results indicate significant upregulation of several cytoplasmic antioxidant and stress-related enzymes (e.g. GSTs, catalases, peroxidases, ascorbate biosynthesis, peroxiredoxins, heat shock proteins). These evidences suggest that apoplastic, cell-wall bound GGT activity is responsible for cell redox coordination and that its silencing evokes a generalised alert response.



## **ABSTRACTS:**

**SESSION II - *Sulfur transport, assimilation and signalling in plants.***



## THE PHYLOGENY OF THE PLANT SULFATE TRANSPORTER GENE FAMILY: AN UPDATE.

Peter Buchner and Malcolm Hawkesford

*Rothamsted Research, West Common, Harpenden AL5 2JQ, United Kingdom*

Sulfur is an essential element for all living organisms. Different mechanisms of sulfate translocation through the plasmamembrane into the cytoplasm are known in nature. In bacteria most of the sulfate transport systems have been identified as ABC-type sulfate transporters belonging to the ATP-binding cassette (ABC) superfamily, where sulfate transport is energized by hydrolysis of ATP. The typical sulfate transporter found in plants belongs to the proton-coupled transport system. These transporters act as proton:sulfate symporters and the gene family is a member of the MFS superfamily. Related genes may be also identified in a number of microbial species, algae and fungi. A third mechanism is the sodium coupled sulfate transport: sodium-sulfate transporter also belong to the MFS superfamily and are found in bacteria, non-vascular plants and mammals.

The rapid progress in genome sequencing for a wide range of organism including model plant species such as *Arabidopsis* and important crop plant species as well as lower plant species, single and multi cellular algae, yeast, fungi and bacteria facilitates the drawing of a comprehensive picture of the phylogeny of these large gene families. An overview and update of the phylogeny of the sulfate transporter gene family in plants and other eukaryotic species will be discussed in relation to their functional diversification and relevance in the evolutionary context.

## F-BOX PROTEIN, EBF<sub>2</sub> IS A COMPONENT OF THE SULFUR DEFICIT SIGNALING PATHWAY IN ARABIDOPSIS

Anna Wawrzyńska, Agnieszka Sirko

*Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, POLAND*

SLIM<sub>1</sub>, a member of the EIN<sub>3</sub>-like (EIL) family of transcription factors in Arabidopsis, is a key positive switch in sulfur deficiency perception. However, the exact signaling cascade leading from sensing to activating the expression of the SLIM<sub>1</sub>-dependent gene set resulting in sulfur metabolism reprogramming remains uncharacterized. We have demonstrated direct binding of SLIM<sub>1</sub> to the 20-nt DNA sequence (UPE-box). Heterologously expressed SLIM<sub>1</sub> (in leaves of *Nicotiana benthamiana*), contrary to its homologue from tobacco, NtEIL<sub>2</sub>, induces reporter gene, controlled by promoter with UPE-box, irrespectively of the sulfur nutritional status. This observation suggests that an additional and uncharacterized Arabidopsis protein, not present in heterologous environment regulates SLIM<sub>1</sub> and assures sulfur-specific transcriptional response. The best described member of EIL family is EIN<sub>3</sub>, a transcription factor controlling the expression of ethylene-responsive genes. EIN<sub>3</sub> is transcribed constitutively, therefore its activity is regulated at posttranscriptional level by two F-box proteins, EBF<sub>1</sub> and EBF<sub>2</sub>, leading to quick ubiquitylation and degradation in the absence of the signal. Using yeast two-hybrid system we observed strong interaction of SLIM<sub>1</sub> with EBF<sub>2</sub>, but not with EBF<sub>1</sub>. When both SLIM<sub>1</sub> and EBF<sub>2</sub> are transiently expressed in *Nicotiana benthamiana* leaves, SLIM<sub>1</sub> is not able to induce reporter gene transcription. This result indicates that EBF<sub>2</sub> is a missing factor responsible for negative regulation of SLIM<sub>1</sub> in the conditions of sulfur sufficiency by targeting it to the 26S proteasome.

Acknowledgments: This work was supported by the Polish Ministry of Science and Higher Education (grant nr 1194/B/PO1/2008/35)

## INTERPLAY OF SLIM<sub>I</sub> AND MIR<sub>395</sub> IN THE REGULATION OF SULFATE ASSIMILATION IN ARABIDOPSIS

Colette A. Matthewman<sup>1</sup>, Cintia G. Kawashima<sup>2</sup>, Siqi Huang<sup>2</sup>, Bok-Rye Lee<sup>1</sup>, Naoko Yoshimoto<sup>3,4</sup>, Anna Koprivova<sup>1</sup>, Ignacio Rubio-Somoza<sup>5</sup>, Marco Todesco<sup>5</sup>, Tina Rathjen<sup>2</sup>, Kazuki Saito<sup>3,4</sup>, Hideki Takahashi<sup>4</sup>, Tamas Dalmay<sup>2</sup>, and Stanislav Kopriva<sup>1</sup>

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MicroRNAs play a vital role in plant development and response to environmental stimuli. MicroRNA<sub>395</sub> (miR<sub>395</sub>) is strongly induced in response to sulfur deficiency. In Arabidopsis, miR<sub>395</sub> targets transcripts from two gene families: three ATP sulfurylase isoforms, ATPS<sub>I</sub>, ATPS<sub>3</sub>, and ATPS<sub>4</sub>, catalyzing the first step of sulfate assimilation, and the low-affinity sulfate transporter, SULTR<sub>2;I</sub>. This is counterintuitive to the known induction of other key components of the sulfate assimilation pathway during sulfur deficiency. We reveal that miR<sub>395</sub> is important for increasing translocation of sulfate to the shoots during sulfur deficiency. Analysis of transgenic lines with altered content of miR<sub>395</sub> and the SULFUR LIMITATION <sub>I</sub> (SLIM<sub>I</sub>) transcription factor show that during sulfur starvation these factors act together to maintain optimal ATP sulfurylase transcript levels and enable increased flux through the sulfate assimilation pathway. Reduced ATP sulfurylase expression affects both sulfate translocation and flux, while reduced expression of SULTR<sub>2;I</sub> limits translocation of sulfate to the shoots. Our results show that miR<sub>395</sub> is integral to the regulation of plant sulfate assimilation.

## A TRANSCRIPTOMIC APPROACH FOR THE IDENTIFICATION OF GENES INVOLVED IN THE RESPONSE TO CHROMIUM AND SULPHUR IN BRASSICA JUNCEA

Michela Schiavon<sup>1</sup>, Giulio Galla<sup>2</sup>, Silvia Quaggiotti<sup>1</sup>, Valentina Telatin<sup>1</sup>, Elizabeth-Pilon-Smits<sup>3</sup>, Markus Wirtz<sup>4</sup>, Ruediger Hell<sup>4</sup>, Gianni Barcaccia<sup>2</sup>, Mario Malagoli<sup>1</sup>.

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<sup>4</sup> Centre for Organismal Studies, University of Heidelberg, University of Heidelberg, 69120 Heidelberg, Germany.

A differential display cDNA-AFLP modified technique was used to identify gene transcripts regulated by hexavalent chromium (CrVI) in relation to sulphur (S) nutrition in *B. juncea* plants grown in the presence of 200 mM sulfate (+S), without sulfate (-S), with 200 mM sulfate plus 200 mM chromate (+S+Cr), or without sulfate plus 200 mM chromate (-S+Cr). The technique also allowed the visualization of the gene transcript expression patterns at different time points (0, 10 min, 1 h, 24 h), and results were further confirmed via Real-Time PCR. Forty-four combinations of degenerate primers designed on conserved sequences of sulphate transporters of other Brassicaceae spp. were assayed, which allowed the detection of 346 Transcript Derived Fragments (TDFs) differentially regulated by Cr and S. Eight TDFs corresponded to sulphate transporters, whose variation in transcript abundance was correlated with the levels of S, sulphate, thiols (cysteine and glutathione), and sulphate uptake rates. Furthermore, a coordinated regulation of sulphate transporters in response to S and Cr supply has been observed. With respect to other TDFs, the analyses PageMan and MapMan revealed a differential gene expression pattern between +S+Cr and -S+Cr plants for several of them, suggesting that many plant responses to Cr stress can vary depending on S availability to plants. A number of genes identified in this study could represent targets of genetic engineering in efforts to increase the capacity of Cr accumulation and tolerance in plant specie employed in phytoremediation.

## CHARACTERIZATION OF THE CHLOROPLASTIDIC PAPS TRANSPORTER IN *ARABIDOPSIS THALIANA*

Gigolashvili Tamara<sup>1</sup>, Ashykhmina Natallia<sup>1</sup>, Ebert Silvia<sup>1</sup>, Titvinidze Sophio<sup>1</sup>,  
Freriegmann Henning<sup>1</sup>, Kopriva Stanislav<sup>2</sup>, Flüge Ulf-Ingo<sup>1</sup>

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3'-phosphoadenosine 5'-phosphosulfate (PAPS) is the universal sulfate donor for the generation of sulfated metabolites like glucosinolates (GS), phenolic compounds, sulfated 12-hydroxyjasmonate and growth regulatory factors like PSYs, PSKs and PGR1-PRG8. Even though PAPS is fundamentally important for stem cell development and the survival of plants, the knowledge about PAPS transport across subcellular membranes is scarce. Recently, we have identified two putative chloroplastidic PAPS transporters in plants. The proteins designated as PAPST<sub>1</sub> and PAPST<sub>2</sub> are suggested to mediate PAPS transport from chloroplasts into the cytosol in exchange with 3'-phosphoadenosine 5'-phosphate (PAP). *PAPST<sub>1</sub>* and *PAPST<sub>2</sub>* are expressed in all organs with the highest expression in developing and mature seeds, primary veins of leaves, the shoot apex and roots. Green fluorescent protein-based analysis demonstrated the targeting of PAPST<sub>1</sub> and PAPST<sub>2</sub> to the chloroplast envelope. Isolation and characterization of a *papst1* knock-out mutant demonstrated that the deficiency in PAPST<sub>1</sub> activity results in 30-50% reduction of GS levels. Moreover, the *papst1/papst2* double homozygous knock-out mutant is not viable. These findings indicate that PAPS-mediated sulfation is of crucial importance to normal growth and development in plants. The severity of the phenotype is similar to that observed in the *tpst1 x tpst2* double knock-out mice mutant that is deficient in sulfation and seriously impaired in postnatal survival. The physiological analysis of the plant transporters is in progress.



## ABSTRACTS:

SESSION III – *The sulphur metabolism regulation.*



## ADENOSINE 5'-PHOSPHATE REDUCTASE (APR<sub>2</sub>) MUTATION IN ARABIDOPSIS IMPLICATES GLUTATHIONE DEPLETION IN SELENATE TOXICITY

Kevron Grant<sup>1</sup>, Nicole Marie Carey<sup>1</sup>, Miguel Mendoza<sup>1</sup>, John Schulze<sup>2</sup>, Marinus Pilon<sup>3</sup>, Elizabeth A.H. Pilon-Smits<sup>3</sup> and Doug Van Hoewyk<sup>1</sup>

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APR<sub>2</sub> is the dominant adenosine 5'-phosphosulfate reductase in Arabidopsis, and converts activated sulfate to sulfite, a vital step in the sulfate reduction pathway. To determine if APR<sub>2</sub> has a role in selenium metabolism, a mutant Arabidopsis line (*apr2-1*) was studied. Sulfur metabolism was perturbed in *apr2-1* plants grown on selenate, as observed by an increase in total sulfur and sulfate, and a two-fold decrease in glutathione concentration. The altered sulfur metabolism in *apr2-1* grown on selenate did not reflect typical sulfate starvation, as cysteine and methionine levels were increased. Knockout of APR<sub>2</sub> also increased the accumulation of total selenium and selenate. However, the accumulation of selenite, and selenium incorporation in protein was lower in *apr2-1* mutants. Decreased incorporation of selenium in protein is typically associated with increased selenium tolerance in plants. However, because the *apr2-1* mutant exhibited decreased tolerance to selenate, it is possible that selenium toxicity can also be caused by selenate's disruption of glutathione biosynthesis leading to enhanced levels of damaging reactive oxygen species.

## ADENOSINE 5'-PHOSPHOSULFATE REDUCTASE, IS REGULATED BY HY5 IN ARABIDOPSIS

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Plant sulfate assimilation is regulated by demand for reduced sulfur as is its key enzyme, adenosine 5'-phosphosulfate reductase (APR). In a genetic screen for mutants lacking this regulation we identified the bZIP transcription factor *LONG HYPOCOTYL 5* (*HY5*) as a necessary component of the regulatory circuit. Regulation of APR activity by the inhibitor of glutathione synthesis, buthionine sulfoximine, or by the precursor of cysteine, *O*-acetylserine, was disrupted in *hy5* mutant. When dark-adapted plants were re-illuminated, the rapid induction of *APR1* and *APR2* mRNA levels was attenuated in *hy5* seedlings, while *APR3* regulation was not affected. Indeed, chromatin immunoprecipitation revealed that *HY5* directly binds to the *APR1* and *APR2* promoters but not to promoter of *APR3*. Accordingly, the regulation of *APR1* and *APR2* by *O*-acetylserine was disturbed in *hy5* roots. *HY5* is also important for the coordination of nitrogen and sulfur assimilation, since unlike the wild type, *hy5* mutants do not undergo a reduction in sulfate uptake and APR activity during nitrogen starvation. Altogether these data show that *HY5* plays an important role in regulation of APR gene expression and of plant sulfate assimilation.

## INVESTIGATIONS OF SULFITE REDUCTASE IN *NICOTIANA TABACUM*

Marcel Naumann <sup>1, 2</sup>, Wolfgang Lein <sup>1, 3</sup>, Stephan Krueger <sup>1, 4</sup>, Holger Hesse <sup>1, 5</sup>, Rainer Hoefgen <sup>1</sup> and Hans-Michael Hubberten<sup>1</sup>

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Sulfate taken up by plants needs to be reduced to become incorporated into various metabolites of different physiological functions. Two enzymes are enabling the reduction steps from sulfate to sulfide. While several studies aimed to clarify the role of adenosine-5'-phosphosulfate (APS) reductase only some works focused in detailed on the properties of sulfite reductase. In most higher plants sulfite reductase is encoded by an single gene and thereby knock out mutants of this essential gene are not available. In a high throughput antisense approach in tobacco which aimed at identifying genes which when moderately inhibited in their expression generate visible phenotypes, sulfide reductase was identified as one of the main targets generating strong chlorosis and necrosis. These plants were investigated in detail and the data obtained exhibited clear differences to observations of studies in *Arabidopsis thaliana* where sulfite reductase expression was similarly affected.

## REPROGRAMMING OF SULPHUR METABOLISM IN *LOTUS JAPONICUS* DURING SYMBIOTIC NITROGEN FIXATION

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Symbiotic nitrogen fixation in legumes takes place in specialized organs called nodules that develop from dedifferentiated root cells after inoculation by specific rhizobia. The rhizobia within the nodules can reduce atmospheric nitrogen to ammonia via nitrogenase activity and provide it to the rest of the plant. Sulfur metabolism seems to be connected with symbiotic nitrogen fixation, since the *Lotus japonicus* mutant of the symbiotic sulphate transporter *sst1* results in development of ineffective nodules (Krusell et al. 2005). However, little is known about the molecular and biochemical mechanisms governing sulphate uptake and subsequent assimilation during symbiotic nitrogen fixation. In order to gain an insight we studied sulphate uptake and metabolism in symbiotic and non-symbiotic organs of the model legume *L. japonicus* either non-inoculated or inoculated with the rhizobium *Mesorhizobium loti* strain R7A or the mutant strain  $\Delta NifH$  that forms ineffective nodules with no nitrogenase activity. External [<sup>35</sup>S] sulphate was fed to *L. japonicus* roots and nodules, and its flux into different sulphur pools such as cysteine, glutathione, homoglutathione and proteins was monitored. Moreover, external [<sup>35</sup>S] sulphate was supplied to the root system of intact plants in order to analyze the sulphate uptake and its distribution into the different parts of the plant. Sulphate and thiol content in addition with the APR activity, the key enzyme of sulphur metabolism, were measured in various organs. These results, in combination with transcript profiling for genes involved in sulphur uptake and metabolism, suggest a global reprogramming of sulphur metabolism, on a whole plant level, during symbiotic nitrogen fixation.

## INVITED LECTURE



## OCCURRENCE AND IMPACT OF SULPHUR COMPOUNDS IN WINE

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Sulphur (S-) compounds play a significant role in grapes, must and wines. Apart from sulphate a broad range of organic S-compounds could be detected at different concentration levels during the winemaking process and in the final wines.

Glutathione, an antioxidant, is protecting wine colour and can contribute to prevent certain wine aroma compounds against oxidation.

Non-volatile aroma precursors in S-cysteine-conjugate or S-glutathione-conjugate form are the basis for volatile thiols which are released during alcoholic fermentation by yeast metabolism. One of the most powerful volatile thiols is 4-methyl-4-sulphanyl-pentan-2-one. These thiols have an important impact on the flavour of wines from certain varieties, e. g. Sauvignon blanc [1].

Volatile S-compounds are also produced during alcoholic and malolactic fermentation. Some of these volatile S-substances, e. g. H<sub>2</sub>S, methanethiol and/or ethanethiol and their contributing disulphides, can cause off-flavours in wine. The main cause is a nutrient deficiency in grape musts which cause stress conditions for yeasts [2].

The formation and fate of volatile S-compounds during the whole winemaking process is reviewed.

- 1 Marullo, P. and Dubourdieu, D. (2010) Yeast selection for wine flavor modulation In: *Managing wine quality*. Volume 2: Oenology and wine quality. Editor: A. G. Reynolds, Woodhead Publishing Limited, 293-345
- 2 Rauhut, D. (2009) Usage and formation of sulphur compounds. In H. König, G. Uden, & J. Fröhlich (Eds.), *Biology of Microorganisms on Grapes, in Must and in Wine* (181-207). Heidelberg, Germany: Springer Verlag GmbH



## ABSTRACTS:

SESSION IV – *The role of sulfite oxidase in plants.*



## **SULFITE OXIDASE IS AN ESSENTIAL COMPONENT IN THE SULFITE HOMEOSTASIS NETWORK UNCOVERED IN TOMATO PLANTS EXPOSED TO STRESS**

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Despite the need of being tightly regulated and the importance for sulfur metabolism, not much is known about the regulation of the cytotoxic sulfite in plants.

By employing environmental stress we show that mutation in tomato *SO* resulted in degradation in total Cys and Met, followed by sulfite accumulation. Sulfite enhancement was accompanied by leaf damage and mortality, dissimilar to the undamaged wild-type plants. Importantly, while the expression of the other known sulfite network components were inhibited in response to stress in wild-type and mutant, *SO* expression in wild-type leaf was significantly enhanced.

Employing sulfite application to unstressed and stressed plants revealed that while unstressed mutants were hardly damaged, environmental stressed mutants were significantly damaged by sulfite application and endogenous sulfite accumulation thereafter, unlike wild-type plants that were barely damaged. In addition, upregulation or downregulation of the expression of sulfite converting and generating network members respectively, were evident already 0.5 or 4h after sulfite application. These results indicate that in the absence of active *SO* the sulfite network is unable to efficiently homeostate endogenous sulfite levels under stress conditions, resulting in sulfite induced leaf tissue damage. However, under normal growth condition sulfite can be efficiently regulated by the sulfite network even when active *SO* is absent. These further indicate that sulfite can be considered as a regulatory sensing metabolite.

## SULFITE DETOXIFICATION MECHANISMS IN RESPONSE TO SO<sub>2</sub>-FUMIGATION IN *ARABIDOPSIS THALIANA*

Domenica Hamisch<sup>1</sup>, Dörte Randewig<sup>2</sup>, Cornelia Herschbach<sup>2</sup>, Monika Eiblmeier<sup>2</sup>, Christian Gehl<sup>1</sup>, Ralf R. Mendel<sup>1</sup>, Heinz Rennenberg<sup>2,3</sup>, and Robert Hänsch<sup>1</sup>

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Air pollution due to sulfur dioxide (SO<sub>2</sub>) is harmful to any creature worldwide, no matter if caused by anthropogenic effects or derived from natural sources. Plants as sessile organisms lack the possibility for escape wherefore they had to develop several protection mechanisms during evolution. In general, SO<sub>2</sub> enters plant cells through the stomata and is rapidly converted into sulfite. Subsequently, toxic effects caused by disruption of disulfide bonds (sulfitolysis) and incidental modification of protein structures direct to plant death.

SO<sub>2</sub> fumigation experiments (600 ppb) gave insights in plant defense mechanisms by analyzing alterations in sulfur-metabolism related enzyme activities and those of sulfite oxidase (SO) using wild type, SO overexpressing (35S::SO) and SO knock out (SO-KO) *Arabidopsis* plants. In response to SO<sub>2</sub> a remarkable expansion of sulfate and a significant increase of the glutathione-pool were observed in wildtype and 35S::SO. Changes were connected with a negative feedback inhibition of APR. SO-KO mutants were consistently negatively affected upon SO<sub>2</sub>-exposure and showed symptoms of injury with necrotic spots. While in SO-KO mutants the sulfate pool was kept constant, thiol-levels strongly increased due to SO<sub>2</sub>.

In summary, our studies deciphered a complex regulatory network driven by co-regulation of APR and SO to control the sulfur assimilation pathway and likewise to stabilize the S-distribution into organic sulfur compounds.

## SULFUR METABOLISM AT DIFFERENT CONCENTRATIONS OF SO<sub>2</sub> IN *POPULUS TREMULA* X *P. ALBA*

Dörte Randewig<sup>1</sup>, Domenica Hamisch<sup>2</sup>, Cornelia Herschbach<sup>1</sup>, Monika Eiblmeier<sup>1</sup>, Christian Gehl<sup>2</sup>, Ralf R. Mendel<sup>2</sup>, Heinz Rennenberg<sup>1,3</sup>, and Robert Hänsch<sup>2</sup>

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Wild type poplar plants and transgenic plants either overexpressing Arabidopsis sulfite oxidase (SO) or down regulated in SO activity by RNAi were fumigated with three different concentrations of SO<sub>2</sub>. Gas exchange parameters, i.e. CO<sub>2</sub> assimilation rates, stomatal conductance, transpiration rates and the SO<sub>2</sub> uptake rates, activities of enzymes of the sulfate reduction pathway and SO activity as well as sulfate and thiol levels were quantified. In contrast to SO<sub>2</sub> fumigated Arabidopsis plants, SO<sub>2</sub> fumigation mediated sulfate increase was not abolished in SO down-regulated poplar lines. Only one of these lines showed decreased CO<sub>2</sub>-assimilation and CO<sub>2</sub>-respiration rates resulting from stomata closure after SO<sub>2</sub> exposure. Whereas SO was the only enzyme responsible for sulfite to sulfate conversion in Arabidopsis, this seems to be different in poplar. Thiol analyses will show, whether reduced sulfur pools are enhanced in response to SO<sub>2</sub> fumigation in poplar and, thus, function as an additional sink for SO<sub>2</sub>-sulfur. In addition, activities of enzymes of the sulfate assimilation pathway will supply a deeper insight into the postulated co-regulation of APS reductase and sulfite oxidase.



## ABSTRACTS:

SESSION V - *Sulfur in environment and in agriculture.*



## THE LONG TERM VARIATIONS OF $\text{SO}_4^{2-}$ AVAILABILITY CONTRIBUTED TO THE RADIATION AND SUCCESS OF CHLOROPHYLL A+C ALGAE IN THE OCEANS

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In the Mesozoic, a dominance shift occurred in the phytoplankton inhabiting the continental shelf: chlorophyll a+c microalgae became prominent in the place of chlorophyll a+b algae and cyanobacteria. In parallel, seawater sulfate increased markedly. We tested the hypothesis that the sulfate increase facilitated the rise to dominance of chlorophyll a+c algae. We cultured the cyanobacterium *Synechococcus* sp., the green alga *Tetraselmis suecica*, the diatom *Thalassiosira weissflogii*, the dinoflagellate *Protoceratium reticulatum*, and the coccolithophorid *Emiliani huxleyi* at 1, 5, 10, 20 and 30 mM  $\text{SO}_4^{2-}$ . *Synechococcus* and *Tetraselmis* growth rates and C:S ratios showed no major changes in response to  $[\text{SO}_4^{2-}]$ . The three chlorophyll a+c algae had higher growth rates, lower C:S and higher S quotas with higher  $[\text{SO}_4^{2-}]$ , but only up to 10 mM. In the presence of the ciliate predator *Euplotes* sp., *Tetraselmis* and *Thalassiosira* showed a higher growth rate and lower C:N and C:S; *Synechococcus* growth was unaffected or lower. We also grew *Tetraselmis*, *Thalassiosira*, *Protoceratium*, and *Synechococcus* in conditions approximating modern, earlier Paleozoic and Proterozoic seawater. In monospecific cultures, all algae exhibited their highest specific growth rates in the Proterozoic treatment. In mixed culture, *Thalassiosira* outgrew the other species in modern seawater, but in Paleozoic medium the chlorophyll a+b alga *Tetraselmis suecica* prevailed. *Synechococcus* grew best in Proterozoic seawater, but did not outgrow eukaryotes in any treatment. We conclude that the long term increase in seawater  $[\text{SO}_4^{2-}]$  may have facilitated the evolutionary expansion of chlorophyll a+c phytoplankton, although not necessarily to the exclusion of other factors.

## PERSPECTIVES FOR SULPHUR USE EFFICIENCY IN AGRICULTURE

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Compared to other macronutrients, the use efficiency of sulphur is low - in the Danish case 25%. In a situation where the sulphur nutrition of plants is expected to be balanced, this indicates an extensive loss of sulphur. Arable land has a sulphur surplus of 20 kg S ha<sup>-1</sup> and for animal production it is currently 5 kg ha<sup>-1</sup>. Thus, there is a need for increasing the efficiency and this presentation gives an overview of the options. Arable production: 1) there is a considerable potential for catch crops to reduce this sulphate leaching, 2) sulphur application generally has a considerable safety margin. A closer match of sulphur with plant demand is needed e.g. site-specific application, 3) there is evidence that low nitrogen availability gives a higher sulphur fertilizer efficiency in plants which can be utilised in low-input farming, 4) farmers are recommended not to reduce the sulphur level in mineral fertilizer in the year of manure application, but availability varies and there is a need to investigate rapid analysis for sulphate in manure. Livestock production: The surplus from animal production is expected to be emitted as gaseous sulphur mostly from animal manure. Except for the loss of plant-available sulphur from the manure, the consequence may be the development of malodorous, volatile, sulphur-containing compounds. Technical solutions are being investigated but another option is to reduce sulphur inputs in feed either directly by reducing the content of sulphur amino acids or indirectly by establishing a closer match of sulphur with plant demand in the field.

## IMPACT OF S FERTILIZATION AND FUNGAL PATHOGENS ON THE RELEASE OF GASEOUS SULFUR COMPOUNDS BY OILSEED RAPE

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Uptake and release of gaseous sulfur (S) compounds are related to several biotic and abiotic factors. The plant S status and fungal infections seem to play a key role in metabolic processes involving gaseous S components. Hydrogen sulfide ( $H_2S$ ) is either released or taken up by the plant in relation to the need for S or the excess of reduced S inside the plant. In comparison, plants generally act as a sink for carbonylsulfide (COS). In a greenhouse experiment the  $H_2S$  and COS exchange of oilseed rape was investigated in relation to the S status of the crop and fungal infection with *Sclerotinia sclerotiorum*. Thiol contents were determined to unravel possible links to the release of gaseous S compounds. The experiments revealed that  $H_2S$  emissions were closely related to the S nutritional status as well as to infections by fungal pathogens. S fertilization caused a change from  $H_2S$  consumption by S deficient oilseed rape plants to a release of  $H_2S$  but fungal infection caused an even stronger increase in  $H_2S$  emissions which was 45-times higher than with S fertilization. Oilseed rape acted as a sink for COS as long as the plants were healthy. Fungal infection caused a shift from COS uptake to COS release. It remains speculative and is subject to further research if the release of  $H_2S$  and COS in response to fungal infection is a mechanism to combat the pathogen or if other functions are important such as the release of excess S or a role as signal molecules or regulators.

## MODELLING THE ALLOCATION OF NUTRIENTS BETWEEN THE ROOT AND SHOOT OF YOUNG S-DEPRIVED MAIZE PLANTS

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Thirteen-day-old maize plants were exposed to S-deprivation; subsequently their nutritional status was monitored for ten days. Plotting the  $-S$  values for each nutrient against the corresponding control ones, the  $-S$  values deviated in a power-type fashion. This holds true for both the  $-S$  shoots and roots, the corresponding plots of which differed from each other. The alteration in the allocation of each nutrient between roots and shoots due to S-deprivation is approached in terms of a power function. The experimental curvature was analyzed through the value of the function's exponent. Two models were formulated and tested through regression analysis. Within the first model, the deviation of the  $-S$  variable (amount of nutrient) from the corresponding control was followed by a power equation. Whereas, within the second model, the alteration of the variable was examined in relation to the alteration of the corresponding dry mass in both control and  $-S$  shoots and roots. Both models produced the same outcomes. In relation to the impact of S-deprivation on dry mass, for the shoot the relative impact was: Ca (45.3%) >  $\text{NO}_3$  (18.9%) > Mg (17.2%) > Mn (14.1%) > W = P = K = Fe = Cu > N (-4.3%) >  $\text{NH}_4$  (-4.7%) > Zn (-12%) > B (-21.4%). For the root, the relative impact was: N = K = Ca = Zn = B > P (-12.7%) >  $\text{NO}_3$  (-14.7%) >  $\text{NH}_4$  (-18.4%) > W (-27.8%) > Mn (-34.4%) > Mg (-37.5%) > Fe = Cu (40.5%). Regression analysis failed in the case of iron.

## **ABSTRACTS:**

**SESSION VI - *Serine acetyltransferase: a key actor on plant sulphur assimilation regulation.***



## TWO TRANSCRIPTION FACTORS ARE PUTATIVE PARTNERS OF ARABIDOPSIS CYTOPLASMIC SERINE ACETYLTRANSFERASE.

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Biosynthesis of cysteine in higher plants is a two step process, which is tightly controlled at various levels. In the first step serine acetyltransferase (SAT) catalyzes the formation of *O*-acetylserine, that is a backbone of cysteine subsequently synthesized by the action of *O*-acetylserine(thiol)lyase (OAS-TL). The formation of the cysteine synthase complex (CSC) by SAT and OAS-TL has been hypothesized to regulate cysteine synthesis, since it results in changed activities of both enzymes. Interestingly, SAT and OAS-TL activities are unevenly distributed between cytosol, plastids and mitochondria. This subcellular compartmentalization is in contrast to provision of sulfide that takes place exclusively in plastids, therefore the process of cysteine synthesis requires fine regulation. In order to address a potential function of the CSC within the regulation of total sulfur homeostasis, we designed a yeast two-hybrid approach that used the entire cytoplasmic CSC to search for interacting partners, instead of single SAT and OAS-TL proteins. This approach identified two putative partners of the CSC, which were both annotated as transcription factors. A more detailed analysis resolved that both candidate proteins interact with SAT<sub>5</sub> but not with OAS-TL<sub>A1</sub>, indicating that CSC formation is not a prerequisite for these interactions. Furthermore, both candidate proteins were able to interact with organellar localized SATs suggesting that the interaction domain is present in a structurally conserved region of the SAT protein. The possible biological role of these interactions will be discussed.

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## CHARACTERIZATION OF THE SERINE ACETYLTRANSFERASE FAMILY FROM *VITIS VINIFERA*

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In plants the synthesis of cysteine is catalyzed by the enzyme O-acetylserine(thiol)lyase (OASTL), from the substrates O-acetylserine (OAS) and sulfide (S<sup>2-</sup>). OAS is produced from serine by serine acetyltransferase (SAT) and S<sup>2-</sup> is the product of sulfate activation/reduction pathway. SAT and OASTL form a protein multiplex (cysteine synthase complex) responsible for the two final steps of cysteine synthesis, associated with a regulatory role in sulfate metabolism. Following the release of *Vitis vinifera* genome, four *SAT* (*Serat*) genes were identified based on sequence homology to other plant species. Some distinct characteristics of the *VvSAT* genes were observed, in particular a strong up-regulation of *VvSAT2;2* mRNA under sulfur deficiency. The biochemical characterization of the four *Vitis vinifera* SAT isoforms revealed SAT activity, although with different intensities. The test of SAT/OASTL interaction showed that two *Vitis* isoforms (*VvSAT1;1* and *VvSAT2;1*) were able to interact with *Arabidopsis thaliana* AtOASTL, in contrast to *VvSAT2;2* which did not interact with AtOASTL. The reason for this lack of interaction apparently consists in the *VvSAT2;2* amino acid sequence. It lacks a complete C-terminus that is responsible for the interaction with OASTL in other SATs. The biological significance of such a distinctive difference will be discussed.

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## FUNCTIONAL ANALYSIS OF *SERAT* GENE FAMILY IN *ARABIDOPSIS*

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Serine acetyltransferase (*SERAT*), which catalyzes *O*-acetylserine (OAS) formation, plays a key role in cysteine biosynthesis. In *Arabidopsis*, the *SERAT* gene family comprises five members. T-DNA knockouts studies revealed functional redundancy in the *SERAT* gene family under normal growth condition [1]. However, their tissue specific expression patterns and specific stress responses as deduced from public micro array data suggest that the individual *SERAT* isoforms might possess distinct functions. To obtain hints for elucidating the specific *SERAT* functions, we grew *serat* mutants under several growth conditions scoring their specificities of gene expression. Further, we performed enzymatic activity assays and metabolite profiles which focus on sulfur related metabolism using combinations of several analytical platforms. From these results, we will discuss the function of individual *SERAT* isoforms.

[1] Watanabe et al. (2008). Comparative genomics and reverse genetics analysis reveal indispensable functions of the serine acetyltransferase gene family in *Arabidopsis*. Plant Cell. 20: 2484-2496.

## THE ROLE OF CYCLOPHILIN IN ACTIVATION OF CHLOROPLAST SERINE ACETYLTRANSFERASE UNDER OXIDATIVE STRESS

Anna Speiser<sup>1</sup>, Monika Mierzwińska<sup>2</sup>, Anna Wawrzyńska<sup>2</sup>, Markus Wirtz<sup>1</sup>, Agnieszka Sirko<sup>2</sup>, Rüdiger Hell<sup>1</sup>

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Cyclophilin CYP20-3 belongs to the eukaryotic protein family of immunophilins and assists in folding and modification of proteins by peptidyl-prolyl-*cis/trans* isomerase activity. CYP20-3 is the sixth most abundant protein in the chloroplast stroma and the only member of this protein family in *Arabidopsis thaliana* that is localized to this compartment. Recently, CYP20-3 was reported to interact *in vitro* with the plastidic serine acetyltransferase (SAT1; Serat2;1). In addition, the corresponding *cyp20-3* loss of function mutant was less resistant to different oxidative stress conditions. Hence, the hypothesis was put forward that CYP20-3 activates SAT1 by triggering the association with plastidic *O*-acetylserine-(thiol)-lyase to form the cysteine synthase complex in order to provide cysteine and glutathione for improved defense against reactive oxygen species (ROS) [1]. This hypothesis was challenged by extended *in vivo* analysis of two different *cyp20-3* T-DNA insertion lines along with the SAT1 knockout mutant *serat2;1* under several ROS-inducing conditions. Neither differences in total SAT activity nor steady state thiol levels were observed under high light stress between wild type and mutant genotypes. Furthermore, ROS formation was triggered with rose bengal, paraquat and sodium chloride. In response to all treatments wild type plants showed visible symptoms of chlorosis confirming the efficiencies of the treatments. However, *cyp20-3* and *serat2;1* mutants showed no signs of increased sensitivity in comparison to the wild type. Based on the confirmed *in vitro* interaction between CYP20-3 and SAT1 using a yeast two-hybrid approach, it is reasonable to speculate that CYP20-3 cyclophilin may function as a chaperone that assists in protein folding and assembly but has no function in activation of chloroplast SAT under the tested stress conditions.

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[1] Dominguez-Solis J. R., He Z., Lima A., Ting J., Buchanan B.B., and Luan S., (2008) PNAS, 105, 16386-16391

## POSTER PRESENTATIONS:

### (P1) ANALYSIS OF SULPHATE DEFICIENCY AND OAS RESPONSIVE GENES IN *ARABIDOPSIS*

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### (P2) AUXIN-MEDIATED CHANGES IN EXTRACELLULAR GLUTATHIONE AND ASCORBATE METABOLISM IN PEA ROOTS-REGULATION OF ROOT ELONGATION BY APOPLASTIC REDOX STATUS

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### (P3) EVALUATION OF NATURAL DIVERSITY IN *ARABIDOPSIS THALIANA* ACCESSIONS FOR NITROGEN AND SULPHUR USE EFFICIENCY.

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### (P4) TRANSCRIPTIONAL REPROGRAMMING OF BACTERIAL AND PLANT SULPHUR UPTAKE AND METABOLISM DURING SYMBIOTIC NITROGEN FIXATION

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**(P5) USING AN AMPHIDIPOID SPECIES TO STUDY SULFATE TRANSPORTER REGULATION.**

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**(P6) EFFECTS OF SELENIUM ADDITION ON THE QUALITY OF HORTICULTURAL PRODUCTS.**

Pezzarossa B<sup>1</sup>, Rosellini I<sup>1</sup>, Malorgio F<sup>2</sup>, Mensuali A<sup>3</sup>, Ferrante A<sup>4</sup>, Remorini D<sup>5</sup>, Massai R<sup>5</sup>.

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**(P7) LOW SELENIUM CONCENTRATION EXERTS BENEFICIAL EFFECTS ON TOMATO PLANTS WITHOUT INTERFERING WITH SULPHUR ASSIMILATION**

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**(P8) DETERMINATION OF SULFITE IN TOMATO LEAVES**

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**(P9) ORGAN-SPECIFIC AND DEVELOPMENTAL REGULATION OF  
SULFITE REDUCTASE PROMOTER IN PLANTS**

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**(P10) ACCUMULATION OF SELENIUM IN *ULVA LAETEVIRENS*  
ARESCHOUG AND EFFECTS ON OXIDATIVE STRESS-RELATED  
METABOLISM AND ULTRASTRUCTURE**

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**(P11) EFFECT OF ION SULFATE AND PROTEIN CONTENT ON HAZE  
POTENTIAL OF WHITE WINES**

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**(P12) GLUTATHIONE DETERMINATION IN SOME ITALIAN WHITE  
GRAPE VARIETIES**

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## **ABSTRACT POSTER PRESENTATIONS:**



## **(P1) ANALYSIS OF SULPHATE DEFICIENCY AND OAS RESPONSIVE GENES IN *ARABIDOPSIS***

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O-acetyl-L-serine (OAS) has been suggested to be a marker for sulphur deficiency and a positive regulator of sulphur deficiency responsive genes. We identified key genes regulating sulphur metabolism in a screen for sulphate deficiency- and OAS-responsive genes using the array data originating from two experiments: In the first experiment, two transcription factors (MYB and zinc finger) were selected based on the response that up-regulated and down-regulated these genes under sulphate starvation and after sulphur re-addition, successively (Nikiforova *et al.*, 2006). In the second experiment, three genes (zinc finger, U-box and kinase), which were significantly up-regulated with OAS accumulation in a time-course experiment using SAT (serine acetyltransferase) inducible over-expressor lines (Hubberten *et al.*, unpublished data), were selected. T-DNA knock out mutants of these genes and their homologous genes were isolated and grown under several nutrient regimes. In this poster, phenotypic and metabolic changes that occur upon induced nutrient stress in these mutant lines, are presented and discussed.

## (P2) AUXIN-MEDIATED CHANGES IN EXTRACELLULAR GLUTATHIONE AND ASCORBATE METABOLISM IN PEA ROOTS- REGULATION OF ROOT ELONGATION BY APOPLASTIC REDOX STATUS

Marija Vidović<sup>1</sup>, Filis Morina<sup>1</sup>, Biljana Kukavica<sup>2</sup>, Antonio Masi<sup>3</sup>, Sonja Veljović Jovanović<sup>1</sup>

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Auxin-mediated ROS generation in apoplast has been correlated with cell wall loosening and root elongation. It has been shown that auxin signalling is affected by redox processes and that inhibition of root growth due to depletion of glutathione is caused by alterations in auxin homeostasis (Koprivova et al. 2010). We have previously shown that inhibition of root elongation, induced by high concentrations of indole 3-acetic acid (IAA), was accompanied by hydroxyl radical generation in pea root extracts (Kukavica et al, 2007). We further examined the mechanism of the auxin-mediated changes in the redox status of root apoplast, which is related to the regulation of cell wall loosening and stiffening. *Pisum sativum* L. plants were grown hydroponically and treated with 1 and 20 mg/l IAA for 10 days. Length of primary and lateral roots progressively decreased with IAA treatment, with significant morphological changes at 20 mg/l IAA. Although it is widely accepted that apoplastic redox status is mainly controlled by ascorbate and ascorbate oxidase (AO), we detected thiols in apoplast as well, implicating that GSH may contribute to the redox status as discussed in Ferretti et al. (2009). While the content of reduced ascorbate increased in apoplast of treated plants, the changes in thiols' concentrations were as follows: cysteine and g-glu-cys decreased with the treatment, while GSH increased at 1 mg/l IAA, but decreased 100-folds at 20 mg/l IAA. As free IAA can be inactivated either by formation of IAA conjugates with glutathione or by oxidative breakdown by POD oxidase activity, we determined the activities of POD, IAA oxidase POD activity and GST. Significant changes in abundance of the ionically bound POD, isoforms with IAA-oxidase activity, were observed. MDHAR activity decreased at 1 mg/l IAA and increased at 20 mg/l IAA. Apoplastic MnSOD and cell wall bound AO increased with auxin treatment. Soluble proteins in extracellular washing fluid were extracted from the roots of control and treated plants and their profile compared using 2D PAGE and diagonal electrophoresis. The role of apoplastic redox processes in auxin signalling related to the growth inhibition is discussed.

### References:

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**(P<sub>3</sub>) EVALUATION OF NATURAL DIVERSITY IN *ARABIDOPSIS THALIANA* ACCESSIONS FOR NITROGEN AND SULPHUR USE EFFICIENCY.**

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Plant growth and development ultimately depends upon environmental features, such as temperature, light intensity, availability of water and essential minerals. Nitrogen (N) and sulphur (S) serves as very important components for plant development. Improving plant N and S use efficiency requires a better knowledge of the regulation of plant N metabolism. This could be achieved using *Arabidopsis thaliana* as a model genetic system, and taking advantage of the natural variation available among ecotypes. In our experiment, we looked for natural variation in plant response to varying N and S nutritions within a core-collection of accessions. We imposed different N and S conditions, e.g. normal supply (N+S+) and various limited supplies (N-S-), and measured different morphological and metabolic traits. The aim of our study is to reveal the plant adaptation to an imbalance of exogenous N and S sources. Comparing the metabolite contents with gene expression levels in the different accessions will allow us to characterize the physiological adaptation. This system biology approach should give useful tools for selection of plants which will be less N and S demanding.

## (P4) TRANSCRIPTIONAL REPROGRAMMING OF BACTERIAL AND PLANT SULPHUR UPTAKE AND METABOLISM DURING SYMBIOTIC NITROGEN FIXATION

Chrysanthi Kalloniati<sup>1</sup>, Panagiotis Krompas<sup>1</sup>, Georgios Karalias<sup>1</sup>, Cornelia Herschbach<sup>2</sup>, Heinz Rennenberg<sup>2</sup> and Emmanouil Flemetakis<sup>\*</sup>

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Symbiotic nitrogen fixation by intracellular rhizobia within legume root nodules involves the reduction of atmospheric N<sub>2</sub> to ammonia by the bacterial enzyme nitrogenase. In the case of *Mesorhizobium loti*, the microsymbiont of the model legume *Lotus japonicus*, many of the genes required for the establishment of an effective symbiosis reside on the symbiosis island, a chromosomally integrated element that can be transferred between mesorhizobia in the environment, converting them into strains able to nodulate *Lotus* species. The *M. loti* symbiosis island also contains a cysteine synthase and a methionine synthase indicating a possible importance of sulphur metabolism in the symbiotic nitrogen fixation. In order to gain insight in the sulfur metabolism during symbiotic nitrogen fixation, we identified bacterial and plant genes involved in sulphate uptake, transport, reduction and assimilation. Transcript accumulation of these genes using Real Time qRT-PCR was studied in symbiotic or free-living bacteria *M. loti* strain R7A or the mutant strains  $\Delta NifA$  and  $\Delta NifH$ ; both mutant strains form ineffective nodules with no nitrogenase activity. Furthermore, we studied the relative transcript levels of the plant genes in symbiotic and non-symbiotic organs of *L. japonicus* plants either non-inoculated or inoculated with *M. loti* strain R7A,  $\Delta NifA$  and  $\Delta NifH$ . According to the results, most of the bacterial genes involved in sulphur metabolism are upregulated in effective nodules and systemic changes in the expression profile of the plant genes are taking place during symbiotic nitrogen fixation.

## (P<sub>5</sub>) USING AN AMPHIDIPOID SPECIES TO STUDY SULFATE TRANSPORTER REGULATION.

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Plant sulfate uptake and systemic distribution are processes mediated by specific transporters whose activity is finely tuned to control sulfur fluxes in the plants depending on their metabolic needs, and their sulfur nutritional status. Cd exposure deeply affects sulfur metabolism and increases the root capability to take up sulfate, as a consequence of the increased plant sulfur needs depending on phytochelatin biosynthesis. Such a behavior is similar to that of sulfur starved plants, although in this case the increased capability to take up sulfate does not derive from increased plant sulfur needs but from the need to maintain the homeostasis of sulfur fluxes along the assimilatory pathway. Both the responses are mainly controlled at transcriptional level and have often been indicated as resulting from the same, although controversial, nutritional signal.

We think that the use of amphidiploid species in the transcriptional control studies of sulfate transporters could be useful in order to underline possible multi-signalling regulatory pathways. In fact, the complexity of the amphidiploid genome, mainly concerning the existence of duplicated genes, may have led to the accumulation of mutations, which otherwise would have been eliminated by natural selection, that could give precious information on the transcriptional regulation mechanisms. Here we discuss the possibility that Cd and sulfur starvation modulate the expression of a high affinity sulfate transporter of *Brassica juncea*, an amphidiploid species carrying both the chromosomal sets of the ancestral parents *Brassica nigra* and *Brassica campestris*, through independent and partially overlapping signals.

## (P6) EFFECTS OF SELENIUM ADDITION ON THE QUALITY OF HORTICULTURAL PRODUCTS.

Pezzarossa B<sup>1</sup>, Rosellini I<sup>1</sup>, Malorgio F<sup>2</sup>, Mensuali A<sup>3</sup>, Ferrante A<sup>4</sup>, Remorini D<sup>5</sup>, Massai R<sup>5</sup>.

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Selenium (Se) is an essential micronutrient for humans and other animals, and a beneficial nutrient for many plants. Se has a close similarity in properties to sulphur and it can substitute for S in biochemical systems. The Se uptake occurs through the same binding sites in the plant root as sulphate. Due to its antioxidative role, Se can delay plant senescence, decrease postharvest losses of agricultural plants, and, at the same time, have positive effects on human health.

We investigated: i) the effects of Se addition on the quality of chicory and lettuce; ii) the effects of the foliar and fruit application of Se on fruit growth and ripening in peach and pear.

i) The addition of 0.5 and 1.0 mg Se L<sup>-1</sup> to the nutrient solution of chicory and lettuce grown in floating system resulted in an increase in the leaf Se concentration, which had a positive effect on the plant yield. Selenium was effective in decreasing the production of ethylene and PAL activity, consequently improving the quality of leafy vegetables and the shelf life. The amount of Se accumulated in plants grown in the nutrient solution containing 0.5 mg Se kg<sup>-1</sup> could provide the rational Se intake for human nutrition in accordance with the recommended dietary allowance (RDA) guidelines.

ii) Foliar and fruit spraying of 1.0 mg Se L<sup>-1</sup> solution to peach and pear resulted in an increase in Se concentration both in leaves and fruit. The enhanced selenium concentration affected the shelf life of fruit delaying the reduction in flesh firmness and fruit ripening, thus positively affecting fruit storage.

## **(P7) LOW SELENIUM CONCENTRATION EXERTS BENEFICIAL EFFECTS ON TOMATO PLANTS WITHOUT INTERFERING WITH SULPHUR ASSIMILATION**

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Selenium (Se) is an essential micronutrient for humans and animals, poisonous if ingested at relatively high concentration. The toxic effect of Se to plants is mainly due to the interference with sulphur (S) metabolism, caused by their close similarity in chemical properties. In particular, selenate competes with sulphate for plant uptake through the sulphate transport system. In some regions in the world, characterized by low soil content of selenium, the diet does not provide adequate amount of this element with adverse consequences for health. Therefore, the biofortification of plants with Se is attaining interest.

Based on that, tomato seedlings (*Solanum lycopersicum* L.) were grown in modified Hoagland nutrient solution for 40 days. Plants were then exposed in triplicate to 0, 5  $\mu$ M or 10  $\mu$ M of Na<sub>2</sub>SeO<sub>4</sub> in the presence of 1mM MgSO<sub>4</sub> for 24h and 5 days.

The Se concentrations utilized in this study did not induce stress in tomato plants and a faster growth was evidenced in plants exposed to 10  $\mu$ M Se than in the control. Tomato plants accumulated Se in roots and leaves, with higher values measured in plants supplied with 10  $\mu$ M Se after 5 days. In plants of this condition, the level of sulphur also increased. The sulphate content measured at 5 days was greater in roots of Se treated plants compared to the control, while in leaves higher values were recorded after 24h. Cysteine content in leaves was lower in plants exposed to Se, especially following 5 days of exposure to 10  $\mu$ M Se, whereas no variation was observed in roots. With respect to glutathione, no significant differences were observed among treatments.

In conclusion, the low selenium concentrations used in this study seem to exert beneficial effects on plant growth without affecting sulphur assimilation.

## (P8) DETERMINATION OF SULFITE IN TOMATO LEAVES

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Sulfite detection in plant tissue is a very complicated procedure, due to the up to 70% sulfite losses upon extraction and derivatization with monobromobimane [1]. A sensitive enzymatic assay for sulfite determination in wild type and sulfite oxidase compromised tomato plants was employed using chicken sulfite oxidase. The level of the detected sulfite was further supported by a modified fuschin-based detection method and by additionally employing both, purified plant SiR and OAS-TL to generate cysteine. The detected sulfite varied only by 2 to 9% among the methods. Importantly, the sulfite calibration curves determined in the presence or absent of plant sulfite, were linear for all three detection methods, exhibiting correlation coefficients higher than 0.998.

[1] Tsakraklides G. et al, 2002. Plant J 32:879-889.

## **(P9) ORGAN-SPECIFIC AND DEVELOPMENTAL REGULATION OF SULFITE REDUCTASE PROMOTER IN PLANTS**

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Sulfite reductase (SiR) is an essential part of sulfur assimilation pathway and catalyzes the reduction of sulfite to sulfide. The expression pattern of SiR in *Arabidopsis* plants was studied. A 2.2-kb fragment containing SiR promoter was cloned and fused with Dronpa (a GFP-like protein) and GUS genes to direct expression of the reporter genes. Homozygous transgenic plants of *Arabidopsis thaliana* (ecotype Col-o) were selected after *Agrobacterium*-mediated transformation and expression of the reporter genes was studied. Organ-specific expression of SiR was demonstrated by Western blotting with specific antibodies. The SiR promoter was active in all organs, albeit not equally expressed, starting from the early stages of plant development. Thus, high level of Dronpa and GUS was observed in root tip and developing siliques. Also the elevated activity of SiR promoter was associated with bundles located in flowering stem and leaf vessels. The expression of the reporter genes was found to be highly increased in phloem zone and S-cells of stem bundles. Our data indicate a semi-constitutive, organ-specific profile of SiR expression that probably associating with demand in sulfur assimilation.

## **(P10) ACCUMULATION OF SELENIUM IN *ULVA LAETEVIRENS* ARESCHOUG AND EFFECTS ON OXIDATIVE STRESS-RELATED METABOLISM AND ULTRASTRUCTURE**

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Selenium (Se) occurs in the aquatic environment principally under two oxidation states, Se(IV) selenite and Se(VI) selenate. Se accumulation in algae can be also affected by the presence of sulfur (S), which is a well-known antagonist of selenate in the form of sulfate.

In the current study the impact of selenium (Se) on the green macroalga *Ulva laetevirens* Areschoug was investigated. The alga was provided for 10 days with concentrations of selenate ( $\text{Na}_2\text{SeO}_4$ ) ranging within 0-100  $\mu\text{M}$ . Se accumulation in the seaweed was linearly related to the given selenate concentration and, apparently, this relationship was not affected by the high sulfate concentration ( $\sim 10 \text{ mM}$ ) recorded in the seawater used as the growth medium. Se increased the activity of oxidative stress-related enzymes (superoxide dismutase, SOD, and catalase, CAT), even when selenate was supplied to *U. laetevirens* at low concentration (2.5  $\mu\text{M}$ ). This indicates that the antioxidative defence system plays a pivotal role in overcoming selenium-derived oxidative stress-damages in the macroalga *U. laetevirens*. Also, selenate exposure did not cause morphological and ultrastructural alterations in *U. laetevirens* cells, and the function and ultrastructure of chloroplasts was unchanged compared to the control seaweeds.

## **(P11) EFFECT OF ION SULFATE AND PROTEIN CONTENT ON HAZE POTENTIAL OF WHITE WINES**

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Protein haze can form in white wines after bottling. It has been shown that the proteins responsible for this problem are pathogenesis-related proteins from grape. However, the hazing potential of a wine does not seem to correlate with its total protein concentration, suggesting the involvement of other wine components. Recently it has been proposed that sulfate ion is an essential factor that is required for the formation of protein haze in white wines. However, the effect of sulfate was studied only in a model solution using purified wine proteins, and a study on the relation between sulfate content and protein stability in real wines is lacking. In the present work 64 unfined white wines were analysed for their protein heat stability, concentration and sulfate content and the correlations existing among these parameters were established. The protein content had a high positive correlation (0.79) with the protein turbidity formed after the heat test. Unexpectedly the sulfate concentration showed a negative correlation (-0.47) with the hazing potential of the wine. On the other hand, a 40% sulfate content reduction, as obtained by precipitation with barium chloride in 5 wines, reduced (from 12 to 80%) the protein haze formed by heating in all cases. The effect of the barium chloride treatment on the other wine parameters (pH, ethanol, protein and polyphenol contents) was investigated and only a slight reduction in proteins and polyphenols was observed. These data suggest that more studies are needed to understand the role of sulfate in wine protein hazing.

## (P12) GLUTATHIONE DETERMINATION IN SOME ITALIAN WHITE GRAPE VARIETIES

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Glutathione (GSH) is the most abundant non-protein thiol compound widely present in living organisms. In wine production, glutathione from grapes plays an important role in preventing oxidative spoilage of white wines. Hydroxycinnamic acids, especially caftaric acid, are the first substrates to be oxidized by the natural polyphenol oxidase (PPO), being thus involved in the oxidative browning of the grape juice. GSH interferes with this process by trapping the caftaric quinones produced by oxidation in the form of 2-S-glutathionyl caftaric acid (GRP) thus limiting the must browning. In addition GSH may play a role in protecting volatile thiols, which are important aroma compounds, during the aging of bottled white wines.

The GSH content in berries of eight autochthonous Italian white grape varieties has been measured by HPLC and compared with both the yeast-available nitrogen (YAN) and total nitrogen content (TN) of the berries. The GSH content showed a very high correlation with YAN. In particular the variety Manzoni bianco showed a GSH content more than three times higher than that found in the other varieties. This variety showed also the highest TN and YAN.

From a technological point of view this is interesting because the glutathione content of the final wine is known to correlate with the YAN content of the juice, for the simple reason that if juice YAN is low then GSH is consumed by the yeast as the nitrogen source. Therefore, due to the high content of YAN of the juice, Manzoni bianco wine should contain also a high concentration of GSH, and this may result in less SO<sub>2</sub> need for protection from wine oxidation.

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