

free-running conditions. Different sulfur fertilization was applied to analyze the effects on pathogenesis-related compounds. Results from these experiments could help to optimize the use of fertilizer and if applicable reduce the amount of fungicides/pesticides.

### 19) The effect of the continuous light in combination with sulfur deprivation on the chlorophyll levels and carotenoids in young maize leaves

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Light fulfills two significant roles in plant growth. At first, light drives photosynthesis by providing energy and second it is perceived by several photoreceptors, thus activating signal pathways. Continuous light changes plant physiology by affecting both roles, thus creating difficulties in identifying the factors that are responsible for injuries under such treatment. As far as we know, the effect of continuous light in combination with plant's nutritional status or nutritional deficiency on its physiology is poorly studied. In particular, there are no references with regard to sulfur deficiency. Towards this direction, the responses of *Zea mays* plants to light environment in combination with nutrition were studied in four treatments; C: normal photoperiod & complete nutrient solution, Cc: continuous light & complete nutrient solution, -S: normal photoperiod & nutrient solution without sulfur, -Sc: continuous light & nutrient solution without sulfur. Plants were grown for seven days under normal photoperiod condition and then the treatment was applied for 3 weeks. The photon flux density was not modified during this period. The effect of the above mentioned cases on growth was monitored via fresh mass measurement, whilst the corresponding effect on the photosystems antennas was followed by determining the extractable levels of Chl *a*, Chl *b* and carotenoids from leaf lamina or sheath, by means of dimethyl sulfoxide. Our results showed that the treatments affected the time of organ appearance as well as their presence itself. The ratio of Chl *a/b* as well as the ratio of carotenoids to total chlorophyll proved to be useful response indicators to each treatment. The dynamic of adjustments presented by the sheaths (Sh) were different than the corresponding ones presented by the laminas (L).

Under continuous light and complete nutrition, the influence focused on L<sub>4</sub>, L<sub>5</sub>, L<sub>6</sub>, whilst L<sub>7</sub> did not occur. Sheaths appearance was not affected except for Sh<sub>5</sub>. Injuries due to this condition focused on youngest leaf from L<sub>4</sub> onwards and Sh<sub>4</sub> onwards. The lamina overall average of Chl *a/b* ratio was 4.1 (an increase by 13.9%), whilst in sheaths it was 2.7 (decreased by 6.9%). The lamina average Car:Chl ratio was 2.1 (decreased by 4.5%), whilst in sheaths it was 3.1 (increased by 10.7%).

A two days delay was observed in laminas L<sub>5</sub>, L<sub>6</sub>, L<sub>7</sub> and sheaths Sh<sub>2</sub>, Sh<sub>3</sub> during the treatment of sulfur deficiency under normal photoperiod. No injuries were caused in laminas. The average Chl *a/b* ratio of the laminas was 3.8 (increased by 5.6%), whilst the average one in sheaths was 2.6 (decreased by 10.4%). In laminas, the average of Car:Chl ratio was 2.5 (increased by 13.6%), whilst in sheaths the corresponding average was 2.9 (increased by 3.6%).

With regard to treatment with continuous light combined with sulfur deficiency, the appearance of organs took place at the same time as in control plants, with the exception of L<sub>7</sub> and Sh<sub>2</sub>. This fact indicates that the deficiency eliminated the effect

of continuous light. Aging and collapsing was observed at the oldest organs L<sub>0</sub>, L<sub>1</sub>, Sh<sub>0</sub>, Sh<sub>1</sub>. In laminas, the average value of Chl *a/b* ratio was 3.8 (increased by 5.6%), whilst in sheaths the average was 2.7 (decreased by 6.9%). At the end of the experiment, in laminas the average of Car:Chl ratio was 2.7 (increased by 22.7%), whilst in sheaths the corresponding average was 2.8 (as in control plants).

### 20) Aerenchyma formation in maize leaves during sulfate deprivation

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Aerenchyma is the term given to plant tissues containing enlarged gas spaces exceeding those commonly found as intracellular spaces. So far, aerenchyma formation under nutrient deficiencies and especially under nitrogen- or phosphorus- or sulfur deficiency has been reported only in the adventitious roots of maize by lysis of cortical cells. Seven-day-old maize plants were grown in a hydroponics setup for nineteen days under sulfate deprivation against plants grown under full nutrition and samplings were taken at day 17 and 26 from sowing (day 10 and day 19 of the deprivation respectively). Samples from the fresh laminas of the 2<sup>nd</sup> leaves were fixed and embedded in paraffin. Sections were received with microtome from the top, middle and base of the lamina and stained with safranin-fast green. The dry mass and water amount, the sulfate and total sulfur contents and the specific surface area of the 2<sup>nd</sup> leaf lamina and the transpiration rate of the plant were determined.

Under the circumstances we observed the presence of enlarged spaces in this lamina of the S-deprived plants, a fact that to our knowledge has not been reported so far. More specific, on the 10<sup>th</sup> day under the deprivation, the cross sections from the top of the 2<sup>nd</sup> leaf lamina of the S-deprived plants, revealed larger substomatal chambers compared to the control plants under full nutrition. In the middle of the S-deprived plants lamina-enlarged spaces appeared among the vascular bundles probably caused by lysis of mesophyll cells. These enlarged spaces stretched from the upper to the lower epidermis or between the stoma and the epidermis with equal frequency of appearance, whilst they appeared fewer times between the upper and the lower stoma. The percentage of the aerenchyma in relation to the total cross section area reached 4.9%. On the base of this lamina enlarge gas spaces appeared too, however to a very small extent, since the percentage of the aerenchymatous area was 0.3% of the total area of the section. On this day, the 2<sup>nd</sup> leaf of the S-deprived plants contained a significantly lesser amounts of sulfate, organic sulfur and total sulfur by 74%, 38% and 48% respectively compared to control plants. The S-deprived lamina's dry mass and water amounts were as in control. The specific surface area of the lamina (dry mass per lamina surface area) of the S-deprived plants was less by 19% compared to control plants. The S-deprived plants presented a larger transpiration rate by 28% than the control plants. These data indicate that on the tenth day of deprivation, aerenchyma may be formed in maize leaves in response to sulfur deficiency following the described pattern between the vascular bundles.

On the 19<sup>th</sup> day under the deprivation, such enlarged spaces appeared only in the middle of the lamina of the S-deprived plants 2<sup>nd</sup> leaf and the percentage of this aerenchyma reached just the 0.7% of the total cross section area. On this day, the 2<sup>nd</sup> leaf of the S-deprived plants presented less amounts of organic

and total sulfur by 68% and 63% respectively in comparison with control plants, whilst the corresponding percentage of the sulfate was 14% more than control plants. Furthermore, the S-deprived lamina presented more dry mass and water amount by 10% and 19% respectively. The specific surface area was as that of control plants and the transpiration rate of the S-deprived plants was decreased by 16% compared to control plants. It is concluded that somehow an alleviation process took place between the first and the second experimental day under the S-deprivation, a fact which to our knowledge has not been reported so far, too.

## 21) Metabolic state of Arabidopsis overexpressing SDI1

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O-acetyl-L-serine (OAS) provides the carbon backbone for cysteine synthesis and is assumed to positively regulate sulfur deficiency-responsive genes as its amount increases upon sulfur limitation. Therefore, mRNA accumulation of a number of sulfur responsive genes might be controlled by OAS. Recently this hypothesis was strengthened by global co-expression analysis under conditions of variable OAS levels with constant sulfur status. The function and the signaling cascade regulating the identified OAS cluster genes (*SDI-1*, *SDI-2*, *LSU1*, *LSU2*, *ChaC* and *SHM7*) are mostly unknown and need further elucidation. Functional characterization of SDI (sulfur-deficiency-induced 1 and 2) is the focus of this study as these candidate genes are prominently expressed upon sulfate deprivation, which is always coupled to OAS accumulation, and upon sulfate deprivation independent OAS accumulation. To promote our understanding of signaling processes and of the actual function of these genes, knock-out and double knock-out as well as overexpresser lines were produced. These lines are under investigation mainly employing transcriptomic and metabolomic approaches. GC/TOF measurements revealed significant increases of most amino acids in lines over-expressing SDI, most prominently alanine, asparagine and serine, as well as the polyamines putresine and spermidine. The levels of most sugars, sugar alcohols and organic acids (especially citrate, succinate, fumarate and malate) were significantly reduced in the same lines. While SDI double knock-out lines have reduced Cys levels, SDI1 overexpressers were found to have significantly elevated Cys levels and also accumulate more sulphate than WT plants under non sulphur limiting conditions. Besides the metabolic changes, SDI overexpresser lines display severe morphological phenotypes. Six-week-old plants grown under either shortday or longday conditions are much smaller in size than WT and they show delayed bolting and flowering and reduced seed production.

## 22) Making wealth out of sulfides and its consequences in Lavrion peninsula, Greece

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The history and the environmental issues of Lavrion are closely connected to sulfur and this connection was the subject of an excursion during the 4<sup>th</sup> Sulphyton Workshop. Lavrion is a town inhabited by more than 10,000 people, situated fifty five kilometers from Athens in the eastern part of Lavreotiki peninsula. Lavreotiki is located in the southeastern tip of Attica, it covers a surface of approximately 200 square kilometers where the places of Thorikos, Kamariza, Plaka, Botsaris Valley, Cavity Soureza Valley, Megala Pefka, Dimoliaki etc. are situated. Most of these places are remarkable centers of cultural and scientific tourism with ancient mines and ore washing plants, the Ancient Theatre of Thorikos, the natural National Park of Sounion, and the temples of Neptune and of Athena at Sounion. Lavreotiki was the center of considerable mining and smelting activities from ancient to recent times, due to its rich mineral wealth, which consists of argentiferous mineral deposits as polymetallic sulphides and iron ore. For this reasons, the history of Lavrion mines is directly connected with the history of Greece.

Many important metal ores are sulfides: argentite (silver sulfide), cinnabar (mercury sulfide), galena (lead sulfide), molybdenite (molybdenum sulfide), pentlandite (nickel sulfide), realgar (arsenic sulfide), stibnite (antimony sulfide), sphalerite (zinc sulfide), pyrite (iron disulfide), and chalcopyrite (iron-copper sulfide). There are mainly two types of mineralization in the substratum of Lavreotiki: a primary mineralization of mixed sulfide ores of basic metals, such as lead, zinc, iron and copper, which has been intensely exploited and a limited metallifery of Fe-Mn ores. A number of other sulfide minerals (e.g. Cu, As) and a variety of sulfur salts also take part in the ore mineral suite of the area. The accessory minerals that prevail are fluorite, calcite, barite, quartz and dolomite. Sulfide mineralization is mainly hosted within the carbonate formations.

The ancient Greeks extracted mainly silver and lead from the minerals of Lavrion, and especially from galena ore, with remarkable techniques. The galena ore was enriched in washing units by using water under pressure in order to remove the light and poor in metal parts of the ore. The enriched galena was converted to the oxide form (PbO + Ag) by the process called reduction roasting that produced lots of sulfur dioxide emissions in the atmosphere. The PbO + Ag chemical compound called "litharge" was directed into a furnace, where it melted with carbon (a process called reduction melting) and produced a PbAg alloy. The Pb-Ag alloy was next placed into a fireproof ceramic cup and heated in a specially constructed furnace where air was blowing under pressure. At 900 °C the alloy melted and lead was oxidizing whilst silver was not oxidizing. Then the alloy formed two separate layers, a heavy one containing silver in the bottom of the cup and a lighter containing litharge in the top. Litharge was removing and the process was continuing until the silver in the bottom was pure (over 99%). The precious metal was then ready for coins construction.

The mining history of Lavrion began at Thorikos, a settlement to the north of Lavrion and one of the most ancient industrial areas in Europe. The peak of mining and smelting activities was between the 6<sup>th</sup> and 4<sup>th</sup> centuries BC, and especially during the 5<sup>th</sup> century BC, the Golden Age of Athens. After the 4<sup>th</sup> century BC the exploitation declined. Mining and smelting activities continued intermittently until the 1<sup>st</sup> century AD. During the Roman and Early Byzantine period (2<sup>nd</sup> century BC – 6<sup>th</sup> century AD) it was sporadic and of small scale. Lavrion area between the 6<sup>th</sup> and 19<sup>th</sup> century was abandoned, losing all of its old glory. During the period 1864–1989 mining and metallurgical activities exploited the remaining deposits and the huge piles of mineral and metallurgical wastes, rich in Pb, Zn, Cu, Fe and other mineral elements. Enormous quantities of wastes from these activities, were deposited in heaps around Lavrion area,