5) Alterations of seed yield and quality in sulphur-limited *Brassica napus* L.

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Sulphur (S) limitation reduces seed yield and quality of various crops such as cereals, mainly used for producing flour, or oilseed rape, a high S demanding plant cultivated for its nutritional value for animal and human feeding. In this work, we describe the impacts of S restriction applied at the bolting (LS32), early flowering (LS53) or start of pod filling (LS70) stages on *Brassica napus* seed composition. For this purpose, lipids were analysed by Near-infrared spectroscopy and two-dimensional electrophoresis were performed on total proteins extracted from mature seeds. The major S compounds of mature seeds were also determined. The reduction of protein quality observed for all LS seeds was related to a reduction of S-rich seed storage protein (SSP) accumulation (as Cruciferin Cru4) at benefit of S-poor SSP (as Cruciferin BnC1). Through this adaptive response, the protein contents of LS70 and LS53 seeds were not affected, but it was reduced for LS32 seeds. The reduction of lipid content in LS53 and LS32 seeds was primarily associated with a reduction of C18 derivates. The ω6/ω3 ratio was increased in LS53 and LS32 seeds. Modulations of proteins associated with lipid storage and carbohydrate metabolism (reduction of caleosines, glyoxysomal malate synthase, thiazole biosynthetic enzyme THI1; accumulation of citrate synthase) could be involved in the alteration of lipid composition of LS53 and LS32 seeds. The accumulation of proteins associated with stress response and a lower level of glutathione in LS53 and LS32 seeds may decrease seed resistance to biotic/abiotic stresses during conservation and germination.

6) How abiotic stress affects glucosinolate biosynthesis in plants

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The glucosinolate (GSL) content is an important quality parameter in plants such as mustard and nasturtium. Both crops are used as spices and in phytopharmaceutical products. Agrotechnical measures such as sulfur fertilization are well known to increase the concentration of sulfur-containing secondary compounds in plants and contribute to the compliance with minimum quality demands. The worldwide scientific interest in using GSLs and their degradation products as anti-carcinogenic agents and reported beneficial health effects of GSL-containing vegetables give reason to maximize the GSL content in harvest products. Yet, another politically promoted objective is to increase the acreage of medicinal plants that are grown in Germany. A beneficial side effect is that biodiversity on agricultural soils will improve. It was the aim of the presented experiments to increase the GSL content in different plants by triggering stress response in the plants.

The impact of different stress parameters on the growth and GSL content of *Tropaeolum majus*, *Sinapis alba* and *Brassica juncea* was investigated in greenhouse experiments. Drought stress (soil water content of 4–8 volume % in comparison to 12–18 volume % in the control), salt application (2 mg NaCl per day with irrigation water) and methyljasmonate (MeJA) spray application (4 ml of a 200 µMol l^{-1} MeJA) were investigated. Different plant features were recorded to evaluate the impact of the treatments on plant performance and stress response. For this purpose evapo-transpiration, biomass development, specific leaf weight, plant pigments, plant thiols, GSL content and GSL yield were determined. Plants were harvested three times during the vegetation period.

Stress response was triggered by marginal doses of stress factors in order to increase the GSL content without negatively affecting crop and GSL yield. Drought and MeJA application reduced biomass production in all three crops by 25–40% while moderate salt applications slightly increased yield.

In all three plant species marginal drought stress and/or the application of MeJA increased the concentration of the prevailing GSLs on a dry weight basis. In *Tropaeolum majus* the glucotropaeolin concentration increased on an average by 21% with drought and by 31.5% after MeJA application at all sampling dates. But only MeJA application increased also the glucotropaeolin yield by 10% while drought and salt application reduced the glucotropaeolin yield. Similar results were found for sinalbin and sinigrin in *Sinapis alba* and *Brassica juncea*, respectively. Drought and MeJA increased the sinalbin concentration in *Sinapis alba* but MeJA was the only elicitor that yielded a 1.7-fold increase of the sinalbin yield in comparison to the control. In *Brassica juncea* MeJA increased the sinigrin concentration and resulted in a 2.4-fold higher sinigrin yield. Salt applications did not affect the GSL concentration significantly.

It can be concluded that MeJA application proved to be a suitable measures to increase the GSL content and yield of *Tropaeolum majus*, *Sinapis alba* and *Brassica juncea*. Though slight drought stress increased the GSL concentration, it reduced biomass production and GSL yield. Marginal salt application showed no effect on the GSL content, while crop yield was slightly increased.

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7) The impact of sulfate, hydrogen sulfide and sulfur dioxide on glucosinolate metabolism in Brassica species

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Glucosinolates are secondary sulfur compounds, especially found in *Brassicaceae*, which may function in plant defense against insects, herbivory and pathogens and have anti-carcinogenic properties. The content of the glucosinolates varies strongly between species, cultivars, developmental stage and may be

affected by the plant sulfur supply/status. In addition to sulfate taken up by the roots, *Brassica* species are able to utilize atmospheric sulfur gasses, *viz*. H_2S or SO_2 taken up by the leaves as sulfur source for growth. In the current study the impact of H_2S or SO_2 on glucosinolate metabolism was studied in two *Brassica* species characterized by a high (*B. juncea*) and low (*B. rapa*) glucosinolate content. 10-day-old seedlings were grown on a 25% Hoagland nutrient solution containing 0.5 mM sulfate for 3 days and subsequently transferred to fresh 25% Hoagland solution at 0 mM sulfate (−S) or 0.5 mM sulfate (+S) and exposed to 0.25 µl l^{-1} H_2S or SO_2 for 7 days. At an ample sulfate supply, exposure of both species to H_2S or SO_2 hardly affected content and composition of glucosinolate. H_2S or SO_2 exposure resulted in a slight decrease in expression of APS reductase expression whereas that of APS kinase and ATP sulfurylase remained unaffected. Sulfate-deprivation of plants resulted in a decreased biomass production and glucosinolate content. Expression of APS reductase was strongly enhanced in sulfate-deprived plants but expression of both APS kinase and ATP sulfurylase hardly changed. When sulfate-deprived plants were exposed to H_2S or SO_2, plant growth was restored, however, the glucosinolate content remained lower than that of sulfate-sufficient plants. Moreover, the expression of APS reductase was partially down-regulated again, whereas expression of APS kinase and ATP sulfurylase remained unaffected.

8) Effect of S-limitation on osmotic potential components in oilseed rape leaves: towards the development of early indicators?

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Oilseed rape (*Brassica napus*) is a S demanding crop, S-limitation resulting in a reduction of yield and of nutritional quality of seeds. Optimization of S fertilization requires to identify indicator of S nutrition that could be used early in the growth cycle.

In this study, we examined the effects of S-limitation on osmotic potential components of oilseed rape leaves. Plants were grown at vegetative stage and submitted to S-limitation (S+: 508.7 µM SO_4^{2-} or S−: 8.7 µM SO_4^{2-}) and were harvested at 0, 1, 2, 3, 7, 13, 24 and 34 days of S-limitation. Each plant was sampled as old leaves, new leaves emerging after S-limitation, roots and petioles.

Plant growth was maintained during the first 13 days of S-limitation as a result of massive internal sulfate mobilization mostly from leaves and its subsequent assimilation. This was at least partly compensated for by an accumulation of malate, nitrate, chloride and phosphate in leaves and to a lesser degree in roots. More surprisingly, leaf osmotic potential decreased after two days of S-limitation. Other compounds such as amino acids, soluble sugars and cations will be quantified in order to evaluate their contribution to the leaf osmotic potential. Additional data (^{15}N-nitrate uptake, nitrate reductase activity, transcript levels of sulfate and nitrate transporters) show that under S-limitation, osmotic potential is affected earlier that growth or N metabolism suggesting that field measurements of leaf ion contents, acting as osmoticum, could be used as early indicators for S fertilization management.

9) Elucidating the molecular components that allow *Salicornia* and *arcocornia* to thrive in high sulfate and sulfide levels

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Salicornia and *Sarcocornia* are extreme halophytes that grow on sea water with high sulfide and sulfate levels and have been recently introduced as new crops for extreme salt conditions such as in the level of Dead Sea water. We are exploring the mechanism/s that allows these plants to cope with sulfate and sulfide levels, which are toxic to many other plants. We grew the plants at different NaCl, sulfate and sulfide levels and we are scanning for relevant genes and proteins that might be related to plant resistance to those stresses.

10) The role of sulfite reductase in sulfite homeostasis

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Using *Arabidopsis* and tomato plants with modified SiR expression, we invastigated the role of SiR in various metabolic processes in plants. We observed that resistance to ectopically applied SO_2/sulfite was a function of SiR expression levels and that plants with reduced SiR expression exhibited higher sensitivity than the wild type. The sulfite sensitive mutants accumulated sulfite and showed a decline in glutathione levels. In contrast, mutants that over-express SiR were more resistant to sulfite toxicity; exhibiting little or no damage. Resistance to high sulfite application was manifested by fast sulfite disappearance and increase in glutathione levels. The notion that SiR plays a role in the protection of plants against sulfite was supported by the rapid up-regulation of *SiR* transcript and activity within 30 min of sulfite injection into Arabidopsis and tomato leaves. Our results indicate that, in addition to participating in the sulfate assimilation reductive pathway, SiR also plays a role in sulfite homeostasis together with sulfite oxidase and the other members of the sulfite network.

11) Regulatory Network of SO_2 detoxification

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