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Effect of Sulfur Nutrition on Glutathione Content in Sugar Beet Plants in Relation with Aphids Infestation

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With 3 Figures

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Summary

ZELENÁ E., ZELENÝ F., WONISCH A. & TAUSZ M. 2011. Effect of sulfur nutrition on glutathione content in sugar beet plants in relation with aphids infestation. – Phyton (Horn, Austria) 50 (2): 319–327, with 3 figures.

The effects of sulfur (S) supply combined with nitrogen (N) on plant growth and biosynthesis of glutathione (GSH) in relation with aphids infestation were studied in young sugar beet (*Beta vulgaris*) plants infested with black bean aphids (*Aphis fabae*). Both nutrients applied separately stimulated growth of leaves but their effects were amplified when applied together. Single N fertilization enhanced significantly reproduction of aphids, addition of S reduced this effect to about 30 %. The contents of GSH in leaves on variants without S were very low (about 5 nmol/g FW) and manifold increased when S was added (up to about 550 nmol/g FW in middle youngest leaves). Sulfur supplied plants had about 5 times more GSH in youngest leaves than in oldest. The positive effect of S on resistance of plants to aphids manifested itself only in sugar beet supplied with N. We suspect GSH may function in

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prevention of toxic saliva action of aphids on metabolism of nitrogen in host plant and thus influence the quality of their food.

Zusammenfassung

ZELENÁ E., ZELENÝ F., WONISCH A. & TAUSZ M. 2011. Effect of sulfur nutrition on glutathione content in sugar beet plants in relation with aphids infestation. [Der Einfluss von Schwefelernährung auf den Glutathiongehalt von Zuckerrüben im Verhältnis zum Blattlausbefall.]. – Phyton (Horn, Austria) 50 (2): 319–327, mit 3 Abbildungen.

Die Auswirkungen von Schwefelzugaben (S) in Kombination mit Stickstoff (N)-Gaben auf das Wachstum und auf die Biosynthese von Glutathion (GSH) im Verhältnis zum Blattlausbefall wurde an jungen Zuckerrüben, die durch mit Schwarze-Bohnen Blattläuse infiziert waren, getestet. Beide Nährstoffe getrennt gegeben förderten das Wachstum der Blätter. Wurden beide Nährstoffe gemeinsam zugesetzt, verstärkte sich dieser Effekt. N alleine förderte signifikant die Vermehrung der Blattläuse, S verringerte den Effekt um 30 %. Ohne S war der GSH-Gehalt sehr gering (ca. 5 nmol/g Frischgewicht). Er stieg um ein Vielfaches nach Zugabe von S (bis zu ca. 550 nmol/g Frischgewicht). Nach S-Gaben hatten die jüngsten Blätter 5-mal mehr GSH als die ältesten. Der positive Effekt von S auf die Blattlausresistenz zeigte sich nur bei Zuckerrüben, die auch N bekamen. Wir sind der Ansicht, GSH verhindert, dass die Blattläuse einen giftigen Einfluss auf den N Stoffwechsel der Wirtspflanzen – der dann die Qualität ihrer Nahrung verändert – nehmen können.

Introduction

Aphids are sucking insects that can cause plant growth reductions and malformations thus damaging crops. Moreover, they are important vectors of virus diseases.

As for phloem feeders, the limiting factor is nitrogen whereas the surplus of sucrose they exude in honeydew. Improved N nutrition and namely over-fertilization with N therefore often increase infestation of crops with aphids. Earlier work showed that this adverse effect of N can be balanced by S supplement. Balanced S nutrition increased not only N utilization for plant growth, but also plant resistance against aphids (ZELENÁ & ZELENÝ 2003, ZELENÁ & al. 2004).

A possible explanation for such an effect may be directly related to primary S metabolism. A low S/N ratio that limits protein synthesis, may improve the nutritional quality of plant material for aphids by increasing free amino acids and amides. Experiments with artificial aphid food confirmed that increased free amino acids and amides increased fecundity of aphids (KLINGAUF 1987, KARLEY & al. 2002).

S-containing metabolites often play an important direct role in defense against pathogens and herbivores. In this respect, the concept of “Sulfur-induced resistance” – SIR (SCHNUG & al. 1995) or, as formulated more recently, “Sulfur-enhanced defense” – SED (HELL & al. 2005) is of interest. Secondary S compounds, such as alliins and glucosinolates, are harmful for many generalist pests, but can even act as attractants for specialist pests (WALLSGROVE & al. 1999, HELL & KRUSE 2007).

Any herbivore effect on plants is not only dependent on plant chemistry determining nutritional quality, but also on plant defense reactions. Aphids deprive plants of photosynthates, and also evoke a defense reaction, perhaps triggered by their saliva. Aphids feeding on plants secrete two kinds of saliva. Gelling saliva forms a protective sheet around the stylet and watery saliva with a number of enzymes as pectinase, peroxidase, phenoloxidase may protect the aphids from harmful plant secondary metabolites and trigger defense responses through the production of reactive oxygen species (JIANG & MILES 1993, DE VOS & al. 2007).

In connection with S nutrition, low molecular weight thiol glutathione (GSH), may have important role in plant defense against aphids and other sucking insects pests. Biosynthesis of GSH depends on supply of plants with S (DE KOK L.J. & al. 1981). GSH might play a dual role in plant response to aphid attack. Due to its capacity to inactivate reactive oxygen species (ROS) via thiol-disulphide redox reactions and to detoxify xenobiotics by conjugation, GSH may have a role in inactivation of toxic saliva injected into the plant. In its function as a cellular redox buffer and participant in cellular signaling processes, it may have a role in coordinating further plant responses upon attack.

In the present study we treated sugar beet (*Beta vulgaris*) plants with different levels of N and S nutrition before subjecting them to aphids (*Aphis fabae*) attack to test whether improved S nutrition increased tissue GSH concentrations and whether changes in GSH concentrations correlate with infestation of plants with aphids.

Material and Methods

Sugar beet (*Beta vulgaris,* L.) cv. Granada seedlings were grown in growth chambers at constant conditions of day/night temperature 25/20 °C, 250 µmol photons $m^{-2}.s^{-1}$ (400–700 nm), photoperiod 16/8 h light/dark on topsoil with total content of C 1%, N 0,2%, S 0,015% and well supplied with other nutrients. Nutrients were mixed into the soil before the sowing, S in the form of sodium sulfate and N in the form of ammonium nitrate at doses 0,1 g / kg (S resp. N) and 0,2 g / kg (2S, resp. 2N) dry soil in following variants: C – unfertilized, N, 2N, S, N+S N+2S. The seedlings were grown in pots containing 300g dry soil (1 plant / pot), watered with distilled water using check weighting. Every variant had 8 replicate.

Twenty-three days after sowing half of plants from every variant were treated with aphids. Black bean aphids (*Aphis fabae*) reared on *Vicia faba* plants were transferred onto the sugar beet leaves, 5 adult females per plant. The next day the number of feeding aphids was checked and in case of missing aphids were replaced. Plants were harvested 30 days after sowing. Aphids were sampled and counted. Fresh matter of leaves was recorded and leaves were gently washed and immediately frozen at –50 °C.

Total glutathione was determined as described in KRANNER & GRILL 1993. In brief, frozen leaf material was ground in liquid N and extracted in 0.1 mol l^{-1} HCl with addition of polyvinylpyrrolidon. Thiol groups were labeled with the specific fluorescent dye monobromobimane, and separation performed on a gradient HPLC with fluorometric detection.

Statistical analysis: The results are presented as a mean value of 4 replications in each variant and were analyzed using Student-t test at $P \leq 0.05$.

Results

Both nutrients N and S stimulated leaf growth of sugar beet seedlings. Their effects were amplified when applied simultaneously. The differences between variants increased over time. At time of aphids treatment, plants supplied with S alone or in combined variants with N had about 30% greater fresh weight of leaves. In contrast N without S did not stimulate plant growth to this time. At harvest (30 days old plants) all fertilized plants had significantly stimulated growth, but the bigger stimulatory effect of S manifested itself. Plants in unfertilized controls formed less leaves (Fig. 1).

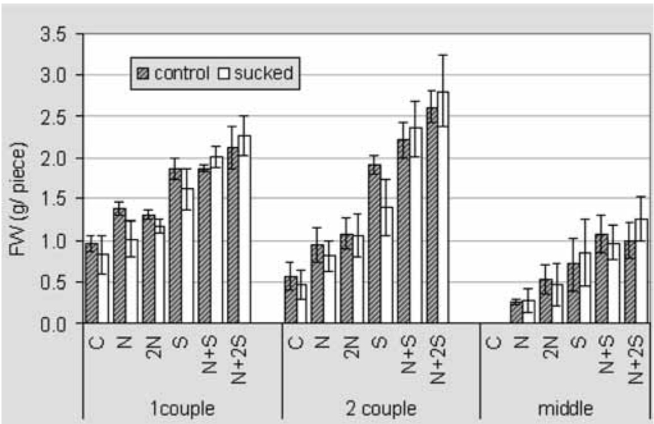

Fig. 1. Effect of N and S fertilization on growth of different age leaves from control and aphids infested plants. Data expressed as mean values of FW (g/piece) and confidentiality intervals at $P \leq 0.05$.

There were big differences in reproduction of aphids on particular variants (Fig. 2). Numbers of aphids on N variants were much higher than on all other variants. Single N fertilization enhanced the number of aphids to about 60 per plant. The doubled N dose did not further increase the infestation of plants. Sulfur applied alone did not influence reproduction of aphids, but added to N, it reduced the stimulatory effect of N to about

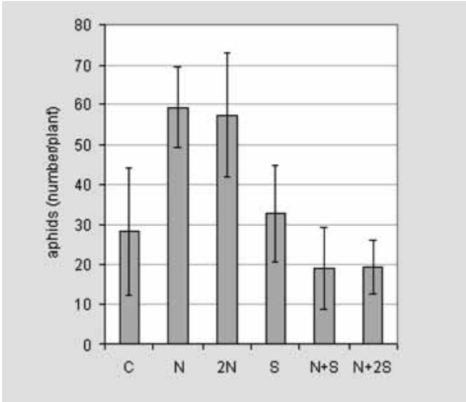

Fig. 2. Effect of N and S fertilization on infestation of sugar beet plants with aphids *Aphis fabae*. Data expressed as mean values of number of aphids/plant and confidentiality intervals at $P \leq 0.05$.

30%. Generally (after 7d of feeding) aphids did not cause any visible symptoms of damage of plants and in most cases any significant changes of leaves growth. (Fig. 1).

Improved S nutrition significantly increased GSH concentrations in sugar beet, up to 550 nmol/g FW in the middle – youngest leaves. There was a negative correlation between GSH content and leaf age, the youngest leaves contained about 5 times more GSH than the oldest. Unfertilized with S variants (C, N and 2N) had only about 5 nmol GSH /g FW and there were no differences between leaves of different age. Aphids after 7 days of feeding caused only insignificant changes of GSH contents except in oldest leaves on S (decrease) and N+2S (increase) variants (Fig. 3 a, b, c).

Discussion

Exposure of plants to various abiotic stresses trigger an accumulation of GSH in plant tissues (TAUSZ 2001, DE KOK & TAUSZ 2001). Increases of GSH content are part of plant responses to pathogens and may help to prevent damages caused by different microorganism (FOYER & RENNENBERG 2000, GULLNER & KÖMIVES 2001). Less information is available on the role of GSH in plants damaged by pests, namely insects.

The presence of GSH in insects themselves was found to decrease effect of pesticides, because conjugation with GSH is an important detoxification mechanism for xenobiotics. Hence, high concentrations of GSH in their host plant may assist pests to survive on pesticide treated plants (SCHNUG & SATOR 2001).

On the other hand, GSH present in leaves may increase resistance and defense capacity of plants against sucking insects. MILES & OERTLI 1993

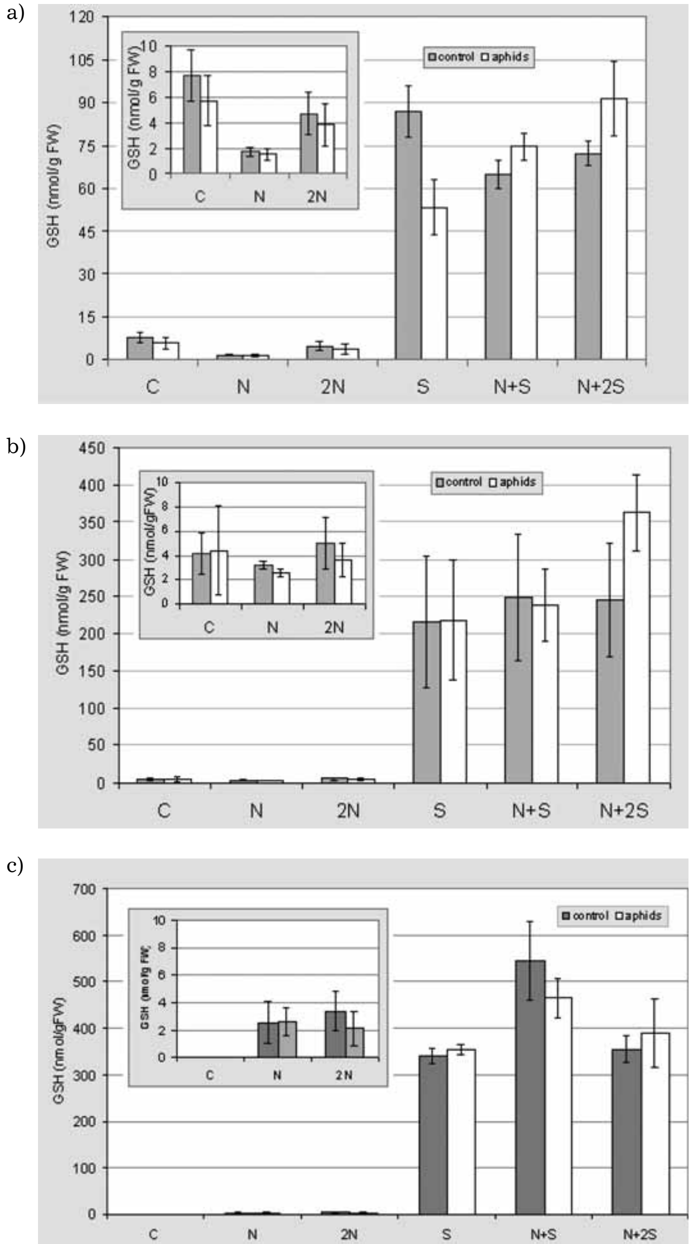

Fig. 3. Effect of N and S fertilization on glutathione content of different age leaves from control and aphids infested plants – a) 1^{st}couple, b) 2^{nd}couple c) middle leaves. Data expressed as mean values of GSH contents (nmol/g FW) and confidentiality intervals at $P \leq 0.05$.

found that the infiltrations of GSH and ascorbate into the stems of alfalfa cultivars depressed the reproductive rate of spotted alfalfa aphid (*Therioaphis trifolii maculata*) and blue green aphid (*Acyrthosiphon kondo*) feeding on those plants. According to the authors the redox system in plants controls oxidation rates during the responses of plants to attack by sucking insects. Antioxidants, such as ascorbate and GSH counteract oxidizing enzymes from aphids saliva and enhance the effectiveness of the plant defensive system. Salivary oxidases of insects may hasten oxidation of monomeric phenolics and quinones that are deterrent to insects.

In our study GSH content was significantly increased in sugar beet seedlings in all variants supplied with S. The differences between S fertilized and unfertilized variants were more significant in younger leaves, which is in agreement with data from literature (HARTMANN & al. 2000). Sulfur deficiency in control and N fertilized plants was manifested as a sharp decrease in GSH content in leaves. Only plants fertilized with N alone had significantly increased numbers of aphids. However there was no clear negative correlation between aphid infestation with GSH content across all variants. Plants fertilized with S alone had high content of GSH, but the number of aphids on them did not differ from the control. Controls plants had low content of GSH, yet also little infestation with aphids. We speculate that a shortage of N substances in leaves both on C and S variants decreased the quality of food for aphids.

Aphids caused mostly insignificant decrease of plants growth. Except of 1^{st} couple of leaves, where they caused decrease on S variant and increase on N+2S variant, they did not influence significantly nor content of GSH in leaves. The increase of GSH content after aphids action is in agreement with finding of ZECHMANN & al. 2009 in a study with sugar beet and *Aphis fabae*, that aphid attack strongly affected GSH content in leaf cells. This result was obtained using immunogold labeling of glutathione. GSH contents were higher in all cell compartments of cells close to the aphids stylets penetration site. Despite such localized response, overall leaf contents of GSH seemed in our study to be less affected by aphids feeding. The decrease of GSH content in leaves as consequence of aphids feeding on variant supplied only with single S, may be in fact the consequence of N shortage for new GSH biosynthesis.

It has to be taken into account that the number of aphids per plant was moderate and the period of sucking short. Large differences in GSH contents between particular variants may have masked potential changes in response to aphid attack. GSH was very low in S deficient plants and appeared excessively high in S fertilized plants. Hence, small changes of GSH content caused by aphids action may have been masked, or those plants were either deficient or luxuriously supplied with GSH and therefore did not respond to the treatment. Moreover, during 7 days of growing leaf biomass changed significantly and the number of feeding aphids per unit biomass at the time of harvest significantly decreased in variants with S.

In summary, we can conclude that improved S nutrition significantly increased tissue GSH concentrations in sugar beet leaves. In conditions of sufficient N supply of plants, improved S supply and increased GSH level, seemed to have a repressing effect on infestation of plants with aphids. We suspect GSH may function in prevention of toxic aphids saliva action on metabolism of nitrogen in host plant and consequently influence quality of food for aphids.

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