Impact of elicitors on glucosinolate production in plants and exudates of turnip (*Brassica rapa*)

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Zusammenfassung

Glucosinolate sind charakteristische sekundäre Pflanzenstoffe der *Brassicaceae*. Ausgewählte Glucosinolate besitzen nachweislich antikanzerogene, antioxidative sowie antimikrobielle Eigenschaften.
Die Extraktion von Glucosinolaten erfolgte bisher aus pflanzlichen Rest- bzw. Abfallmaterial (z.B. Brokkoli-Stiele), in denen oft nur sehr niedrige Konzentrationen an gewünschten, gesundheitsfördernden Gesamtglucosinolaten vorhanden sind. Zudem variiert der Gehalt an Gesamt- und Einzelglucosinolaten je nach Sortenwahl, Anbauverfahren und Erntetermin sehr stark, so dass das Rohmaterial eine sehr unterschiedliche Qualität hinsichtlich der Glucosinolatgehalte und des Glucosinolatmusters aufweist.
Durch die Wahl der *Brassica*-Art kann über die Ausnutzung des genetischen Potentials im ersten Schritte die Gehaltshöhe an Gesamt- und gewünschten, d. h. gesundheitsfördernden Einzel-glucosinolaten gezielt beeinflusst werden. Aufgrund dessen wurde die Teltower Rübe (*Brassica rapa* L. var. *rapa pygmea teltoviensis*) ausgewählt, weil dies in Vergleich zu anderen *Brassicaceae* einen höheren Gehalt an gewünschten Glucosinolaten wie z. B. Glucobrassicin, Gluconasturtiin und Neoglucobrassicin aufweist.
Wurzelexsudate sind im Vergleich zu Pflanzenextrakten relativ einfache Stoffgemische. Diese Eigenschaft der Wurzelexsudate kann den Aufwand und damit die Kosten für die Einengung und Reinigung zur Gewinnung der Glucosinolate minimieren. Pflanzen wurden in hydroponischen und aeroponischen Systemen kultiviert, so dass durch kontinuierliche Rhizosekretion die Glucosinolate ohne aufwendige Extraktionsverfahren aus den Wurzelexsudaten gewonnen werden konnten. Diese Technologie ermöglicht einen neuen und effizienten Weg für ein "Phytomanufacturing" zur Gewinnung von Glucosinolaten. Es wurde zudem eine Methode zur Separation der Glucosinolate vom Nährmedium und zu ihrer Anreicherung entwickelt.
Für ein ausgeprägtes Wurzelwachstum und damit für eine verstärkte Glucosinolatausbildung ist die Entwicklung eines geeigneten Anbausystems von entscheidender Bedeutung. Daher wurden hydroponische und aeroponische Systemen für Rübe konzipiert und verglichen.
Pflanzenwachstum, Rhizosekretion sowie auch die Glucosinolatsynthese können durch die Applikation verschiedener Nährlösungszusammensetzungen und durch die Anwendung von Elicitoren gesteuert werden. Um das Sekundärwurzelwachstum und Glucosinolatesynthese zur stimulieren, wurde eine spezielle Nährlösung entwickelt. Die Beeinflussung der Genexpression durch gezielte Elicitorenverwendung in den verschiedenen Schritten der Glucosinolatesynthese ermöglicht Veränderung der Gehalte und des Glucosinolatprofils in der Pflanze sowie in den Exsudaten. Als Elicitoren wurden Salicylsäure und Methyljasmonat ausgewählt. Da die Reaktion der Pflanzen auf die Elicitoren, und folglich die Erhöhung der Glucosinolatgehalte im Pflanzengewebe sowie in den Exsudaten kurzzeitig, und die Erhöhung der Glucosinolatsynthese in Abhängigkeit vom Entwicklungsstadium unterschiedlich hoch sein kann, wurde der Elicitoreneinfluss bei verschiedenen Entwicklungsstadien der Rübenpflanzen auf den Glucosinolatgehalt untersucht.
Durch die Optimierungen des Kulturmanagements erhörte sich der Glucosinolatgehalt in den Pflanzen und auch in den Exsudaten. Die Ergebnisse, die während dieser Forschungsarbeit gefunden worden, können in weiteren Untersuchungen für die Gewinnung von sekundären Metaboliten aus Pflanzen durch Wurzelsekretion genutzt werden.

Abstract

Glucosinolates are a group of phytochemicals found in plants of the family *Brassicaceae.* Due to their anti-carcinogenic, antioxidative, and antimicrobial properties glucosinolates have generated considerable interest for the food and pharmaceutical industry.
For the extraction of glucosinolates the plant wastes (*ex.* broccoli stalks) are used, but they often have low concentrations of health-promoting glucosinolates. Moreover, the content of total as well as individual glucosinolates depends strongly on plant variety, cultivation, harvesting and storage conditions, which influence the quality of the initial row material for glucosinolates manufacturing.
The content and profile of glucosinolates can be matched through the selecting of *Brassica* plants with proper genetic potential. Turnip (*Brassica rapa* L. var. *rapa pygmea teltoviensis*) was selected because of its high level of desirable glucosinolates, particularly glucobrassicanapin, gluconasturtiin, and neglucobrassicin, as compared to other *Brassicaceae*.
Glucosinolates could be produced by continuous rhizosecretion from the roots of soil-free grown plants. Root exudates are relatively simple mixtures in comparison to plant tissues, which allow reducing the costs and procedure of their extraction and purification. Moreover, to the advantages of rhizosecretion belongs its continuity. This technology offers a novel and efficient way of glucosinolate "phytomanufacturing". In the course of investigation it was developed the method of glucosinolate extraction from the growing medium.
To increase the yield of glucosinolates, it is necessary to activate their synthesis in plant tissues and to alter their exudation from plant roots.
For enhancing root growth, and consequently root surface, the optimal soil-free system had to be constructed. It was compared different, particularly hydroponic and aeroponic systems were compared.
Plant growth, glucosinolate synthesis, and rhizosecretion intensity can be regulated by creating special plant nutrition status and elicitor application. To increase the yield of glucosinolates from plant tissues and exudates it was composed special nutrient solution.
The way to modulate the profile of individual glucosinolates is to influence the expression of genes, involved in phytochemical synthesis by the elicitor application. Salicylic acid and methyl jasmonate were selected based on the previous investigations.
Plant response on elicitor application showed to be decreased with the time. This made important to study the kinetic of plant reaction on elicitor treatment and its dependence on the stage of elicitor application.
Through the development of the complex technology of turnip plant cultivation it was accessed the enhancement of total and individual, particularly the content of health-promoting glucosinolates in plants and exudates.
This study makes the contribution in developing of the approaches for designing the novel strategies for the production and isolation of phytochemicals.

LIST OF ABBREVIATIONS

SA	salicylic acid
MJ	methyl jasmonate
DIR	Delayed Inducible Resistance
RIR	Rapidly Inducible Resistance
SAR	Systemic Acquired Resistance
ARR	Age Related Resistance
JA	jasmonic acid
PR	patogenesis related
H1H	hydroponic with Hoagland solution
H2H	hydroponic with two times increased Hoagland solution
H2H2S	hydroponic with two times increased Hoagland solution and two times increased sulfur
AD1H	aeroponic with defensor with Hoagland solution
AD2H	aeroponic with defensor with two times increased Hoagland solution
AD2H2S	aeroponic with defensor with two times increased Hoagland solution and two times increased sulfur
AS1H	aeroponic with sprayers with Hoagland solution
AS2H2	aeroponic with sprayers with two times increased Hoagland solution
AS2H2S	aeroponic with sprayers with two times increased Hoagland solution and two times increased sulfur
HSA_0	hydroponic (with two times increased Hoagland solution, two times increased sulfur) with salicylic acid, applied at the beginning of the experiment
HMJ_0	hydroponic (with two times increased Hoagland solution, two times increased sulfur) with methyl jasmonate, applied at the beginning of the experiment
$ADSA_0$	aeroponic with defensor (with two times increased Hoagland solution and two times increased sulfur) with salicylic acid, applied at the beginning of the experiment
$ADMJ_0$	aeroponic with defensor (with two times increased Hoagland solution and two times increased sulfur) with methyl jasmonate, applied at the beginning of the experiment
$ASSA_0$	aeroponic with sprayers (with two times increased Hoagland solution and two times increased sulfur) with salicylic acid, applied at the beginning of the experiment
HSA_{15}	hydroponic (with two times increased Hoagland solution, two times increased sulfur) with salicylic acid, applied on 5^{th} day of the experiment
HSA_{20}	hydroponic (with two times increased Hoagland solution, two times increased sulfur) with salicylic acid, applied on the 20^{th} day of the experiment
HSA_{25}	hydroponic (with two times increased Hoagland solution, two times increased sulfur) with salicylic acid, applied on the 25^{th} day of the experiment

Some botanical and common name of plants:

Brassica rapa var. *rapa pygmeae teltoviensis*	- teltower turnip
Brassica rapa var. *rapifera*	- turnip
Brassica rapa	
Brassica napus	- oilseed rape
Brassica alba	- white mustard
Brassica nigra	- black mustard
Brassica oleracea var. *capitata alba*	- white cabbage
Brassica oleracea var. *italica*	- broccoli

Brassica oleracea var. *botrytis*	- white cauliflower
Brassica oleracea var. *Gommifera*	- Brussel sprouts
Raphanus sativus var. *sativus*	- radish
Nicotiana tabacum	- tabac
Lupinus luteus	- lupine
Arabidopsis thaliana	- arabidopsis
Solanum tuberosum	- potato
Rubia cordifolia	- indian madder
Isatis tinctoria	- woad

1 INTRODUCTION

Plants are valuable source of phytochemicals, many of which can be used as additives for functional foods as well as basic compounds for nutraceuticals and pharmaceuticals (Mulabagal and Tsay, 2004; Vanisree *et al*., 2004). More then 25 % of all pharmaceuticals contain active ingredients extracted from higher plants (Freese and Calpan, 2004). The aging population and ever-growing demand for better pharmaceuticals should foster the use of green plants as sources of new drug discovery and manufacturing (Fontanel and Tabata, 1987).

Besides direct extraction from plants and chemical synthesis to provide those compounds or derivatives with similar uses, plant cell cultures has been developed as promising alternative for producing metabolites that are difficult to be obtained by chemical synthesis or plant extraction (Zhao *et al*., 2005). However, it is often difficult to induce plant cells to produce desirable phytochemicals in sufficient quantities (Knorr, 1994). Probably the reason in many cases is that the growth without tissue differentiation is incompatible with the expression of phytochemical pathways (Wielanek and Urbanek, 1999).

The other problem is the high operating costs for the production and purification of phytochemicals, ranging averagely from 100 to 300 USD g^{-1} (DePalma, 2003). About 40 % of these costs are incurred for the production of phytochemicals and the rest for their recovery and purification. One of the possible ways to simplify the procedure of phytochemical extraction is the increase of their secretion into the culture medium (Knorr, 1994), and then obtaining them from exudates (Gleba *et al*., 1999). The root secretion (rhizosecretion) offers a simplified and more cost effective method for phytochemical isolation from growing medium rather than from complex tissue extracts. The future challenge for rhizosecretion lies in the successful development of cost-effective technologies for collection of biologically active molecules secreted by roots, and in their large-scale implementation. Hence, for cases when the conditions of plants growth can be optimized for rhizosecretion, plants root exudates can be used as a continuous source of valuable phytochemicals (Gaume *et al*., 2003).

Last years the glucosinolates, biologically active substances found in plants of the family *Brassicaceae,* have generated considerable pharmacological interest due to their human health-promoting effects, particularly anti-carcinogenic properties (Schreiner *et al*., 2000; Talalay and Fahrey, 2001). Based on the chemical structure of the side chain, glucosinolates can be divided into different classes such as aliphatic, aromatic, and indole (Fahrey *et al*., 2001). Glucosinolates, reported to be the most effective messengers against cancer development, are indole glucosinolate glucobrassicin and their derivates (Bonnesen *et al*., 2001). In addition, aromatic gluconasturtiin and glucotropaeolin are both also considered to be the strong anticarcinogens (Johnson, 2002). Aliphatic isothyocyanates, derivative from glucoalyssin (Mithen and Toroser, 1995) and glucoraphanin (Fahrey *et al.,* 1997) induce detoxification enzymes participating in cancer preventing processes.

The effects of glucosinolates on the quality of human food have encouraged interest in their natural biosynthetic pathways, and in the possibility of manipulating of their level in plants (Mithen *et al*., 2000). The profile of metabolites in plants is possible to influence with the endogenous transgenic manipulation of the signal pathway or with the exogenous addition of signal molecules (Baldwin, 1996). Manipulating of metabolite profile with transgenic technique is more complicated, because many phytochemicals are not directly gene products but, rather, the products of complicated biosynthetic pathways requiring many enzymatic-mediated steps.

However, the concentration of particular phytochemicals can be changed by overexpressing enzymes participating in metabolic pathways (Knorr, 1994).
Since the major roles of glucosinolates in plants are to protect them from attack of insects, herbivores and pathogens, some strategies for glucosinolate production based of this principle have been developed (Zhao *et al*., 2005). Accumulation of phytochemicals often occurs in plants subjected to stresses including various signaling molecules or elicitors (Dörnenburg and Knorr, 1995). Elicitation of hydroponically grown roots may increase the content of defense substances in plants and their exudates; moreover, it adds another unexplored dimension to the chemical diversity normally hidden in silent parts of the plant genome (Gaume *et al*., 2003).
Moreover, by exuding of glucosinolate from plants under controlled conditions and under the absence of plant damage it is possible to avoid the converting of glucosinolates into their hydrolysis products by the enzyme myrosinase, stored in plant tissues separately with glucosinolates and acting in case of tissue destruction.
The main aim of the present investigation was to develop the technology for turnip growing in soil-free systems, optimised for receiving of high yield of glucosinolates from plants and their exudates. This included the optimization of soil-free systems for plant growth; modification of nutrient solution for enhancing of turnip growth and increase glucosinolate content in plants and exudates; treatment the plants with elicitors, selection of more effective elicitor and optimal time for its application as well as development of the technology for recovery of exuded glucosinolates from the growing medium.
Turnip was selected for our studies because of its high level of glucosinolates as compared to other *Brassicaceae* (Schonhof *et al*., 2000).
The important task was to compare the intensity of glucosinolate production in plant parts and in exudates of turnip. The glucosinolate profile in leaves, secondary roots, and exudates of turnip had not been studied before and their content and profile differed form these for primary roots. It was also examined the content of glucosinolate in the parts of plants that have grown after elicitor treatment (primary roots) and compared to these of the directly (secondary roots) and non-directly (leaves) treated parts. Finally, it was established the differences in plant reaction of elicitor application on different stages of plant development.
This study could make the contribution in developing the approaches for designing the novel strategies for the production and isolation of glucosinolates.

2 REVIEW OF LITERATURE

2.1 Rhizosecretion as a technology for receiving the phytochemicals

2.1.1 Ability of plants to exude

Apart from the function of plant roots as organs for nutrient uptake, they also release a wide range of compounds into the root environment (Neumann and Roemheld, 2002). In annual plant species 30-60 % of the photosynthetically fixed carbon is translocated to the roots, and up to 70 % of it can be released into the rhizosphere. According to Merbach *et al*. (1999) the amount of root-borne compounds released into soil is 11-20 % of net CO_2-assimilation or 13-32 % of the ^{14}C incorporated into the plants. 5-6% of ^{15}N assimilated by plants are realized as root-borne N compounds. Root-born C and N compounds found in the root zone are mainly (60-80 %) water soluble. The water soluble extracts consist mainly of neutral (carbohydrates) and acid fraction (organic acids), while the basic fraction (amino acids) made up a small portion only.

Roots have the remarkable ability to secrete a vast array of low and high molecular weight molecules into the rhizosphere in response to biotic and abiotic stresses - in a process termed rhizosecretion (Bucher, 2001). Root secrete compounds should have a wide spectra of biological activities including protection against biotic and abiotic stresses (Walker *et al*., 2003).

While the evolution of plant shoots followed primarily "introverted" paths by perfecting physical barriers between themselves and the environment, roots had to be more "extroverted" in their relationship with soil (Gleba *et al*., 1999). This requirement created a unique set of biological mechanisms of plant defense.

Plants produce a compositionally diverse array of more then 100 000 different low molecular weight compounds (Bais *et al*., 2004). The rich diversity of phytochemicals arises because of selection for improved defence mechanisms against a broad array of microbes, insects and plants. The plant-borne compounds around roots are extremely heterogenous in their origin and composition (Merbach *et al*., 1999). Depending on root zone and root age, root-borne compounds originate from soluble extracts, from gelatinous substances from the root surface (mucilage, mucigel), from cell lysates, and from dead or sloughed off root cells. But since the separation and quantification of root-borne substances is extremely difficult, generally a chemical characterization is done by the rough separation according to their solubility in water and other solvents.

The functional importance of exuded compounds includes:

- influence on nutrient availability (García *et al*., 2001),
- activity and turnover of microbes (Bais *et al*., 2004),
- turnover of soil organic matter (Heim et al., 2001),
- protection against pathogens and herbivores (Akram and Hussain, 1987),
- inhibition of growth of competing plant species (Walker *et al*., 2003a).

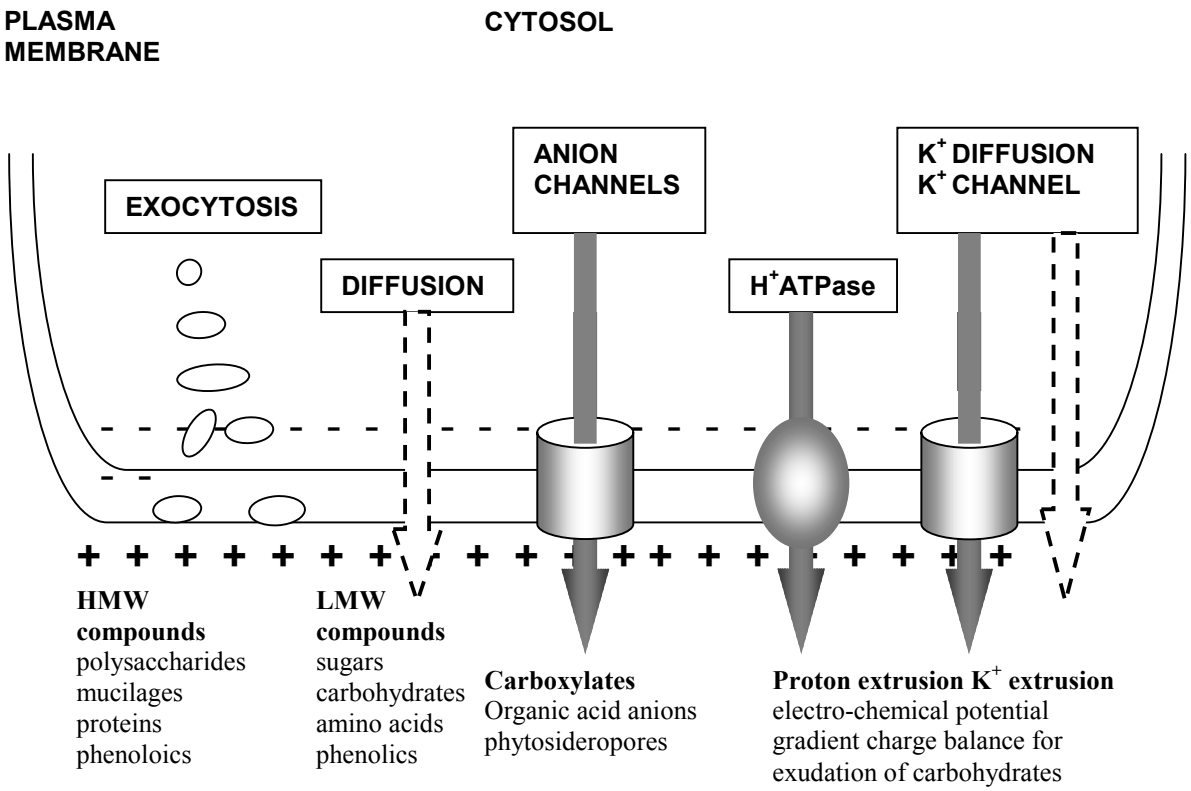
2.1.2 Ecological role of plant exudates

Bioactive compounds, exuded from plant roots belong to a group of phytochemicals, which are known to play a major role in the adaptation of plants to their environment (Mulabagal and Tsay, 2004). Root-root, root-microbe, and root-insect communications are likely continuous occurrences in the biologically active soil zone. This communication can be either positive

(symbiotic) to the plant, such as the association of epiphytes, mycorrhizal fungi, and nitrogen-fixing bacteria with roots or negative to the plants, including interactions with parasitic plants, pathogenic bacteria, fungi, and insects (Walker *et al*., 2003). Root exudates act as messengers that communicate and initiate biological and physical interactions between roots and soil organisms. The area of soil surrounding a plant root represents a unique physical, biochemical, and ecological interface between the roots and the external environment (Gatehouse, 2002). The rhizosphere is in part regulated by the root system itself through chemicals exuded into the surrounding soil.
A little is known about compounds in root exudates. Some resent works has brought light on the subject. Isoflavonoids and flavonoids presented in the root exudates of a variety of legume plants activate the *Rhizobium* genes responsible for the nodulation process and, possibly, for vesicular-arbuscular mycorrhiza colonization (Jones *et al*., 2004). Flavonoids, rosmaric acid, naphtoquinones, and shikonin from root exudates play an important role in root-microbe interaction (Bais *et al.*, 2004). A variety of plants produce herbicidal allelochemicals that may inhibit growth and germination of neighboring plants. It has been reported that flavonoids, ρ-hydroxy acids, quinines, and cytokinins arte used by plants to regulate the rhizosphere to the detriment of neighboring plants (Gransee and Wittenmayer, 2000).

2.1.3 Mechanisms of rhizosecretion

Although root exudation clearly represents a significant carbon cost to the plants, the mechanisms and regulatory processes controlling root secretion are just now beginning to be examined (Walker *et al*., 2003).
One mechanism by which plants cope with pathogens is the production of a “front line” of detached, living cells called border cells (Bais *et al*., 2004). These border cells exude a diverse array of biological chemicals that influence the behavior of fungi and bacteria. Border cells and their associated products can contribute up to 98% of the carbon-rich material that is released by plants as root exudates.
Root exudates can be released passively (diffusates) or actively (secretions) from the root cells.
Very little is currently known about the molecular mechanisms for the trafficking of phytochemicals. In at least some plants, channels are likely to be involved in the secretion of organic acids normally present at high levels in the cytoplasm. A good example is provided by the exudation of citrate, malate, and related organic acids by maize and wheat in response to high Al_3^+ concentrations (Neumann and Roemheld, 2002).
Root exudation of amino acids and sugars generally occurs passively via diffusion and may be enhanced by stress factors affecting membrane integrity, such as nutrient deficiency (K, P, Zn), temperature extremes, or oxidative stress.
According to Roshchina and Roshchina (1993) root exudates include low-molecular-weight phytochemicals as well as high-molecular-weight compounds (Figure 2.1). Low-molecular-weight compounds such as amino acids, organic acids, sugars, phenolics, and various other phytochemicals are believed to comprise the majority of root exudates (Walker *et al*., 2003). To high molecular weight compounds belong mucilage and proteins. Release of the major low-molecular-weight organic compounds of root exudates is a passive process along the steep concentration-gradient that usually exists between the cytoplasm of intact root cells (millimolar range) and the external solution (micromolar range) (Neumann and Roemheld, 2000).



HMW - high-molecular-weight, LMW - low-molecular-weight

Figure 2.1. Transport of exudates through cell membrane of plants (Neumann and Roemheld, 2002)

Direct diffusion through the lipid bilayer of the plasmalemma (Figure 2.1) is determined by membrane permeability, which depends on the physiological state of the root cell and on the polarity of the exuded compounds, facilitating the permeation of lipophilic exudates. At the cytosolic pH of approximately 7.1-7.4, more polar intracellular low-molecular-weight organic compounds such as amino acids and carboxylic acids usually exist as anions characterized with low plasmalemma permeability.

A positive charge gradient, which is directed to the outer cell surface as a consequence of a large cytosolic K^+ diffusion potential and of plasmalemma ATPase-mediated proton extrusion, promotes not only uptake of cations from the external solution but also the outward diffusion of carboxylate anions.

Root exudates often include phenylpropanoids and flavonoids, presumably synthesized on the cytoplasm surface of the endoplasmic reticulum (Winkel-Shirley, 2001). Although the mechanisms by which these compounds are transported from the endoplasmic reticulum to the plasma membrane are not known, it is possible that they are transported by endoplasmic reticulum-originating vesicles that fuse to the cell membrane and release their contents (exocytosis).

Also the involvement of membrane transporters such as ABC transporters might be responsible for the secretion of compounds (Martinoia *et al.*, 2002). ABC transporters use ATP hydrolysis to actively transport chemically and structurally unrelated compounds from cells. Thus, *Arabidopsis thaliana* contains 53 putative ABC transporter genes. Most of ABC transporters are localized in the vacuolar membrane and are believed to be responsible for the intercellular sequestration of cytotoxins. *Arabidopsis thaliana* ABC transporters AtPGP1, localized to the plasma membrane is involved in cell elongation by actively pumping auxin from its site of synthesis in the cytoplasm to appropriate cells. Plasma membrane ABC transporters might be

involved in the secretion of defense metabolites, and their expression may be regulated by the concentration of these metabolites.

2.1.4 Rhizosecretion of glucosinolates

Glucosinolates can be exuded by the roots of *Brassica* plants and then may be hydrolised by microbial myrosinase to release the isothiocyanates and other degradation products (Matthiessen, 1995). Bryant (2003) reported that *Brassicas* release glucosinolates into the soil, which further break down to isothiocyanates. The excreted glucosinolates can significantly suppress soil-born pathogens; reduce weeds such as pigweed, lambsquarter, and barnyardgrass, as well as several nematode species.
However, the recent studies, provided by Gimsing *et.al*. (2005) showed that glucosinolates are rather stable in soil.
Potter *et al*. (1998) reported about the high level of sinigrin (over 160 nmol g^{-1} soil) in the case of *Brassica caritana* amendment which showed slow anti-nematode activity.
Elliott and Stowe (1971) wrote about the discovering of glucosinolates in culture medium of *Isatis tinctoria*. They suggested three possible modes of releasing glucosinolates into medium: the compounds may be actively secreted from roots into the medium, they may be released from dead cells which are sloughed off into medium, or they may be released from the cut end of the excised root. Later they proved that actively growing healthy roots release glucosinolates.
Brown *et al.* (1994) showed that fresh tissues may release products more slowly than those physically disrupted prior to amendment, such as occurs by tissue crushing, air drying, freeze drying, or grinding. Lower maximum concentrations are thus likely to be produced from fresh tissue, but the period of release is potentially extended.
Lifetimes of glucosinolate products in the environment are generally short, which is an advantage when considering environmental impact (Brown and Morra, 1997). Glucosinolates and there degradation products isothiocyanates remain in soil for as little as a few days to a few weeks. The stability of individual glucosinolates varies strongly. In the studies of Brown and Morra (1997) 90 % of the isothiocyanates observed in rapeseed meal-amendment soil disappeared within 24 h, whereas only 60 % of allyl isothiocyanates amended in the form of an aqueous phase mixture to the same soil was lost during this time.
Nine glucosinolate degradation products, including five isothiocyanates, three nitriles, and one oxalozidine were identified in field soils extracted after *Brassica napus*. The most abundant products were 2-phenylethyl isothiocyanates (from gluconasturtiin). Isothiocyanates have also been collected in the rhizosphere of a few plants (Borek *et al.,* 1995). Also Brown *et al.* (1994) showed that the isothiocyanates were produced as the dominant product regardless of soil characteristics.
It seems that water prevents the destruction of glucosinolates and their products in soil. Methyl isothiocyanates has reported solubility in water of 7.6 mg ml^{-1} (Borek *et al.*, 1996). Increased water content increased the half-life of allyl isothiocyanates. Thus, continually wet conditions, especially when combined with cold temperatures, could result in increasing isothiocyanates lifetimes. However, no correlation between soil pH and allyl isothiocyanates or thiocyanates disappearance was observed for soils ranging in pH from 4.35 to 9.10.
Choesin and Boerner (1991) suggested that the transporting of glucosinolates from one part of plant to other and perhaps their secretion from root into the growing surrounding is the selective process. Chen *el al.* (2001a) showed that the difference between the relative glucosinolate content in phloem and leaves is stipulated by the selection for specific glucosinolates loaded into phloem.

2.1.5 Molecular farming

Compounds exuded from roots of hydroponically grown plants are not mere leakage products, but are evolutionarily perfected signaling and defense molecules synthesized in response to various environmental signals (Borisjuk *et al*., 1999).
Knowing the fact that *Brassica* plants can exude glucosinolates into the growing medium we suggested collecting the glucosinolates from the root exudates of hydroponically grown plants.
Root-exuded compounds can be effectively collected in a specially constructed hydroponic system (Gaume *et al*., 2003). In addition, the purification of individual compounds is a relatively simple task as compared to the tissue extraction methods. Gleba *et al.* (1999) attempted to obtain the heterologous proteins from *Nicotiana tabacum* rhizosecretes: green fluorescent protein of the jellyfish *Aequorea victoria*, human placental secreted alkaline phosphatase, and xylanase from the thermophylic bacterium *Clostridium thermocellum*.
Swarup *et al*.(2004) showed that the plants of *Arabidopsis thaliana* exuded until 41.5 % of synthesized in plant tissues flavonols into the growth surrounding. In addition it was figured out, that exudates content also antocyanins, phytoalexins, glucosinolates, lignin precursors, conjugated glucosides of several phenolic compounds.
The biochemical analysis of root exudates from 120 plant species, provided by Gleba *et al*. (1999), can be summarized as following:
- Each plant exudes a distinct set of compounds, which is a unique biochemical fingerprint for a given species. The extraction and purification of phytochemicals from the biochemically complex plant tissues is a laborious and expensive process that presents a major obstacle to large scale manufacturing. However, these *in vitro* systems (cell or hairy root culture) may be expensive, slow growing, unstable, and relatively low yielding. The non-destructive rhizosecretion process may provide high yields of phytochemicals over the lifetime of plant and facilitate their downstream purification, combining the advantages of the whole plant and *in vitro* protein expression systems.
- Root exudates are relatively simple mixtures, in comparison to solvent extracts of plant tissue, which makes the isolation of the active molecules an easier task.
- Root exudates are devoid of pigments and tannins, known to interfere in activity screens and do not contain large quantities of biologically inert structural compounds.
- Chemical composition of root exudates is very different from that of conventional methanolic extracts of root tissue.
- Rhizosecretion can be operated continuously without destroying the plant, thus, producing a higher total yield of the phytochemicals over the plant life.

It exist the technology of glucosinolate separation from plant tissues, based of the biorefining of cruciferous oilseed crops or aqueous enzyme-aided extractions, which is also called "Green Chemistry" technique (Bagger *et al*., 2003). The separation occurs in aqueous emulsion without the use of organic solvents and it allows oilseeds to be transformed into such products as lipids, oil, and protein products, carbohydrates, special fibres, and various types of low-molecular-weight compounds, including glucosinolates and derived substances (Figure 2.2). "Green chemistry" is the improved technology of separation, because it uses for degradation of plant cell walls enzymes instead of chemical solvents. Separation of the rape seed constituents occur during decanting, sieving, centrifugation and flash chromatography separation of *Brassica napus* during fractioning of the 1st generation into oil, syrup, protein, and hulls, and following the 2nd generation, were the outlet substances are oil, emulsifiers, surfactants, proteins, bioactive substances (among them glucosinolates), ampiphillic lipids, and protein fiber mix (Bagger *et al*., 1999).

We suggested that by using of exudates as a source of glucosinolates it is possible to shorten this process and avoid the stage of cell wall degradation as well as enzyme inactivation, milling, centrifugation, and decanter separation (Figure 2.2).

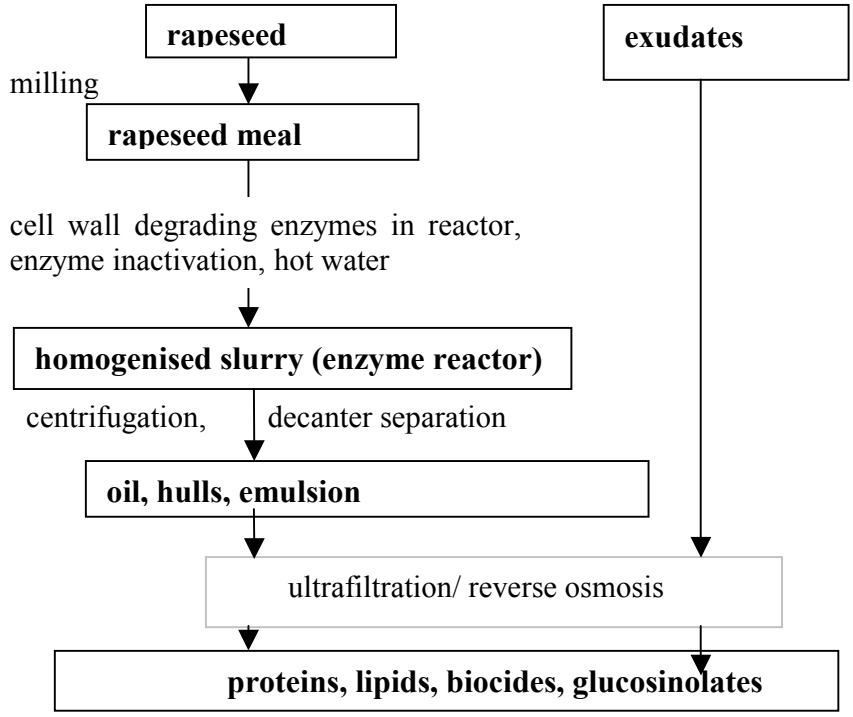


Figure 2.2. Comparison "Green chemistry" (Bagger *et. al*, 1999) and the technology for receiving glucosinolates from exudates

In open (flowing culture system) the exudates may be removed from the circulating nutrient solution. Collection of root exudates in solution generally yields much diluted solution of exudates (Engels *et al*., 2000). This problem may be solved by the development of technology for exudates concentration by decreasing of volume of trap solution *e.g*. by freeze-drying or rotevaporation (depends on the stability of the compounds). The volume restriction may lead to high salt concentrations which can result in precipitation of exudates. We suggest that interfering salts could be removed by ion exchange resigns prior to volume reduction. Low- and high-molecular-weight compounds can be separated by ultrafiltration.

2.1.6 Ways for increasing rhizosecretion

Rhizosecretion can be affected by multiple factors, such as light intensity, temperature, nutritional status of plants (Lasserre *et al*., 2003), activity of retrieval mechanisms, stress factors, sorption characteristics of the growth medium (Engels *et al.*, 2000).

It is possible to assume, there are some different ways to increase the yield of exuded phytopharmaceuticals. The most reliable of them are:

- to increase the absorptive root surface area (Bucher, 2001). Cluster roots are functionally linked with the excretion of large amounts of organic chelators and enzymes;
- to increase the content of phytochemicals in plants, which can lead to enhance of there content in exudates: this is possible to realize by elicitor application (Kiddle *et al*., 1994). It has been observed that exudate chemical diversity as well as quantity of certain compounds can be greatly increased by the

elicitation process. Elicitors mimic the effects of stresses on plant roots, activating biochemical defense systems and resulting in quantitative and qualitative changes in the composition of the exudates (Gaume *et al*., 2003). Walker *et al.* (2003) have shown that chemical or biological elicitors, acting as natural stresses, stimulate roots to exude an array of compounds not detected in the constitutively expressed exudates of *Arabidopsis thaliana*.

2.2 Glucosinolates as biologically active substances

2.2.1 Chemical structure, classification and properties

Glucosinolates are a class of about 120 thioglucosides that are characteristic of the *Brassicaceae* plants and related families in the order Capparales (Kjaer, 1976). They consist of a ß-D-thioglucose reduced group, a sulfonated oxime moiety, and a variable side-chain derived from amino acids (Figure 2.3) (Mithen *et al.*, 2000).

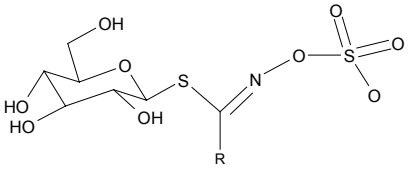


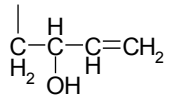
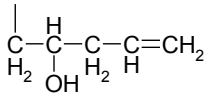
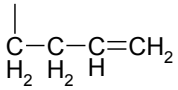
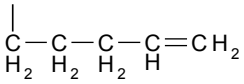
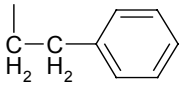
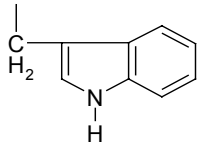
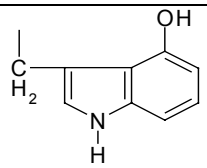
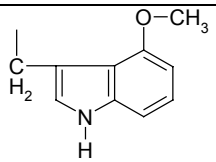
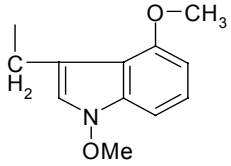
Figure 2.3. General formula of glucosinolates

Based on the chemical structure of their side chain, glucosinolates can be subdivided into three classes: aliphatic glucosinolates (derived from methionine), aromatic glucosinolates (from phenylalanine and possibly from tyrosine), and indole (derived from tryptophan) (Bennett and Wallsgrove, 1994; Mithen, 2001) (Table 2.1).
The sulfate group imparts strongly acidic properties and consequently glucosinolates occur in nature as anions counterbalanced by cations. The cation is usually potassium, being one of the most abundant cations in plant tissues. The sulfate group and the thioglucose moiety impart nonvolatile and hydrophilic properties to all glucosinolates, the R group is variable in properties from lipophilic to hydrophilic (Fahrey *et al*., 2001). The variable structure of the side chain confers a variety of chemical properties, influencing parameters such as volatility, miscibility, and membrane transmissibility (Potter *et al*., 1998).
Glucosinolates in *Arabidopsis thaliana* have been found to be highly enriched in certain sulfur containing S-cells, which are called giant cells that line the phloem between the vascular bundles and the endodermis (Adreasson *et al*., 2001).

2.2.2 Biological functions in plants

Glucosinolates and there breakdown products act as allelochemicals and play a role in plant defenses, moreover, they inhibit microbial growth (Glenn *et al*., 1988), intermediate in biosynthesis of indole phytoalexins (Wynnegriffiths *et al*., 1994), or stimulate oviposition and feeding by insects (Mewis *et al*., 2005). Glucosinolates also act as feeding deterrents for polyphagous herbivores and feeding stimulants for crucifer-specialists. For example, among *Brassica rapa* pests, glucosinolates deter feeding by polyphagous grey field slug and stimulate feeding of the oligophagous cabbage stem flea beetle (Bartlet *et al*., 1999). Particularly indole are known to be the most potent class of glucosinolates in stimulating several crucifer-feeding insects. The traditional use of crucifers as green manures is partly based upon the toxic nature of the glucosinolate degradation products, which serve to reduce the soil inoculum of pathogens and pests for subsequent horticultural crops (Mithen *et al.*, 2000). High levels of glucosinolates have been shown experimentally to impose metabolic stress on generalist invertebrate herbivores, and to cause impaired growth.

Table 2.1. Structure and classification of glucosinolates

Subclasses of glucosinolates		
Trivial name	**R-group**	**Structure of R-group**
1. Aliphatic		
progoitrin	2-hydroxy-3-butenyl	
gluconapolieferin	2-hydroxy-4-pentenyl	
gluconapin	3-butenyl	
glucobrassicanapin	4-pentenyl	
2. Aromatic		
gluconasturtiin	2-phenylethyl	
3. Indole		
glucobrassicin	3-indolylmethyl	
4-hydroxy-glucobrassicin	4-hydroxy-3-indolylmethyl	
4-methoxyglucobrassicin	4-methoxy-3-indolylmethyl	
neoglucobrassicin	1-methoxy-3-indolylmethyl	

In the case of *Brassica rapa*, high glucosinolate content is associated with reduced grazing by slugs and pigeons, and there is evidence that in certain habitats the exposure of wild populations of *Brassica oleracea* to herbivores imposes selection pressure for increased level of aliphatic glucosinolates (Raybould and Mozes, 2001). However, other invertebrate herbivores which specialize on *Brassicas* have become adapted to the presence of glucosinolates.

Indole glucosinolates can be converted into indoleacetic acid and may thus contribute to active auxin levels in plants.

Glucosinolates may be a sink for nutrients like nitrogen and sulfur. Fenwick and Heany (1983a) reported that glucosinolates are probably used by the plants as a sulfur source, since

there are already some indications that double-zero *Brassica rapa* is more sensitive to sulfur deficiency than single-zero plants. However, Fieldsend and Milford (1994) suggested that glucosinolates contained only a small proportion of the crop total sulfur and that they were unlikely to be a major source of recyclable sulfur, even under conditions of severe deficiency.

2.2.3 Glucosinolates in human health promotion

As components of human food, the biological activities of glucosinolates and their hydrolysis products have generated considerable pharmacological interest. Depending on glucosinolate composition and on the prevalence of hydrolysis products, consumption of glucosinolates has been linked with a reduced risk of developing cancer (isothiocyanates), but in some cases with goitrogenic effects (thiocyanates) (Figure 2.8). Moreover, glucosinolates and their breakdown products are responsible for the bitter taste as of *Brassica nigra*; they also contribute to the characteristic flavours of *Brassica* vegetables (Charron *et al*., 2004).
Glucosinolates are accompanied in plant tissues by the destructive enzyme myrosinase, but in the absence of myrosinase humans can efficiently convert glucosinolates through the action of microflora of gastrointestinal tract (Talalay and Fahey, 2001).
Boiling and microwave cooking lead to extensive loss of glucosinolates through enzyme-mediated degradation, thermal degradation, and leaching into the cooking water (Slominski, 1989).

Anti-carcinogenic properties of glucosinolates
Brassica vegetables such as *Brassica oleracea* var. *botrytis*, *Brassica oleracea* var. *capitata alba*, *Brassica oleracea* var. *italica*, and *Brassica rapa var. rapifera* contain high level of glucosinolates, of which the degradation products have strong anticarcinogenic properties (Zhang, 1994).
Zhao *et al*. (2001) reported about the protective effects of *Brassica oleracea* var. *italica* against lung cancer and colorectal polyps. According to Zhang and Talalay (2001) glucosinolates and their destruction products isothiocyanates protect against tumor development in liver, mammary gland, and forestomach. They target mammalian Phase 1 and Phase 2 metabolizing enzymes and their coding genes, resulting in decrease carcinogen-DNA interactions and in increased carcinogen detoxification (Figure 2.4).
There are 3 ways of chemical prevention of cancer by glucosinolates:
- Stimulation of anti-poisoning enzymes. The beginning of cancer development starts with activating of consumed carcinogen by Phase 1 enzymes (monooxygenases) that metabolize lipophilic procarcinogens, often converting them to highly carcinogenic epoxides (Longcope, 1994). Studies on sinigrin have shown it suppresses Phase 1 enzymes. Also isothiocyanates such as phenylethyl isothiocyanate (from gluconasturtiin) and benzyl isothiocyanate (from glucotropaeluin) modify the balance of Phase 1 and 2 enzymes that are expressed in liver, and in epithelial cells including these of the colon (Misiewicz *et al*., 2004).
- Suppression of cancer cell growth. The described above stage of syntheses of highly carcinogenic epoxides can be blocked with Phase 2 enzymes. Glucosinolates and there isothiocyanates activate Phase 2 enzymes, which belong to glutathionetransferase family and increase the detoxification of chemical carcinogens. Phase 2 enzymes metabolize the products of Phase 1 and form inactive conjugates (Johnson, 2002).

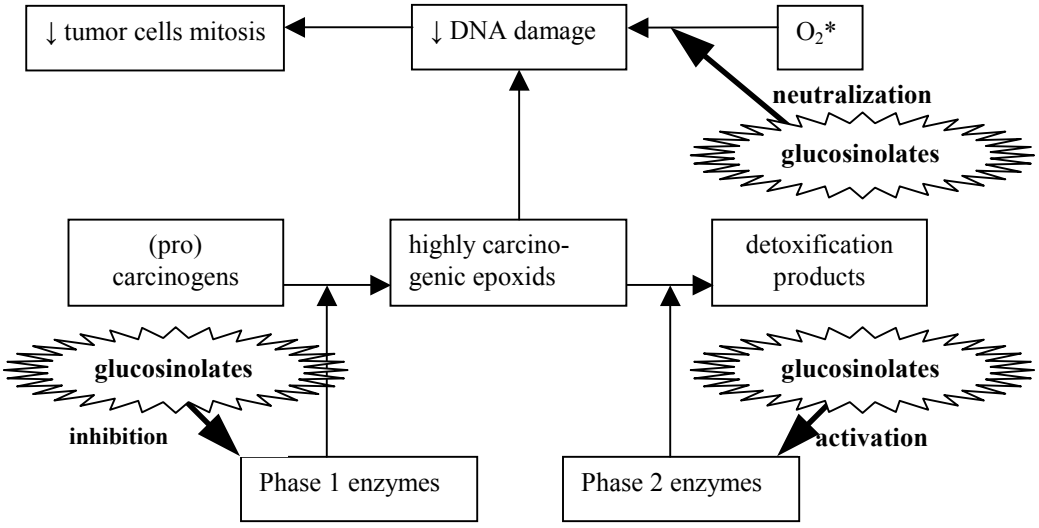


Figure 2.4. Anticarcinogenic action of glucosinolates

Isothiocyanates also inhibit mitosis and stimulate apoptosis in human tumor cells. This second effect raises the possibility that in addition to blocking DNA damage, isothiocyanates may selectively inhibit the growth of tumor cells even after initiation by chemical carcinogens (Sones, 1984). The ability of isothiocyanates to induce Phase 2 enzymes may be linked to their ability to suppress the proliferation of preneoplastic cells. Phenylethyl isothiocyanate (derivative of gluconasturtiin), inhibits the action of lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (McDanell and McLean, 1988).

Thornalley (2002) reported that natural isothiocyanates derived from aromatic and aliphatic glucosinolates are effective chemoprotective agents that block chemical carcinogenesis and prevent several types of cancer. For example, the isothiocyanate of sulforaphane inhibits Phase 1 enzyme-mediated activation of pro-carcinogens, induces Phase 2 detoxification enzymes such as quinonereductase and glutathione-S-transferase in hepatoma cells, and blocks mammary tumor formation in rats (Posner, 1994).

Talalay and Zhang (1996) showed that glucosinolates also induced increased activities of enzymes involved in the detoxification and conjugation of carcinogens for elimination: glutathione S-transferases, quinone reductase, epoxide hydrolase and UDP glucuronosyl transferase. Inhibition of tumor growth in pre-clinical development by isothiocyanates may contribute to the association of decreased cancer incidence with dietary glucosinolate consumption.

- **Neutralization of reactive oxygen radicals (indirect way).** Glucosinolates act as antioxidants and neutralize reactive oxygen radicals.

Neuhauser *et al*. (2003) wrote that 64 % of the case-control studies showed an inverse association between consumption of *Brassica* vegetables and risk of cancer. This may be circumvented by prophylactic therapy particularly for people with otherwise unavoidable high risk of developing tumours that are susceptible to chemoprevention by isothiocyanates.

Flavour and taste

Glucosinolates and their breakdown products are important determinants of flavor and taste of *Brassicas* (Mithen *et al.*, 2000). Allyl isothiocyanate is largely responsible for the characteristic hot flavours of mustard and horseradish, and the glucosinolates sinigrin and progoitrin confer bitterness on *Brassica* vegetables. Bitterness in some *Brassicas* is due to high levels of progoitrin and gluconapin glucosinolates (Fenwick, 1983).

Goitrogenic effect

The presence of glucosinolates in seeds of oilseed cruciferous crops significantly reduces the quality of the seed meal left following oil extraction. This is largely due to the presence of certain glucosinolates, which degrade to give goitrogenic products (Mithen *et al*., 2000).
The most notable of these is oxazolidine-2-thione derived from progoitrin which accumulates in the seeds of *Brassica napus*. In addition to alterations to the size, structure, and function of the thyroid, feeding rapeseed meal can lead to damage to the liver and kidneys (Holst and Williamson, 2004) (Figure 2.8).

Bioavailability

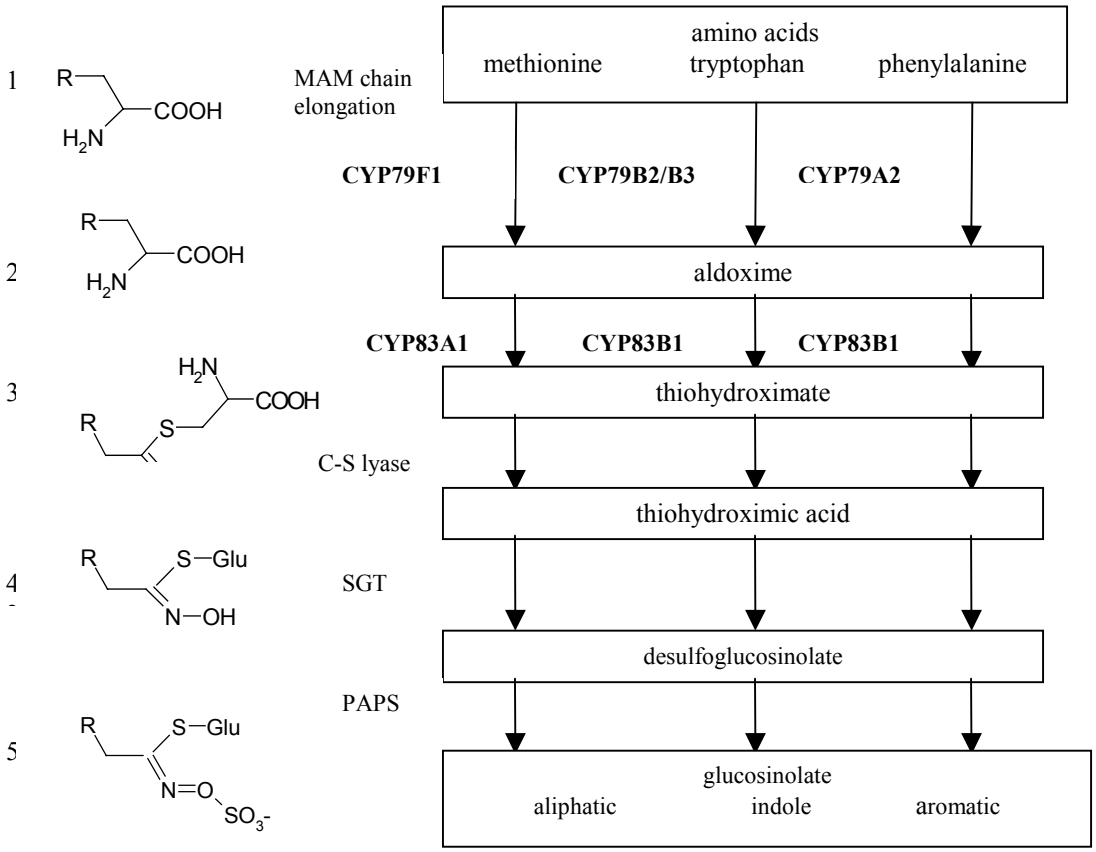
If vegetables are eaten raw, both intact glucosinolates and active myrosinase are ingested simultaneously, which enables the breakdown of the glucosinolates to occur within the alimentary tract (Johnson, 2002). Some of the ingested glucosinolates are also broken down in colon by bacterial microflora, which also expresses myrosinase activity.
If plant myrosinase is deactivated, glucosinolates can be metabolised by intestinal bacterial enzymes (Mithen *et al.*, 2000).
Glucosinolate degradation also occurs to some extent in the upper digestive tract, probably by spontaneous chemical degradation, since no myrosinase activity has ever been found in digestive tissues (Jongen, 1996). The acid environment of the stomach is known to convert indole-3-carbinol, a major hydrolysis derivative of glucobrassicin, into a range of polycyclic aromatic condensation products.

2.2.4 Glucosinolate biosynthesis

Despite the diversity of glucosinolate side-chains, only seven of these structures correspond directly to a protein amino acid (alanine, valine, leucine, isoleucine, phenylalanine, tyrosine and tryptophan) (Bak *et al.*, 1996). The diversity of glucosinolates is produced by the combination of several protein amino acids, variation in chain-elongation for some of these, and secondary modifications of the glucosinolate side chain and thioglucose moiety (Fahey *et al.*, 2001; Chen and Andreasson, 2001) (Figure 2.5).

Glucosinolate biosyntesis can be represented as following:

The amino acid is hydroxylated to the N-hydroxyamino acid in the presence of oxygen and NADPH. The N-hydroxyamino acid is decarboxylated to give the aldoxime.
The conversion of amino acids to aldoximes is a key step in glucosinolate biosynthesis. This step is catalyzed by cytochromes P450 from the CYP79 family (Xu *et al.*, 2001). Three genes are involved in aldoxyme formation. They convert methionine (CYP79F1/F2), phenylalanine (CYP79A2), and tryptophan (CYP79B2/B3) to the corresponding aldoximes (Halkier and Du, 1997). Cytochrome P450 mono-oxygenases convert aliphatic and aromatic amino acids as well as tryptophan to their corresponding oximes (Mikkelsen *et al.*, 2000). The conversion of chain-elongated forms of methionine and phenylalanine is catalyzed by flavin-containing monooxygenases while the conversion of tryptophan to indole acetaldoxime, as the precursor of indole glucosinolates, is mediated by plasma membrane bound peroxidases (Mithen *et al.*, 2001). The specificity for each of these enzymes for the particular amino acid substrate may provide one point in the biosynthetic pathway where different classes of glucosinolates could be independently regulated, and is thus of interest to genetic modification approaches which may seek to up or down regulate specific glucosinolates.



MAM – methylthioalkylmalate synthase, SGT - S-glucosiltransferase, PAPS - 3'-phosphoadenosine-5'-phosphosulfate

Figure 2.5. Glucosinolate biosynthesis in *Arabidopsis thaliana* (according to Mikkelsen *et al.*, 2003; Halkier and Du, 1997; Chen *et al.*, 2003).

The conversion step in the chain elongation of methionine is catalyzed by methylthioalkylmalate synthases: MAM1 which is responsible for dihomo-methionine formation and MAM2 participating homo-methionine formation). They control whether methionine is chain-elongated with one or two methylene groups (Mikkelsen *et al.*, 2002). CYP79F1 gene encodes cytochrome P450, the enzyme catalyzing metabolism of short-chain methionine derivatives in the biosynthesis of aliphatic glucosinolates (Tantikanjana *et al.*, 2004). Heterologous expression of CYP79F1 showed that the enzyme was able to convert homo-, dihomo-, trihomo-, tetrahomo-, and pentahomo-methionine to the corresponding aldoximes (Naur *et al*., 2002). Cytochrome P450 of CYP79A2 participates the conversion of phenylalanine into aromatic glucosinolates, and CYP79B2 as well as CYP79B3 into indole glucosinolates (Kliebenstein *et al*., 2005).
The next step in glucosinolate synthesis is the reduction of aldoximes to thihydroximates. CYP83B1 has been shown to have high affinity for tryptophan- and phenylalanine-derived aldoximes (Bak and Feyereisen, 2001), although it does metabolize the aliphatic aldoximes with very low affinity. CYP83A1 has high affinity for the aliphatic aldoximes although it does metabolize the aromatic aldoximes with low affinity (Bak and Feyereisen, 2001).
The thiohydroximate is then formed by introduction of sulfur, where sulfur donor is cysteine or methionine. Thiohydroximic acid is formed by cleavage of the S-alkylthiohydroximate in reaction thought to be catalyzed by C-S lyase (Wallsgrove and Bennett, 1995). The post-oxime

enzymes are specific for the functional group, and mostly unspecific for the side chain. They include a single gene family C-S lyase that cleaves the C-S bound to produce a thiohydroxymic acid, a thio-glucosiltransferase (UGT74B1) that is likely to have functional homologues, and finally three sulfotransferases ST5a, ST5b, and ST5c, of which ST5a has indole desulfoglucosinolate as preferred substrate (Piotrowski *et al*., 2004). C-S lyase is identified recently as the *SUPERROOT1* gene product (SUR1) (Piotrowski *et al*., 2004).
Thiohydroximic acid converts to desulphoglucosinolates in presence of SGT (S-glucosyl-transferase or UDPG-thiohydroximate-glucosyltransferese) (Piotrowski *et al*., 2004).
The final step in the biosynthesis of glucosinolate core structure is catalyzed by desulpho-glucosinolate: PAPS sulfotransferase, or PAPSST (3-phosphoadenosin-sulfate: desulpho-glucosinolate sulphotranspherase), which convert desulphoglucosinolates to glucosinolates (Piotrowski *et al*., 2004). The transfer of the active sulfate group from PAPS to acceptor molecules is catalysed by sulfotransferases. Three subgroups of sulfotransferases have been identified in plants: the steroid sulfotransferases from *Brassica napus*, hydroxyjasmonic acid-specific sulfotransferase from *Arabidopsis thaliana*, and an additional sulfotransferase (RaR047, At2g03760) has been cloned from *Arabidopsis thaliana*, and its mRNA level was found to be up-regulated by pathogens and SA. Kliebenstein *et al*. (2005) represented the following classification of sulfotransferases: AtST5a (acts preferably on tryptophan- and phenylalanine-derivatives), AtST5b and AtST5c (both act preferably on methionine-derivatives).
After biosynthesis of the core structure, glucosinolates are subjected to secondary modifications (Mikkelsen *et al.*, 2003). These include: oxidations of the side chain sulfur to sulfinyl or sulfonyl compounds and loss of the methylthiogroup (which leads to the formation of a terminal double bond), hydroxylation, methoxylation, glucosylation, sulfation (Wallsgrove and Bennett, 1995).

Peculiarities of aliphatic glucosinolate synthesis
Following the biosynthesis of methylthioalkyl glucosinolates from methionine, the side-chain may undergo various modifications. The suggested pathway involves an initial oxidation to methylsulphinylalkyl, followed by the removal of the methylsulphinyl group and desaturation, which results in formation of alkenyl glucosinolates, and subsequent hydroxylation to give hydroxyalkenyl glucosinolates (Mithen *et al.*, 2001).
In *Brassica napus* two loci *Gsl-oh-C* and *Gsl-oh-A* regulate the hydroxylation of several alkenylglucosinolates (Figure 2.6). Genetic studies lead to the proposal of a model where the initial oxidation of methionine-derived glucosinolates is regulated by alleles of the *Gsl-oxid* loci, and the oxidation to alkenyl glucosinolates is regulated by alleles of the *Gsl-alk* loci (Mithen *et al.*, 1995). Three genes, *AOP1, AOP2,* and *AOP* encode 2-oxoglutarate-dependent dioxygenases (Kliebenstein *et al.*, 2002). No function is assigned to AOP1, but recombinant AOP2 was shown to catalyze the conversion of glucoiberin and glucoraphanin to the corresponding alkenylglucosinolates. Furthermore, recombinant AOP3 is shown to catalyze the conversion of glucoiberin to glucoibeverin.

Peculiarities of aliphatic glucosinolate synthesis
The first committed step in synthesis of the core structure of indole glucosinolates in *Arabidopsis taliana* is the conversion of tryptophan to indolaldoxime, a step catalyzed by the substrate-specific cytochromes P450 CYP79B2 and CYP79B3 (Hansen and Halkier, 2005). Of the two oxime-metabolising enzymes CYP83A1 and CYP83B1, the latter the substrate specifity towards indole and aromatic oximes, which are oxidized to an active oxime proposed to be either aci-nitro or nitrile oxime, that is subsequently conjugated to a sulfur donor, most likely cysteine. Indole alkaloid camalexin, found in *Arabidopsis thaliana*, derives from indolealdoxime produced by CYP79B2 and CYP79B3.

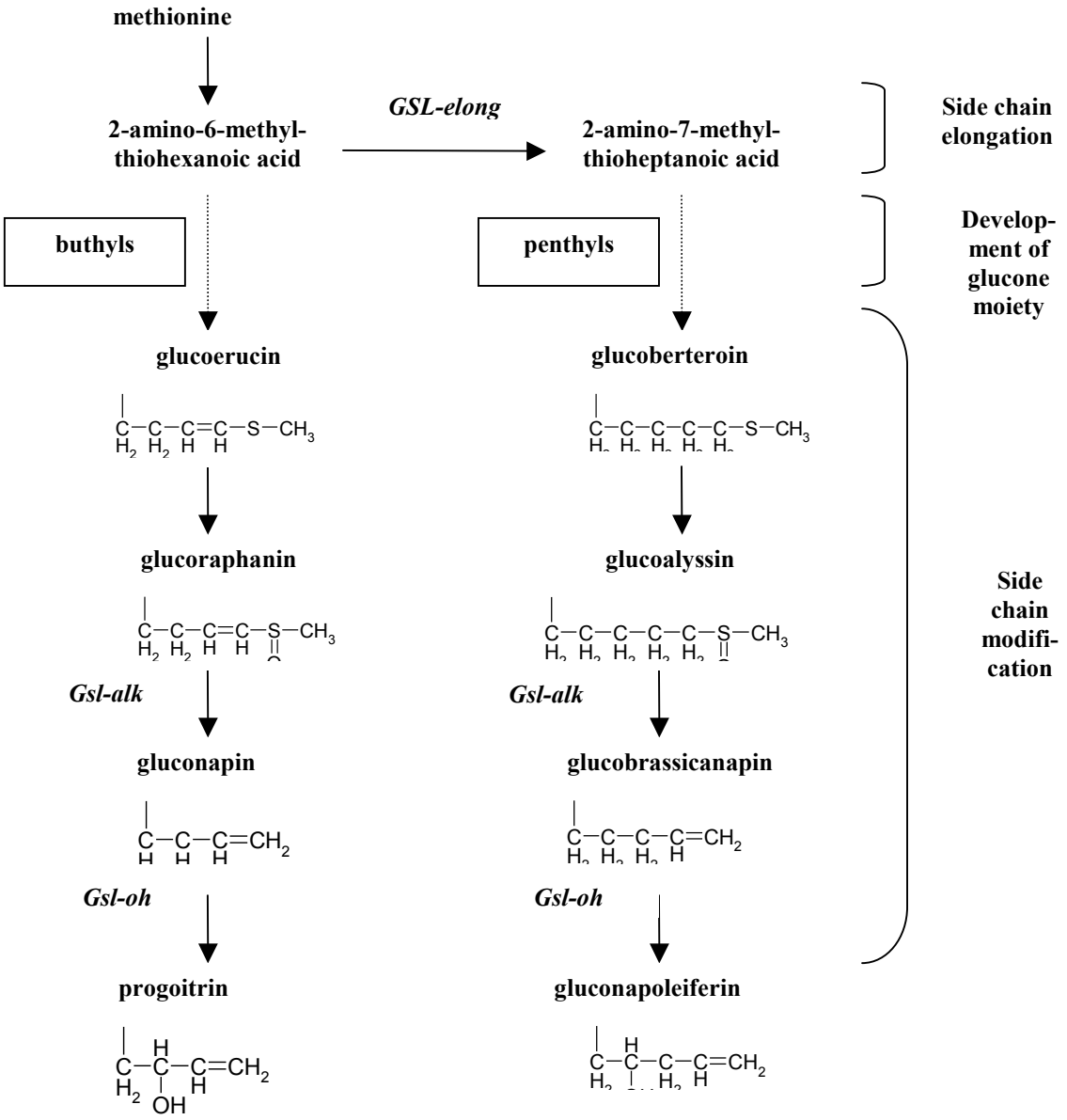
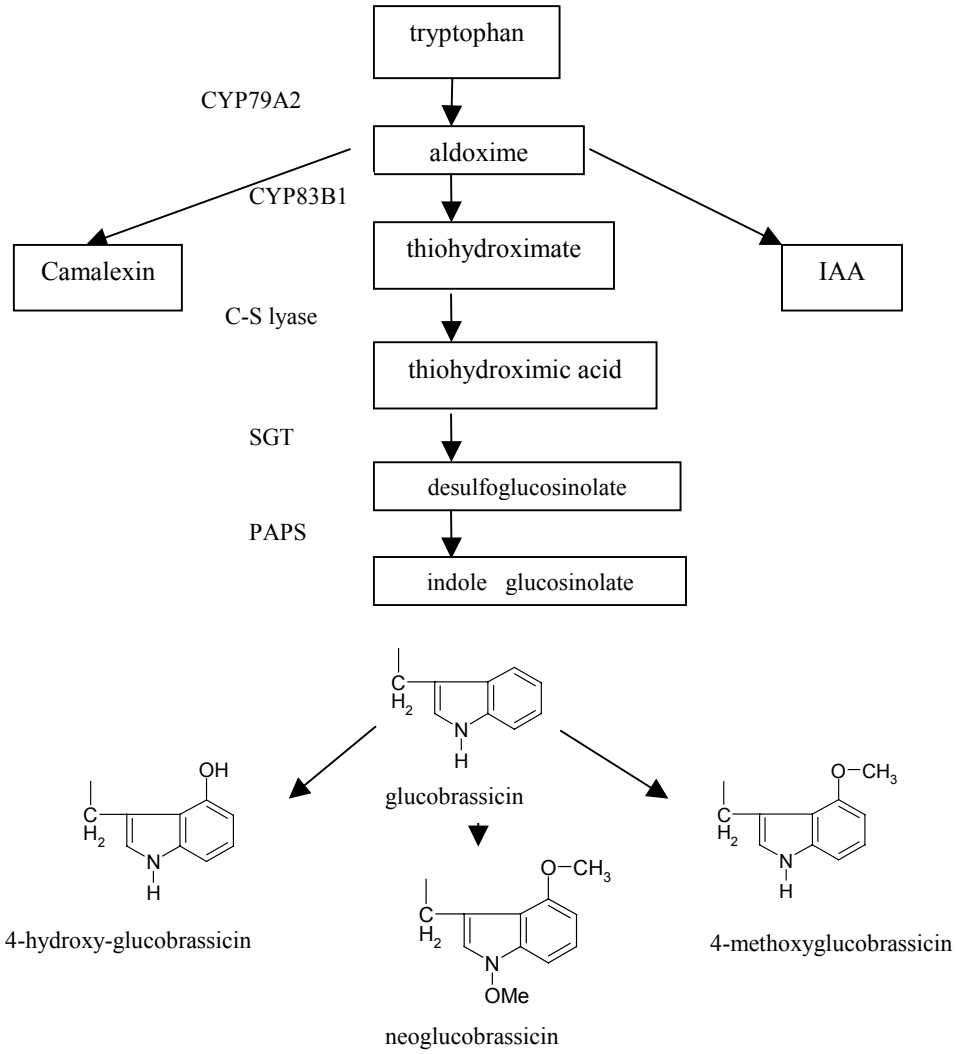


Figure 2.6. Side chain elongation and modification of aliphatic glucosinolates in *Brassicaceae* (according to Hill *et al.,* 1987; Magrath *et al.,* 1994)

Brader *et al*. (2001) showed that treatment of plants with *Erwinia carotorova* incduces *CYP79B3*, but not *CYP79B2*. In addition, *CYP79B3* knockout mutants contain a lower level of indole glucosinolates as compared to *CYP79B2* knockout mutants (Zhao *et al*., 2002). This suggests that *CYP79B3* and *CYP79B2* have differential rates, which may be part of the regulation of indolealdoxime into the different biosynthetic pathways: indole glucosinolates, camalexin or indoleacetic acid (Hansen and Halkier, 2005).
Kliebenstein *et al*. (2001) reported that QTLs controll the characteristic methoxylation on either the 1 or 4 positions on indole glucosinolates have been identified.



SGT - S-glucosiltransferase, PAPS - 3'-phosphoadenosine-5'-phosphosulfate, IAA – indolacetic acid

Figure 2.7. Modification of indole glucosinolates in *Brassicaceae* (according to Mithen, 2000; Hansen and Halkier, 2005)

Glucosinolate degradation

Glucosinolates remain chemically stable within the cytoplasm until brought into contact with the enzyme myrosinase following tissue disruption (Mithen *et al.*, 2001). After physical damage of plant tissue, glucosinolates are broken down by myrosinase, releasing glucose and a complex variety of biologically active products (Bennett *et al.,* 1996).

The myrosinase-glucosinolate system is regarded as a defense system against herbivores. Myrosinase (β-thioglucoside glucohydrolase, EC 3.2.3.1) catalyses cleavage of glucosinolates to aglucons, that decompose to isothiocyanates, thiocyanates, nitriles, and epithionitriles (Figure 2.8). Myrosinase is mainly found in special idioblasts of myrosin cells, are anatomically characterized by high protein content in the vacuole.

Rauth (2002) showed by immunocytochemical analysis, that myrosinase localized exclusively in myrosin cells in the phloem parenchyma, whereas no myrosin cells were detected in the ground tissue of *Arabidopsis thaliana*. Myrosin cells are found to be different from the companion cells and the glucosinolate-containing S-cells present in *Arabidopsis thaliana*. Myrosinase-binding protein is found in every myrosin cells except those of the ground tissue. In general, glucosinolates and myrosinase are thought to be brought together to interact, either by transport mechanism or by tissue disruption, *e.g.*

wounding caused by insect herbivore, breaking cellular boundaries (Andreasson *et al*., 2001). The ground tissue initial cells in *Arabidopsis thaliana* may not have the capacity to form idioblasts, and therefore myrosinase-containing cells are lacking in this tissue.

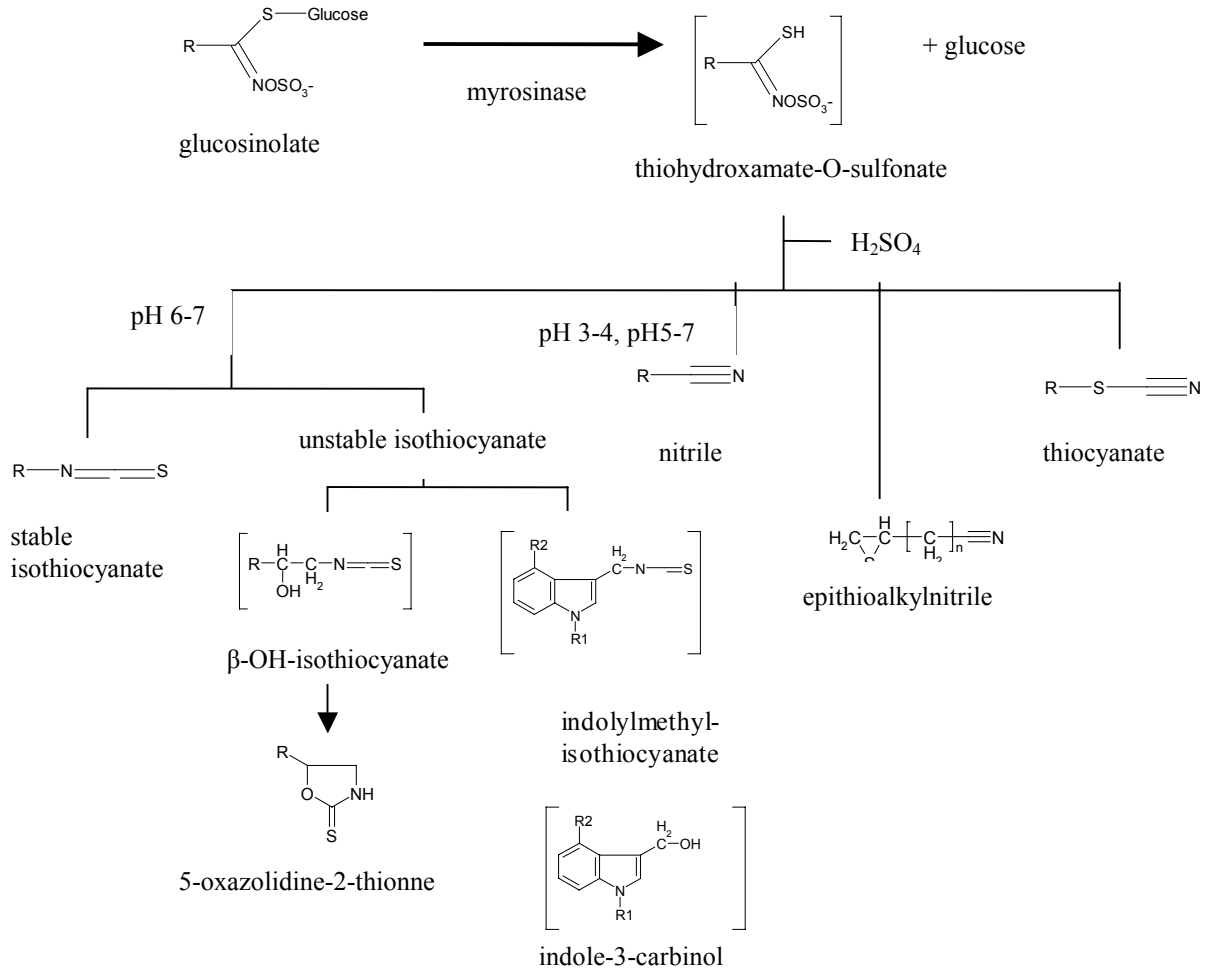


Figure 2.8. Formation of glucosinolate hydrolysis products (according to Holst and Williamson, 2004)

During germinating myrosinase-building protein disappears outside myrosin cells, concominant with a dramatic decrease of mainly aliphatic glucosinolates (This means that myrosinase degrades preferably aliphatic glucosinolates).
Glucosinolates are transported by the phloem. The glucosinolate and myrosinase containing cells may have cytoplasm connection, but there is a need for tonoplast transporters because both glucosinolates and myrosinase are reported to be located in vacuoles. Charron and Sams (2004) reported that some myrosinase could be observed in vacuoles of non-myrosin cells of cotyledons.
Splitting of glucosinolates leads to the formation of a wide range of biologically active compounds (Bonnesen *et al.*, 2001; Holst and Williamson, 2004) including isothiocyanates (which can have powerful antithyroid effects and interfere with the synthesis of necessary thyroid hormones), oxazolidine-2-thiones (depress growth and increase the incidence of goiters; they inhibit thyroid function by blocking the incorporation of iodine into thyroxine precursors and by suppressing thyroxine secretion from the thyroid), nitriles (depress growth,

cause liver and kidney lesions, and in severe cases: bile duct hyperplasia, and megalocytosis of tubular epithelium in the kidney), thiocyanates (inhibit iodine uptake by the thyroid leading to reduced iodination of tyrosine and resulting in decreased production of the important thyroid hormone thyroxine), epithionitriles, and indolyl compounds (Figure 2.8).
The patterns of products vary with the structure of the side-chain and the reaction condition. At low pH the formation of the nitrile is favored, whereas neutral or high pH favors the formation of the isothiocyanate (Bones and Rossiter, 1996). Conversion to nitriles is enhanced in the presence of ferrous ions. Aglucones from glucosinolates, which contain β-hydroxylated side-chains as for example goitrin form the oxazolidine-2-thiones. Indole glucosinolates also form unstable isothiocyanates, which degrade to the corresponding alcohol and may condense to form diindolylmethane. At more acidic pH, indole glucosinolates can form indolyl-3-acetonitrile and elemental sulfur. This nitrile has auxin activity, and can also be converted to indole-3-acetic acid.
Chemical structure of glucosinolate products is important for their biological activity. Small changes to side-chain structures can have significant effects. For example, while methylthioalkyl glucosinolates produce volatile and pungent isothiocyanates (the major flavor compound in *Raphanus sativus* is 4-methylthiobutyl isothiocyanate (from glucoraphanin); methylsulphinylalkyl glucosinolates (*ex*. glucoalissin) produce non-volatile isothiocyanates with relatively mild flavors, such as those found in *Brassica oleracea* var. *italica*. Removal of the methylsulphinyl group and the addition of a double bond results again in a volatile isothiocyanate. Finally, addition of a hydroxyl group to gluconapin and glucobrassicanapin results in the spontaneous cyclisation of the unstable isothiocyanate and the production of a non-volatile product.
Fahrey *et al*. (1997) reported that glucosinolates are very stable and present in plant tissues in much higher concentrations as their hydrolysis products. Under carefully controlled conditions designed to extract glucosinolates completely, which prevent myrosinase activity, plants have been shown to contain most exclusively glucosinolates.
According to Borek *et al.*, (1996) myrosinase may be also realized to soil via root exudation. Myrosinase exhibits the highest activity at the buffer pH of 7.0 and greatly decreases when pH is higher or lower than this point in soils. However, it was reported the different pH optima for myrosinase: for that from *Brassica oleracea* var. *capitata alba* it is 8.0; from *Brassica alba* and *Brassica napus* it ranges from 4.5 until 4.9. Myrosinase activity increases with the increasing temperature up to 40^0 C, and start to decrease above 40^0 C (Al-Turki and Dick, 2003).

It has been believed that the glucosinolates will be hydrolyzed or degraded quickly in soil, but recently Gimsing et al (2005) proved the availability of eight glucosinolates in soil extracts after incorporation of *Brassica juncea* and *Brassica napus*.

2.2.5 Regulation of glucosinolate biosynthesis

The content and profile of glucosinolates in plant tissues may depend on:
- endogenous factors - genotype and cultivars (Schonhof *et al*., 2004), developmental stage of plant and plant age (Fenwick and Heany, 1983b), plant part (Van Etten, 1979);
- exogenous factors - agronomic factors as nutrient supply, particularly the fertilization with sulfur and nitrogen (Schreiner *et al*., 2004), plant injuries or stress, *e.g*. insect attacks, elicitors (Rothe *et al.*, 2004).

For the creating of products, enriched with desirable phytochemicals it is important to establish the interaction between the genotypical and ecophysiological effects on synthesis of phytochemicals (Schreiner, 2005).

2.2.5.1 Endogenous factors

Genotype and cultivars

Genetical is the most important factor, influencing on the biochemical status of plants. Environmental and physiological factors may modify the expression of genes, participating in phytochemical synthesis, but the genetic background is the major determinate (Schreiner, 2005). Schonhof *et al*. (2000) showed that genotype influenced the content of glucosinolates, but not their pattern.

Capparaceae comprises roughly 350 genera and perhaps 3.500 species (Spinks *et al*., 1984). It includes vegetables (*Raphanus sativus*, *Brassica oleracea*, *Brassica rapa*), ornamental species (stock, sweet alyssum), familiar condiment sources as well as basic cultures for production of edible and industrial oils.

There is a wide variation in the distribution of glucosinolates among plants. Glucotropaeolin has been found in most glucosinolate-producing plants, whereas indolylmethyl glucosinolate (glucobrassicin) rarely occurs outside *Capparaceae* and is a family-specific glucosinolate (Horbowicz, 2002). Indole glucosinolates are found in *Brassicaceae*, *Tropaeolaceae*, *Bataceae*, and *Resedaceae* (Griffiths *et al*., 2001). The chain-elongated glucosinolates are restricted to *Brassicaceae*, *Capparaceae*, and *Resedaceae*.

An extensive variation in glucosinolate profiles is seen in *Brassicaceae* where *Brassica napus* has approximately 30 glucosinolates (Li *et al.,*1999a) and *Arabidopsis thaliana* has 34 different glucosinolates in 39 different ecotypes (Gross *et al.*, 2000). However, most plants have a limited number of main glucosinolates (usually six) with a few others presented in trace amounts (Rauth, 2002).

The comparison of the profiles of main glucosinolates in *Brassica* vegetables shows, that the general content and distribution of sub-classes of glucosinolates is individual for each species. For example, the main glucosinolate in *Raphanus sativus* seeds is glucoraphanin, whereas *Brassica juncea* seed is dominated by progoitrin. *Brassica oleracea* seeds contain mainly gluconapoleiferin, whereas *Brassica napus* seeds contain gluconapoleiferin, gluconapin, and glucobrassicanapin (Brown and Morra, 1997).

The highest content of glucosinolates was reported to be in *Brassica rapa* var. *rapa pygmeae teltoviensis* or teltow turnip (Schreiner *et al*., 2005) (Table 2.2).

Table 2.2. Distribution profile of the major glucosinolates in *Brassicaceae* (Schreiner, 2005; Schonhof *et al*., 2000)

Species	Glucosinolates, mg $100g^{-1}FW$	% of total glucosinolates		
		aliphatic	aromatic	indole
Brassica rapa L. var. *rapa*	21-340	30 - progoitrin 12 - glucobrassicanapin	30 – gluconasturtiin	
Brassica rapa L. var. *rapa pygmeae teltoviensis*	790-890	18 - glucobrassicanapin 11 - progoitrin	46 – gluconasturtiin	20
Brassica oleracea L. var. *capitata alba*	26-275	25 – glucoiberin 22 - sinigrin		20
Brassica oleracea L. var. *italica*	40-340	47 – glucoraphanin		44
Brassica oleracea L. var. *botrytis*	14-208	22 – glucoiberin 25 - sinigrin		39
Raphanus sativus L. var. *sativus*	4-218	90- glucoraphasatin		

It may be explained by the intensive synthesis of glucosinolates in turnip tissues, but also probably by the activity of turnip myrosinase. Fahrey *et al*. (2001) wrote that the myrosinase activity in turnip reached 0.3 μmol min^{-1} mg^{-1} protein, while in radish it reached 10.5 μmol min^{-1} mg^{-1} protein, and in daikon sprouts 280 μmol min^{-1} mg^{-1} protein.
The high content of glucosinolates in turnip tissues was the reason for choosing this plant as an object of our investigations.

Turnip
Teltow turnip has a well-documented history regarding the cultivation in the Teltow area and their unique quality – mostly reported in terms of culinary appreciation (Brückner *et al*., 2005). Cultivation of teltow turnip in Brandenburg is has been disregarded for many decades. It was known already in 18 century and was considered as a delicious vegetable (Schonhof *et al*., 2000). In the last ten years the interest in this traditional product arose again.
The typical attributes of teltow turnip are given in Tables 2.3 and 2.4.

Table 2.3. Metrical characteristics and appearance of teltow turnip (Schonhof *et al*., 1999; Brückner *et al*., 2005)

Metrical characteristics	Weight – 20 - 40 g, length - 50 – 75 mm, diameter – 25 - 40 mm, length and respective diameter
Shape	Slim, longitudinal shape or rotund swelling at the neck
Rooting	High amount of secondary roots, distribution of insertions
Vertical indentation	Number and position f parallel to growth axis
Horizontal stripes	Number and position of thickened, corked or discolored bands
Dry weight	15.6 -18.6 %

Table 2.4. Biochemical characteristics of turnip, $100g^{-1}$ FW (Schonhof *et al*., 1999)

Main compounds, g		Minerals, mg		Vitamins, mg	
Protein	1.0	Potassium	240	Vitamin C	20
Fat	0. 2	Calcium	50	Vitamin A	0.06
Carbohydrates	5.3	Phosphorus	30	Vitamin B_1	0.04
Sucrose	3.9	Magnesium	7	Vitamin B_2	0.05
Fructose	0.4	Iron	7	Niacin	0.067
Glucose	0.8				

Plants of turnip form flat, round form, thick turnip with of hard consistence and small leaves. It is biennial plant, which succeed in full sun in a well-drained soil. Turnip grows best in highly fertile soil with pH 5.5 - 6.8 (Vogel, 1996), however it tolerates an annual average temperature range of 3.6 to 27.4°C and a pH in the range of 4.2 to 7.8 (Shattuk and Wang, 1993).
Plants are very easily grown, especially when young. Accumulation of 60-70 % of biomass in turnip root takes place between 49-56 days after sowing. During the period between 42-66 days of vegetation, it is admitted an increase (2-3 folds) in glucose and fructose contents of roots, accompanied by an increase in activities of acid and alkaline invertases (Gupta *et al.*, 2001).
Leaves and roots of turnip can be eaten raw or cooked. Roots often used as a cooked vegetable, the young roots can also be grated and eaten in salads; they have a slightly hot flavor (Brückner *et al*., 2005).

Leaves or stems of turnip are folk remedy for cancer (Shattuck and Mayberry, 1998). The crushed ripe seeds are used as a poultice on burns. The root when boiled with lard is used for breast tumors. A salve derived from the flowers helps against skin cancer.

Developmental stage of plant

Numerous authors have shown that glucosinolate concentrations vary within plants of a single species, and that fluctuations occur with plant age. According to Rangkadilok *et al*. (2002), the glucosinolate content is much higher in old plants.

Examined plant part

Li *et al.* (1999) admitted that the total content and spectrum of individual glucosinolates varied widely within plant parts. There is no correlation between the glucosinolate content in leaf, stem, and root. It appears that glucosinolate synthesis and accumulation is under tissue-specific control.

Generally, it is high in seeds (up to 10 % of dry weight), whereas the levels in leaves, stems, and roots are approximately ten times lower (Smith *et al.,* 1991).

Also Rothe *et al.* (2004) reported that the regulation and occurrence of individual glucosinolates is independent in plant parts and tissues. Nevertheless, the glucosinolate profiles of leaf and stem of *Brassica rapa* are nearly identical with an aliphatic to indole glucosinolate ratio of about 2 : 3, wile the root glucosinolate profile differs substantially from that of leaf and stem. The 4-methoxyglucobrassicin is the main glucosinolate in all three tissues of *Brassica rapa*; however, the highest content is measured in the root. Glucobrassicin is the major glucosinolates in leaf and stem, while gluconasturtiin is remarkable aromatic glucosinolate in root, but it does not occur in leaf or stem.

The major glucosinolate in seed of *Raphanus sativus* is not detected in its leaves or roots, whereas glucoraphanin is found in all tissues of *Brassica juncea* (Brown and Morra, 1997).

Rosa (1997) reported, there was a very high difference between the total glucosinolate levels in the aerial part of the *Brassica oleracea* plant 0.581 mmol 100 g^{-1} of dry weight and roots 2.124 mmol 100 g^{-1}. Large differences between seed, leaf, and root glucosinolate profiles of several *Brassicas* have been also described by Sang *et al.* (1984).

Rosa and Rodrigues (1998) showed that total and individual glucosinolate levels have very high significant differences between the two plant parts of *Brassica oleracea*. Whilst the glucosinolate patterns of the aerial part of the plant and of the roots remain the same, levels of major glucosinolates in aerial part are 0.23 mmol 100 g^{-1} (of dry weight) for glucoiberin and 0.072 for sinigrin; in roots, gluconasturtiin and neoglucobrassicin show the highest average concentrations, with 0.67 and 0.41 mmol 100 g^{-1}, respectively.

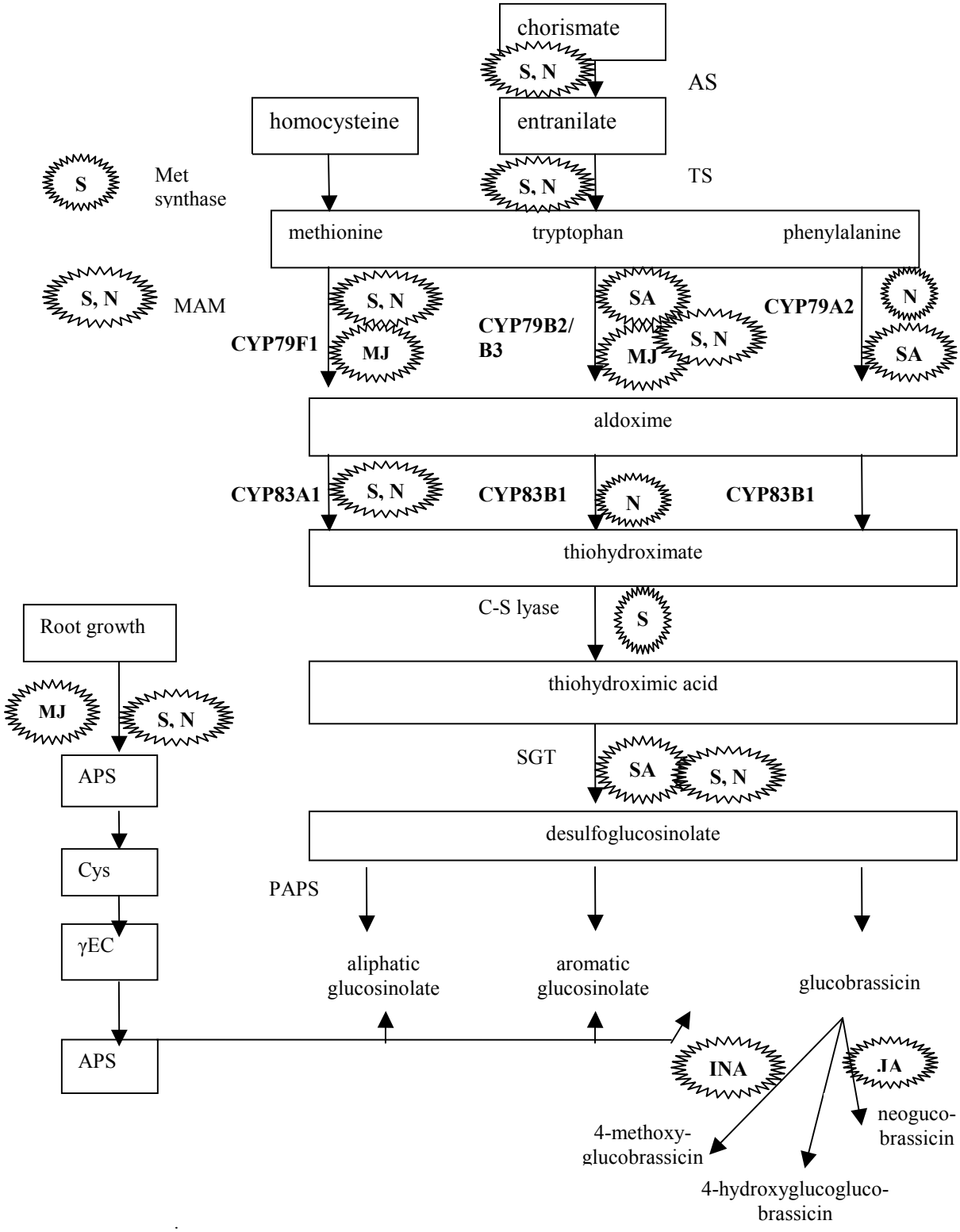
Smith and Griffiths (1988) showed for a number of *Brassica rapa* cultivars, that glucosinolate content of the stem was significantly higher than that of the leaf. For both leaf and stem, progoitrin amounted on average to over 50 % of total glucosinolates and the other alkenyl glucosinolates to 40 % and 30 % of the total glucosinolates found in leaves and stems, respectively. Gluconasturtiin and glucobrassicin are present at comparatively low levels in both leaves and stems of *Brassica napus* as compared with progoitrin.

2.2.5.2 Exogenous factors and metabolic engeneering of glucosinolates

A number of chemical and physical factors like media component, pH, temperature, and aeration affecting production of phytochemicals (Mulabagal and Tsay, 2004). Kliebenstein *et al*. (2005) assumed that nitrogen and sulfur belong to abiotic factor, mostly determining the glucosinolate profile in plants.

Consequently, the manipulation of the nutrient supply and physical conditions is perhaps the most fundamental approach for altering levels of specific glucosinolates in plants.
Nutrient supply influences expression of the genes of CYP79 family (involved in aldoxyme formation), entcoding cytochrome P450 enzymes. They are the key enzymes in glucosinolates biosynthesis and modulation of their activity causes changes in glucosinolates profile (Mikkelsen *et al.*, 2002) (Figure 2.9).
Modification of the glucosinolate side chain is of particular importance because of the physico-chemical properties and the biological activity of the glucosinolate degradation products are determined by the structure of the side chain (Wittsok and Halkier, 2002). Overexpression, and downregulation of CYP79 genes, entcoding P450 enzymes, makes it possible to generate custom designed glucosinolate profiles (Petersen *et al.*, 2001). Many cytochrome 450 genes are involved in pathways regulating the levels of endogenous signal molecules, and many of those in the biosynthesis of plant natural products are expressed primary after induction, e.g. under conditions of stress (Halkier *et al.*, 2005). It is found that CYP79 genes can be strongly induced by the elicitors like JA (jasmonic acid), this strongly influence the total level of indole glucosinolates (Mikkelsen *et al*., 2003). Recently, it has been found that neoglucobrassicin accumulation was strongly induced in response to JA treatment. Treatment with 2,6-dichloroisonicotinic acid (a functional homologue of SA (salicylic acid)) resulted in accumulation of 4-methoxyglucobrassicin.
Also the gene entconding γ-glutamatcysteinsynthase is up-regulated in roots under the influence of sulfur and nitrogen (Hirai *et al*., 2004), and MJ (methyl jasmonate) (Halkier *et al.*, 2005). This gene is responsible for cell division in roots meristem, influencing root biomass growth and consequently increases of root surface, which influence root functions of nutrient uptake as well as phytochemical exudation.

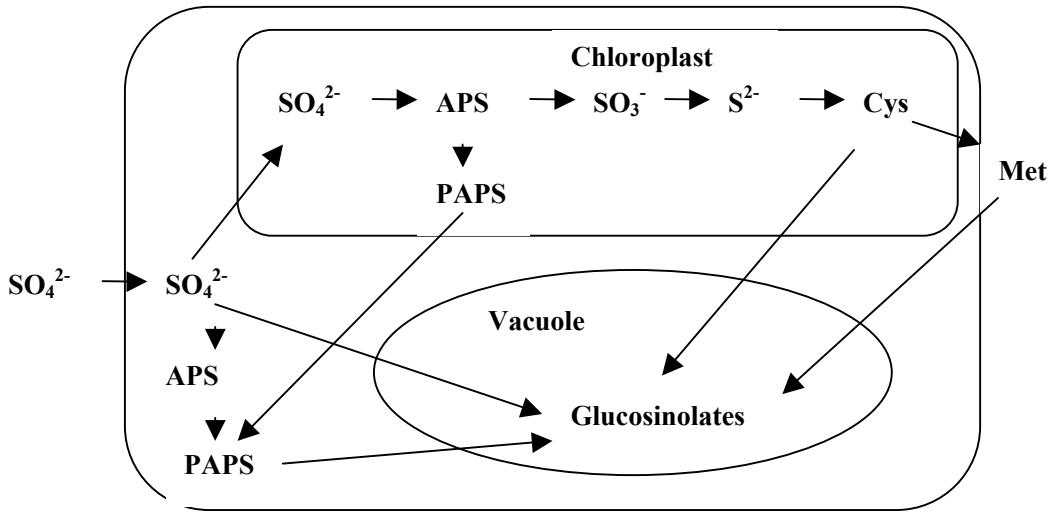
Nutrient supply
Within any particular variety of vegetable, the glucosinolate content is influenced greatly by growing conditions (Mithen *et al.*, 2000).
Fertilizer application exerts a significant effect on glucosinolate content. In the experiments of Vallejo *et al.* (2003) was shown that not all classes of glucosinolates are affected by the fertilization. The highest aliphatic glucosinolate level is obtained under the rich fertilization, and oppositely, total indole and aromatic glucosinolates are not influenced strongly by the fertilization.
Especially important for glucosinolates is sulfur supply, however in plant tissues sulfur represents only one fifteenth of nitrogen part. Ciska *et al*. (2000) wrote that among agronomic factors the intensity of sulfur fertilization is of primary importance for glucosinolate conent in plants and its sufficient supply can lead to 2-3 fold increase in glucosinolate content for *Brassica rapa*. One glucosinolate molecule contains two or three sulfur and one nitrogen atom, hence glucosinolate can play role as sulfur storage source (Hirai *et al*., 2004).
Saito (2004) represented the way of inorganic sulfur utilization and its involvement in glucosinolate synthesis as following: the inorganic sulfur or sulfate ion is fixed into cystein by the sulfur assimilation pathway in plants (Figure 2.10). Thereafter cystein is converted to methionine. The form of sulfur found in xylem and phloem sap is also primarily sulfate, which means that the sulfur is translocated through the plant mostly *via* unmetabolized sulfate.



S - sulfur, N - nitrogen, SA – salicylic acid, MJ – methyl jasmonate, JA – jasmonic acid, INA – 2,6-isonicotinic acid (SA homologue), MAM – methylthioalkylmalate synthase, APS – adenosine-5'-phosphosulfate, Cys – cystein, γEC - γ-glutamylcysteine, SGT - S-glucosiltransferase, PAPS - 3'-phosphoadenosine-5'-phosphosulfate, APS – sulfurylase, AS – antranilate synthase, TS – tryptophan synthase.

Figure 2.9. Modulation of glucosinolate synthesis according to Mikkelsen *et al.* (2002), Petersen *et al.* (2001), Halkier *et al.* (2005), and Hirai *et al*. (2004).

Subsequently, sulfate is subjected to activation to adenosine-5'-phosphosulfate (APS) for further conversion. The major assimilatory pathway is reduction of APS to SO_3^- and then to S^{2-}, then formation of serine, yielding cystein. A branch point in this pathway is converting of APS to 3'-phosphoadenosine-5'-phosphosulfate (PAPS). Cystein is the central compound for production of a variety of metabolites containing reduced sulfur, such as methionine, reduced glutation and glucosinolates.
Sulfur assimilation can be regulated by the number of factors as nutrient supply, stage of plant development, stress conditions. Particularly signaling pathways involving MJ and auxin seem to be involved in the sulfur stress response (Saito, 2004). Several studies of transcriptome analysis suggest that MJ and auxin are involved as signals of sulfur-deficiency stress. Indeed, MJ induces a cluster of sulfur assimilation genes. Auxin signaling is presumable involved through activation of nitrilase leading in changing in root morphology. Primary and secondary metabolic pathways, involving amino acids, carbohydrates, and glucosinolates are modulated in response to sulfur availability. The level of glucosinolates decreases rapidly by nutrient, particularly sulfur deficiency (Hirai *et al*., 2004). Glucosinolates, which contain two or three sulfur atoms per molecule, are regarded as storage and possibly mobilizing forms of assimilated sulfur in response to acute sulfur deficiency.



APS – adenosine-5'-phosphosulfate, PAPS - 3'-phosphoadenosine-5'-phosphosulfate, APS – sulfurylase, Cys – cystein, Met - methionine.

Figure 2.10. Sulfur assimilatory metabolism in the subcellular compartments of plant cells (according to Saito, 2004)

The synthesis of individual glucosinolates in roots and leaves is differently influenced by sulfur deficiency, as it was shown by Hirai on the example of Arabidopsis thaliana, grown in nutrient sufficient conditions and under the nitrogen and sulfur deficiency.
Schonhof *et al*. (2002) have shown that sulfur is important element in synthesis of amino acids cystein and mehionin, which are precursors in glucosinolate synthesis. They showed the direct dependence between the sulfur supply and the content of glucosinolates in *Brassica* vegetables. Increase of sulfur supply enhances mostly aliphatic glucosinolates, while the content of indole glucosinolates is practically not changed.

Plant stress

Stress factors tend to increase glucosinolate content, presumably because of their function as stress responding defense compounds.

The overview about the influence of stress factors, particularly elicitors, on phytochemical production is given in the chapter 2.3.

2.3 Elicitors

2.3.1 Regulation of phytochemical biosynthesis

Elicitors are molecules that stimulate defense or stress-induced responses in plants (Radman *et al.*, 2004). They trigger signal cascades that activate several defense responses in plants such as reinforcement of cell walls (Dörnerburg and Knorr, 1995), induction of pathogenesis-related proteins (Kunkel and Brooks, 2002), or synthesis of phytochemicals (Mikkelsen *et al*., 2000).

The enhance of the content of phytochemicals involved in defense reaction of plants as well as detection of novel ones upon elicitation suggests that elicitation this may be a functional tool of technology for phytochemical production.

Wu and Lin (2002) suggested that the treatment of plants with biotic and abiotic elicitors causes the defense response similar to those by pathogen infection or environmental stimuli and can be one of the most effective ways to improve the yield of phytochemicals.

Plants have developed a number of strategies to defend themselves against pathogen and herbivores (Bennett *et al.*, 1994). Treatment of plants with elicitors causes accumulation of a range of plant defensive phytochemicals, such as cyanogenic glycosides, glucosinolates (Zhao *et al.*, 2005), alkaloids, phenols, phytoalexins, terpenes (Radman *et al.*, 2004). Dörnerburg and Knorr, (1995) showed that application of cell wall pectin isolated from suspension culture of *Morinda citrifolia* led to maximizing of antraquinone production in *Solanum tuberosum*.

Elicitor-induced plant defense responses involve a cascade of physiological events in the challenged cells, such as changes in membrane potential and ion fluxes, the production of active oxygen species, the activation of defense-related genes, and the synthesis of secondary metabolites (Wu and Lin, 2002). The increase in membrane ion fluxes, Ca^{2+} influx and K^{+} efflux/H^{+} influx exchange and the production of active oxygen species such as H_2O_2, which is refereed to as the oxidative burst, are two early and important events in plant defense.

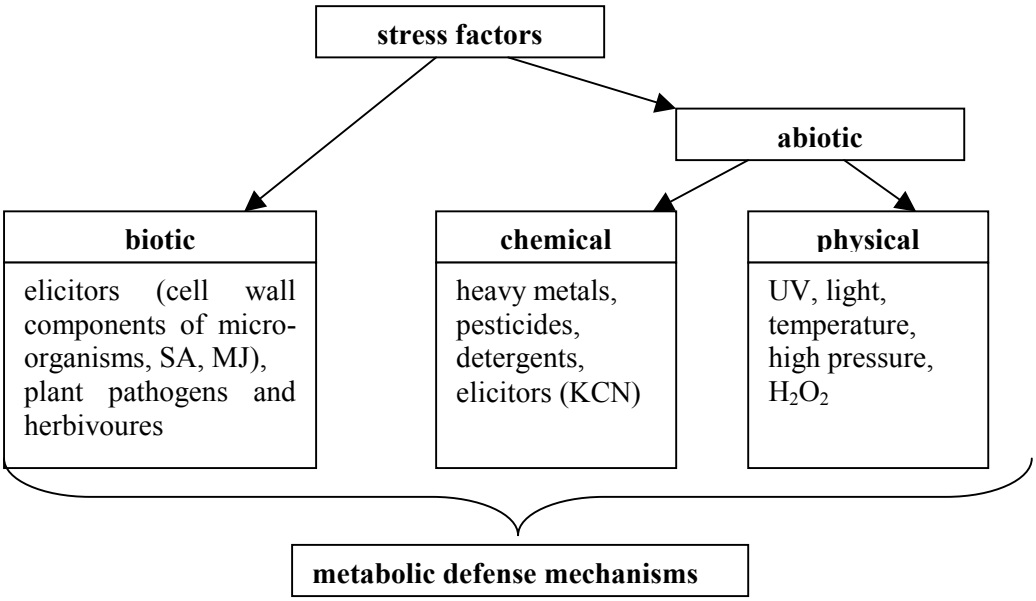
It has been found that polysaccharides and proteins produced by invading plant pathogens act as elicitors, triggering the synthesis of phytoalexins in plant tissues surrounding the infection site (Cipollini, 2002). In this context, elicitation is perceived as a potentially useful approach for enhanced and extended production of desirable phytochemicals. Elicitors, when introduced in small concentrations to a living cell system, initiates or improve the biosynthesis of specific compounds. The increased production of the phytochemicals through elicitation cultures has opened up a new area of research which could have important economical benefits for bioindustry. Poulev *et al.* (2003) mentioned that elicitation is the relative ease scale-up process, which opens an opportunity for the introduction of useful novel metabolites as well as enhancement of the production of commercially useful phytochemicals.

The type and structure of elicitors varies greatly (Radman *et al.*, 2003). They may be formed inside or outside plant cells, and are distinguished as endogenously or exogenously inducers. Depending of their origin, they are classified as biotic or abiotic (Dörnerburg and Knorr, 1995) (Figure 2.11).

Abiotic elicitors are the substances of non-biological origin. The causes of the abiotic stress can be of chemical of physical nature, among them are:

- chemicals like inorganic salts, heavy metals, some chemicals that disturb membrane integrity (Radman *et al*., 2003),
- physical factors like mechanical wounding, ultraviolet irradiation, high salinity, high or low osmolarity, extreme temperature, drought, ozone or high pressure (Dörnerburg and Knorr, 1995).

Biotic stress can be caused by bacterial, viral or fungal attack as well as by elicitors of biological origin, which include:
- polysaccharides derived from plant cell walls (pectin or cellulose), microorganisms (chitin or glucans) and glycoproteins (Dörnerburg and Knorr, 1995),
- low-molecular-weight organic acids (Radman *et al*., 2003),
- low-molecular-weight phytochemicals produced by plants in response to physical damage, fungi or bacteria attack (Thaler *et al*., 2002),
- proteinkinases.



SA - salicylic acid, MJ - methyl jasmonate

Fig. 2.11. Classification of elicitors according to Dörnerburg and Knorr (1997)

2.3.2 Plant defense mechanism

Rickauer *et al.* (1997) based on the character of physical or chemical changes distinguished three classes of plant responses to herbivores or infection: inducible responses, inducible resistance, and inducible defense.
Inducible plant responses are classified by their speed and extent within the plant as:
- **Delayed Inducible Resistance (DIR)** - influences herbivores in the next generation or year
- **Rapidly Inducible Resistance (RIR)** - influences current herbivores or pathogens during their lifetime, it can be subdivided into:

- **Localized RIR** - restricted to areas around herbivore or pathogen attack, may be due to a hypersensitive response of localized cell death to prevent pathogen spread.

- Systemic Acquired Resistance (SAR) - the entire plant responds to herbivore or pathogen attack. Plants possess inducible defense systems to withstand attack by pathogens and pests. SAR is long lasting and effective against a broad spectrum of viral, fungal, and bacterial pathogens (Verberne *et al*., 2003).

- Besides the SAR, host plants may also acquire resistance to pathogens in response to endogenous stimuli associated with their own development (Hugot *et al.*, 2004). The occurrence of a transition from susceptibility to resistance during development is a phenomenon, occurring in

plants in case of viruses, Bacteria, fungi, and oomycetes. Recently Kus *et al*. (2002) reported about the **Age-related Resistance (ARR).**

ARR has been observed in many plant species but little is known about the biochemical or molecular mechanisms involved in this response. *Arabidopsis thaliana* becomes more resistant or less susceptible to virulent *Pseudomonas siryngae* as plants mature (bacterial growth reduction of 10- to 100-fold). ARR is a distinct defense response, unlike the SAR. It exist the relationship between plant age and disease resistance: elder plants display increased resistance or reduced susceptibility to pathogens, as it was described by Kus *et al*. (2002) for wheat and tobacco plants. It was also observed a correlation between the plant age and the accumulation of phytoalexin by *Nicotiana tobacco* in response to *Verticillum albo-atrum* infection, constitutive accumulation of terpenoids or capsidiol accumulation in older cotton plants in response to infection with *Phytophtora capsici*. Although ARR is documented from a pathological point of view, Hugot *et al.* (2004) described the genetic and molecular bases of disease control during plant development.

A large number of genes involved in resistance responses, among these are genes encoding pathogenesis-related (PR) proteins. Thus, according to Kliebestein *et al*. (2002) there are 413 genes participating in SAR in *Arabidopsis thaliana*. Moreover, the differential expressions of these genes are 2.5-fold or more. Also older leaves of flowering tobacco accumulate specific PR proteins (PR-1, PR-2, and PR-3), and this correlates with increased resistance to viral and fungal pathogens (Kus *et al*., 2002).

As exogenous application of salicylic acid (SA) results in expression of the same set of SAR marker genes and the induction of resistance, it was assumed that SA functions as a phytohormone, produced at the site of infection and transported to the systemic tissues to activate SAR (Yalpani *et al.,* 1993). SAR leads to broad-spectrum systemic resistance after an initial "immunizing" infection and is associated with SA accumulation and *PR-1* expression (Yalpani *et al.,* 1991). ARR response in tobacco was also associated with 5-fold increase of SA (Yalpani *et al.,* 1993).

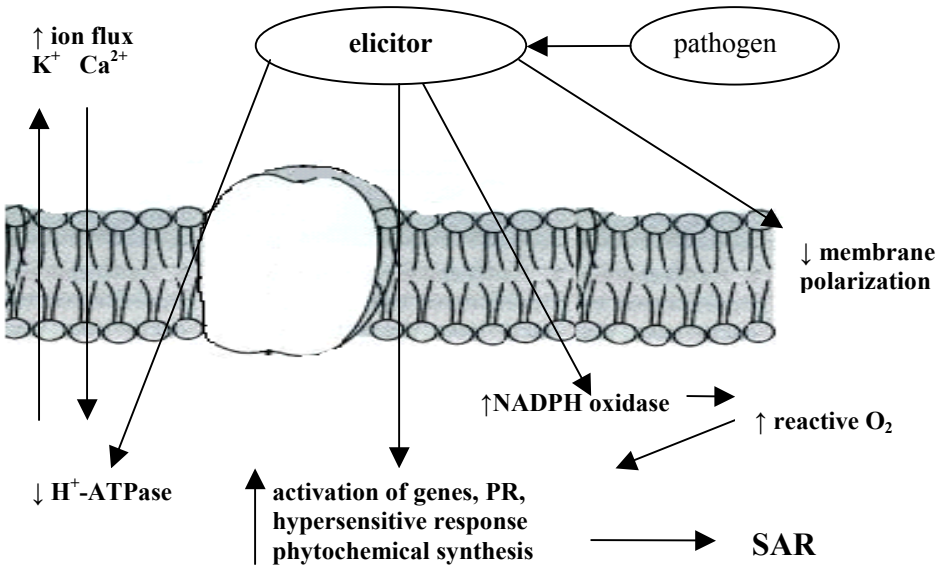
There are some similarities and differences between SAR and ARR (Hugot *et al.*, 2004). In the case of SAR, SA is required for activation of the signaling transduction pathway leading to resistance to resistance against *Phytophtora syringae* in Arabidopsis *thaliana*. However, unlike SAR, *Arabidopsis thaliana* ARR to *Phytophtora syringae* does not depend on a functional *NPR1* gene (Hugot *et al.*, 2004) and is not caused by constitute *PR-1* gene expression in mature leaves (Kus *et al*., 2002).

Many types of plant chemicals may be involved in inducible responses (Rickauer *et al.*, 1997). The best-studied are proteins, SA derivatives, JA (jasmonic acid) derivatives, carbohydrates and related compounds (the role of carbohydrate related compounds in SAR is probably mediated via the SA or JA pathways and their signaling molecules), SA and JA pathways may be interrelated by a phenomenon called biochemical cross-talk. This introduces additional complexity into the SAR response by potentially allowing for simultaneous activation by a single elicitor, or interference between the JA and SA pathways.

The defense response is a both time and space controlled complex signaling network leading to host defense-genes expression (Farmer, 2000). The nature of the physical injury and the nature of pathogen-encoded molecules are two variables that modulate which set of defense-related genes will be expressed and to which extend.

From a pathogenesis point of view, many elicitors may act as avirulence determinants of a plant genetic system that refers to a general response of gene-to-gene resistance in plant innate immunity, in which plant resistance genes confer resistance to pathogens matching avirulence gene by specific recognition events. Elicitors of avirulence determinants must be recognized by plant receptor localized to the plasma membrane or the cytoplasm before initiating signaling pathways, which lead to defense reaction such as synthesis of PR-proteins or defense phytochemicals. Molecular recognition and physical interaction between elicitor signal molecules and specific plant receptors are complex processes but are required for specific elicitor signal transduction. By

causing changes in receptor conformation or activation of receptor kinases, elicitors subsequently or indirectly activate their corresponding effectors, such as ion channels, lipases and kinases, which then transducer the elicitor signal to downstream defense responses (Kunkel and Brooks, 2002).
Elicitors effect enzymes of secondary metabolism, oxidative burst, phytoalexin signal transduction and anion channels. The first step of the defense response to a pathogen is the recognition of pathogen elicitors. Subsequent activation of transcription factors leads to induction of plant defense genes and biosynthesis of endogenous secondary signals (Dörnerburg and Knorr, 1995) (Figure 2.12).



PR - pathogenesis-related proteins, SAR - systemic acquired resistance

Figure 2.12. General mechanism for biotic elicitation (according to Dörnerburg and Knorr, 1995)

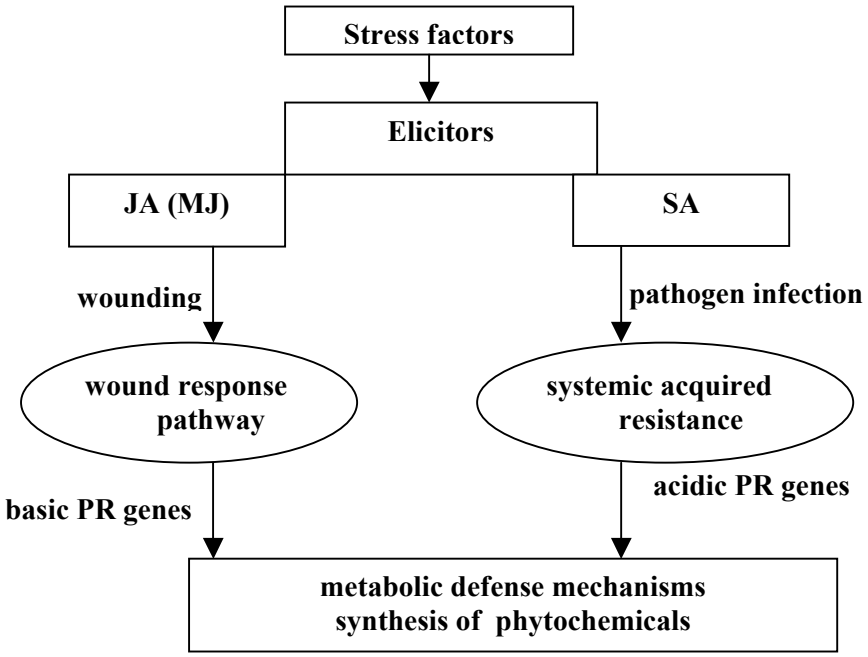
The general cellular process and regulatory principle for activation of plant phytochemical biosynthesis is that, an extracellular of intracellular signal is perceived by a receptor on the plasma membrane or endomembrane; the elicitor signal perception initiates a signal transduction network that lead to activation of *de novo* biosynthesis of transcription factors, which regulate the expression of biosynthetic genes involved in plant secondary metabolism. The resulting enzymes catalyze the biosynthesis of target phytochemicals (Halkier and Du, 1997).
A general mechanism for the elicitation in plants may be summarized on the basis of elicitor-receptor interaction. When a plant is challenged by the elicitor rapid array of biochemical responses occur. These include (Dörnerburg and Knorr, 1995; Radman *et al.*, 2003):

1) Binding of the elicitor to a plasma membrane receptor.
2) Changes in ion fluxes across the membrane: Ca^{2+} influx to the cytoplasm from the extracellular environment and intracellular Ca^{2+} reservoirs; stimulation of K^+ efflux.
3) Rapid changes in protein phosphorilation patterns and protein kinase activation, mitogen-activated protein kinase stimulation.
4) Synthesis of secondary messengers and diacylglycerol mediating intracellular Ca^{2+} release, nitric oxide and octadecanoid signalling pathway.
5) Cytoplasm acidification caused by H^+-ATPase inactivation and decrease in membrane polarization; extracellular increase of pH has been reported in elicitor-treated plant tissues.
6) Activation of NADPH oxidase responsible for reactive oxygen stress and cytosol acidification.

7) Cytoskeleton reorganization.
8) Production of reactive oxygen stress messengers such as the O_2 and H_2O_2 that might have a direct antimicrobial effect as well as contributing to the generation of bioactive fatty acid derivatives and being involved in the cross-linking of cell-wall-bound proline-rich proteins. H_2O_2 can act as a secondary messenger and it is involved in the transcriptional activation of defense genes.
9) Accumulation of PR proteins.
10) Cell death at the infection site (hypersensitive response), structural changes in the cell wall (lignifications of the cell wall, callus deposition).
11) Transcriptional activation of the corresponding defense-response genes.
12) Synthesis of JA and SA as secondary messengers.
13) Systemic acquired resistance.

2.3.3 Role of salicylic acid and methyl jasmonate in plant defense reaction

JA and SA are both important components of signal transduction cascades activating plant defense responses against herbivore and pathogen attack (Preston *et al*., 1999). SA and JA are known to play key roles in plant responses such as defense against abiotic stresses, wounding and exposure to ozone as well as defense against insect and microbial attack (Kunkel and Brooks, 2002) (Figure 2.13).



PR - pathogenesis-related proteins, JA – jasmonic acid, MJ - methyl jasmonate, SA - salicylic acid

Figure 2.13. Defense signaling pathways according to Klessing and Malamy (1994).

The synthesis of JA arises from a pathway which is called the octadecanoid pathway, because the starting material is the common plant fatty linolenic acid (Baldwin, 1996). This pathway is remarkably similar to pathways responsible for the synthesis of prostaglandins, prostacyclins, leukotrienes, and thromboxanes in animals, whose synthesis originates from the fatty acid,

arachidonic acid. In animal systems, these potent secondary messengers play a complex suite of roles, many of which related to pain and inflammatory responses to wounding. Similarly, jasmonates in plants mediate many defensive responses to wounding. The exogenous addition of jasmonates to plants has been found to induce a growing number of wound- or herbivore-induced chemical defenses. All of the jasmonate-induced responses that have been studied at the molecular level involve induced changes in gene transcription or translation.

Mechanical wounding or wounding by insects or herbivores triggers the wound response pathway where the signaling is mediated by plant regulators JA (Sembdner and Parthier, 1993). JA and its volatile methyl ester MJ (methyl jasmonate) have long been observed to be transducers of elicitor signals for the production of phytochemicals. Induction of plant phytochemicals accumulation by MJ is not limited to certain types of metabolites, but induces a wide variety of plant products including terpenoids, flavonoids, and alkaloids (Sudha and Ravishankar, 2003). Therefore, JA signaling pathway is generally regarded as an integral signal for biosynthesis of many phytochemicals. Also because many elicitors stimulate endogenous JA biosynthesis in plants, the JA signaling pathway is regarded as a transducer or mediator for eliciting signaling, leading to accumulation of phytochemicals. It has been shown the elicitor-induced indole alkaloids accumulation in *Candeas roseus* (Zhao *et al.*, 2005), activation of indole glucosinolates biosynthesis in *Arabidopsis thaliana* (Brader *et al*., 2001).

SA (2-hydrobenyoic acid) is a well-known inducer of SAR in plant-pathogen interaction (Klessing and Malamy, 1994), but it is not a universal inducer for production of plant defense metabolites. SA quickly accumulates at the site of infection during the pathogen attack and plant hypersensitive reaction, and it spreads to other parts of the plant to induce a wide range of defense responses. SA induces gene expression related to biosynthesis of phytochemicals in plants. For example, indole alkaloids in *Candeas roseus* cell culture can be induced by acetilsalycilic acid, an analogue of SA (Zhao *et al.*, 2005). In *Rubia cordifolia* cultures, both MJ and SA strongly induced antraquinone phytoalexin production (Bulgakov *et al*., 2002).

The terminal step in the defense-signaling cascade is the activation of defense pathogenesis-related genes that encode PR-proteins. There are several groups of PR-proteins and different signaling molecules, such as SA, JA or ethylene, can induce their expression. Generally, genes encoding acidic PR-proteins (acidic PR-1, PR-2 and PR-3 gene families) are induced by SA while JA induces genes encoding basic PR-proteins (basic PR-1, PR-2, PR-5 and PR-6 gene families).

A notable difference between these two cascades is their different kinetics of elicitation. After leaf wounding, JA concentrations in wounded leaves increase rapidly and transiently, with pools waxing and waning within minutes. JA and MJ burst precedes the activation of several defense-related genes (Creelman *et al*., 1997) and the accumulation of plant defense compounds, including proteins, alkaloids (Baldwin, 1996), flavonoids and phenolics (Mizukami *et al*., 1993). In comparison, SA displays a slower, more attenuated response to tobacco mosaic virus inoculation, with systemic increases occurring several days after inoculation and lasting for several weeks (Raskin *et al*., 1992).

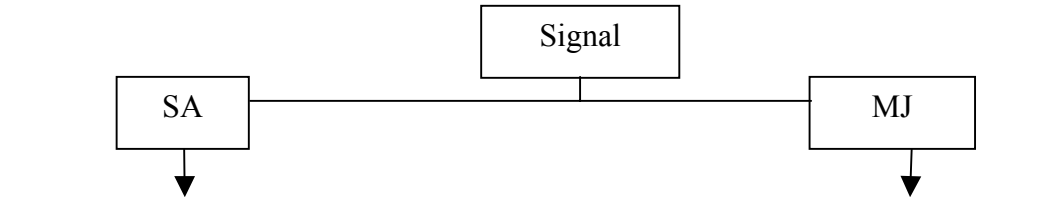
JA and SA pathways are mutually antagonistic. Treatment of plant with JA resulted in inhibited expression of SA-dependant genes (Thaler *et al*., 2002).

Sudha and Ravishankar (2003) reported that MJ and SA were both found to stimulate the anthocyanin production in the callus cultures of *Daucus camta*. The highest levels of anthocyanin were observed in the cultures treated with 200 μM SA and 0.01 μM MJ. The MJ and SA treatments resulted in higher activity of Ca^{2+}ATPase suggesting that the enhancement of anthocyanin by SA and MJ could be mediated through the involvement of the calcium channel. The treatment of the callus cultures with SA was found to result in marginally higher titers of endogenous polyamines whereas MJ resulted in lower levels of polyamines as compared to the control. The SA treatment was found to result in lower ethylene production and the treatment with MJ stimulated the ethylene production.

2.3.4 Salicylic acid and methyl jasmonate influence on glucosinolate synthesis

Wallsgrove *et al.* (1999) characterized the glucosinolate accumulation in vegetative tissues of *Brassica napus* as a dynamic process, influenced by stresses such as tissue damage by herbivores and fungal infection. In contrast, certain glucosinolates appear to be necessary for *Plasmodiophora brassicae* (clubroot) infection of *Brassica napus*, and increased indole or aromatic glucosinolate content is associated with successful infection.
Brassica napus leaves can also synthesize SA, as it was shown by Kiddle *et al*. (1995), but it may not be required for glucosinolate induction: mechanical damage alone is sufficient to cause *Brassica napus* leaves to accumulate glucosinolates and SA can be synthesized in response to wounding.
Glucosinolate profiles can be altered by treatments with elicitors (Mikkelsen *et al*., 2000; Poulev *et al*., 2003). Application of either SA or MJ can dramatically increase the glucosinolate content in plants (Kiddle *et al*., 1994). SA and MJ serve as signaling molecules induced by pathogen infestation (Doughty *et al*., 1995) and mechanical wounding (Bodnaryk *et al*., 1994). Treatment of plants with SA and JA results in increased amounts of glucosinolates, although Kiddle *et al*. (1994) reported that JA induces mainly indole glucosinolates in leaves, whereas SA indole glucosinolates also in roots of *Brassica nigra* and *Brassica oleracea*.
Doughty *et al*. (1995) showed that JA and MJ at μmol doses induced large, sustained, systemic increases (up to 20-fold) in the concentration of specific indole glucosinolates in cotyledons and leaves of *Brassica napus* (glucobrassicin) and *Brassica rapa* (1-methoxy-glucobrassicin), which comprised 90 % of the total glucosinolates in treated leaves.
Glombitza *et al*. (2004) explained the increase of glucosinolate synthesis under SA and MJ influence as their affecting the transcription of several genes involved in synthesis. Furthermore in many species of *Capparales*, the application of MJ and SA increased contents of indole and aliphatic glucosinolates (Bodnaryk *et al*., 1994; Doughty *et al*., 1995). Kiddle *et al*. (1994) reported that SA also caused an accumulation of aromatic glucosinolate gluconasturtiin (2-phenylethylglucosinolate) in *Brassica napus*. Bennett and Wallsgrove (1994) showed that indole glucosinolates in *Brassica napus* and *Arabidopsis thaliana* can be induced by SA and MJ. Doughty *et al.* (1995) reported that indole glucosinolates can be accumulatd systemically in plants treated with JA, a signal molecule associated with response to wounding, herbivory and infection.
Attempts to individually induce the aliphatic, aromatic, or indole glucosinolates with either SA or MJ demonstrated that different controls operate on each of their biosynthetic pathways. Van Dam *et al*. (2003) reported that in several plant species, SA and MJ responses are systemic through the plants, thus the interaction between above-ground and below-ground induced defenses are likely to occur. Wallsgrove *et al*. (2003) showed that, in leaves of *Brassica napus* cultivar "Bienvenu" the treatment with *Sclerotinia* caused 10-20 fold increase in aromatic and indole glucosinolates in 3rd, infected leaves, and in 7th leaves the systemic increase caused only 25-99% increment. In the *Brassica napus* lines "Cobra" and "Capricon" the 3rd leaves had a similar localized increase in glucosinolate, but systemic effects were much lower (20%).
However, the individual classes of glucosinolates respond differently to the induction treatment. In general, tryptophan-derived indole glucosinolates are more responsive to induction than aliphatic glucosinolates. Mikkelsen *et al*. (2000) pointed out that different indole glucosinolates methoxylating enzymes are influenced by SA and MJ, whereas aliphatic glucosinolates appear to be primarily genetically and not environmentally controlled. Furthermore, Zeng *et al*. (2003) reported that aromatic and indole glucosinolates are responsible for interaction between Brassicaceae and fungi in the contrast to aliphatic glucosinolates.

The accumulation of indole glucosinolates following JA treatment and infection with certain pathogens may be regulated at the transcriptional level. *CYP79B2* and *CYP79B3* can be induced by MJ treatment, while *CYP79B2* by wounding (Mikkelsen *et al*., 2003) and by infection with *Pseudomonas syringae* (Hull *et al*., 2000) (Figure 2.14). *CYP79B3,* but not *CYP79B2,* was induced by treatment with JA as well as a cultural filtrate of *Erwinia carotovora* (Brader *et al*., 2001). This suggests specific but different roles for CYP79B2 and CYP79B3, which may not be physiologically redundant genes.
CYP79A2 converts phenylalanine to its aldoxime in the biosynthesis of benzylglucosinolate (Wittstock and Halkier, 2000). This is reflected in the expression pattern of *CYP79A2*. The expression pattern is very similar to the infection pattern of the bacterial pathogen *Xanthomonas campestris*. This suggests that CYP79A2 may function as a 'watchdog' to minimize the chance and the extent of infection by producing benzylglucosinolate at the possible entry points *of Xantomonas campestris*. *Arabidopsis thaliana* expressing a 35S::CYP79B2 construct was shown to accumulate up to five-fold more indole glucosinolates than the wild type (Mikkelsen *et al.*, 2000).



	CYP79B2	+++
	CYP79B3	++
	CYP79F1	+
	CYP79F2	+
	glucobrassicin	+++
+	4-hydroxy-glucobrassicin	
	neoglucobrassicin	++++

Figure 2.14. Signal transduction pathways and their effects on glucosinolate biosynthetic genes and products (Mikkelsen *et al.*, 2003)

However, elicitor influence on glucosinolate content depends also on plant species and cultivars. Loivamaki *et al.* (2004) represented the data that glucosinolate concentration in *Brassica napus* cultivar "Tuli" is naturally higher than in cultivar "Valo", but "Valo" reacted to MJ more strongly as "Tuili". It looks likely MJ raised the glucosinolate concentration in plants above a certain threshold level.

2.2.5 Elicitor influence on root exudation

Gleba *et al*. (1999) postulated that chemical as well as physical elicitors stimulate roots of various plants to exude phytochemicals in much higher quantities as non-elicited plants. Moreover, roots of elicited plants exude an array of compounds not detected in the exudates of non-elicited plants. The same elicitor triggers the production of different compounds in different plant species.
Walker *et al.* (2003) reported that after roots of *Arabidopsis thaliana* had been elicited with SA, JA, chitosan, and two fungal cell wall elicitors, 289 phytochemicals were quantified in the root

exudates. Several of these compounds exhibited a wide range of antimicrobial activity against both soil-borne bacteria and fungi at the concentration detected in the root exudates.

Kneer *et al.* (1999) showed that roots of hydroponically cultivated *Lupinus luteus*, transferred into water secreted minor amounts of genistein (about 0.05 mg 100 g^{-1} FW). Secretion of genistein from roots (rhizosecretion) was stimulated dramatically from 0.1 to over 1.0 mg 100 g^{-1} FW of roots by SA applied at concentration of 800 μM. Increased levels of genistein in root exudates corresponded to greater amounts of genistein in root tissue. Moreover, it was shown that mostly the elicitor-induced rhizosecretion of genistein occurres during the first day, followed by a gradual decline. Further addition of elicitor treatments has little effect on genistein rhizosecretion, indicating that the induction of rhizosecretion by the identified elicitors is a once only event.

It can be hypothesized that elicitor treatment of hydroponically grown plants can result in quantitative and qualitative changes in the composition of the exudates and this fact can be used as a tool for the technology for phytochemical manufacturing, particularly receiving them from plants as well as plant exudates. The important step for our investigations is to find the optimal elicitor and its concentration for increasing the quantity of glucosinolates in plants and their exudates.

3 MATERIALS AND METHODS

3.1 Design of experiments and conditions of plant growth

The object of our investigations was turnip (*Brassica rapa* L. var. *rapa pygmea teltoviensis*). The plants of turnip were cultivated in August-September 2001, April-May, June-July and October-November 2002, in February-March and December 2003, and in January 2004 in the greenhouse of the Institute for Vegetable- and Ornamental Crops, Grossbeeren/Erfurt e.V. in Grossbeeren, Germany. The analyzing of plant material and exudates had been provided in the Department Quality of the Institute for Vegetable- and Ornamental Crops in Grossbeeren and in the Department of Food Biotechnology and Process Engineering at the Technical University of Berlin.
The research program consisted of 3 experiments, schematically represented in Table 3.1.

Table 3.1. Schematic presentation of experimental design

<table>
<tr><th>Expe-riment</th><th>Description of the experiment</th><th>Sys-tem</th><th colspan="3">Treatments</th></tr>
<tr><td rowspan="3">1.</td><td rowspan="3">In this experiment it was provided:
- installation and comparison of different systems for plant growth: hydroponic (H), aeroponic with defensor (AD), and aeroponic with sprayers (AS)
- comparison of nutrient solutions: initial Hoagland (1H), two times concentrated Hoagland (2H) and two times concentrated Hoagland + two times increased sulfur concentration (2H2S)</td><td>H</td><td>H1H</td><td>H2H</td><td>H2H2S</td></tr>
<tr><td>AD</td><td>AD1H</td><td>AD2H</td><td>AD2H2S</td></tr>
<tr><td>AS</td><td>AS1H</td><td>AS2H</td><td>AS2H2S</td></tr>
<tr><td rowspan="3">2.</td><td rowspan="3">Two times concentrated Hoagland solution + two times increased sulfur concentration (2H2S) was selected for this experiment. It was studied the application of elicitors:
- salicylic acid (SA_0)
- and methyl jasmonate (MJ_0),
applied at the beginning of experiment</td><td>H</td><td>H</td><td>HSA_0</td><td>HMJ_0</td></tr>
<tr><td>AD</td><td>AD</td><td>$ADSA_0$</td><td>$ADMJ_0$</td></tr>
<tr><td>AS</td><td>AS</td><td>$ASSA_0$</td><td>$ASMJ_0$</td></tr>
<tr><td rowspan="2">3.</td><td rowspan="2">Hydroponic system with two times concentrated Hoagland solution + two times increased sulfur concentration, and SA is selected for this experiment.
In this experiment it is compared application of SA on:
- 0 day (HSA_0),
- 15^{th} day (HSA_{15}),
- 20^{th} day (HSA_{20}),
- and 25^{th} day (HSA_{25}).</td><td>H</td><td colspan="2">H</td><td>HSA_0</td></tr>
<tr><td colspan="2">HSA_{15}</td><td>HSA_{20}</td><td>HSA_{25}</td></tr>
</table>

According to the goals of our studies, it was provided the following investigations:
- installation of aeroponic and hydroponic systems and their comparison;
- optimization of plant nutrition;
- development of the method of glucosinolate separation and concentration from growing medium;

- determination of physiological and biochemical parameters of plants: dynamic of growth, fresh weight, dry matter, and shoot-root index; dynamic of profile and content of total and individual glucosinolates in all parts of plants, particularly in their leaves, primary, and secondary roots as well as in exudates.

3.1.1 Preparation of plant seedlings

Seeds of turnip were germinated inside Grodan rockwool cubes (5 cm width x 5 cm depth x 5 cm height) placed inside standard greenhouse plastic trays (52 cm width x 35 cm depth x 7 cm height) (Figure 3.1a). Seeds were watered with half concentrated Hoagland solution through overhead misting systems and were germinated until the roots of seedlings had emerged 2-4 cm out of the bottom of cubes (15-20 days, 2 leaves) (Figure 3.1b). Cubes were then inserted at 10 x 11 cm intervals into the holes of polystyrene plates (1 m x 1 m large, 4.5 cm thick) that were installed in hydroponic and aeroponic systems (Figure 3.1c).

a) seedlings in plastic trays b) seedlings in plastic trays c) plants in aeroponic, 1st day

Figure 3.1. Germination of seeds and preparation of plants for the experiment

3.1.2 Greenhouse conditions for plant growing

Plants were grown under 12 hrs photoperiod (from 8 AM until 8 PM) and hν 400 µmol m^{-2} s^{-1}. The quantum sensors (Model Li-190 SZ, LI-COR Lincoln, Nebraska, USA) were used for measuring the photosynthetic active radiation. When the natural radiation outside the green house exceeded 450 µmol m^{-2} s^{-1} plants were shaded using overhead curtains. As a source of artificial light the lamps SRG 140 (Philips, Germany) were used. This type of lamps promotes plant growth with the light of natural spectrum (Bakker *et al*., 1995). The lamps were installed 1.5 m above the systems and covered about 2 m^2 of surface.
The air temperature in greenhouse was 16°C during the day and 12°C at night, while in root chambers it exceeded the air temperature at approximately 1-2°C. The greenhouse was vented when the temperatures exceeded the norm.
The humidity in greenhouse achieved 60 %. It was supported by the fog system HNS (Type 7, Osberma, Engelskirchen, Germany) with sprayers HNS (Type 07.1, Osberma, Engelskirchen, Germany).
The temperature and humidity in greenhouse were registered with aspirations psychrometr AT (Type 77001, Henrich Zeiseniss Wasser Technik, Herrsching, Germany).

The temperature in roots zone inside aeroponics systems was registered with psychrometr PT 100 (Type WTE 10, Geraberg GmbH, Geraberg, Germany).
The temperature, light, and humidity conditions in growth chamber were controlled with RAM CC 600 equipment (Type DT 500, Ltd Rowville, Victoria, Australia). The measurements were provided every minute and the average meaning was saved once pre hour.
All the above described conditions were maintained constantly during the vegetative period in all the seasons.

3.1.3 Nutrient solution

One of the most important environmental factors, which can play key role in plant growth and development, is nutrition. It exist a number of solutions, which are used for growing *Brassicaceae* in hydroponics (Maloupa, 2002), but there is no solution, adapted for growing turnip in hydroponics as well as in aeroponics. We have selected an optimal solution for providing satisfied growth of plants and later modified this solution for enhancing glucosinolate content in plants as well as in exudates.
It is possible to stimulate the growth of *Brassicaceae* by enhancing nitrogen (stimulate root growth and decrease shoot-root ratio) and sulfur supply (stimulate glucosinolate synthesis) (Zhao, 1994). Moreover, the modification of the nutrient solution according to these aspects can lead to changing in exuded glucosinolate quantity and profile.
As the initial solution for turnip growing we choose the Hoagland solution (Table 3.2), which is usually used for growing plants in hydroponics (Bugbee, 1995).

Table 3.2. Hoagland solution and its modifications

Elements, g 100 l^{-1}		1H	2H	2H2S
A	$Ca(NO_3)_2$	41.00	82.00	82.00
	KNO_3	30.31	60.62	60.62
	FeChe	1.54	3.08	3.08
B	KH_2PO_4	13.60	27.20	27.20
	$MgSO_4\,7H_2O$	22.62	49.24	98.48
	H_3BO_3	0.1427	0.2854	0.2854
	$MnCl_2$	0.0416	0.0832	0.0832
	$ZnSO_4$	0.0061	0.0122	0.0122
	$CuSO_4$	0.0026	0.0052	0.0052
	H_2MoO_4	0.0080	0.0160	0.0160

A, B – vessels for storage of concentrated nutrient solution
1H - Hoagland solution, 2H - two times concentrated Hoagland solution, 2H2S - two times concentrated Hoagland solution + two times increased sulfur concentration

The 100 times concentrated solution was stored in 2 different vessels – “A” and “B” to avoid the precipitation of calcium and iron with phosphate and sulphate (Sonneveld, 2002). For the preparation of dissolved nutrient solution it was used water from reverse flow osmosis system. This water is free from ions and has nearly null-EC, which is necessary to avoid the interactions between the chemicals from water and nutrient solution. At the same time it makes easy to control EC and pH of nutrient solution in water systems (Voogt *et al*., 1997).

pH has been measured with the electronic pH meter GPHR (Model 1400A, Greisinger, Germany). The pH of Hoagland solution was *ca*. 6.2, after the modification it decreased until *ca.* 5.3, but was increased to 6.2 by adding of bascal (approximately 10 ml per 100 l of nutrient solution). As during growth plants removed nutrients from the solution, the pH rose slowly and became more alkaline. When pH reached *ca*. 7.2, it was decreased until 6.0-6.2 by adding 25 % H_3NO_4 (approximately 50 ml per 100l of nutrient solution).
For EC measurements it has been used the conductivity meter GMh (Model 3410, Greisinger, Germany). The EC of Hoagland solution is 1.3 mS cm^{-1}. The total nutrient concentration of 1.5 mS cm^{-1} in the root environment aims at optimal nutrient uptake in order to achieve maximum yields (DeKreij *et al*., 1999), but in our experiments with 2H it reached ca. 2.6, which was the optimal EC for turnip.
Therefore, for turnip in hydroponic and aeroponic, total nutrient concentration was increased, because during growing of turnip at Hoagland solution it was observed the chlorosis of leaves (data not shown). According to this fact the first step of nutrient solution modification was the increase of Hoagland solution concentration with keeping the balance between the elements: the concentration of all nutrients was increased concentrated 1.5 times (1.5H) and two times (2H). Also Krumbein *et al*. (2001) wrote that turnip needs high amounts of sulfur fertilizers in complement of nitrogen. This was the reason for two times increment of sulfur concentration in our experiments.

3.1.4 Plant elicitation

The concentrations of SA 800 μM (138 μg l^{-1}) and MJ 100 μg l^{-1} (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) were chosen according to recommendations of Doughty *et al.* (1995), Furmanova (1997), Sudha and Ravishankar (2003). The elicitors were added into nutrient solution: at the beginning of plant growth in the aeroponic and hydroponic systems in the experiment with SA and MJ as well as SA on the 15^{th}, 20^{th} and 25^{th} days of plant growth (Table 3.1). Root application was chosen because the previous studies, provided by Van Dam *et al*. (2003) had shown that leaf treatment with elicitors, particularly MJ and SA, increased glucosinolate level in shoots, but not in roots, whereas root application influenced metabolite content in roots as well as in shoots.

3.1.5 Technical characteristics of hydroponics and aeroponics

According to the previous researches it was suggested to growth the plants in hydroponics for receiving their exudates producing (Borisjyk *et al*., 1999). We have taken also aeroponics with the aim to minimize the quantity of solution in system. Our suggestion was to simplify the process of exudate extraction.
There were the reasons for choosing the proper systems:
- in water systems the nutrients are dissolved directly in water and the plants can receive the desired amount of nutrients directly to the root;
- pH and EC are easy to maintain;
- in water systems plant growth and yield per area can be increased, while pests and diseases decreased (Jones, 1983).

In our experiments there were installed three different systems: hydroponics (with floating platform and aeration system), aeroponic with pressure sprayers and aeroponic with atomizer fogger or defensor.

Floating hydroponic system

In hydroponic with floating platform and aeration system the plants were anchored into the floating platform placed directly on the surface of the nutrient solution (Figure 3.2). The hydroponic was aerated by blowing compressor (Model 40A-10011, Hiblo, Germany), which bubbled air through the nutrient solution at a flow rate of about 100 ml min^{-1} by each of 10 aeration tubes.

The dimensions of hydroponic were 1 m x 1 m, each system contained 100 l of nutrient solution and 56 plants.

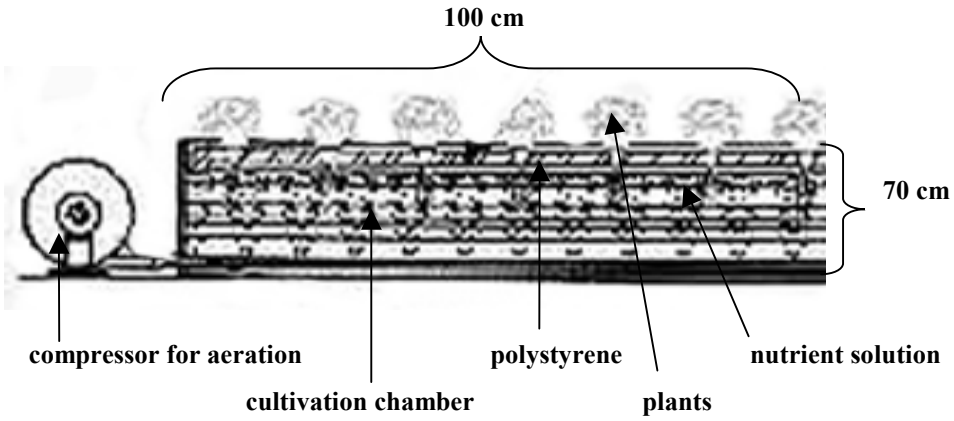


Figure 3.2. Construction of floating hydroponic system

Aeroponic systems

In aeroponic system the plants are supplied with nutrients through humidified air, the roots remain suspended and immersed in the growing chamber, where they are sprayed with a mist of nutrient solution at short intervals, usually every few minutes (Maloupa, 2002). The solution is taken from its supply tank by a pump controlled by an extremely precise timer calibrated for very short irrigating cycles, ranging from seconds to minutes (Jensen, 1997).

Aeroponic with sprayers

In aeroponic with sprayers the sprayers bathe the roots in a film of nutrient solution, which keeps near 100 % relative humidity to prevent drying of plant roots.

The construction of the system, we used in our studies is shown at Figure 3.3.

In aeroponic with sprayers (Type Dan Fogger 7800, Dan Sprinklers, Kibbutz Dan, Israel), installed in our experiments (Figure 3.4), the nutrient solution was pumped with two-stages centrifugal pump CH-60 (Model A-A-CVBV, Grundfos, Erkrath, Israel) from a storage tank via filter Bitron 15 (type G15T8, Oase, Berlin, Germany) to the cropping bed, from which it returned to the tank.

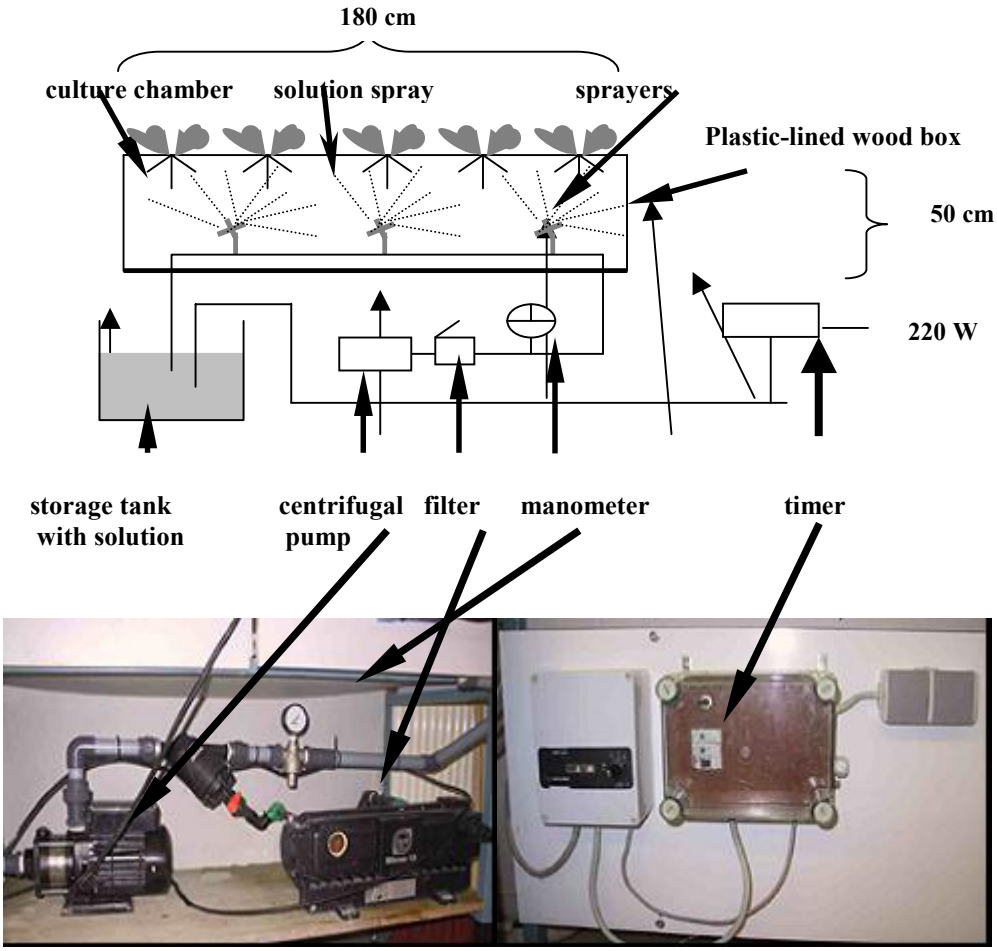


Figure 3.3. Construction of aeroponic with sprayers

In our experiments aeroponic with sprayers sprayed solution constantly, without interval, which is not typical for such type of aeroponic, but it was stipulated by the peculiarities of turnip root structure and sensibility to lack of water supply. The pressure in pump was 2 bars (although usually 4 bars is used), otherwise it caused mechanical damage of turnips roots.
The dimensions of aeroponic with sprayers were 0.9 m x 1.8 m, and 0.5 m height. It contained 100 l of nutrient solution and 126 plants were cultivated there.

Figure 3.4. Plants in aeroponic with sprayers

Aeroponic with defensor

Aeroponic with defensor, installed for our studies, is shown on the Figures 3.5 and 3.6.

Figure 3.5. Plants in aeroponic with defensor

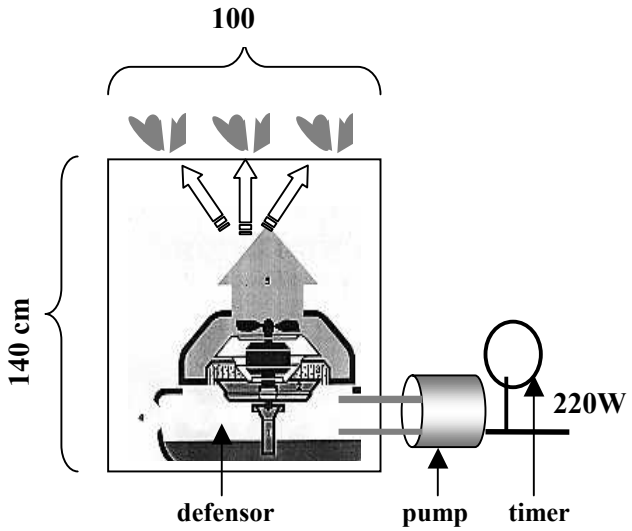


Figure 3.6. Construction of aeroponic with defensor

It consisted of defensor (Type 505, Axair AG, Pffaeficon, Germany), the rotation atomiser, which produces finest fog: the particles of water are 5-10 μm (Figure 3.7); pump (Type 157, EAW Electronic, Dresden, Germany), and timer, switching the pump on for every 15 minutes with 30 minutes interval.

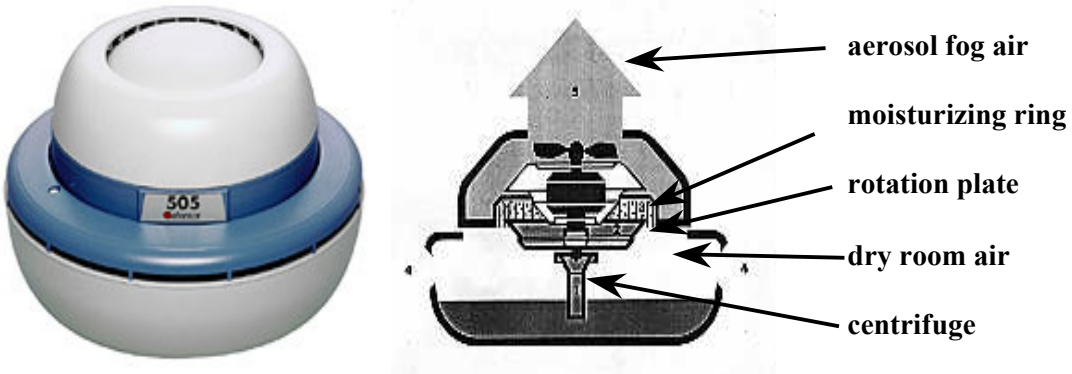


Figure 3.7. Construction of defensor 505

The dimensions of aeroponic with defensor were 1 m x 1 m, and 1.4 m height. It contained 25 l of nutrient solution and 56 plants were cultivated there.

3.2 Sampling of plant material and exudates

3.2.1 Sampling and analyzing of plants

The duration of the experiment was 30 days. At each harvesting date 10 plants from each treatment have been taken ones per 10 days for 1st experiment and once per 5 days for 2nd and 3rd experiment. Plants were randomly selected and harvested between 09:00 and 11:00 AM. Sampling was carried out at the same time to minimize differences due to daily fluctuations of the plant glucosinolate content (Rosa, 1997). Plants were separated into three parts: leaves (leaf blades and petioles), primary (or bulbous) roots, and secondary (or active fine) root.

Fresh and dry weight
Fresh weight was determined; the plant material was frozen at –28°C in deep freezer (Type Denley CFC-Free, Life Science International LTD., Denmark) and dried in freeze-drier Alpha (Type 1-4, Christ, Osterode am Harz, Germany). Dry weight was then determined and the plant material was ground.

Length of secondary roots
The length of root hairs was calculated after scanning of fresh secondary roots and analysing of scanned surface with the software image analyser Zeiss (Type KS 400, Version 3.0, Carl Zeiss Vision, Munich, Germany). From each treatment it was selected three roots and from each taken duplicated samples from upper, middle and down parts of root systems. The fresh weight of each sample was 100 mg.

3.2.2 Sampling of exudates

Knowing the fact that *Brassica* plants can exude glucosinolates into the growing medium we suggested collecting the glucosinolates from the root exudates of hydroponically grown plants. Moreover, it is known, that the rhizosecretion offers a simplified method for the isolation of phytochemicals from simple hydroponic medium rather than from complex plant extracts.
Extraction and determination of glucosinolates from plat tssues have been performed for many years, but there have been no published methods for the extraction and quantification of glucosinolates from growing medium.
Four replicates of 1 liter solution containing the plant root exudates were taken from each system every 10 days for the 1st experiment and every 5 days starting on day 10 for the 2nd and 3rd experiments (Table 3.1). During sampling the roots were additionally washed with the solution from the growing system by use of the compressor (Type 3590, Agrotop GmbH, Obertraubling, Germany). In sampled solution 20 μM l^{-1} $AgNO_3$ was added to prevent the glucosinolate destruction by myrosinase, which can also been exuded into growing medium and its activity can be stopped with $AgNO_3$ (Al-Turki and Dick, 2003).

Figure 3.8. ProScale System

After sampling, nutrient solution was changed in each system. The samples were pre-filtered using paper filters (N 604, Schleicher and Schuell, Dassel, Germany).
The principle of ultrafiltration has been used for the preparation of exuded samples. Exudate samples were processed with the ProScale System (Millipore GmbH, Eschborn, Germany) (Fig. 3.8), a bench top development Tangential Flow Filtration (TFF) system, used for fluid processing. The ultrafiltration is a pressure driven membrane process used to concentrate, separate, and purify the solution.
The high-pressure bench consisted of Helicon-RO-4 Cartridges containing Nanomax-95 Membrane. The membrane was a thin semi-permeable polymeric material that retained macromolecules and allowed smaller dissolved molecules to pass through.
It was provided the selecting and concentrating compounds with molecular weights 250-2000 Daltons and removing monovalent salts from the solution.
The samples were then transfused into 600 ml glass flacks (Christ, Osterode am Harz, Germany), frozen at -28°C, and freeze-dried.
As it is possible to see from the Figure 3.9, the procedure of exudates samples preparation needs fewer steps as the procedure of plant material preparation.
The additional step in the process of preparation the samples of exudates for HPLC analyzes is the pre-filtration and concentration of the liquid. From the other side, the shortening of the procedure of exudates samples preparation as comparing to that of plant materials is in avoiding the steps of material grounding and extracting.

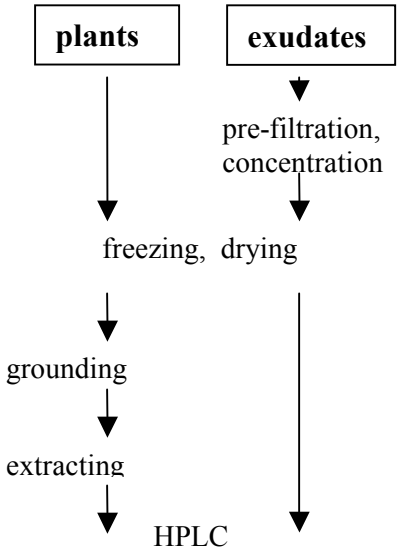
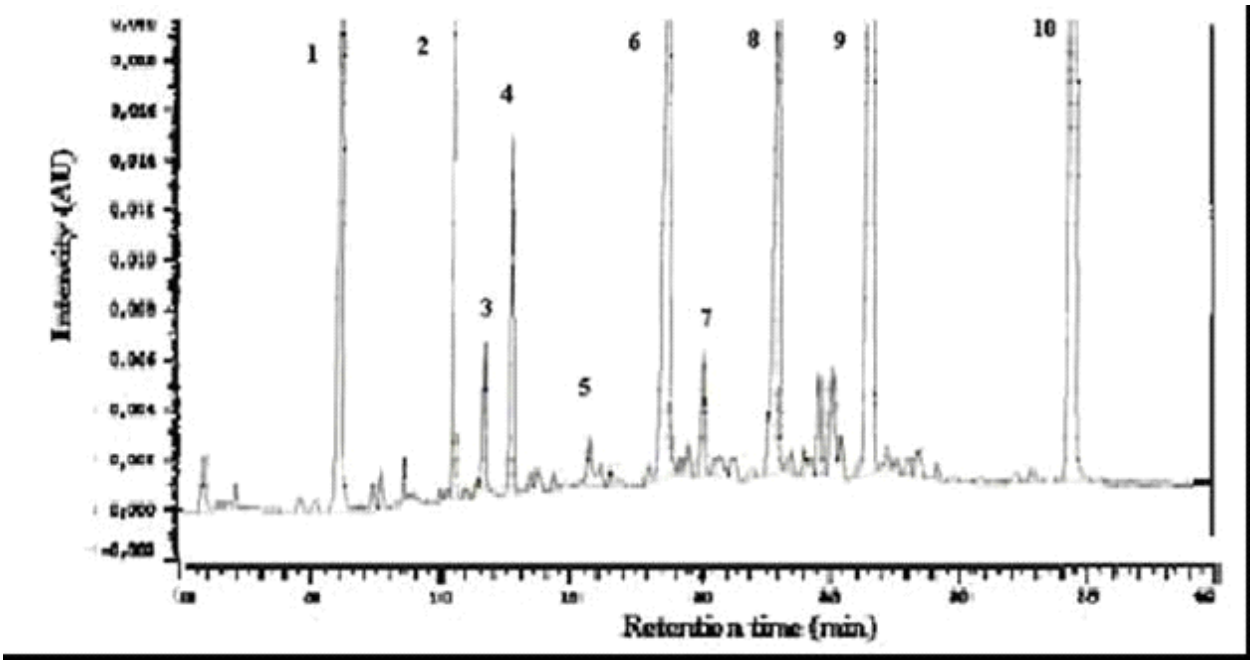


Fig. 3.9. Preparation of plant and exudates material for HPLC analysis

3.3 Glucosinolate extraction and determination by HPLC

A modified HPLC method of Lange *et al.* (1991) improved by Krumbein *et al.* (2001) was used for glucosinolate determination. Freeze-dried sample material (0.5 g of plant or dried substances from 1 l of solution with exudates) was heated to and incubated at 75°C for 1 min, extracted twice with 4 ml of a methanol/water mixture (v/v=7:3) and then, after adding 1 ml

0.4 M barium acetate, centrifuged at 4000 rpm for 10 min. The extracted material was pooled and made up to 10 ml. From this, 5 ml of the extract was applied to a 250 µl DEA-Sephadex A-25 ion-exchanger (acet-activated, Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) and rinsed with 10 ml bi-distilled water. Next, 250 µl of a purified solution of aryl sulfatase (Boehringer-Mannheim GmbH, Mannheim, Germany) was applied and left for 12 h before the desulfo-compounds were flushed with 5 ml bi-distilled water.
Desulfoglucosinolate analysis was conducted by HPLC (Merck HPLC pump L-7100, DAD detector L-7455, automatic sampler AS-7200 and HPLC Manager-Software D-7000) using Spherisorb ODS2 column (5 µm, 250 x 4 mm). A gradient of 0-20% acetonitrile in water was selected from 2 to 34 min, followed by 20% acetonitrile in water until 40 min, and then 100% acetonitrile for 10 minutes until 50 min. Determination was conducted at a flow of 1.3 ml min^{-1} and a wavelength of 229 nm. Sinigrin (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) and glucotropaeolin (AppliChem GmbH, Darmstadt, Germany) were used as standards. Individual glucosinolates were identified by comparison of their retention times with individual glucosinolates in standard reference materials of oilseed rape (BCR-190R and BCR-367 R) (Wathelet *et al*., 1991) (Linsinger *et al.*, 2001). Glucosinolate content was calculated using sinigrin as internal standard and the response factor of each compound relative to sinigrin. Determination of glucosinolates was performed in duplicate and the concentrations of glucosinolates were expressed as mg $plant^{-1}$. The data represent the dynamic of the glucosinolate content in plants and exudates during the experiment as well as collected in exudates during the 30 days of experiment.
The profile of glucosinolates in turnip consists of at least 10 glucosinolates (Figure 3.10 and 3.11, Table 3.3).

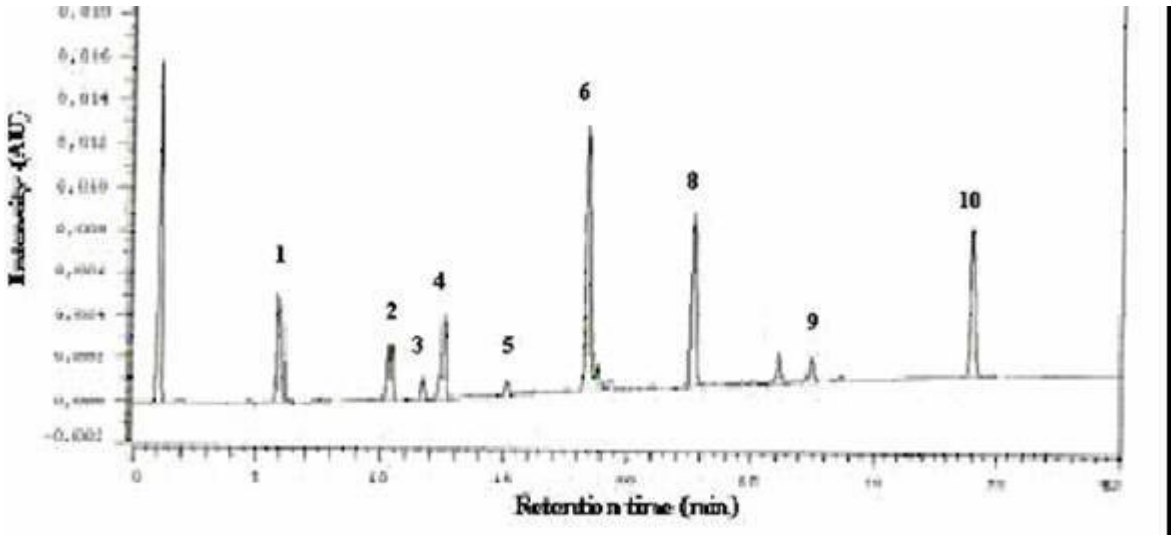


1 – progoitrin, 2 – gluconapoleiferin, 3 – glucoalyssin, 4 – gluconapin, 5 - 4-hydroxy-glucobrassicin, 6 – glucobrassicanapin, 7 – glucobrassicin, 8 – gluconasturtiin, 9 - 4-methoxy-glucobrassicin, 10 – neoglucobrassicin
Figure 3.10. HPLC chromatogram of glucosinolate profile in primary roots of turnip

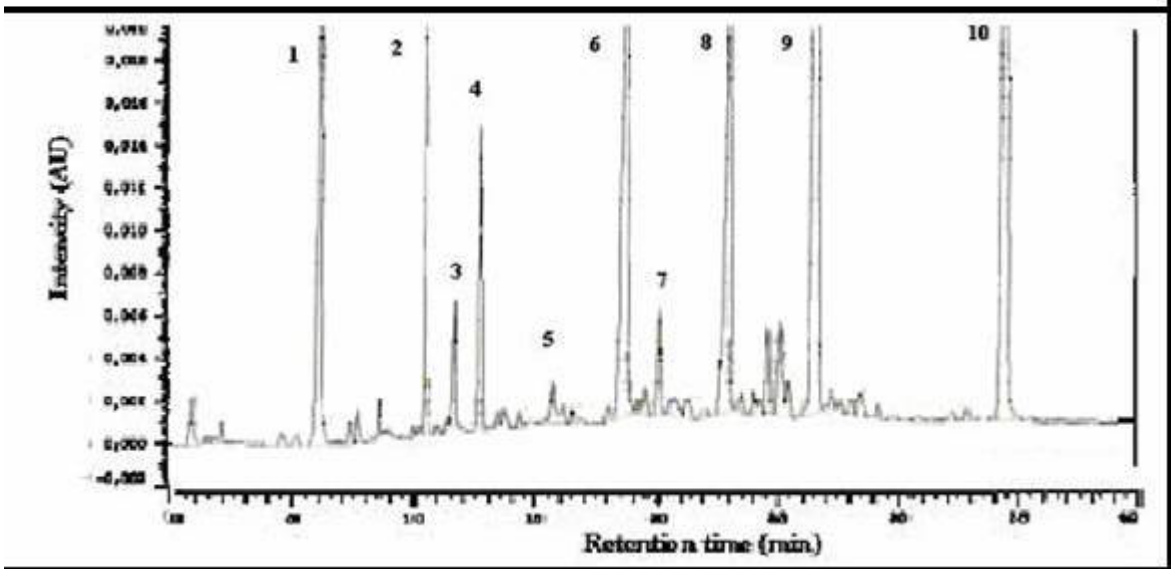
Table 3.3. Glucosinolates presented in turnip

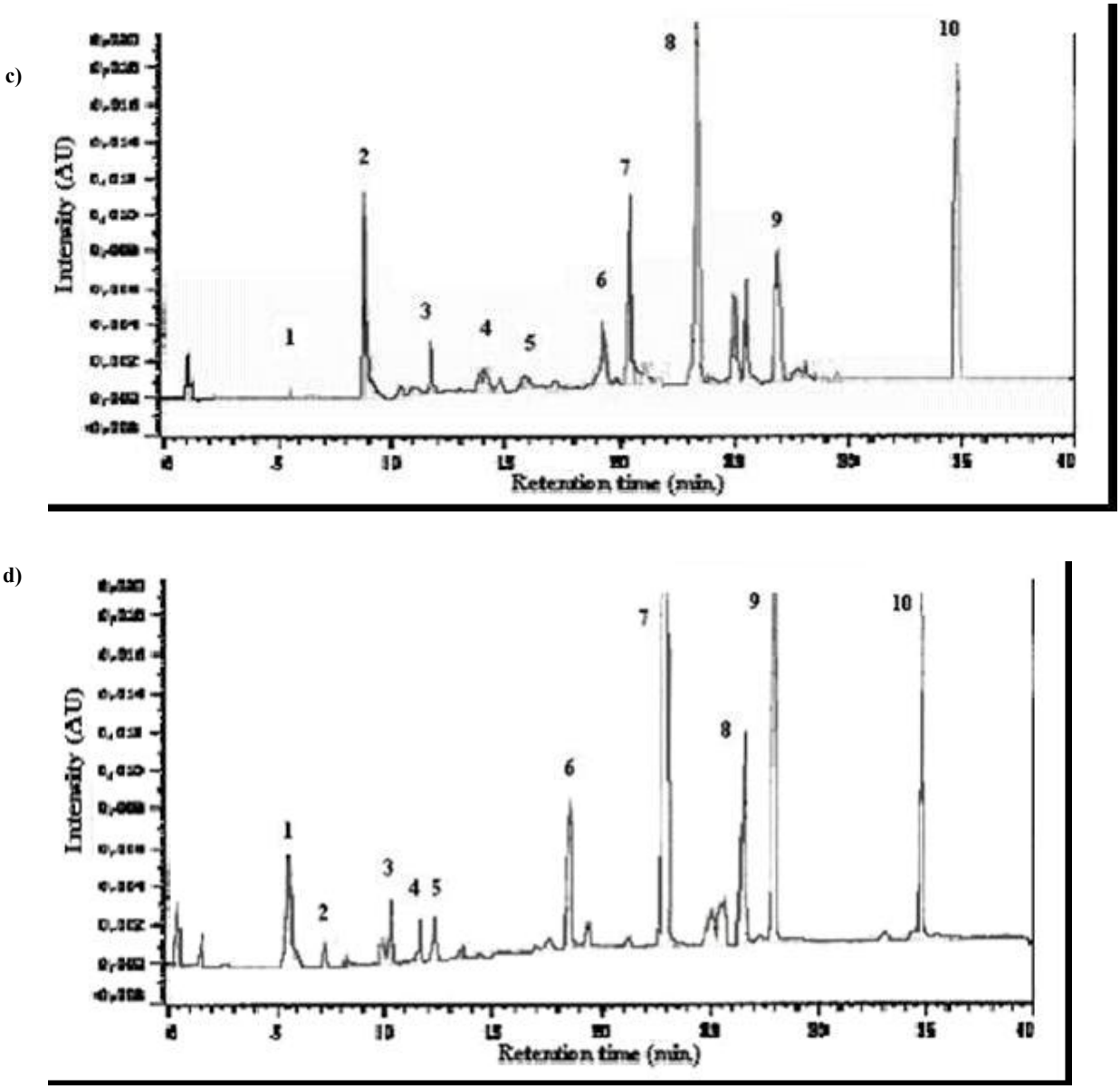
Glucosinolate		Retention time	Response factor
1	Progoitrin	5.9	1.09
2	Gluconapoleiferin	10.5	1.00
3	Glucoalyssin	11.8	1.00
4	Gluconapin	12.7	1.11
5	4-OH-glucobrassicin	18.9	0.28
6	Glucobrassicanapin	18.6	0.93
7	Glucobrassicin	22.8	0.29
8	Gluconasturtiin	26.3	0.95
9	4-methoxy-glucobrassicin	27.5	0.25
10	Neoglucobrassicin	34.2	0.20

a)



b)





1 – progoitrin, 2 – glucoraphanin, 3 – gluconapoleiferin, 4 – glucoalyssin, 5 – gluconapin, 6 - 4-hydroxy-glucobrassicin, 7 – glucobrassicanapin, 8 – glucobrassicin, 9 – gluconasturtiin, 10 - 4-methoxy-glucobrassicin, 11 – neoglucobrassicin
a - leaves (dilution 1:10), b - primary roots (dilution 1:10), c - secondary roots (dilution 1:10), d - exudates of turnip (dilution 1:100)

Figure 3.11. Glucosinolate profile

Individual glucosinolate content in mg $100g^{-1}$FW was calculated according to the time of retention and peaks intensity, using sinigrin as an external standard and the response factor of each compound relative to sinigrin.

$$Cgsl = \frac{Sgsl \cdot RFgsl}{Sis \cdot RFis} \cdot \frac{Nis}{W} \cdot DW$$

Cgls - content of individual desulfoglucosinolate, mg 100 g^{-1} FW,
Sgsl - square of peaks of desulfoglucosinolate,

Sis - square of peaks of internal standard,
RFgsl - response factor of desulfoglucosinolate,
RFis - response factor of internal standard,
Nis - quantity of internal standards in mg ml^{-1},
W - weight of sample, g
DW - dry weight of sample, %

It was also calculated the content of glucosinolates per plant (in leaves, secondary, and primary roots):

$$Vgslpl = \frac{Cgsl \cdot FW}{100g}$$

Cglspl - content of individual desulfoglucosinolate, $plant^{-1}$
Cgls - content of individual desulfoglucosinolate, mg $100g^{-1}$ FW
FW - fresh weight of plant (leaves, primary, and secondary roots), g^{-1}.

The content of glucosinolates in exudates of one plant was calculated through the formula:

$$Cgslex = \frac{Cgsl \cdot Vol}{Npl}$$

Cgsl ex - content of individual desulfoglucosinolate in exudates, $plant^{-1}$
Cgsl - content of individual desulfoglucosinolate in solution in whole system, l^{-1}
Vol - volume of solution in system, l
Npl - quantity of plants in system

3.2.6 Statistical analysis

The fresh weight, dry matter, and total as well as individual glucosinolate contents in plants and exudates were analyzed using descriptive statistic, analysis of variance, and least significant differences were calculated with the Tukey's Honest Significant Difference test (HSD, significance level $\alpha \leq 0.05$). All statistical analyses were performed with Statistica™ for Windows™ (Version 6.1, Statsoft Inc.).
The presented data points are the means of:

- 4 replications for HPLC analyses of glucosinolates,
- 10 replications for 1st experiment and 5 replications for 2nd and 3rd experiment for measurement of fresh and dry weight,
- 18 replication for analyzing of secondary root length (scanned).

4 RESULTS AND DISCUSSIONS

4.1 Influence of nutrient supply on plant growth and glucosinolate content in plants and exudates of turnip

During this experiment it was compared the impact of three soil-free systems (hydroponic, aeroponic with defensor, and aeroponic with sprayers) as well as influence of three different nutrient solutions on turnip growth and glucosinolate content in plants and exudates (Table 3.1). The reason for modification of the initially selected Hoagland solution was the observing of the leaf chlorosis for plants, grown in systems with this solution.

4.1.1 Total glucosinolate content

Nutrient supply and type of system clearly showed the influence on total as well as individual glucosinolate content in plants and their exudates. Moreover, it differed between parts of plants and changed during plant growth. The major part of glucosinolates on the beginning of the experiment was measured for leaves and then with plant growth it became higher in secondary roots and in appeared after 10^{th} day primary roots.
On 10^{th} day H1H leaves had 1.1 mg $plant^{-1}$ of glucosinolates (73 % of these in plants), while H2H2S 1.2 mg $plant^{-1}$ (75 %), and H2H2S 1.5 mg $plant^{-1}$ (78 %) (Table 4.1). On 30^{th} day it changed to 3.2 mg $plant^{-1}$ for H1H (25 % of these in plants) as well as for H2H2S (22 %) and for H2H2S 3.9 mg $plant^{-1}$ (22 %). In contrast to hydroponically grown plants, in AS1H leaves total glucosinolate content reached 4.9 mg $plant^{-1}$, while in AS2H and AS2H2S it decreased during the experiment (Attachment, Table 4.1b). This could be explained by differences in growth and development of plants from hydroponic and aeroponic systems. It includes enlargement of root system, slowing down the glucosinolate synthesis in leaves, as it also was written by Rosa (1997), transportation of glucosinolates from leaves to roots (Chen *et al.*, 2001) as well as differences in nutrient needs during the plant growth (Carlson *et al*., 1987).
The total glucosinolate content for 2H2S leaves on 10^{th} day was higher as compared to 1H and 2H, then on 20^{th} day was lower as for two other systems and on 30^{th} day exceeded again that for 1H and 2H. Also Hirai *et al*. (2004) mentioned, that the insufficient sulfur supply cause the decrease of aliphatic glucosinolate content in leaves, but not in roots of *Arabidopsis thaliana*, which can be explained by activation of methionine-synthase in roots under the sulfur deficiency and absence of this effect in leaves. The same dynamic was also observed for plants from AD, however AS2H2S leaves on 30^{th} day had less glucosinolates as these from AS1H and AS2H.
From the table 4.1 it is possible to see, that glucosinolate content in secondary and primary roots did not correspond to that in leaves. In addition, Rosa and Rodrigues (1998) reported that the total and individual glucosinolate levels showed highly significant differences between the different plant parts.
H1H secondary roots on 10^{th} day had 37 % of glucosinolates contented in leaves. However, it changed during the vegetation and on 30^{th} day total glucosinolate content in H2H2S secondary roots reached 125 % of these in leaves. On 10^{th} day leaves of H2H had lower content of glucosinolates as H2H2S, but they were characterized by 2-fold higher glucosinolate content in secondary roots as H1H and H2H2S.

Table 4.1. Influence of plant nutrition on total glucosinolate content in plants and exudates of turnip from hydroponic (mg plant^{-1})

Date	Treatment	Leaves	Secondary roots	Primary roots	Exudates
10 days	H1H	$1.1^{a} \pm 0.3$	$0.4^{a} \pm 0.1$		$0.4^{a} \pm 0.1$
	H2H	$1.2^{a} \pm 0.2$	$0.8^{b} \pm 0.2$		$0.7^{b} \pm 0.1$
	H2H2S	$1.5^{a} \pm 0.2$	$0.4^{a} \pm 0.0$		$0.7^{a} \pm 0.2$
20 days	H1H	$2.8^{a} \pm 0.3$	$2.1^{a} \pm 0.2$	$2.9^{a} \pm 1.1$	$1.0^{a} \pm 0.2$
	H2H	$2.9^{a} \pm 0.2$	$3.8^{b} \pm 0.3$	$3.5^{a} \pm 0.7$	$1.9^{b} \pm 0.3$
	H2H2S	$2.4^{a} \pm 0.4$	$3.2^{b} \pm 0.3$	$4.8^{b} \pm 0.4$	$1.5^{ab} \pm 0.5$
30 days	H1H	$3.2^{ab} \pm 0.7$	$2.4^{a} \pm 0.7$	$6.3^{a} \pm 0.9$	$1.2^{a} \pm 0.4$
	H2H	$3.2^{a} \pm 0.1$	$3.6^{b} \pm 0.4$	$7.4^{ab} \pm 1.1$	$1.8^{b} \pm 0.2$
	H2H2S	$3.9^{b} \pm 0.3$	$4.9^{c} \pm 0.8$	$9.0^{b} \pm 0.6$	$2.7^{c} \pm 0.3$
Total	H1H				$2.6^{a} \pm 0.4$*
	H2H				$4.1^{b} \pm 1.2$*
	H2H2S				$4.6^{b} \pm 0.6$*

H1H - hydroponic with Hoagland solution; H2H - hydroponic with two times concentrated Hoagland solution; H2H2S - hydroponic with two times concentrated Hoagland solution and two times increased concentration of sulfur. The differences are compared for each treatment. Values followed by the same letter are not significantly different. *Sum of glucosinolates exuded during 30 days

According to Dhankar *et al*. (1993) increase of glucosinolate concentration in roots and decrease in leaves could be explained by their transport in order to give more glucosinolates, which then could be degraded and play a role of source of sulfur for roots. Sulfur deficiency could cause gene induction of glucosinolate biosynthetic enzymes and possibly translocation of glucosinolates from leaves to roots.

As also leaves, H and AD secondary roots were more influenced by 2H on 20th day of experiment, but on 30th day for 2H2S treatments, the content of glucosinolates in secondary roots was maximal as compared to 1H and 2H. Decrease of glucosinolate content in 2H secondary roots on 30th day as compared to 20th could be explained by the deficiency of sulfur and consequently by glucosinolate degradation by myrosinase in order to meet the sulfur demand for sulfur-containing primary metabolites (Hirai *et al*., 2004). The same dependence was also measured on 30th day for AS and AD (Attachment, Table 4.1a and 4.1b). Different reactions of roots expressed in changes in glucosinolate synthesis caused by aging, which correlates with the metabolic activity of root (Walker *et al*., 2004). Changing of glucosinolate content could be explained by the gradual maturation of root tissues and variation of their metabolic activity.

Primary roots of turnip appeared only after 10th day of the experiment. That explains why the first measurements for them are shown starting from 20th day. On 30th day around 50 % of plant glucosinolates were concentrated in primary roots. Supply of plants with 2H increased glucosinolate content by 1.1 mg plant^{-1} for H2H as compared to H1H, and additional application of sulfur led to its increase by 1.6 mg plant^{-1} as compared to H2H (Table 4.1). Similar tendencies were also measured for both aeroponics. (Attachment, Tables 4.1a and 4.1b).

As it is possible to see, the glucosinolate content varied between plant organs, which also have been reported by Fahrey *et al*. (2001). Rauth (2002) also wrote that the concentration of different glucosinolates varies widely among different developmental stages of plants as well as among different organs.

The total glucosinolate content in primary roots increased rapidly to the end of experiment, while in leaves it decreased. Fahrey *et al*. (2001) assumed that plant age is a major determinate of the qualitative and quantitative glucosinolate composition in plants. This may be explained by translocation of glucosinolates between the aerial parts of the plant and its roots (Rosa, 1997).

Heaney *el al.* (1983) reported that the application of sulfur fertilizer leads to increase in glucosinolate content. Also Booth and Walker (1992) approved that glucosinolate content can be increased by sulfur application, even when yield is not affected, and the glucosinolate content responds more to sulfur application as to application of other elements. It was also written that sulfur supply is the most noticeable factor, influencing glucosinolate content in plants. However, Fieldsend and Milford (1994) showed that glucosinolates contain only a small proportion of the crop total sulfur and that they are unlikely to be a major source of recyclable sulfur, even under conditions of severe sulfur deficiency.

By study of the total glucosinolate content in aboveground part of *Brassica* plants Heaney *et al.* (1983) showed that the increase nitrogenous fertilizer application leads to increase in total glucosinolate content. However, contrasting results have been reported about the influence of nitrogen, ranging form the negative to a positive. Finally, Zhao *et al.* (1994) represented that the balance between nutrient supply, particularly nitrogen and sulfur played an important role in the regulation of glucosinolate biosynthesis.

The effect of increased sulfur concentration in nutrient solution on total glucosinolate content was more noticeable for plants from aeroponic as from hydroponic. In general, plants from both of aeroponics had more glucosinolates as plants from hydroponics. The content of exuded glucosinolates depended on nutrient supply, as it is possible to see from the Figure 4.1, on stage of plant development (Figure 4.2), and on type of growing system (Figure 4.3). The content of excreted during 30 days glucosinolates for H2H2S reached 2.6 mg plant^{-1} (26 % of these in plants), while for H2H 4.1 mg plant^{-1} (29 %), and for H2H2S 4.6 mg plant^{-1} (26 %) (Table 4.1).

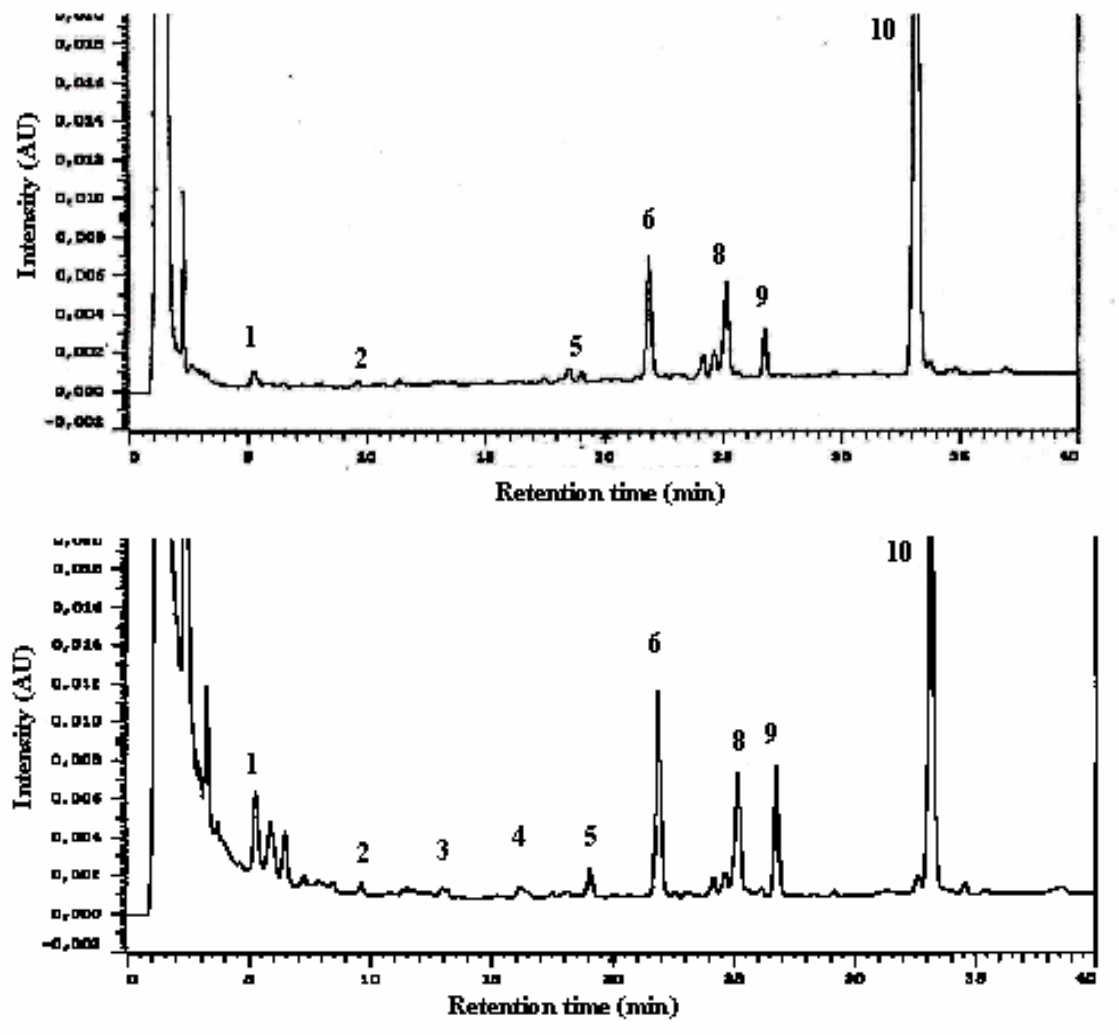
The type of system for plants growing also influenced on glucosinolate content in exudates. During 30 days AD1H plants exuded 2.2 mg plant^{-1} of glucosinolates (19.4 % of these in plants), while AD2H 3.6 (24%), and AD2H2S 5.9 mg plant^{-1} (30%) (Attachment, Table 4.1a). AS1H plants exuded 2.4 mg plant^{-1} (15.3 % of these in plants), AS2H 3.1 mg plant^{-1} (16 %), and AS2H2S 5.0 mg plant^{-1} (23 %) of glucosinolates (Attachment, Table 4.1b).

The dependence of the content of exuded glucosinolates on the growing system and nutrient supply may be explained by the influence of these factors on the total length of secondary (fine) roots (Table 4.2). It depended strongly on the type of system and on nutrient supply. Root length of AS1H plants on 30th day reached 61 m plant^{-1}, which was 1.4-fold more as for H1H plants and 1.4-fold less as for AD1H. At this time, H2H plants increased their root length to 56 m plant^{-1}, which was 1.3-fold more as for H1H plants, but 1.2-fold less as for H2H2S.

The increase of root length of AS plants was proportional to that for H plants, but AD developed much longer root system. The length of AD1H secondary roots reached 86 m plant^{-1}, with increase of nutrient supply roots became 1.8-fold longer as compared to AD1H, adding of sulfur led to 1.2-fold increase of root length.

a)

b)



a) hydroponic, initial Hoagland solution; b) hydroponic, two times concentrated Hoagland solution with two times increased concentration of sulfur

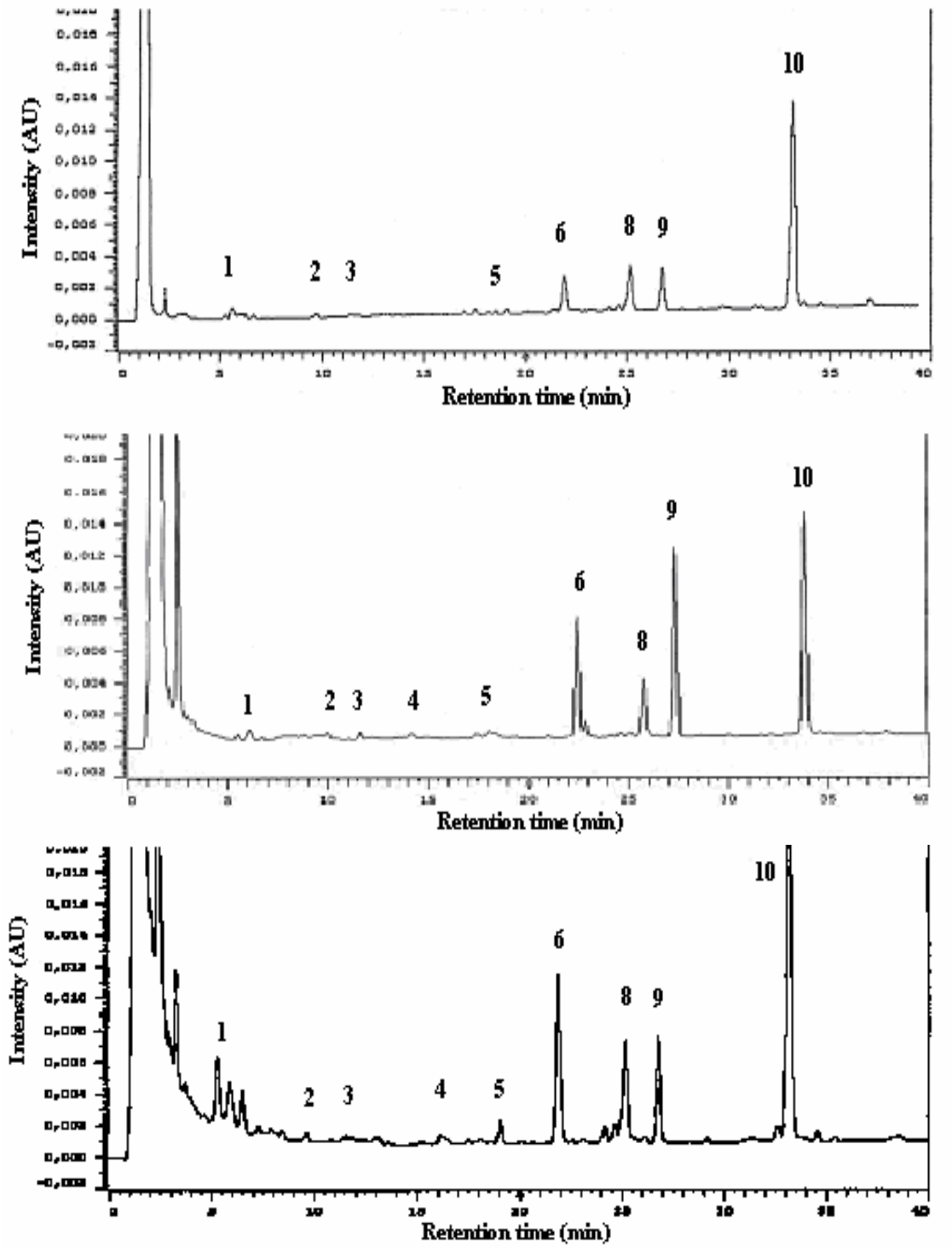
1 – progoitrin, 2 – gluconapoleiferin, 3 – glucoalyssin, 4 – gluconapin, 5 - 4-hydroxy-glucobrassicin, 6 – glucobrassicanapin, 7 – glucobrassicin, 8 – gluconasturtiin, 9 - 4-methoxy-glucobrassicin, 10 - neoglucobrassicin

Figure 4.1. HPLC profile of glucosinolates recovered on 30th days from H1H and H2H2S root exudates

a)

b)

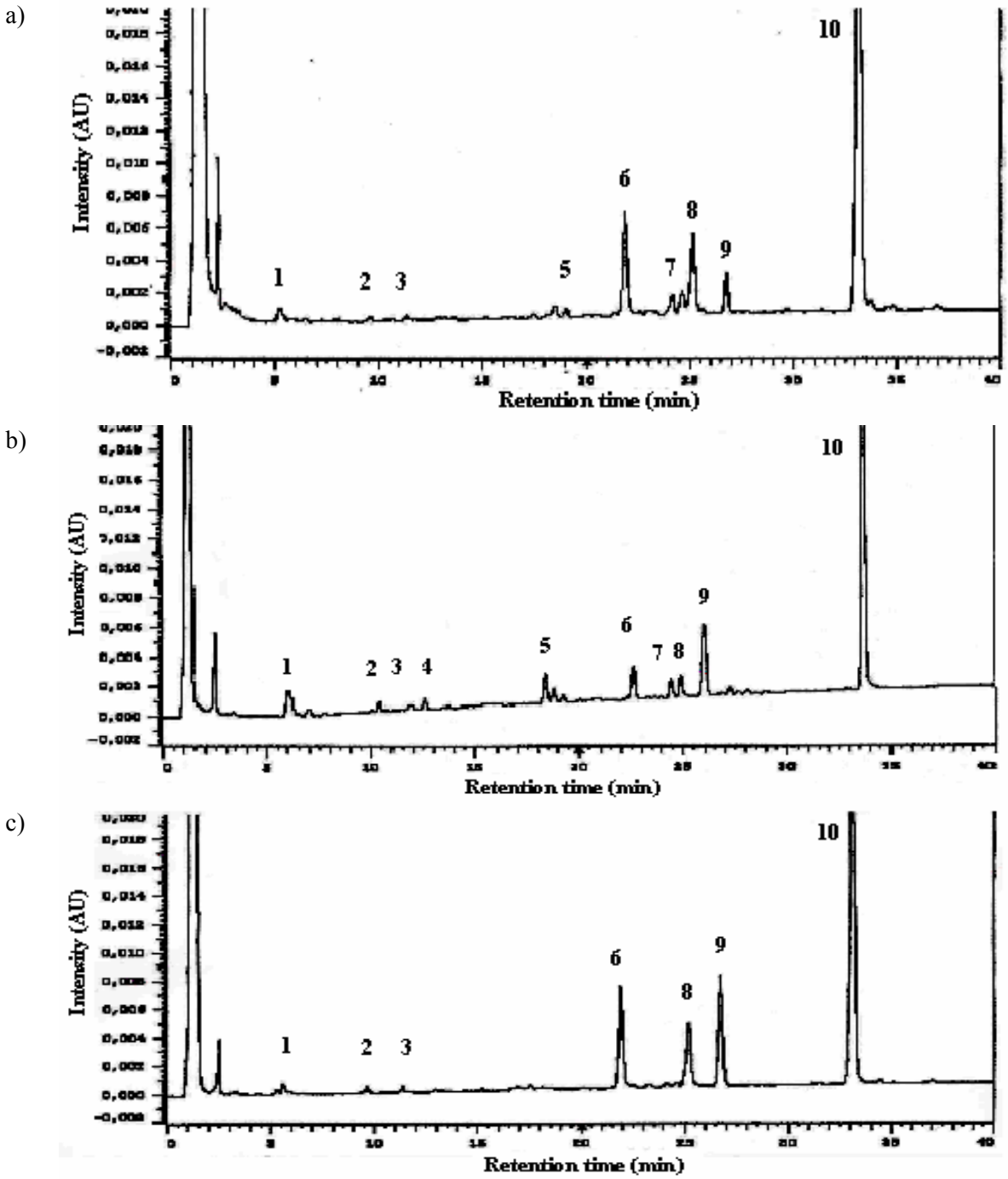
c)



a) 10 days; b) 20 days; c) 30 days

1 – progoitrin, 2 – gluconapoleiferin, 3 – glucoalyssin, 4 – gluconapin, 5 - 4-hydroxy-glucobrassicin, 6 – glucobrassicanapin, 7 – glucobrassicin, 8 – gluconasturtiin, 9 - 4-methoxy-glucobrassicin, 10 - neoglucobrassicin

Figure 4.2. HPLC profile of glucosinolates recovered from 2H2S root exudates on 10th, 20th, and 30th day of experiment



a) H1H - Hydroponic, Hoagland solution; b) AS1H – Aeroponic with sprayers, Hoagland solution; c) AD1H – Aeroponic with defensor, Hoagland solution

1 – progoitrin, 2 – gluconapoleiferin, 3 – glucoalyssin, 4 – gluconapin, 5 - 4-hydroxy-glucobrassicin, 6 – glucobrassicanapin, 7 – glucobrassicin, 8 – gluconasturtiin, 9 - 4-methoxy-glucobrassicin, 10 - neoglucobrassicin

Figure 4.3. HPLC profile of glucosinolates recovered during the period between 20th -30th day of experiment from root exudates of plants grown in different systems

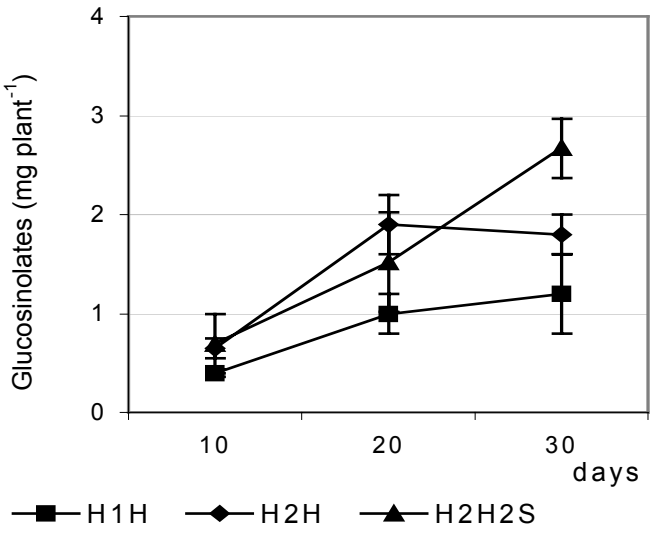
Table 4.2. Influence of plant nutrition on the length of turnip secondary roots on 30th day, m plant^{-1}

System	1H	2H	2H2S
H	$43^{a} \pm 7$	$56^{ab} \pm 14$	$68^{a} \pm 3$
AS	$61^{a} \pm 10$	$74^{a} \pm 9$	$77^{a} \pm 12$
AD	$86^{a} \pm 11$	$158^{b} \pm 16$	$183^{b} \pm 20$

1H - Hoagland solution; 2H - two times concentrated Hoagland solution; 2H2S - two times concentrated Hoagland solution and two times increased concentration of sulfur; H – hydroponic, AD - aeroponic with defensor; AS - aeroponic with sprayers. The differences are compared for each treatment. Values followed by the same letter are not significantly different. Values followed by the same letter are not significantly different.

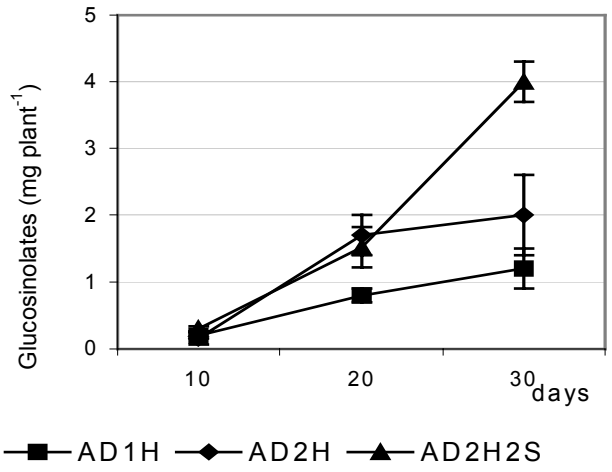
Berdanek *et al*. (2005) wrote that roots possess special means to modify the chemistry and, consequently, the biology of the surrounding rhizosphere by secreting root-specific, biologically active metabolites whose chemical nature and quantity depends on the nature and intensity of signals perceived from the environment.

In spite of changing the concentration of nutrient solution, the content of exuded glucosinolates did not essentially change during the first 10 days: H1H plants 0.4 mg plant^{-1} and H2H as well as H2H2S 0.7 mg plant^{-1}.

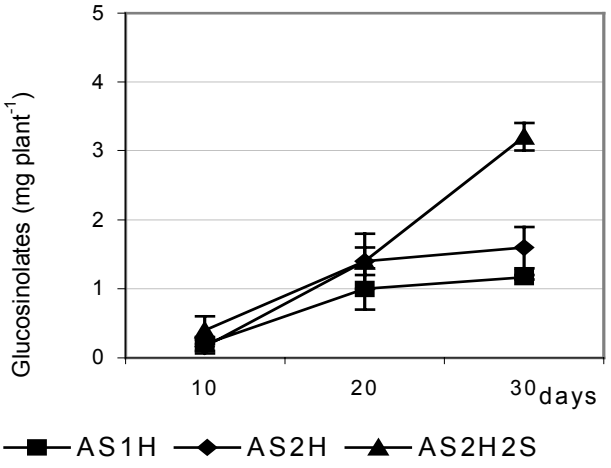


H1H - hydroponic with Hoagland solution; H2H - hydroponic with two times concentrated Hoagland solution; H2H2S - hydroponic with two times concentrated Hoagland solution and two times increased concentration of sulfur

The impact of nutrients on glucosinolates in exudates was more essential for hydroponic as for aeroponic systems: during the first 10 days the highest total glucosinolate content in exudates was for AD2H2S 0.3 and for AS2H2S 0.4 mg plant^{-1}, which was 2.5 and 1.4-fold lower as during the same period for H2H2S (Figure 4.4). Increase of glucosinolates in exudates from 2H could be explained by the observation, made by Shattuck and Wang (1993). Rich nitrogen supply increases the cuticle conductance, the conductance of root border cells also increases, and this could lead to intensification of rhyzosecretion.



AD1H - aeroponic with defensor with Hoagland solution, AD2H - aeroponic with defensor with two times concentrated Hoagland solution, AD2H2S - aeroponic with defensor with two times concentrated Hoagland solution and two times increased concentration of sulfur



AS1H - aeroponic with sprayers with Hoagland solution, AS2H - aeroponic with sprayers with two times concentrated Hoagland solution, AS2H2S - aeroponic with sprayers with two times concentrated Hoagland solution and two times increased concentration of sulfur

Figure 4.4. Influence of plant nutrition on the kinetic of glucosinolates in exudates of turnip

During the next 10 days, the content of exuded substances increased for H1H to 1.0 mg plant^{-1}, in H2H to 1.9 mg plant^{-1}, and in H2H2S to 1.5 mg plant^{-1}.

During the last 10 days for H1H it reached 1.2 mg plant^{-1} (0.2 mg plant^{-1} more then during the previous 10 days), for H2H it decreased slightly as compared to 20th day and was 1.8 mg plant^{-1}, and in H2H2S it increased by 1.2 mg plant^{-1} and reached 2.7 mg plant^{-1}.

This could be connected with more intensive developmentof secondary roots under the increased nutrient supply as well as intensification of exudation process.

By calculation the quantity of exuded glucosinolates per 1 g of secondary roots, it is possible to see, that between 20th and 30th days 1 g (fresh weight) of H1H secondary roots exuded 0.11 mg of glucosinolates, while from H2H and H2H2S 0.17 mg g^{-1}.

Slowing down the intensity of glucosinolate exudation for plants from 1H and 2H systems could be explained by altering of roots, and consequently by decrease of their metabolic activity (Walker *et al*., 2003; Jones *et al*., 2004).

The same tendencies were observed for both aeroponics (Figure 4.4). During 30 days AD2H2S plants exudates 2.7 mg plant^{-1} of glucosinolates and AS2H2S plant 3.2 mg plant^{-1}.

4.1.2 Individual glucosinolates in plants and exudates

Glucosinolate profile was influenced by the nutrient supply of plants as well as by the type of system. Changing of composition of aliphatic, aromatic, and indole glucosinolates in all parts of plants and their exudates during the growth period is shown in Table 4.3 and Attachment, Tables 4.3a and 4.3b.
The main class of glucosinolates in leaves, primary roots, and exudates for all treatments was aliphatic, while for secondary roots indole. The smallest part represented aromatic glucosinolates for leaves and roots of turnip as well as for exudates. On 30th day of experiment aliphatic, aromatic, and indole glucosinolates for H1H leaves composed 48 : 12 : 39 %. More as a half of the glucosinolates in H1H primary roots and exudates belonged to aliphatic, while indole composed only 19 and 22 %, respectively. H1H secondary roots had 46 % of indole glucosinolates, while 42 % of aliphatic.

Table 4.3. Influence of plant nutrition on composition of aliphatic, aromatic, and indole glucosinolates in plants and exudates of turnip from hydroponic on 30th day

Object	Treatment		Aliphatic	Aromatic	Indole
Leaves	H1H	mg plant^{-1}	$1.6^{b} \pm 0.3$	$0.4^{b} \pm 0.1$	$1.3^{a} \pm 0.3$
		%	48	12	39
	H2H	mg plant^{-1}	$1.4^{a} \pm 0.4$	$0.1^{a} \pm 0.1$	$1.4^{a} \pm 0.2$
		%	48	3	48
	H2H2S	mg plant^{-1}	$2.6^{c} \pm 0.1$	ND	$1.3^{a} \pm 0.1$
		%	67	0	33
Secondary roots	H1H	mg plant^{-1}	$1.0^{a} \pm 0.2$	$0.3^{a} \pm 0.1$	$1.1^{a} \pm 0.0$
		%	42	13	46
	H2H	mg plant^{-1}	$1.0^{a} \pm 0.1$	$1.0^{b} \pm 0.1$	$1.6^{b} \pm 0.1$
		%	28	28	44
	H2H2S	mg plant^{-1}	$1.3^{a} \pm 0.2$	$1.2^{a} \pm 0.4$	$2.3^{a} \pm 0.2$
		%	27	25	48
Primary roots	H1H	mg plant^{-1}	$3.5^{a} \pm 0.1$	$1.6^{b} \pm 0.3$	$1.2^{a} \pm 0.2$
		%	56	25	19
	H2H	mg plant^{-1}	$4.0^{b} \pm 0.1$	$1.7^{b} \pm 0.3$	$1.7^{b} \pm 0.3$
		%	54	23	23
	H2H2S	mg plant^{-1}	$6.8^{c} \pm 0.2$	$0.7^{a} \pm 0.2$	$1.4^{a} \pm 0.2$
		%	76	8	16
Exudates	H1H	mg plant^{-1}	$1.5^{a} \pm 0.3$*	$0.6^{a} \pm 0.2$*	$0.6^{a} \pm 0.1$*
		%	56	22	22
	H2H	mg plant^{-1}	$1.9^{a} \pm 0.3$*	$1.2^{b} \pm 0.2$*	$1.0^{b} \pm 0.2$*
		%	45	30	25
	H2H2S	mg plant^{-1}	$2.1^{b} \pm 0.3$*	$1.2^{a} \pm 0.3$*	$1.6^{a} \pm 0.3$*
		%	43	24	33

H1H - hydroponic with Hoagland solution; H2H - hydroponic with two times concentrated Hoagland solution; H2H2S - hydroponic with two times concentrated Hoagland solution and two times increased concentration of sulfur. The differences are compared for each treatment. Values followed by the same letter are not significantly different. * Sum of glucosinolates exuded during 30 days.

Increase of nutrient supply led to decrease the part of aromatic glucosinolates in leaves, while increase of indole, but in secondary roots, the part of aromatic glucosinolates increased in the contrast to aliphatic.
In the experiments of Vallejo *et al.* (2003) was also shown that total aliphatic glucosinolate levels are significantly affected by fertilization in the contrast to the total aromatic. However, little is known from the literature about the influence of environmental factors on the profile of individual glucosinolates.
Sulfur application resulted in a larger response of aliphatic glucosinolates than indole. In H2H2S as compared to H2H the part of aliphatic glucosinolates increased for leaves and primary roots. For secondary roots and exudates, the part of indole glucosinolates slightly increased. Interesting is that in leaves under the increased content of sulfur disappeared aromatic glucosinolates, in primary roots and exudates their part was also reduced, but not in secondary roots (Table 4.3).
Josefsson (1970) also wrote that sulfur application increases aliphatic and decreases indole glucosinolates. Vallejo *et al.* (2003) pointed that sulfur nutrition influences much more on the content of aliphatic than indole glucosinolates and explained this by activation of the synthesis of methionine, the sulfur amino acid that have the function of precursor in syntheses of aliphatic glucosinolates.
Biosynthesis of aliphatic glucosinolates requires the participation of two sulfur-containing compounds: cysteine and 3-phospho-adenosyl-5-phosphosulphate (PAPS) (Zhao, 1993). In addition to these two compounds, methionine is also required in the biosynthesis of aliphatic glucosinolates, which makes the glucosinolate syntheses sensitiv to the plant sulfur status.
Indole glucosinolates are influenced strongly by environmental factors but some heritable variation for concentration has been found and is likely to be result of 2-3 genes in *Brassica napus* (Raybould and Moyes, 2001). In contrast, aliphatic glucosinolates are under strong genetic control and the genetics of their side-chain structure and concentration are genetically dependent.

4.1.2.1 Aliphatic glucosinolates in plants and exudates

The major part of aliphatic glucosinolates in plants on 10^{th} day of the experiment was in leaves: for H1H 0.6 mg $plant^{-1}$ (85 % of these in plants), for H2H2S 0.8 mg $plant^{-1}$ (90 %) (Table 4.4). Rauth (2002) also reported that leaves exhibit greater concentration of aliphatic glucosinolates on the beginning of plant growth, while accumulation of aliphatic glucosinolates in roots started when the plants growth stabilized.
On 30^{th} day the main part of aliphatic glucosinolates was concentrated in primary roots: for H1H it reached 3.5 mg $plant^{-1}$ (70 % of these in plants), while in leaves 1.6, and in secondary roots 1.0 mg $plant^{-1}$. Application of 2H lead to 1.2-fold increase of aliphatic glucosinolates in primary roots as compared to 1H, while in leaves slightly decreased, and in secondary roots did not change (Table 4.4). The similar distribution of aliphatic glucosinolates was also found for AD2H2S (Attachment, Table 4.4a). Primary roots of AS2H2S plants had 5.2 mg $plant^{-1}$ of aliphatic glucosinolates, while leaves 2.6, and in secondary roots 0.8 mg $plant^{-1}$ (Attachment, Table 4.4b).
Tantikanjana *et al.* (2004) wrote that aliphatic glucosinolates are derived from methionine, and they are synthesized through varying steps of chain elongation and modification. The CYP79 family of cytochrome P450 enzymes catalyzes the first step of glucosinolate formation: oxidation of amino acids to aldoximes. The P450 enzyme of CYP79F1 uses short-chain derivatives of methionine as substrates and overexpression of CYP79F1 genes in *Arabidopsis*

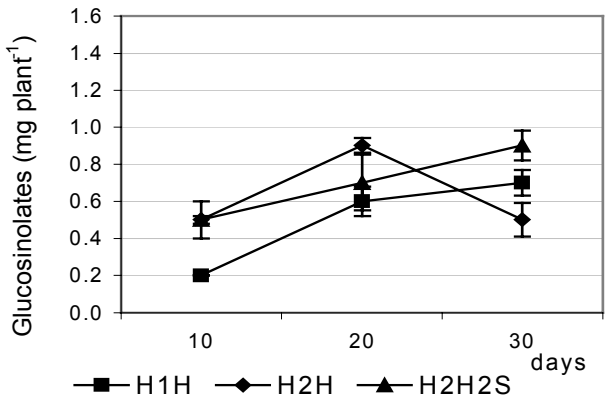
thaliana leads to increases in short-chain glucosinolates levels (which are the only aliphatic glucosinolates found in turnip plants). CYP79F1 participates in conversion of short-chain methionine derivatives (di- and trihomomethionine) into aliphatic aldoximes (Mikkelsen *et al*., 2000). The highest expression level of CYP79F1 genes was observed in roots, while it was normalized in leaves of *Arabidopsis thaliana* (Glombitza *et al*., 2004).

Table 4.4. Influence of plant nutrition on aliphatic glucosinolate content in plants and exudates of turnip from hydroponic (mg plant^{-1})

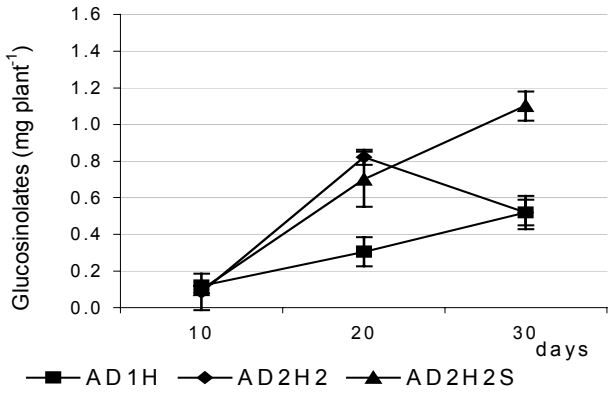
Treatment		Leaves	Secondary roots	Primary roots	exudates
10 days	H1H	$0.6^{ab} \pm 0.1$	$0.1^{a} \pm 0.1$	-	$0.2^{a} \pm 0.0$
	H2H	$0.5^{a} \pm 0.1$	$0.2^{a} \pm 0.1$	-	$0.5^{a} \pm 0.1$
	H2H2S	$0.8^{b} \pm 0.1$	$0.1^{a} \pm 0.0$	-	$0.5^{b} \pm 0.0$
20 days	H1H	$1.2^{a} \pm 0.3$	$0.9^{b} \pm 0.0$	$1.1^{a} \pm 0.1$	$0.6^{a} \pm 0.1$
	H2H	$1.2^{a} \pm 0.3$	$1.0^{b} \pm 0.1$	$1.0^{a} \pm 0.1$	$0.9^{a} \pm 0.0$
	H2H2S	$1.6^{b} \pm 0.2$	$0.5^{a} \pm 0.1$	$1.6^{b} \pm 0.1$	$0.7^{a} \pm 0.2$
30 days	H1H	$1.6^{b} \pm 0.3$	$1.0^{a} \pm 0.2$	$3.5^{a} \pm 0.1$	$0.7^{a} \pm 0.1$
	H2H	$1.4^{a} \pm 0.4$	$1.0^{a} \pm 0.1$	$4.0^{b} \pm 0.1$	$0.5^{a} \pm 0.1$
	H2H2S	$2.6^{c} \pm 0.1$	$1.3^{a} \pm 0.2$	$6.8^{c} \pm 0.2$	$0.9^{b} \pm 0.1$
Total	H1H				$1.5^{a} \pm 0.3$*
	H2H				$1.9^{a} \pm 0.3$*
	H2H2S				$2.1^{a} \pm 0.3$*

H1H - hydroponic with Hoagland solution; H2H - hydroponic with two times concentrated Hoagland solution; H2H2S - hydroponic with two times concentrated Hoagland solution and two times increased concentration of sulfur. The differences are compared for each treatment. Values followed by the same letter are not significantly different. *Sum of glucosinolates exuded during 30 days

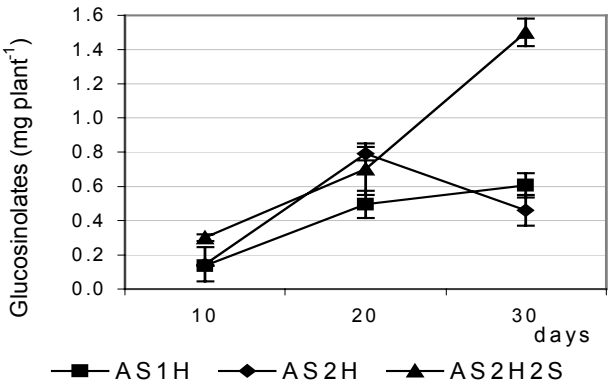
Influence of sulfur on aliphatic glucosinolate content was stronger as 2H. Primary roots of plants from H2HS had 6.8 mg plant^{-1} of aliphatic glucosinolates (2.8 mg plant^{-1} more then H2H), leaves 2.6 mg plant^{-1} (1.2 mg plant^{-1} more then H2H), and secondary roots 1.3 mg plant^{-1} (0.3 mg plant^{-1} more then H2H) (Table 4.4). In 2H2S secondary roots on 30th day aliphatic glucosinolate content increased by 30% as compared to 2H, while in 2H2S leaves it was increased by 85 % as compared to 2H leaves (Table 4.4).
Also Hirai *et al*. (2004) wrote about the difference in reaction of leaves and roots of Arabidopsis thaliana on nitrogen and sulfur deficiency. In contrast to leaves, in roots under the nutrient deficiency CYP79F1 as well as CYP83A1, both participating in aliphatic glucosinolate synthesis, are up-regulated, which cause increase in aliphatic glucosinolate content in roots. The up-regulation of these genes is not registered in leaves, which could explain more intensive synthesis of aliphatic glucosinolates in leaves under the sufficient nutrient supply.



H1H - hydroponic with Hoagland solution; H2H - hydroponic with two times concentrated Hoagland solution; H2H2S - hydroponic with two times concentrated Hoagland solution and two times increased concentration of sulfur



AD1H - aeroponic with defensor with Hoagland solution, AD2H - aeroponic with defensor with two times concentrated Hoagland solution, AD2H2S - aeroponic with defensor with two times concentrated Hoagland solution and two times increased concentration of sulfur



AS1H - aeroponic with sprayers with Hoagland solution, AS2H - aeroponic with sprayers with two times concentrated Hoagland solution, AS2H2S - aeroponic with sprayers with two times concentrated Hoagland solution and two times increased concentration of sulfur

Figure 4.5. Influence of plant nutrition on the kinetic of aliphatic glucosinolates in exudates of turnip

H2H2S plants exuded 2.1 mg plant^{-1} of aliphatic glucosinolates during 30 days; AD2H2S plants 1.9, and AS2H2S 2.4 mg plant^{-1} (Figure 4.5). Aliphatic was the major class of glucosinolates for H2H2S and AS2H2S exudates, while for AD2H2S indole glucosinolates made the majority.

The content of aliphatic glucosinolates in exudates of 1H plants in all systems increased sustainable in the course of the experiment. During the first 10 days H1H plants exuded 0.2 mg plant^{-1} of aliphatic glucosinolates, the next 10 days 0.6 mg plant^{-1}, and the last 10 days 0.7 mg plant^{-1}. Interesting, that 2H plants exuded nearly the same quantity of aliphatic glucosinolates, as 1H plants, but on 20th day their content increased rapidly and exceeded that for 1H as well as for 2H2S: for H2H it reached 0.9 mg plant^{-1}, while for H2H2S 0.7 mg plant^{-1}.

However, the next 10 days H2H plants exuded 0.5 mg plant^{-1} of aliphatic glucosinolates, while H2H2S 0.9 mg plant^{-1}. By calculation the quantity of exuded aliphatic glucosinolates per 1 g of secondary roots, it is possible to see, that during 30 days of experiment 1 g (fresh weight) of H1H secondary roots exuded 0.14 mg g^{-1} of aliphatic glucosinolates, while H2H 0.16 and H2H2S 0.14 mg g^{-1}. In aeroponics it was observed the same tendencies. The highest content of aliphatic glucosinolates was measured for AS2H2S on 30th day 1.5 mg plant^{-1} of exuded glucosinolates, while for AS2H it became lower as for AS1H (Figure 4.5).

Individual aliphatic glucosinolates

Turnip has a restricted profile of aliphatic glucosinolates, which include gluconapin, progoitrin, glucobrassicanapin, and gluconapoleiferin. Gluconapin belongs to butenyl glucosinolates and could be hydroxylated to progoitrin. Glucobrassicanapin is pentenyl glucosinolate, which hydroxilate to gluconapoleiferin (Naur *et al*., 2003).
Enhance of nutrient supply for turnip caused the increase of aliphatic glucosinolates mostly in primary roots. On 30th day of the experiment gluconapin and gluconapoleiferin content was nearly not changed for H2 primary and secondary roots, however application of sulfur increased their contents in primary roots (Table 4.5), which caused the accumulation of 65 % of all gluconapin and 79 % of gluconapoleiferin of plants in primary roots, while for H1H plants it was 53 and 67 %, respectively.

Table 4.5. Influence of plant nutrition on individual aliphatic glucosinolate content in plants and exudates of turnip from hydroponic on 30th day (mg plant^{-1})

Gluco-sinolates	Treat-ment	Content, composition	Leaves	Secondary roots	Primary roots	Exudates*
Gluconapin	H1H	mg plant^{-1}	$0.5^{a} \pm 0.0$	$0.3^{a} \pm 0.0$	$0.9^{a} \pm 0.3$	$0.4^{a} \pm 0.1$*
		%	29	18	53	
	H2H	mg plant^{-1}	$0.5^{ab} \pm 0.1$	$0.2^{a} \pm 0.1$	$1.0^{a} \pm 0.2$	$0.6^{b} \pm 0.0$*
		%	29	12	59	
	H2H2S	mg plant^{-1}	$0.7^{b} \pm 0.2$	$0.2^{a} \pm 0.1$	$1.7^{b} \pm 0.3$	$0.4^{b} \pm 0.0$*
		%	27	8	65	
Progoitrin	H1H	mg plant^{-1}	$0.6^{a} \pm 0.1$	$0.5^{a} \pm 0.2$	$1.3^{a} \pm 0.1$	$0.7^{ab} \pm 0.2$*
		%	25	21	54	
	H2H	mg plant^{-1}	$0.5^{a} \pm 0.1$	$0.5^{a} \pm 0.1$	$1.8^{b} \pm 0.3$	$0.9^{b} \pm 0.0$*
		%	18	18	64	
	H2H2S	mg plant^{-1}	$0.9^{b} \pm 0.0$	$0.6^{b} \pm 0.2$	$2.8^{b} \pm 0.2$	$0.6^{b} \pm 0.1$*
		%	21	14	65	
Glucobras-sicanapin	H1H	mg plant^{-1}	$0.2^{a} \pm 0.2$	$0.1^{a} \pm 0.1$	$0.6^{a} \pm 0.0$	$0.2^{a} \pm 0.0$*
		%	22	11	67	
	H2H	mg plant^{-1}	$0.2^{a} \pm 0.1$	$0.1^{a} \pm 0.0$	$0.5^{a} \pm 0.1$	$0.2^{a} \pm 0.0$*
		%	25	13	63	
	H2H2S	mg plant^{-1}	$0.2^{a} \pm 0.1$	$0.1^{a} \pm 0.1$	$1.1^{a} \pm 0.1$	$0.4^{b} \pm 0.0$*
		%	14	7	79	
Gluconapo-leiferin	H1H	mg plant^{-1}	$0.4^{ab} \pm 0.1$	$0.2^{a} \pm 0.1$	$0.7^{a} \pm 0.1$	$0.1^{a} \pm 0.0$*
		%	31	15	54	
	H2H	mg plant^{-1}	$0.3^{a} \pm 0.0$	$0.2^{a} \pm 0.0$	$0.7^{a} \pm 0.1$	$0.2^{b} \pm 0.0$*
		%	25	17	58	
	H2H2S	mg plant^{-1}	$0.6^{a} \pm 0.2$	$0.3^{a} \pm 0.2$	$1.0^{b} \pm 0.1$	$0.5^{b} \pm 0.1$*
		%	32	16	53	

H1H - hydroponic with Hoagland solution; H2H - hydroponic with two times concentrated Hoagland solution; H2H2S - hydroponic with two times concentrated Hoagland solution and two times increased concentration of sulfur. The differences are compared for each treatment. Values followed by the same letter are not significantly different. * Sum of glucosinolates exuded during 30 days

Progoitrin content in primary roots increased for 2H as well as for 2H2S. 54 % of all progoitrin was in H1H primary roots, while in H2H and H2H2S primary roots it reached 64 and 65 %.
Not strong changes of aliphatic glucosinolate content in all plant organs under the different nutrient supply could be explained by the fact that the concentrations of aliphatic glucosinolate are under strong genetic control with a high heritability ($h^2 = 0.87$ in *Brassica napus*) (Raybould and Moyes, 2001). Two to five independent genes in *Brassica napus* appear to control 71 % of the variation in aliphatic glucosinolate concentration, with *gsl-elong* and *gsl-alk* mapping of QTLs for this trait.
Interesting is also the fact, that in some cases increase of the solution concentration (2H) caused the decrease in aliphatic glucosinolate content. This was measured for progoitrin and gluconapoleiferin in H2H leaves as compared to H1H leaves, for gluconapin in H2H secondary roots, and for glucobrassicanapin in H2H primary roots. This could find the explanation by Zhao *et al*. (1994) that in sulfur deficient plants increasing of nitrogen supply can decrease the relative proportion of aliphatic glucosinolates, with a compensatory increase in that of indole glucosinolates.
Among the aliphatic glucosinolates, progoitrin and gluconapin were the mostly influenced by the increased sulfur supply.
Progoitrin was the most spread aliphatic glucosinolate in plants of turnip. Also Ciska *et al*. (2000) reported that progoitrin was the major glucosinolate in plants of turnip and its part reached 38 % of all glucosinolates. On 10th day of plant growth the major part of progoitrin was concentrated in leaves, but to the end of vegetation up to 70 % of progoitrin was measured in primary roots (Table 4.6).

Table 4.6. Influence of plant nutrition on progoitrin content in plants and exudates of turnip from hydroponic (mg plant^{-1})

Days	Treatments	Leaves	Secondary roots	Primary roots	Exudates
10	H1H	$0.2^{ab} \pm 0.1$	ND	-	$0.1^{a} \pm 0.0$
	H2H	$0.2^{a} \pm 0.0$	ND	-	$0.2^{a} \pm 0.1$
	H2H2S	$0.3^{b} \pm 0.0$	ND	-	$0.1^{a} \pm 0.1$
20	H1H	$0.5^{a} \pm 0.0$	$0.4^{a} \pm 0.0$	$0.4^{a} \pm 0.1$	$0.4^{b} \pm 0.0$
	H2H	$0.5^{ab} \pm 0.1$	$0.5^{b} \pm 0.0$	$0.3^{a} \pm 0.1$	$0.5^{b} \pm 0.1$
	H2H2S	$0.7^{b} \pm 0.1$	$0.3^{a} \pm 0.1$	$0.4^{b} \pm 0.1$	$0.2^{a} \pm 0.0$
30	H1H	$0.6^{a} \pm 0.0$	$0.5^{a} \pm 0.1$	$1.3^{a} \pm 0.2$	$0.2^{a} \pm 0.0$
	H2H	$0.5^{a} \pm 0.1$	$0.5^{a} \pm 0.1$	$1.8^{b} \pm 0.2$	$0.3^{ab} \pm 0.1$
	H2H2S	$0.9^{a} \pm 0.1$	$0.6^{a} \pm 0.0$	$2.8^{c} \pm 0.2$	$0.4^{b} \pm 0.1$

H1H - hydroponic with Hoagland solution; H2H - hydroponic with two times concentrated Hoagland solution; H2H2S - hydroponic with two times concentrated Hoagland solution and two times increased concentration of sulfur. ND - not determined. The differences are compared for each treatment. Values followed by the same letter are not significantly different.

On 20th day it was measured sulfur influence on progoitrin content in leaves, but not in other plant parts or exudates. However, on this stage the content of progoitrin in H2H exudates was higher as for H1H, while lower for H2H2S. On 30th day progoitrin content was not influenced in secondary roots by 2H and 2H2S treatments, but reached 1.8 mg plant^{-1} for H2H primary roots (0.5 mg plant^{-1} more as for H1H) and 2.8 mg plant^{-1} for 2H2S (1.5 mg plant^{-1} more). The same tendency was measured for aeroponics, where the maximal content of progoitrin was on 30th

day in systems with 2H2S (Attachment, Tables 4.5a and 4.5b). In general, H1H plants exuded during 30 days 0.7 mg plant^{-1} of progoitrin, increase of nutrient supply led to 1.3-fold increase in exuded progoitrin, while sulfur application decreased the exuding of progoitrin.

Zhao *et al*. (1994) also found clear influence of sulfur applications on progoitrin content in seeds of *Brassica napus*, while the other individual aliphatic glucosinolates gluconapin, gluconapoleiferin, and glucobrassicanapin were influenced to much lower extend. Zhao (1994) wrote that increase the nitrogen rate increased the relative proportion of progoitrin at the expense of glucobrassicanapin, and to a lesser extent, gluconapoleiferin, in the low glucosinolates cultivar of *Brassica napus* cultivar "Cobra", but at the expense of gluconapin in the high glucosinolates cultivar "Bienvenu". He suggested that a high nitrogen supply favors the hydroxylation step from gluconapin to progoitrin.
Until the 20th day of the experiment the major part of **gluconapin** was measured in leaves of plants in all treatments, however on 30th day the main organ of plants, containing this glucosinolate was primary root (Table 4.7). During the whole experiment, 2H did not influence gluconapin content, but it was increased by sulfur. Gluconapin content for 2H2S leaves and secondary roots was nearly the same as for two other treatments with lower nutrient supply. However, H2H2S primary roots had 1.7 mg plant^{-1} of this glucosinolate, which was 0.8 mg plant^{-1} more as for H1H plants was 1.6 mg plant^{-1} and for exudates 0.8 and 0.7 mg plant^{-1} more as for H1H and H2H, respectively. In exudates gluconapin was identified already on 10th day and its content was the same on 20th day, but on 30th day the glucosinolate was found only in H1H exudates. It could be explained by the intensive exudation of gluconapin in H1H secondary roots. On 30th day the content of gluconapin in H1H secondary roots decreased as compared to previous 10 days, while H2H and H2H2S secondary roots the decrease of gluconapin was not measured. H1H plants exuded during 30 days 0.4 mg plant^{-1} of gluconapin (Table 4.5). Increase of nutrient supply led to 1.3-fold increase of the content of exuded glucosinolate, and in the contrast to progoitrin sulfur application increased the exudation of gluconapin by 1.5-fold as compared to progoitrin. This could be explained by the nature of these glucosinolates. They belong to the pool of buthenyl glucosinolates and progoitrin is the hydroxylated inheritor of gluconapin.

Table 4.7. Influence of plant nutrition on gluconapin content in plants and exudates of turnip from hydroponic (mg plant^{-1})

Days	Treatments	Leaves	Secondary roots	Primary roots	Exudates
10	H1H	ND	ND	-	ND
	H2H	ND	$0.1^{a} \pm 0.0$	-	ND
	H2H2S	ND	$0.1^{a} \pm 0.0$	-	$0.1^{b} \pm 0.0$
20	H1H	ND	$0.1^{b} \pm 0.1$	$0.1^{b} \pm 0.1$	ND
	H2H	ND	$0.1^{b} \pm 0.1$	$0.1^{b} \pm 0.0$	$0.1^{a} \pm 0.0$
	H2H2S	$0.2^{a} \pm 0.1$	$0.1^{a} \pm 0.0$	$0.5^{a} \pm 0.0$	$0.2^{a} \pm 0.1$
30	H1H	$0.4^{a} \pm 0.2$	$0.2^{a} \pm 0.1$	$0.7^{a} \pm 0.2$	$0.1^{a} \pm 0.0$
	H2H	$0.3^{a} \pm 0.1$	$0.2^{a} \pm 0.1$	$0.7^{a} \pm 0.1$	$0.1^{a} \pm 0.0$
	H2H2S	$0.6^{a} \pm 0.1$	$0.3^{a} \pm 0.0$	$1.0^{b} \pm 0.3$	$0.2^{b} \pm 0.0$

H1H - hydroponic with Hoagland solution; H2H - hydroponic with two times concentrated Hoagland solution; H2H2S - hydroponic with two times concentrated Hoagland solution and two times increased concentration of sulfur. ND - not determined. The differences are compared for each treatment. Values followed by the same letter are not significantly different.

It is possible to conclude that it exist a close connection between total aliphatic glucosinolates, like butyls and pentyls as well as also alkenyls and hydroxyalkenyls and under the influence of different environmental factors they could be converted into each other or be synthesized at the costs of other aliphatic glucosinolates. Josefsson (1970) also reported that under the increased sulfur supply among aliphatic glucosinolates the highest increase was measured for progoitrin and lower content for gluconapin.
Until 20th day no **gluconapoleiferin** was measured in leaves for all treatments, and only small amount of this glucosinolate was found in H2H and H2H2S secondary roots as well as only for H2H2S exudates (Table 4.8). On 20th day gluconapoleiferin appeared in secondary roots for all treatments, but still no glucosinolate was identified in H1H and H2H leaves and in H1H exudates. In contrast to progoitrin and gluconapin, enhance of nutrient supply decreased on 30th day gluconapoleiferin content in leaves and did not influence it in other plant parts and in exudates. Sulfur application caused 2-fold increase of glucosinolate in leaves (0.6 mg plant^{-1}) as compared to H2H, 1.5-fold in secondary roots (0.3 mg plant^{-1}), 1.4-fold in primary roots (1.0 mg plant^{-1}), and 2-fold in exudates (0.2 mg plant^{-1}).

Table 4.8. Influence of plant nutrition on gluconapoleiferin content in plants and exudates of turnip from hydroponic (mg plant^{-1})

Days	Treatments	Leaves	Secondary roots	Primary roots	Exudates
10	H1H	ND	ND	-	ND
	H2H	ND	$0.1^{a} \pm 0.0$	-	ND
	H2H2S	ND	$0.1^{a} \pm 0.1$	-	$0.1^{a} \pm 0.0$
20	H1H	ND	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	ND
	H2H	ND	$0.1^{a} \pm 0.1$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$
	H2H2S	$0.3^{a} \pm 0.0$	$0.1^{a} \pm 0.1$	$0.2^{a} \pm 0.1$	$0.2^{b} \pm 0.0$
30	H1H	$0.4^{ab} \pm 0.1$	$0.2^{a} \pm 0.0$	$0.7^{a} \pm 0.1$	$0.1^{a} \pm 0.0$
	H2H	$0.3^{a} \pm 0.0$	$0.2^{a} \pm 0.0$	$0.7^{a} \pm 0.2$	$0.1^{a} \pm 0.0$
	H2H2S	$0.6^{b} \pm 0.1$	$0.3^{b} \pm 0.0$	$1.0^{a} \pm 0.3$	$0.2^{a} \pm 0.1$

H1H - hydroponic with Hoagland solution; H2H - hydroponic with two times concentrated Hoagland solution; H2H2S - hydroponic with two times concentrated Hoagland solution and two times increased concentration of sulfur. ND - not determined. The differences are compared for each treatment. Values followed by the same letter are not significantly different.

On 10th day of the experiment **glucobrassicanapin** was found only in leaves (Table 4.9). Application of 2H and 2S did not influence its content. On 20th day glucobrassicanapin in addition to leaves was also measured for primary roots, where sulfur application led to 2-fold increase in glucosinolate content. On 30th day glucobrassicanapin was found in all parts of plants and in exudates. It was not influenced by 2H, but 2S application lead to 1.9-fold increase in glucosinolate content in primary roots as well as 1.5-fold increase of its content in exudates. n general, during 30 days plants from H1H and from H2H exuded 0.2 mg plant^{-1} of glucobrassicanapin, while H2H2S 0.4 mg plant^{-1}.

Table 4.9. Influence of plant nutrition on glucobrassicanapin content in plants and exudates of turnip from hydroponic (mg plant^{-1})

Days	Treatments	Leaves	Secondary roots	Primary roots	Exudates
10	H	$0.1^{a} \pm 0.0$	ND	-	ND
	HSA_0	$0.1^{a} \pm 0.1$	ND	-	ND
	HMJ_0	$0.1^{a} \pm 0.1$	ND	-	ND
20	H	$0.2^{a} \pm 0.0$	ND	$0.2^{a} \pm 0.0$	ND
	HSA_0	$0.2^{a} \pm 0.0$	ND	$0.2^{a} \pm 0.1$	ND
	HMJ_0	$0.2^{a} \pm 0.1$	ND	$0.4^{b} \pm 0.0$	ND
30	H	$0.2^{a} \pm 0.1$	$0.1^{a} \pm 0.0$	$0.6^{a} \pm 0.1$	$0.2^{ab} \pm 0.1$
	HSA_0	$0.2^{a} \pm 0.0$	$0.1^{a} \pm 0.1$	$0.5^{a} \pm 0.1$	$0.1^{a} \pm 0.0$
	HMJ_0	$0.2^{a} \pm 0.1$	$0.1^{a} \pm 0.0$	$1.1^{b} \pm 0.3$	$0.3^{b} \pm 0.1$

H1H - hydroponic with Hoagland solution; H2H - hydroponic with two times concentrated Hoagland solution; H2H2S - hydroponic with two times concentrated Hoagland solution and two times increased concentration of sulfur. ND - not determined. The differences are compared for each treatment. Values followed by the same letter are not significantly different.

The ratio of butenyl (gluconapin and progoitrin) to pentenyl (glucobrassicanapin and gluconapoleiferin) glucosinolates differed between plant organs: for H1H leaves it reached 1.8, and was lower for secondary and primary roots as well as for exudates (Table 4.10). Enhance of nutrient supply increased this ratio in leaves, but decreased it in secondary roots. However, additional application of sulfur did not influence this ratio. This could mean that the activity of Gsl-elongase, enzyme responsible for chain elongation and the conversion ratio of 2-amino-6-methyl-thiohexanic acid (precursors of butenyl glucosinolates) into 2-amino-7-methyl-thioheptanoic acid (precursor of pentenyl glucosinolates), could be influenced by increased nutrient supply, but not by sulfur.

Moreover, the strongest increase in butenyl glucosinolates was measured for 2H2S leaves (1.6 mg plant^{-1}). This could be explained by increased up-regulation of MAM synthase (enzyme which control the elongation of methionine and intensity of synthesis of butenyl or chain elongation to pentenyl glucosinolates) in leaves, but not in roots, under the rich nitrogen and sulfur supply, as it has been shown by Hirai *et al*. (2004).

For exudates from H1H and H2H2S, the ratio of butenyl to pentenyl glucosinolates was slightly lower as for secondary roots. This means, plants exuded more intensively butenyl glucosinolates as pentenyls. We could suggest that the side chain length of molecule side chain or ecological role of exuded glucosinolates or their products play role in exudation intensity.

Alkenyl glucosinolates (gluconapin and glucobrassicanapin) undergo hydroxylation and conversion to hydroxyalkenyl glucosinolates (progoitrin and gluconapoleiferin). The ratio of hydroxyalkenyl : alkenyl glucosinolates for leaves and primary roots of H1H plant was 1.4 and 1.3, while for secondary roots and exudates it was higher 1.8 and 4.0, respectively (Table 4.10).

Aliphatic glucosinolate side-chain structure is determined by elongation of the initial side-chain and subsequent modifications, such as oxidation, desaturation, and hydroxylation. Variation in the type of modification is often controlled by allelic variation at a single locus (Raybould and Moyes, 2001). For example, single loci controls elongation of the side chain (*gsl-elong*), conversion of methylthioalkylside-chains to sulphinylalkyl side-chains (*gsl-oxid*) and then to alkenyl side chains (*gsl-alk*) and hydroxylation of alkenyl side-chains (*gsl-oh*).

Table 4.10. Influence of plant nutrition on correlation between the subclasses of aliphatic glucosinolates in plants and exudates of turnip from hydroponic

Butenyls/ pentenyls**					
Treatment		Leaves	Secondary roots	Primary roots	Exudates
H1H		1.8 : 1	1.3 : 1	1.2 : 1	1.2 : 1
	mg plant^{-1}	1.1 : 0.6	0.8 : 0.6	2.2 : 1.9	1.1 : 0.9*
H2H		2 : 1	1.2 : 1	1.2 : 1	1.4 : 1
	mg plant^{-1}	1.0 : 0.5	0.7 : 0.6	2.8 : 2.3	1.5 : 1.1*
H2H2S		2 : 1	1.1 : 1	1.2 : 1	1 : 1
	mg plant^{-1}	1.6 : 0.8	0.8 : 0.7	4.5 : 3.9	1.0 : 1.0*
Hydroxyalkenyls/ alkenyls***					
H1H		1.4 : 1	1.8 : 1	1.3 : 1	4 : 1
	mg plant^{-1}	1.0 : 0.7	0.7 : 0.4	2.0 : 1.5	0.8 : 0.2*
H2H		1.1 : 1	2.3 : 1	1.7 : 1	5.5 : 1
	mg plant^{-1}	0.8 : 0.7	0.7 : 0.3	2.5 : 1.5	1.1 : 0.2*
H2H2S		1.7 : 1	3.0 : 1	1.4 : 1	2.8 : 1
	mg plant^{-1}	1.5 : 0.9	0.9 : 0.3	3.8 : 2.8	1.1 : 0.4*

H1H - hydroponic with Hoagland solution; H2H - hydroponic with two times concentrated Hoagland solution; H2H2S - hydroponic with two times concentrated Hoagland solution and two times increased concentration of sulfur. The differences are compared for each treatment, and for each plant organ as well as exudates. Values followed by the same letter are not significantly different. *Sum of glucosinolates exuded during 30 days; **butenyls - gluconapin + progoitrin, pentenyls – glucobrassicanapin + gluconapoleiferin; ***hydroxyalkenyls - progoitrin + gluconapoleiferin, alkenyls - glucobrassicanapin + gluconapin

The ratio of hydroxyalkenyl : alkenyl glucosinolates decreased for H2H leaves, but increase for primary roots, secondary roots, and especially for exudates (until 5.5, which was 2-fold higher as for secondary roots and 5-fold higher as for leaves). This ratio increased for H2H2S leaves and secondary roots as compared to these of H2H, but decreased for exudates (until 2.8, which became lower as for secondary roots). This could mean that the activity of glucosinolate-oxidase, the only enzyme involved in hydroxylation of alkenyl glucosinolates, changed under the influence of nutrients as well as the conversion ratio of alkenyls to hydroxyalkenyils. Magrath *et al*. (1994) explained that the hydroxylation of pentenyl glucosinolates is proportional to the hydroxylation of bytenyl glucosinolates and is regulated by the same *Gls-oh* genes, regulating glucosinolate-oxidase. Zhao *et al.* (1994) wrote that increasing of nitrogen supply strongly favors the hydroxylation of gluconapin into progoitrin.

4.1.2.2 Aromatic glucosinolate (gluconasturtiin)

Only five families within the Capparales order are known to accumulate tryptophan-derived glucosinolates, amoung them also *Brassica* vegetables (Griffiths *et al.*, 2001). Turnip has the only aromatic glucosinolate gluconasturtiin.
The content of gluconasturtiin in leaves varied essentially during the plant growth. For H2H2S leaves it increased until 20th day to 0.2 mg plant^{-1}, and then disappeared to the end of the experiment (Table 4.11). Brown *et al*. (2003) also reported about the disappearing of glucosinolates from leaves, which was explained as a consequence of catabolic processes as

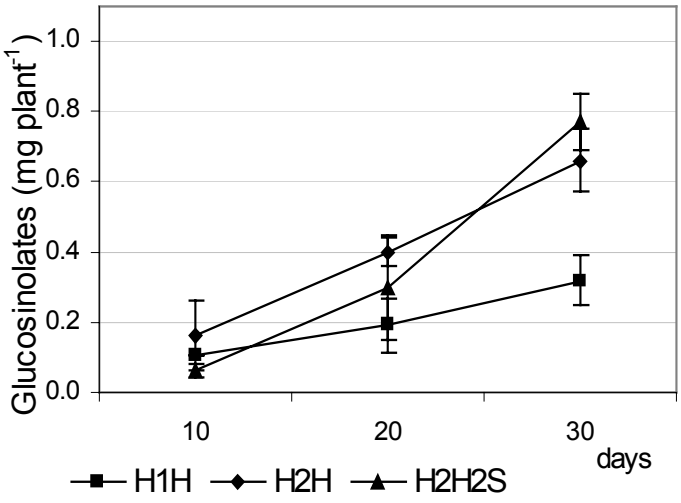
well as transporting. The hydrolysis of glucosinolates by myrosinase after plant wounding is well known (Brown *et al*., 2003), but there is little information about whether or not myrosinase plays role in glucosinolate breakdown in intact plants.
Also for AD2H2S and AS2H2S gluconasturtiin in leaves was extremely reduced until the end of plant growth under 2H2S (Attachment, Tables 4.11a and 4.11b). In systems with 2H gluconasturiin content practically did not increase during the last 10 days of the experiment.
Gluconasturtiin content for H1H secondary roots reached 0.4 mg plant^{-1} on 20th day, but to the end of experiment it became 0.3 mg plant^{-1}. The same dynamic was also measured for H2H, but the content of glucosinolate increased as compared to H1H and reached the maximum with 1.2 mg plant^{-1} on 20th day. For H2H2S the increase of gluconasturtiin was permanent during all the time of the experiment. Wielanek and Urbanek (1999) also showed that the production of aromatic glucosinolates in hairy root cultures of *Tropaeolum majus* paralleled the rapid root growth. By increased sulfur supply, this could be explained by the fact, that phenylalanine and cystein give sulfur to molecule of aromatic glucosinolate.

Table 4.11. Influence of plant nutrition on gluconasturtiin content in plants and exudates of turnip from hydroponic (mg plant^{-1})

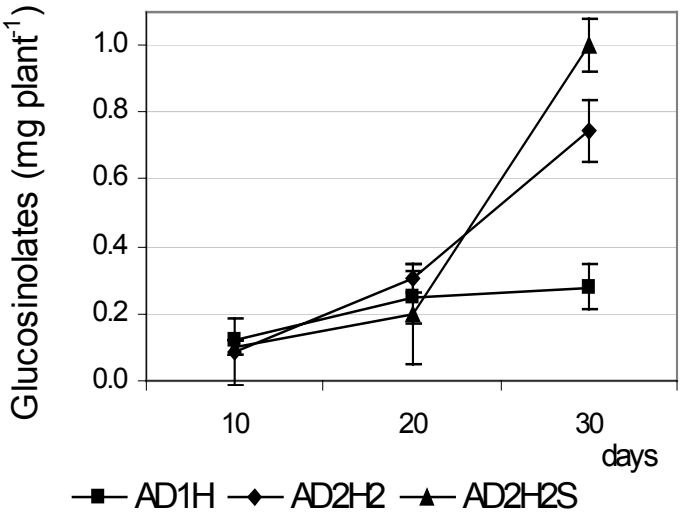
Date	Treatment	Leaves	Secondary roots	Primary roots	exudates
10 days	H1H	$0.1^{ab} \pm 0.1$	$0.1^{a} \pm 0.1$	ND	$0.1^{a} \pm 0.0$
	H2H	$0.3^{b} \pm 0.1$	$0.2^{a} \pm 0.0$	ND	$0.2^{a} \pm 0.1$
	H2H2S	$0.1^{a} \pm 0.1$	$0.2^{a} \pm 0.0$	ND	$0.1^{a} \pm 0.0$
20 days	H1H	$0.6^{b} \pm 0.1$	$0.4^{a} \pm 0.1$	$0.9^{a} \pm 0.1$	$0.2^{a} \pm 0.0$
	H2H	$0.8^{b} \pm 0.1$	$1.2^{b} \pm 0.3$	$1.4^{b} \pm 0.2$	$0.4^{a} \pm 0.2$
	H2H2S	$0.2^{a} \pm 0.2$	$0.9^{b} \pm 0.2$	$1.9^{c} \pm 0.1$	$0.3^{a} \pm 0.1$
30 days	H1H	$0.4^{b} \pm 0.1$	$0.3^{a} \pm 0.1$	$1.6^{b} \pm 0.3$	$0.3^{a} \pm 0.0$
	H2H	$0.1^{a} \pm 0.1$	$1.0^{b} \pm 0.1$	$1.7^{b} \pm 0.3$	$0.7^{b} \pm 0.2$
	H2H2S	ND	$1.2^{b} \pm 0.4$	$0.7^{a} \pm 0.2$	$0.8^{b} \pm 0.1$
Total	H1H				$0.6^{a} \pm 0.2$*
	H2H				$1.2^{b} \pm 0.2$*
	H2H2S				$1.1^{b} \pm 0.3$*

H1H - hydroponic with Hoagland solution; H2H - hydroponic with two times concentrated Hoagland solution; H2H2S - hydroponic with two times concentrated Hoagland solution and two times increased concentration of sulfur. The differences are compared for each treatment. Values followed by the same letter are not significantly different. ND – not determined. *Sum of glucosinolates exuded during 30 days

The content of gluconasturtiin in H1H primary roots increased from 0.9 mg plant^{-1} on 20th to 1.6 mg plant^{-1} on 30th day. The increase, but to the lesser extend was also measured for H2H primary roots. Nevertheless, for H2H2S primary roots it rapidly decreased to the end of experiment and on 30th day became 2.5-fold lower as for H2H (Table 4.11).
Hirai *et al*. (2004) has shown that in case of sulfur deficiency gluconasturtiin content increases for roots, but not for leaves of *Arabidopsis thaliana*.



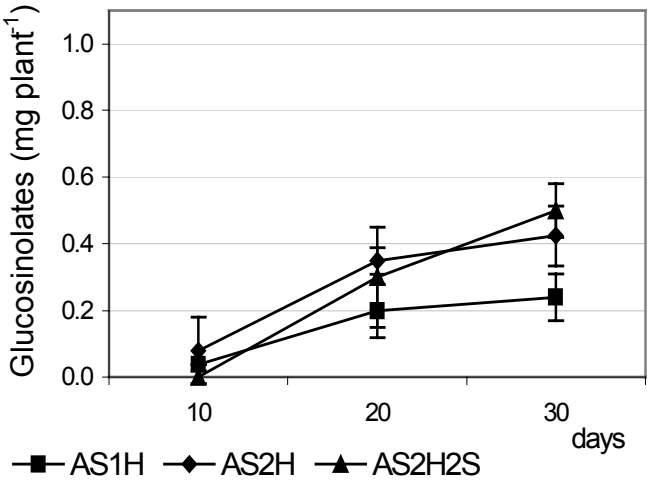
H1H - hydroponic with Hoagland solution; H2H - hydroponic with two times concentrated Hoagland solution; H2H2S - hydroponic with two times concentrated Hoagland solution and two times increased concentration of sulfur



AD1H - aeroponic with defensor with Hoagland solution, AD2H - aeroponic with defensor with two times concentrated Hoagland solution, AD2H2S - aeroponic with defensor with two times concentrated Hoagland solution and two times increased concentration of sulfur

This was explained by the up-regulation of two enzymes antranylate synthase and tryptophan synthase, which increase synthesis of tryptophan precursor of gluconasturiitn and consequently enhance the content of gluconasturtiin. This was measured on 30th day for H2H primary roots in the contrast to these in H2H2S primary roots and for H2H secondary roots on 20th day. The absence of this effect in secondary roots on 30th day could be explained partially by transport of gluconasturtiin, synthesized in primary roots and leaves into the secondary roots.

The content of gluconasturtiin gradually increased in exudates of al l treatments: from 0.1 mg plant^{-1} for H2H2S, AD2H2S, and AS2H2S on 10th day to 1.1, 1.3, and 0.8 mg plant^{-1} on 30^{t} ^{h} day, respectively (Figure 4.6). This was 1.8-time more then in H1H, 2.1-times more then in AD1H, but 1.2-time less as in AS1H.



AS1H - aeroponic with sprayers with Hoagland solution, AS2H - aeroponic with sprayers with two times concentrated Hoagland solution, AS2H2S - aeroponic with sprayers with two times concentrated Hoagland solution and two times increased concentration of sulfur

Figure 4.6. Influence of plant nutrition on the kinetic of gluconasturtiin in exudates of turnip

This probably could be connected with transport of gluconasturtiin from leaves and primary roots into secondary roots and then exuding into the growing medium. This suggestion can be supported by measured decreasing and disappearing of gluconasturtiin in primary roots and leaves of turnip (Table 4.11). By calculation the quantity of exuded aromatic glucosinolate gluconasturtiin per 1 g of secondary roots, it is possible to see, that during 30 days of experiment 1 g (fresh weight) of H1H secondary roots exuded 0.06 mg g^{-1}, while H2H 0.10, and H2H2S 0.07 mg g^{-1}.

4.1.2.3 Indole glucosinolates

The content of indole glucosinolates in leaves slightly increased under the influence of 2H: for H2H leaves on 30th day it reached 1.7 mg plant^{-1}, while for H1H 1.3 mg plant^{-1}. Application of sulfur decreased the indole glucosinolate content in leaves (Table 4.12). The same tendention was observed for aeroponics (Attachment, Tables 4.12a and 4.12b).

Shattuck and Wang (1993) suggested that the increase of nutrient supply, particularly nitrogen lead to activation of pentose phosphate and shikimic acid pathways, causing an increased production of tryptophan and consequently accumulation of indole glucosinolates.

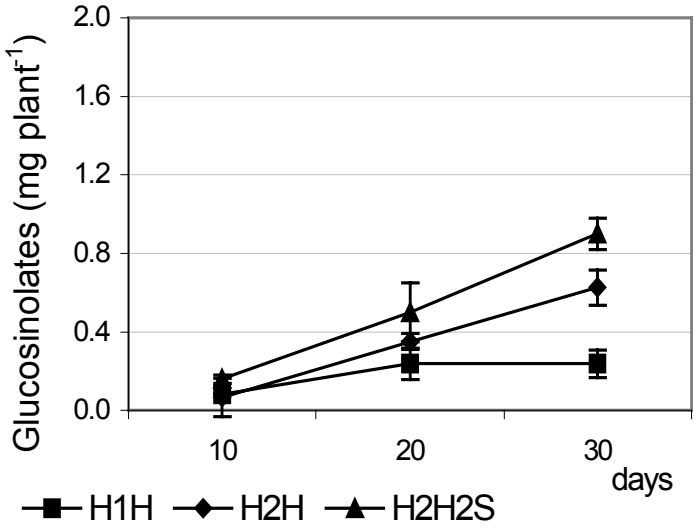
Heaney *et al.* (1983) also showed that in aboveground parts of *Brassica* vegetables grown under increased nitrogenous fertilizer indole glucosinolates are relatively unaffected. This may be explained by increase aliphatic glucosinolate content, because the indole glucosinolate concentration is inversely related to aliphatic glucosinolate concentration. The inverse relationship results from a shift in the amino acid composition, which acts as precursors for the different classes of glucosinolates (Charron and Sams, 2004).

During the experiment the content of indole glucosinolates in H2H2S secondary roots increased from 0.2 mg plant^{-1} on 10th day until 2.3 mg plant^{-1} on 30th day, and became 2.1-fold more then for H1H and 1.4-fold more then for H2H (Table 4.12). The same was also typical for aeroponics (Attachment, Tables 4.12a and 4.12b). Secondary roots of H1H plants had only 85 % of indole glucosinolate content in leaves, but H2H 125 %, and for H2H2S 175 %.

Table 4.12. Influence of plant nutrition on indole glucosinolate content in plants and exudates of turnip from hydroponic (mg plant^{-1})

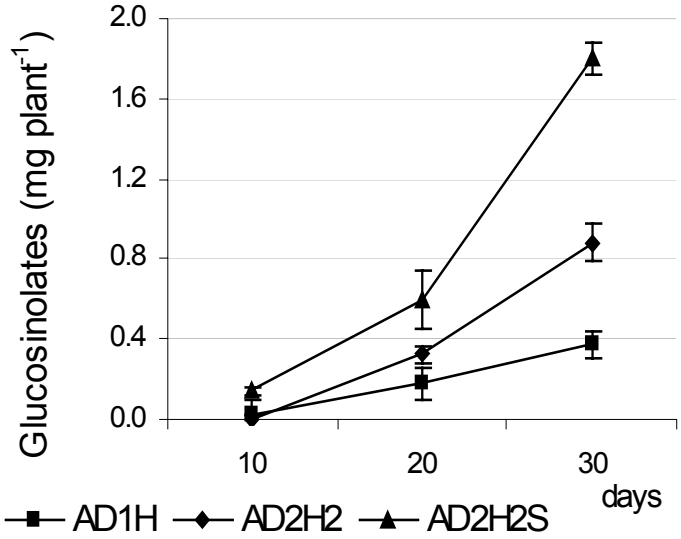
Date	Treatment	Leaves	Secondary roots	Primary roots	Exudates
10 days	H1H	$0.3^{a} \pm 0.0$	$0.1^{a} \pm 0.1$	ND	$0.1^{a} \pm 0.0$
	H2H	$0.6^{b} \pm 0.1$	$0.3^{b} \pm 0.0$	ND	$0.1^{a} \pm 0.0$
	H2H2S	$0.6^{b} \pm 0.1$	$0.2^{a} \pm 0.0$	ND	$0.2^{b} \pm 0.0$
20 days	H1H	$0.8^{a} \pm 0.2$	$0.9^{a} \pm 0.2$	$0.8^{a} \pm 0.0$	$0.2^{a} \pm 0.0$
	H2H	$0.7^{a} \pm 0.2$	$1.6^{b} \pm 0.2$	$1.1^{b} \pm 0.0$	$0.4^{b} \pm 0.1$
	H2H2S	$0.7^{a} \pm 0.1$	$1.8^{b} \pm 0.1$	$1.3^{c} \pm 0.1$	$0.5^{b} \pm 0.0$
30 days	H1H	$1.3^{a} \pm 0.3$	$1.1^{a} \pm 0.0$	$1.2^{a} \pm 0.2$	$0.2^{a} \pm 0.0$
	H2H	$1.4^{a} \pm 0.2$	$1.6^{b} \pm 0.1$	$1.7^{b} \pm 0.3$	$0.6^{b} \pm 0.1$
	H2H2S	$1.3^{a} \pm 0.1$	$2.3^{c} \pm 0.2$	$1.4^{ab} \pm 0.2$	$0.9^{c} \pm 0.1$
Total	H1H				$0.6^{a} \pm 0.1$*
	H2H				$1.0^{b} \pm 0.2$*
	H2H2S				$1.6^{c} \pm 0.3$*

H1H - hydroponic with Hoagland solution; H2H - hydroponic with two times concentrated Hoagland solution; H2H2S - hydroponic with two times concentrated Hoagland solution and two times increased concentration of sulfur. The differences are compared for each treatment. Values followed by the same letter are not significantly different. ND – not determined. *Sum of glucosinolates exuded during 30 days

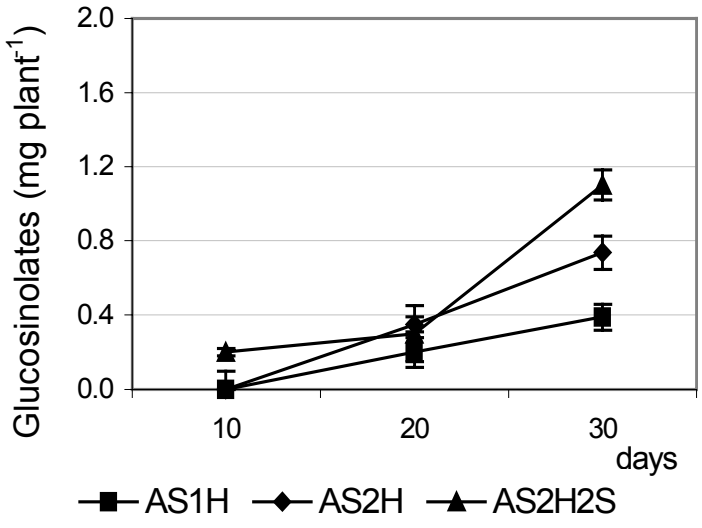


H1H - hydroponic with Hoagland solution; H2H - hydroponic with two times concentrated Hoagland solution; H2H2S - hydroponic with two times concentrated Hoagland solution and two times increased concentration of sulfur

In addition, Elliott and Stowe (1971) reported that indole glucosinolate content of root exceeds that of leaves. It could be explained by more intensive synthesis of indole glucosinolates in root as in leaves. The other possible factor is the transformation of indole glucosinolates from other plant parts (Chen *et al*., 2001a), or decrease of aliphatic glucosinolate content (Charron and Sams, 2004). Increase of indole glucosinolate content in leaves was also measured in all systems, while for 2H2S their content decreased. It may be connected with increase of aliphatic glucosinolates under the influence of sulfur.



AD1H - aeroponic with defensor with Hoagland solution, AD2H - aeroponic with defensor with two times concentrated Hoagland solution, AD2H2S - aeroponic with defensor with two times concentrated Hoagland solution and two times increased concentration of sulfur



AS1H - aeroponic with sprayers with Hoagland solution, AS2H - aeroponic with sprayers with two times concentrated Hoagland solution, AS2H2S - aeroponic with sprayers with two times concentrated Hoagland solution and two times increased concentration of sulfur

Figure 4.7. Influence of plant nutrition on the kinetic of indole glucosinolates in exudates of turnip

First 10 days of experiment H1H and H2H plants exuded 0.1 mg plant^{-1} of indole glucosinolates, while 2H2S twice as much as plants from other systems (Figure 4.7). During the next 10 days the content of exuded indole glucosinolates for 1H reached 0.2 mg plant^{-1}, while for H2H 0.4 and for H2H2S until 0.5 mg plant^{-1}. The influence of 2H and 2S became more obvious on 30th day. The content of exuded glucosinolates for H1H was 0.2 mg plant^{-1}, while for H2H increased to 3-fold as compared to H1H and for H2H2S 4.5-fold.During the whole experiment H1H plants exuded 0.6 mg plant^{-1} of indole glucosinolates, H2H plants exuded 1.4-fold more, and H2H2S 2.5-fold more, which composed 18, 48, and 39 % of these in secondary roots. This means, under the influence of fertilizers the excretion of glucosinolates is more intensive and that it is selective and is not based on leakage from root tissues.

In AD1H and AD2H as well as in AS1H and AS2H no indole glucosinolates were identified during the firs 10 days of experiment. However, during the 30 days AD1H plants exuded 0.6 mg plant^{-1} of glucosinolates, which was half as much as AD2H and 4.3-fold lower as AD2H2S (Figure 4.7).

Interestingly, that in AS2H the increase of nutrient concentration did not increase the content of glucosinolates in exudates and it reached 1.0 mg plant^{-1}, the same as in AS1H. Sulfur application in AS led to excreting 1.8 mg of glucosinolates per plant during the 30 days period.

Individual indole glucosinolates

Four indole glucosinolates were determined in turnip plants and exudates: glucobrassicin and its derivates, particularly neoglucobrassicin, 4-hydroxyglucobrassicin, and 4-methoxy-glucobrassicin.
Modification of the side chain of indole glucosinolates is important, because the physicochemical properties and the biological activity of glucosinolate degradation products are determined to a large extend by the structure of the side chain (Hansen and Halkier, 2005).
On 10th day of experiment **glucobrassicin** was measured only in leaves and was enhanced for 2H, but decreased by sulfur application (Table 4.13). The next 10 days H2H leaves and secondary roots had 1.3 and 1.7-fold more of glucosinolate as these from H1H. On this stage H1H exudates contended no glucobrassicin, while H2H 0.2 and H2H2S 0.3 mg plant^{-1}. On 30th day the content of glucobrassicin in all plant parts did not change for H1H as compared with previous 10 days, but glucobrassicin was already measured in exudates. Glucobrassicin content in leaves of H2H and H2H2S decreased as compared to previous 10 days, while in H2H2S primary roots and exudates it increased, which could be connected with its transportation from secondary roots.
Elliott and Stowe (1971) also measured especially high content of glucobrassicin and neoglucobrassicin in exudates of woad.

Table 4.13. Influence of plant nutrition on glucobrassicin content in plants and exudates of turnip from hydroponic (mg plant^{-1})

Days	Treatments	Leaves	Secondary roots	Primary roots	Exudates
10	H1H	$0.1^{ab} \pm 0.1$	ND	-	ND
	H2H	$0.2^{b} \pm 0.0$	ND	-	ND
	H2H2S	$0.1^{a} \pm 0.0$	ND	-	ND
20	H1H	$0.3^{a} \pm 0.1$	$0.3^{a} \pm 0.1$	$0.2^{a} \pm 0.0$	ND
	H2H	$0.4^{a} \pm 0.1$	$0.5^{b} \pm 0.0$	$0.2^{a} \pm 0.1$	$0.2^{a} \pm 0.1$
	H2H2S	$0.2^{a} \pm 0.1$	$0.3^{a} \pm 0.1$	$0.3^{a} \pm 0.1$	$0.3^{b} \pm 0.1$
30	H1H	$0.3^{ab} \pm 0.1$	$0.3^{a} \pm 0.0$	$0.2^{a} \pm 0.0$	$0.1^{a} \pm 0.0$
	H2H	$0.3^{b} \pm 0.0$	$0.4^{a} \pm 0.1$	$0.2^{a} \pm 0.0$	$0.1^{a} \pm 0.1$
	H2H2S	$0.1^{a} \pm 0.1$	$0.3^{a} \pm 0.0$	$0.3^{a} \pm 0.1$	$0.2^{a} \pm 0.1$

H1H - hydroponic with Hoagland solution; H2H - hydroponic with two times concentrated Hoagland solution; H2H2S - hydroponic with two times concentrated Hoagland solution and two times increased concentration of sulfur. The differences are compared for each treatment. Values followed by the same letter are not significantly different. ND – not determined.

Neoglucobrassicin was the most predominant indole glucosinolate. It was measured in leaves and roots for all systems, and in H2H as well as H2H2S exudates already on 10th day of the experiment (Table 4.14). The next 10 days the influence of 2H and 2H2S treatment was measured for secondary roots: 3 and 5.5-fold more as for H1H. Moreover, on 20th day it was

still presented only in H2H and H2H2S exudates. On 30th day neoglucobrassicin content in secondary roots continued to be much higher as for H1H plants: for H2H 3 and for H2H2S 4.3-fold higher as for H1H, and in primary roots it became for H2H 2 and for H2H2S 3.5-fold higher as for H1H. Application of sulfur led to increase neoglucobrassicin in exudates. During 30 days the highest concentration of neoglucobrassicin was in exudates H2H2S: 0.7 mg plant^{-1}, while for H1H it was 7 and for H2H 3.5-fold lower (Table 4.12). The low content of neoglucobrassicin was also measured in AD1H exudates 0.1 mg plant^{-1}, for AD2H 3, while for AD2H2S 11-fold more (Attachment, Table 4.12a). Also Berdanek *et al*. (2005) reported about the exuding of neoglucobrassicin in high concentration by *Arabidopsis thaliana*.

Table 4.14. Influence of plant nutrition on neoglucobrassicin content in plants and exudates of turnip from hydroponic (mg plant^{-1})

Days	Treatments	Leaves	Secondary roots	Primary roots	Exudates
10	H1H	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	-	ND
	H2H	$0.2^{b} \pm 0.0$	$0.3^{b} \pm 0.0$	-	$0.1^{a} \pm 0.1$
	H2H2S	$0.3^{b} \pm 0.1$	$0.2^{ab} \pm 0.1$	-	$0.2^{a} \pm 0.1$
20	H1H	$0.3^{a} \pm 0.1$	$0.2^{a} \pm 0.1$	$0.3^{a} \pm 0.1$	ND
	H2H	$0.2^{a} \pm 0.0$	$0.6^{b} \pm 0.1$	$0.5^{a} \pm 0.1$	$0.1^{a} \pm 0.0$
	H2H2S	$0.3^{a} \pm 0.1$	$1.1^{c} \pm 0.1$	$0.4^{a} \pm 0.2$	$0.3^{b} \pm 0.1$
30	H1H	$0.2^{a} \pm 0.0$	$0.2^{a} \pm 0.0$	$0.4^{a} \pm 0.1$	$0.1^{a} \pm 0.0$
	H2H	$0.3^{b} \pm 0.0$	$0.6^{b} \pm 0.2$	$0.8^{b} \pm 0.1$	$0.1^{ab} \pm 0.1$
	H2H2S	$0.5^{c} \pm 0.1$	$1.7^{c} \pm 0.2$	$0.7^{b} \pm 0.1$	$0.3^{b} \pm 0.1$

H1H - hydroponic with Hoagland solution; H2H - hydroponic with two times concentrated Hoagland solution; H2H2S - hydroponic with two times concentrated Hoagland solution and two times increased concentration of sulfur. The differences are compared for each treatment. Values followed by the same letter are not significantly different. ND – not determined. *Sum of glucosinolates exuded during 30 days

On 10th day of the experiment **4-hydroxy-glucobrassicin** was measured only in leaves of plants from all systems (Table 4.15). On 20th day the slight increasing of glucosinolate content was measured only for secondary roots and it was presented only in exudates of H2H plants. For 30-days-old H1H plants 4-hydroxy-glucobrassicin became the major glucosinolate, the manifold was in leaves (0.6 mg plant^{-1}), but plants exuded very small amount of this glucosinolate (0.1 mg plant^{-1}) (Table 4.12). Nearly the same was the content of 4-hydroxy-glucobrassicin for all parts of H2H plants, but for H2H2S its concentration was higher for leaves and much lower for secondary roots (5-fold lower as for H2H). However, for AD1H and AS1H 4-hydroxy-glucobrassicin was not dominative glucosinolate in plants, but it also mostly was measured in leaves (Attachment, Tables 4.12a and 4.12b).

No **4-methoxy-glucobrassicin** was measured in plants and exudates on 10th day of experiment (Table 4.16). The next 10 days it was detected in secondary and primary roots, where it was not influenced neither by increased nutrient supply, nor by additional sulfur application. On 30th day 4-methoxy-glucobrassicin appeared in leaves of H1H and H2H and in exudates of H2H and H2H2S. This could be caused by the induction of glucosinolate synthesis in secondary roots, but not by its transportation into secondary roots, because its synthesis was not matched in other plant parts. Interestingly is also the observation, that leaves and primary roots of H2H2S did not

have 4-methoxy-glucobrassicin, even thought this glucosinolate was in these organs for H1H and H2H (Table 4.16).

Table 4.15. Influence of plant nutrition on 4-hydroxy-glucobrassicin content in plants and exudates of turnip from hydroponic (mg plant^{-1})

Days	Treatments	Leaves	Secondary roots	Primary roots	Exudates
10	H1H	$0.1^{a} \pm 0.0$	ND	-	ND
	H2H	$0.2^{a} \pm 0.1$	ND	-	ND
	H2H2S	$0.2^{a} \pm 0.1$	ND	-	ND
20	H1H	$0.2^{a} \pm 0.1$	$0.2^{a} \pm 0.1$	$0.2^{a} \pm 0.0$	ND
	H2H	$0.2^{a} \pm 0.0$	$0.3^{a} \pm 0.0$	$0.2^{ab} \pm 0.1$	$0.1^{a} \pm 0.0$
	H2H2S	$0.2^{a} \pm 0.1$	$0.2^{a} \pm 0.1$	$0.3^{b} \pm 0.0$	ND
30	H1H	$0.6^{a} \pm 0.0$	$0.5^{b} \pm 0.1$	$0.4^{a} \pm 0.0$	$0.1^{a} \pm 0.0$
	H2H	$0.6^{a} \pm 0.2$	$0.5^{b} \pm 0.2$	$0.5^{a} \pm 0.1$	$0.2^{a} \pm 0.1$
	H2H2S	$0.7^{a} \pm 0.1$	$0.1^{a} \pm 0.0$	$0.4^{a} \pm 0.0$	$0.1^{a} \pm 0.1$

H1H - hydroponic with Hoagland solution; H2H - hydroponic with two times concentrated Hoagland solution; H2H2S - hydroponic with two times concentrated Hoagland solution and two times increased concentration of sulfur. The differences are compared for each treatment. Values followed by the same letter are not significantly different. ND – not determined. *Sum of glucosinolates exuded during 30 days

Table 4.16. Influence of plant nutrition on 4-methoxy-glucobrassicin content in plants and exudates of turnip from hydroponic (mg plant^{-1})

Days	Treatments	Leaves	Secondary roots	Primary roots	Exudates
10	H1H	ND	ND	-	ND
	H2H	ND	ND	-	ND
	H2H2S	ND	ND	-	ND
20	H1H	ND	$0.2^{a} \pm 0.1$	$0.1^{a} \pm 0.1$	ND
	H2H	ND	$0.2^{b} \pm 0.0$	$0.2^{a} \pm 0.1$	ND
	H2H2S	ND	$0.2^{a} \pm 0.1$	$0.2^{a} \pm 0.1$	ND
30	H1H	$0.2^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.3^{a} \pm 0.1$	ND
	H2H	$0.2^{a} \pm 0.1$	$0.1^{ab} \pm 0.1$	$0.3^{a} \pm 0.1$	$0.2^{a} \pm 0.0$
	H2H2S	ND	$0.2^{b} \pm 0.0$	ND	$0.2^{a} \pm 0.1$

H1H - hydroponic with Hoagland solution; H2H - hydroponic with two times concentrated Hoagland solution; H2H2S - hydroponic with two times concentrated Hoagland solution and two times increased concentration of sulfur. The differences are compared for each treatment. Values followed by the same letter are not significantly different. ND – not determined. *Sum of glucosinolates exuded during 30 days

The individuality of exudate profiles for each plant species can be supported by the fact that in this experiment 4-methoxy-glucobrassicin in exudates was presented in relatively low concentration as compared to other indole glucosinolates, while in experiment of Berdanek *et al*. (2005) this glucosinolate together with neoglucobrassicin is the most abundant in exudates of *Arabidopsis thaliana*.

As it was already mentioned above, the indole glucosinolate concentration is inversely related to aliphatic glucosinolate concentration (Charron *et al.,* 2004). The inverse relationship results from a shift in the amino acids, which acts as precursors for the different classes of glucosinolates.
In this experiment the ratio of aliphatic to indole glucosinolates differed between plant organs and changed during the experiment. On 10th day the highest proportion of aliphatic to indole glucosinolates in leaves was for H1H treatment, which decreased to the end of experiment, while for H2H2S leaves it increased (Table 4.17). In secondary roots with the increment of nutrient supply, the part of indole glucosinolates was higher as aliphatic during the whole period of plant growth. On 30th day the lowest ratio was measured for secondary roots 0.9 for H1H, and it decreased for H2H and H2H2S until 0.6 as well as for exudates it was decreased from 2.5 for H1H to 1.9 for H2H and 1.3 for H2H2S. The highest proportion of aliphatic : indole glucosinolates was on 30th day for primary roots at all treatments, however as compared to H1H it was 1.2-fold lower for H2H and 2-fold higher for H2H2S (Table 4.17).

Table 4.17. Influence of plant nutrition on correlation between the aliphatic and indole glucosinolates in plants and exudates of turnip from hydroponic

Days	Treatment	Content/ correlation	Leaves	Secondary roots	Primary Roots	Exudates
10	H1H	mg plant^{-1}	0.6 : 0.3	0.1 : 0.1		0.2 : 0.1
			2 : 1	1 : 1		2 : 1
	H2H	mg plant^{-1}	0.5 : 0.6	0.2 : 0.3		0.5 : 0.1
			0.8 : 1	0.7 : 1		5 : 1
	H2H2S	mg plant^{-1}	0.8 : 0.6	0.1 : 0.2		0.5 : 0.2
			1.3 : 1	0.5 : 1		2.5 : 1
20	H1H	mg plant^{-1}	1.2 : 0.8	0.9 : 0.9	1.1 : 0.8	0.6 : 0.1
			1.3 : 1	1 : 1	1.4 : 1	6 : 1
	H2H	mg plant^{-1}	1.2 : 0.7	1.0 : 1.6	1.0 : 1.1	0.9 : 0.4
			1.7 : 1	0.6 : 1	0.9 : 1	2.5 : 1
	H2H2S	mg plant^{-1}	1.6 : 0.7	0.5 : 1.8	1.6 : 1.3	0.7 : 0.5
			2.3 : 1	0.3 : 1	1.2 : 1	1.3 : 1
30	H1H	mg plant^{-1}	1.6 : 1.3	1.0 : 1.1	3.5 : 1.2	0.7 : 0.2
			1.2 : 1	0.9 : 1	2.9 : 1	3.5 : 1
	H2H	mg plant^{-1}	1.4 : 1.4	1.0 : 1.6	4.0 : 1.7	0.5 : 0.6
			1 : 1	0.6 : 1	2.4 : 1	0.9 : 1
	H2H2S	mg plant^{-1}	2.6 : 1.3	1.3 : 2.3	6.8 : 1.4	0.9 : 0.9
			2 : 1	0.6 : 1	4.9 : 1	1 : 1
Total	H1H	mg plant^{-1}				1.5 : 0.6*
						2.5 : 1
	H2H	mg plant^{-1}				1.9 : 1.0*
						1.9 : 1
	H2H2S	mg plant^{-1}				2.1 : 1.6*
						1.3 : 1

H1H - hydroponic with Hoagland solution; H2H - hydroponic with two times concentrated Hoagland solution; H2H2S - hydroponic with two times concentrated Hoagland solution and two times increased concentration of sulfur. The differences are compared for each treatment. Values followed by the same letter are not significantly different. ND – not determined. *Sum of glucosinolates exuded during 30 days

Brown *et al*. (2003) wrote that for leaves of *Arabidopsis thailiana* it is measured the decline in the proportion of aliphatic glucosinolates to indole and that this proportion in roots was lower as in leaves, which was also possible to observe in our experiment. Zhao *et al.* (1994) also reported that sulfur application results in a larger response in the aliphatic group than in the indole group. They suggested that the more sensitive response of aliphatic glucosinolates to the plant sulfur status is occurring probably due to the requirement of methionine in their biosynthesis, as compared to the indole glucosinolates, which are derived from tryptophan. Moreover, the decrease of the proportion of aliphatic to indole glucosinolates for H2H leaves and primary roots as compared to H1H could be explained by the fact that in sulfur deficient plants, increasing of nitrogen supply decreased the proportion of aliphatic glucosinolates, whith a compensatory increase in that of indole glucosinolates.
As it is possible to conclude, plant organs react differently on change in sulfur supply: leaves and primary roots synthetise more aliphatic glucosinolates, while secondary roots more indole, or/and the transfer of indole glucosinolates from leaves and primary roots into secondary roots is more intensive as that for aliphatic glucosinolates.
In exudates the ratio of aliphatic to indole glucosinolates for H1H on 10^{th} day was 2, then it increased to 6, and to the end of vegetation decreased again to 3.5. The lowest ratios were measured on 30^{th} day for H2H and H2H2S. The average ratio for H1H exudates was 2.5, while it decreased with the increase of nutrient supply: for H2H reached 1.9 and for H2H2S 1.3.
It is possible to suggest, that the proportion of aliphatic glucosinolates to indole changes with plant age: it decreases for leaves and increases for primary roots, while does not vary for the secondary roots. For exudates this proportion decreases with the time, which means that plants selectively exude mostly aliphatic glucosinolates on the beginning of their growth, while later they exude more indole glucosinolates.

4.1.3 Fresh weight of plants

The difference in fresh weight of plants from different treatments was possible to observe already at the beginning of experiment. The fresh weight of H1H leaves on 10^{th} day was 5.6 and secondary roots 1.8 g, while for H2H plants it was 1.0 and 0.2 g more and for H2H2S 3.0 and 0.8 g (Table 4.18). Leaves of plants from all hydroponics had 0.2 mg of glucosinolates per 1 g of fresh weight, but for secondary roots the difference was observed: H2H secondary roots had 4 mg g^{-1} of glucosinolates, which were twice as much as for H1H and 4-fold more as for H2H2S. The most intensive growth of plants was measured for AS: AS1H leaves fresh weight on 10^{th} day was 13.2 g and secondary roots 4.7 g, but not for AD1H (Attachment, Tables 4.18a and 4.18b).
On 20^{th} day the difference in fresh weight between treatments increased: H1H leaves weighted 11.2 g, while these from H2H 1.5 and from H2H2S 1.8-fold more. This explains the fact that H1H leaves had 1.5 and 3-fold higher concentration of glucosinolates, as H2H and H2H2S. Fresh weight of secondary roots was more influenced by sulfur as by 2H, but lower concentration of glucosinolates as H2H. Roots had higher concentration of glucosinolates, because they may be more susceptible to pathogen attack, they try to accumulate more glucosinolates against attack (Hirai *et a*l., 2004).

Table 4.18. Influence of plant nutrition on on fresh weight (g) and glucosinolate concentration (mg g^{-1}) in turnip plants from hydroponic

Time	Treat-ment	Fresh weight, g/ concentration of glucosinolates, mg g^{-1}	Leaves	Roots		Leaf : root index
				secondary	primary	
10 days	H1H	g	$5.6^{b} \pm 0.1$	$1.8^{b} \pm 0.2$	ND	3.1
		mg g^{-1}	0.2	0.2		
	H2H	g	$6.6^{b} \pm 0.1$	$2.0^{b} \pm 0.3$	ND	3.3
		mg g^{-1}	0.2	0.4		
	H2H2S	g	$8.6^{b} \pm 0.9$	$2.8^{b} \pm 1.0$	ND	3.1
		mg g^{-1}	0.2	0.1		
20 days	H1H	g	$11.2^{b} \pm 1.9$	$5.6^{b} \pm 1.4$	$3.4^{a} \pm 0.7$	2.0
		mg g^{-1}	0.3	0.9	0.4	
	H2H	g	$16.5^{b} \pm 2.3$	$6.6^{b} \pm 2.2$	$3.4^{a} \pm 1.2$	2.5
		mg g^{-1}	0.2	1	0.6	
	H2H2S	g	$20.3^{b} \pm 2.2$	$8.1^{a} \pm 1.8$	$4.6^{c} \pm 0.4$	2.5
		mg g^{-1}	0.1	1	0.4	
30 days	H1H	g	$16.3^{b} \pm 3.6$	$10.4^{b} \pm 1.1$	$5.6^{a} \pm 0.3$	1.6
		mg g^{-1}	0.2	1.1	0.2	
	H2H	g	$20.0^{b} \pm 3.6$	$12.2^{b} \pm 1.8$	$6.0^{a} \pm 0.9$	1.6
		mg g^{-1}	0.2	1.2	0.2	
	H2H2S	g	$24.5^{a} \pm 1.8$	$15.0^{a} \pm 2.7$	$6.4^{b} \pm 0.7$	1.6
		mg g^{-1}	0.2	1.4	0.3	

H1H - hydroponic with Hoagland solution; H2H - hydroponic with two times concentrated Hoagland solution; H2H2S - hydroponic with two times concentrated Hoagland solution and two times increased concentration of sulfur. The differences are compared for each treatment. Values followed by the same letter are not significantly different.

On 20th day fresh weight of plants from aeroponics was higher as for hydroponics: AS1H leaves 18.9 and for AD1H leaves weighted 17.2 g. In general, application of 2H as well as 2H2S caused increase of plant fresh weight, but not glucosinolate concentration. Enhance of nutrient and particularly sulfur supply increased fresh weight of secondary and primary roots as well as concentration of glucosinolates per fresh weight. The highest concentration of glucosinolates was constantly measured for secondary roots: 1.4 mg g^{-1} for H2H2S, while 0.2 mg g^{-1} for leaves and 0.3 mg g^{-1} for primary roots.

At the end of the experiment fresh weight of AS1H leaves and roots reached 32.9 and 22.0 g (7.6 g primary and 14.4 g secondary roots), which was 12.7 and 7.3 g more than these for AD1H plants and 16.6 and 6.0 g more than for H1H. Vieira *et al.* (1998) also wrote that during the pot and open-field experiments increase fertilization results in increase in fresh weight of *Brassica rapa*.

On 30th day fresh weights of AS2H and AD2H2S leaves were 31.0 and 36.6 g, secondary root 18.7 and 21.9 g, respectively. Fresh weight of H2H and H2H2S leaves was 20.0 and 24.5 g, secondary root weighted 12.2 and 15.0 g, respectively. It confirms, that the increased sulfur supply influenced more essential on fresh weight of leaves than these of roots. Secondary roots in aeroponic were better developed as in hydroponic.

Leaf to root index for all systems and treatments was maximal on 10th day of the experiment, but then dropped until 30th day (Table 4.18, Attachment, Tables 4.18a and 4.18b). Leaf to root index of AD2H and AD2H2S plants reached 3.0 and 2.9 on 10th day, and on 30th day they decreased to 1.1. For H1H and H2H2S on 10th day it was 3.1, and on 30th day became 1.6.

Summary

Nutrient supply and type of system influenced on individual and, consequently, total glucosinolate content in leaves, primary and secondary roots, as well as exudates of turnip. Moreover, it differed between plant parts and changed during plant growth. There are no reliable data in literature on root glucosinolate concentration. According to our results, roots of turnip accumulated a manifold of glucosinolates in comparison to leaves. The major part of glucosinolates on the beginning of the experiment was measured for leaves and then with plant growth it became higher in secondary roots and in appeared after 10th day primary roots.

This experiment has shown that increase of Hoagland solution concentration as well as sulfur supply led to enhance of total glucosinolate content in plants, mostly in secondary roots and from 20th day in primary roots.

Type of growing system influenced on plant root growth, glucosinolate synthesis, transportation of glucosinolates from leaves to roots, and exudation. Particularly, the root length depended strongly on the type of system and on nutrient supply. Root length of AS1H plants on 30th day reached 61 m plant^{-1}, which was 1.4-fold more as for H1H plants and 1.4-fold less as for AD1H. The impact of nutrients on glucosinolate content in exudates was more essential for hydroponic as for both aeroponics. The dependence of the content of exuded glucosinolates on the growing system and nutrient supply could be explained by the influence of these factors on length of secondary roots and probably by changes the cuticle conductance of root border cells under the influence of nutrients, as it was mentioned by Roshchina and Roshchina (1993). Slowing down the intensity of glucosinolate exudation for plants from 1H and 2H systems could be explained by altering of roots, and consequently by decrease of their metabolic activity (Walker *et al*., 2004).

Glucosinolate profile was influenced by the nutrient supply of plants. Increase of nutrient supply led to decrease of aromatic glucosinolates in leaves, while increase of indole. However, in secondary roots aromatic glucosinolates increased in the contrast to aliphatic.

Application of sulfur fertilizing increased aliphatic glucosinolate content, but decreased aromatic and indole glucosinolates in plants, however, it increased the part of indole glucosinolates in exudates.

The major part of aliphatic glucosinolates in plants on 10th day of the experiment was in leaves, but on 30th day the main part of aliphatic glucosinolates was concentrated in primary roots, where the application of 2H lead to 1.2-fold increase of aliphatic glucosinolates as compared to 1H. However, fewer than 2H aliphatic glucosinolate content in leaves slightly decreased, and in secondary roots did not change. Increase of aliphatic glucosinolates under the influence of 2S was stronger as 2H, especially increased were progoitrin and gluconapin.

Aliphatic was the major class of glucosinolates for H2H2S and AS2H2S exudates, while for AD2H2S indole glucosinolates made the majority.

The ratio of butenyl to pentenyl glucosinolates and hydroxyalkenyl to alkenyl glucosinolates glucobrassicanapin in exudates differed of these in plants. They increased for H2H exudates, but under sulfur influence decreased. Plants exuded more intensively butenyl glucosinolates as pentenyl and hydroxyalkenyl as alkenyl, which means, the exudation was selective process for individual aliphatic glucosinolates. This could be explained by ecological role of exuded

glucosinolates and prove the fact that exudation of glucosinolates is an active process, not leaching.

Aromatic gluconasturtiin disappeared from leaves and primary roots under the sulfur influence, while for 2H it increased in secondary roots. The content of gluconasturtiin gradually increased in exudates of all treatments. This probably could be connected with transport of gluconasturtiin from leaves and primary roots into secondary roots and then exuding into the growing medium.

Indole glucosinolate content increased for 2H plants, mostly in secondary and primary roots, but 2H2S caused more intensive exudation of indole glucosinolates. During the whole experiment H1H plants exuded 0.6 mg plant^{-1} of indole glucosinolates, H2H plants exuded 1.4-fold more, and H2H2S 2.5-fold more, which composed 18, 48, and 39 % of these in secondary roots. Under the influence of fertilizers, the excretion of indole glucosinolates was more intensive. Neoglucobrassicin was the most predominant indole glucosinolate, its highest concentration was in exudates from H2H2S.

The ratio of aliphatic to indole glucosinolates differed between plant organs and changed during the experiment: it decreased for leaves and increased for primary roots. This could be explained by the fact that in sulfur deficient plants, increasing of nitrogen supply decreased the proportion of aliphatic glucosinolates, whith a compensatory increase in that of indole glucosinolates.

Plants selectively exuded more aliphatic glucosinolates on the beginning of their growth, while later they exude more indole glucosinolates. Consequently, the yield of glucosinolates from plants differed of that from exudates.

Fresh weight of leaves under the increased nutrient supply was more influenced as that of roots. Roots had higher concentration of glucosinolates as leaves. They might be more susceptible to pathogen attack; consequently, plant roots could be used as a rich source of glucosinolates.

4.2 Influence of salicylic acid and methyl jasmonate on glucosinolate production in leaves, roots, and root exudates of turnip

Chemical diversity and content of exudates could be dramatically enhanced by elicitation process. For increase of glucosinolate content in plants and exudates, we applied such elicitors as salicylic acid (SA) and methyl jasmonate (MJ) on the beginning of plant growth in szstems (Table 3.1).

4.2.1 Total glucosinolate content in plants and exudates of turnip

The response of plants on root treatment with elicitors was inducible, which means that treatment of secondary roots cause also the increment of glucosinolates in leaves. The distribution of glucosinolates in plant organs has been changed under the influence of elicitors. Their application resulted in enhancement of glucosinolate production in plants as compared to non-treated samples (Table 4.19).

Both of elicitors stimulated the glucosinolate production in leaves. Right after elicitor application the stimulation of glucosinolate synthesis in leaves was stronger, as at the end of the experiment. On 10^{th} day leaves contented the manifold of the plant glucosinolates: HSA_0 leaves had 2.7 mg $plant^{-1}$ (67 % of these in plants), HMJ_0 2.9 mg $plant^{-1}$ (74 %), while H leaves 1.5 mg $plant^{-1}$ (75 %) (Table 4.19). Elicitor influence on total glucosinolate content in leaves was also observed in aeroponics (Attachment, Tables 4.19a and 4.19b). On 30^{th} day the effect of elicitors on glucosinolate content in leaves was not so strong as on 10^{th} day: for HSA_0 it reached 4.1 mg $plant^{-1}$ (1.1-fold more as for H) and for HMJ_0 5.6 mg $plant^{-1}$ (1.4-fold more). This could be explained by reducing of elicitor effect as well as by the transport of glucosinolates from leaves into other plant parts as secondary roots (Chen and Andreasson, 2001).

Induction of glucosinolate synthesis in leaves after MJ treatment of roots could be explained by the fact that MJ can diffuse to distal part of the plant via the vapor phase or by intracellular migration, possibly through the phloem (Wasternack and Parthier, 1997). Kiddle *et al*. (1994) showed that SA applying to soil drench to oilseed rape plants also increased the concentration of glucosinolates in their leaves.

Van Dam *et al*. (2003) reported about the systemic induction of glucosinolate synthesis in leaves of *Brassica oleraceae* by root elicitation and in roots after elicitor application to leaves, which in case with MJ was explained by its volatility. Moreover, it has been shown that the reaction of leaves and roots on elicitor treatment was not identical and different individual glucosinolates have been matched.

The content of glucosinolates in secondary roots markedly increased during plant growth for all systems. For H leaves it increased 2.6-folds during 30 days, while for secondary roots 12.2-folds (from 0.4 to 4.9 mg $plant^{-1}$) (Table 4.19). This effect was smaller in aeroponics: for AD secondary roots the glucosinolate content during plant growth increased from 1.0 to 5.6 mg $plant^{-1}$ (5.6-folds) and for AS from 1.5 to 7.1 mg $plant^{-1}$ (4.7-folds) (Attachment, Tables 4.19a and 4.19b). Increase or decrease in glucosinolate content in roots did not correlate with that in leaves, which proves the independency of their syntheses.

Table 4.19. Elicitor influence on total glucosinolate content in plants and exudates of turnip from hydroponic (mg plant^{-1})

Date	Treatment	Leaves	Secondary roots	Primary roots	Exudates
10 days	H	$1.5^{a} \pm 0.2$	$0.4^{a} \pm 0.0$		$0.7^{a} \pm 0.2$
	HSA_0	$2.7^{b} \pm 0.3$	$1.3^{b} \pm 0.1$		$1.6^{b} \pm 0.0$
	HMJ_0	$2.9^{b} \pm 0.6$	$1.0^{b} \pm 0.1$		$2.1^{c} \pm 0.2$
15 days	H	$1.7^{a} \pm 0.1$	$0.8^{a} \pm 0.1$	$3.0^{b} \pm 0.2$	$0.8^{a} \pm 0.1$
	HSA_0	$3.4^{b} \pm 0.2$	$1.6^{b} \pm 0.2$	$2.7^{b} \pm 0.3$	$2.0^{b} \pm 0.3$
	HMJ_0	$4.3^{b} \pm 0.8$	$1.7^{b} \pm 0.2$	$1.5^{a} \pm 0.1$	$1.9^{b} \pm 0.3$
20 days	H	$2.4^{a} \pm 0.4$	$3.2^{a} \pm 0.3$	$4.8^{c} \pm 0.3$	$1.0^{a} \pm 0.1$
	HSA_0	$3.1^{b} \pm 0.2$	$4.8^{b} \pm 0.3$	$3.9^{b} \pm 0.2$	$1.9^{b} \pm 0.3$
	HMJ_0	$5.3^{c} \pm 0.8$	$5.4^{c} \pm 0.3$	$2.3^{a} \pm 0.2$	$1.7^{b} \pm 0.3$
25 days	H	$2.8^{a} \pm 0.1$	$4.4^{a} \pm 0.4$	$6.4^{ab} \pm 1.1$	$1.2^{a} \pm 0.1$
	HSA_0	$4.0^{b} \pm 0.4$	$7.3^{b} \pm 0.7$	$6.8^{b} \pm 0.7$	$1.5^{b} \pm 0.1$
	HMJ_0	$5.6^{c} \pm 0.3$	$7.6^{b} \pm 0.3$	$5.3^{a} \pm 0.8$	$1.3^{ab} \pm 0.1$
30 days	H	$3.9^{a} \pm 0.3$	$4.9^{a} \pm 0.8$	$9.0^{a} \pm 0.6$	$1.5^{ab} \pm 0.2$
	HSA_0	$4.1^{a} \pm 0.4$	$10.2^{b} \pm 2.1$	$8.0^{a} \pm 1.0$	$1.6^{b} \pm 0.1$
	HMJ_0	$5.6^{b} \pm 0.5$	$7.8^{b} \pm 0.8$	$9.9^{a} \pm 1.0$	$1.4^{a} \pm 0.1$
Total	H				$4.6^{a} \pm 0.6$*
	HSA_0				$7.8^{b} \pm 0.7$*
	HMJ_0				$7.3^{b} \pm 1.1$*

H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; HSA_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; HMJ_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + methyl jasmonate applied on the beginning. The differences are compared for each treatment. Values followed by the same letter are not significantly different. *Sum of glucosinolates exuded during 30 days

Kiddle *et al*. (1994) also reported about the increase of glucosinolate content in roots of *Brassica rapa* after elicitor application to the roots of plants, however Van Dam *et al*. (2003) wrote about the decrease of total glucosinolate content in roots of *Brassica nigra* and *Brassica oleracea*. Perhaps, the reaction of plants on elicitor application is individual for different plant species.

Elicitors increased the glucosinolate content in secondary roots, but in contrast to leaves at the end of plant growth SA caused higher effect as MJ. On 30th day the content of glucosinolates for HSA_0 secondary roots reached 10.2 mg plant^{-1}, for HMJ_0 7.8 mg plant^{-1}, which was 2 and 1.6-fold more as for H secondary roots (Table 4.19). At this time total glucosinolate content in $ADSA_0$ secondary roots achieved 10.8 mg plant^{-1} (2-folds more as for AD); for $ASSA_0$ it reached also 10.8 mg plant^{-1} (1.5-fold more then for AS) (Attachment, Tables 4.19a and 4.19b).

The slower increase of glucosinolate content in secondary roots of MJ treated plants as compared to SA may be explained by intensification of exudation process (see below).

Baldwin (1996) showed in experiments with *Nicotiana sylvestris* that MJ, applied to leaves of hydroponically grown plants, activated the wound-induced increase in nicotine. The treatment primarily resulted in increases of *de novo* synthesis largely in the roots; the alkaloid is then transported to the leaf in the xylem stream. This could be extrapolated on our experiments and

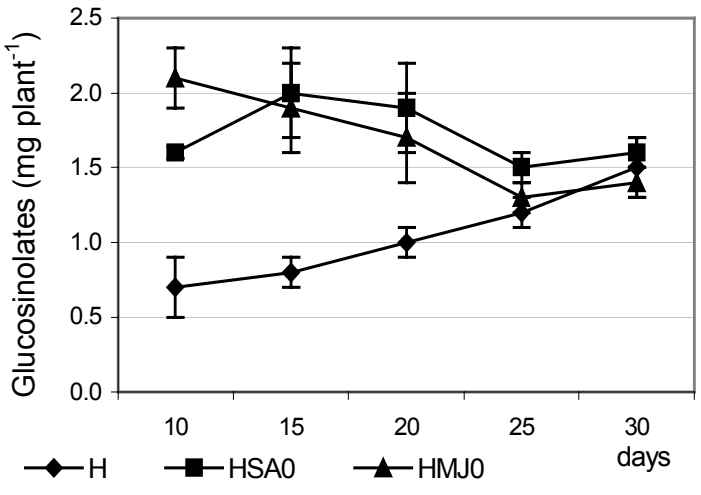
consequently the reaction of turnip on SA application could be the increase of glucosinolates particularly in secondary roots, while MJ in leaves.

Primary roots were not formed on 10th day; on 15th day in H they had 3.0 mg plant^{-1} of glucosinolates (Table 4.19). The content of glucosinolates in primary roots of plants from all systems and treatments increased gradually with plant development and on 30th H primary roots 9.0 mg plant^{-1}. Application of SA decreased the total glucosinolate content in primary roots: on 30th day in HSA_0 it was 1.0 mg plant^{-1} lower as for H. For $ADSA_0$ it was nearly the same as for non-treated plants. This could be explaine by slowing down the process of plant development under SA influence (see 4.3.3). Application of MJ decreased the glucosinolate content in primary roots in the first half of the experiment, but on 30th day HMJ_0 primary roots had 0.9 mg plant^{-1} of glucosinolates more then these from H. This was connected with suppressing of plant growth under MJ treatment on the beginning of plant growth (see 4.3.3).

Elicitor application in both types of aeroponic caused decrease of glucosinolates in primary roots (Attachment, Tables 4.19a and 4.19b).

Glucosinolate content in exudates in systems, where the elicitors have not been applied smoothly enhanced in the course of experiment. The content of exuded glucosinolates also depended on the type of system: during the first 10 days H plants exuded 0.7 mg plant^{-1} of glucosinolates, AD 0.3 mg plant^{-1} and AS 0.4 mg plant^{-1}. During the last 5 days the glucosinolate content in exudates increased until 1.4 mg plant^{-1} for H, 2.1 for AD, and 2.0 mg plant^{-1} for AS (Table 4.19, Attachment, Tables 4.19a and 4.19b).

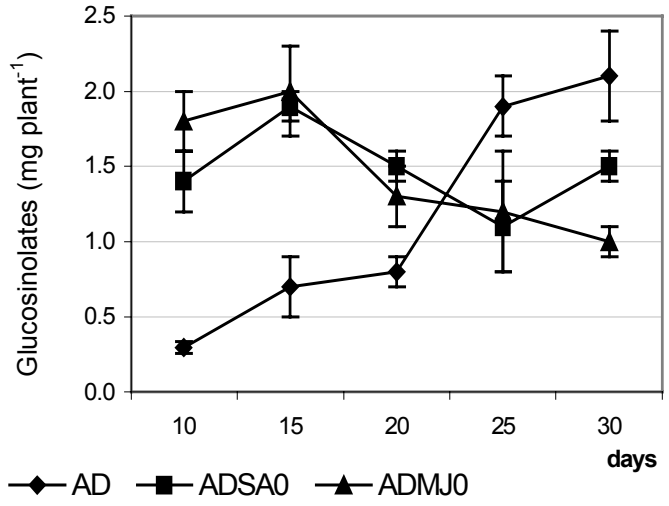
At the beginning of the experiment both elicitors increased total glucosinolate content in exudates, but the exudation of glucosinolates in elicitor-treated systems decreased until the end of experiment (Figure 4.8). Increased glucosinolate content in exudates after the application of SA and MJ was observed already at the beginning of the experiment, which could be explained by defense reaction of plants, caused by the addition of elicitor as it was showed by Baldwin (1996). In addition, Bennett and Wallsgrove (1994) wrote that the increase in glucosinolates in exudates might be due to the release of plant defense substances triggered by the addition of elicitor.



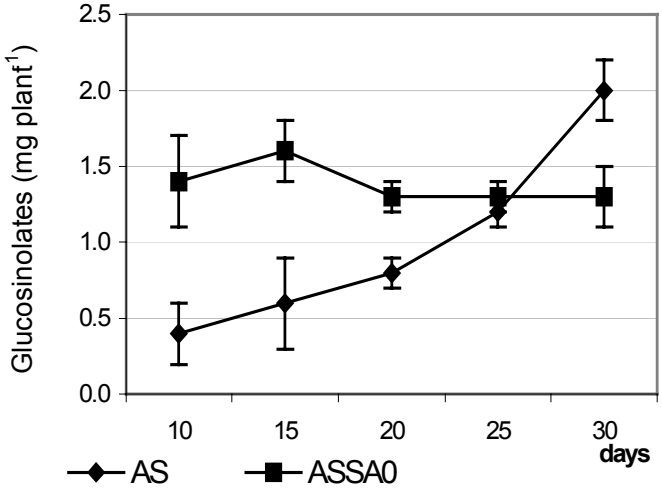
H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; HSA_0 - two times concentrated Hoagland solution + two times increased sulfur + salicylic acid; HMJ_0 - two times concentrated Hoagland solution + two times increased sulfur + methyl jasmonate applied on the beginning

During the first 10 days HSA_0 plants exuded 1.6 mg plant^{-1} glucosinolates (2.1-folds more then H plants) and HMJ_0 2.1 mg plant^{-1} (3-folds more), while to the end of the experiment it decreased rapidly.

The effect of SA and MJ treatment in hydroponics was to observe until the 20th day and on 30th day it dropped until 1.6 and 1.4 mg plant^{-1}, respectively, which did not significantly differed of that for the non-treated variant. During 30 days H plants exuded 4.6 mg plant^{-1} of glucosinolates, which was 26 % of these in plants, HSA_0 7.8 mg plant^{-1} (35 %), and HMJ_0 7.3 mg plant^{-1} (31 %).



AD – aeroponic with defensor, two times concentrated Hoagland solution + two times increased sulfur; $ADSA_0$ - two times concentrated Hoagland solution + two times increased sulfur + salicylic acid; $ADMJ_0$ - two times concentrated Hoagland solution + two times increased sulfur + methyl jasmonate applied on the beginning



AS – aeroponic with sprayer, two times concentrated Hoagland solution + two times increased sulfur; $ADSA_0$ - two times concentrated Hoagland solution + two times increased sulfur + salicylic acid

Figure 4.8. Elicitor influence on the kinetic of total glucosinolate content in exudates of turnip

Also Kneer *et al.* (1999) showed on the example of genistein, exuded from roots of *Lupinus luteus,* that the content of defense compounds decrease some period after the treatment with elicitors. They concluded that plant response to elicitor application is the once only event, which occurs during the first days after treatment, followed by a gradual decline.Treatment with elicitors also increased glucosinolate content in exudates of AD and AD, but in contrast to H the content of exuded glucosinolates between 25^{th}-30^{th} day in elicitor-treated systems was much lower as in non-treated. On 10^{th} day total glucosinolate content in $ADSA_0$ exudates was 1.4 mg $plant^{-1}$ (4.7-folds more then for AD), reached the highest point 1.9 mg $plant^{-1}$ during the next 5 days, and then dropped on 30^{th} day to 1.5 mg $plant^{-1}$ (1.4-fold lower as for non-treated system). During 30 days AD plants exuded 5.9 mg $plant^{-1}$ of glucosinolates (30 % of these in plants), $ADSA_0$ 7.4 mg $plant^{-1}$ (31 %), and $ADMJ_0$ 7.3 mg $plant^{-1}$ (32 %).

There is no common opinion how the glucosinolates can pass through the membranes. Most obvious, they are transported via Ca^{2+} channels, which means, by the increase of ion exchange the glucosinolates efflux increase.

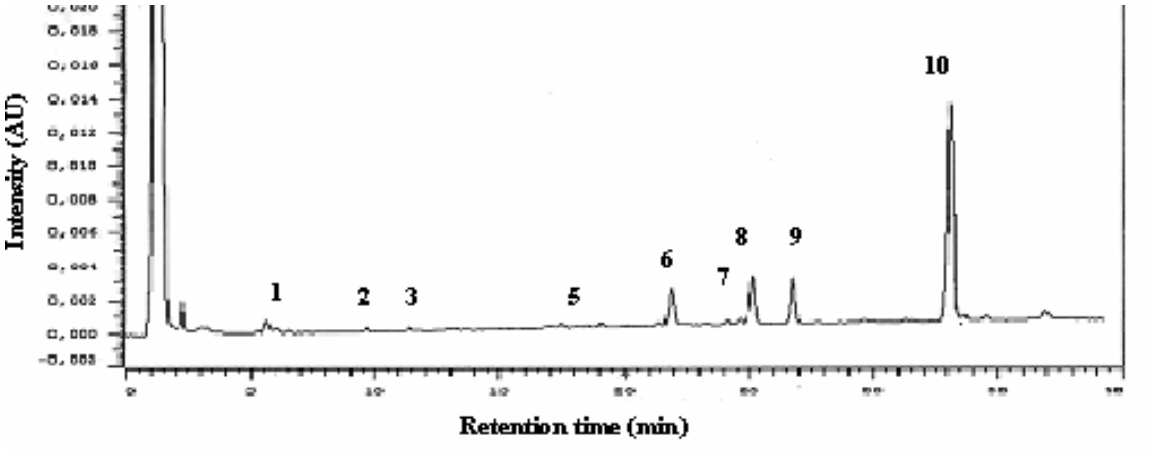
Increase of the part of exuded glucosinolates as compared to their content in plant tissues could be explained also by the defense response of plants, caused by elicitor application. This involve a cascade of physiological events, amount which is also changes in membrane potential and ion fluxes, increase in Ca^{2+} influx and K^+ efflux/H^+ influx exchange (Wu and Lin, 2002). In addition, Gatehause (2002) wrote that wounding response of plants includes Ca^{2+} release from vacuoles and opening of ion channels in the plasma membrane.

As it is possible to conclude, both elicitors increased total glucosinolate content in exudates at the beginning of the experiment. However, MJ caused more essential increment of total glucosinolate content as SA, which might be partially explained by the fact, that SA increases not only the glucosinolate synthesis, but also their decomposition by enhances of the myrosinase activity (Kiddle *et al*., 1994).
In spite of exuding glucosinolates more by treated as non-treated plants, the glucosinolate content in secondary roots of treated plants was higher as for non-treated. This can find the explanation that elicitor-induced rhizosecretion is based on *de novo* synthesis of secondary metabolites and not on elicitor-induced leakage from root tissues (Kneer *et al*., 1999).

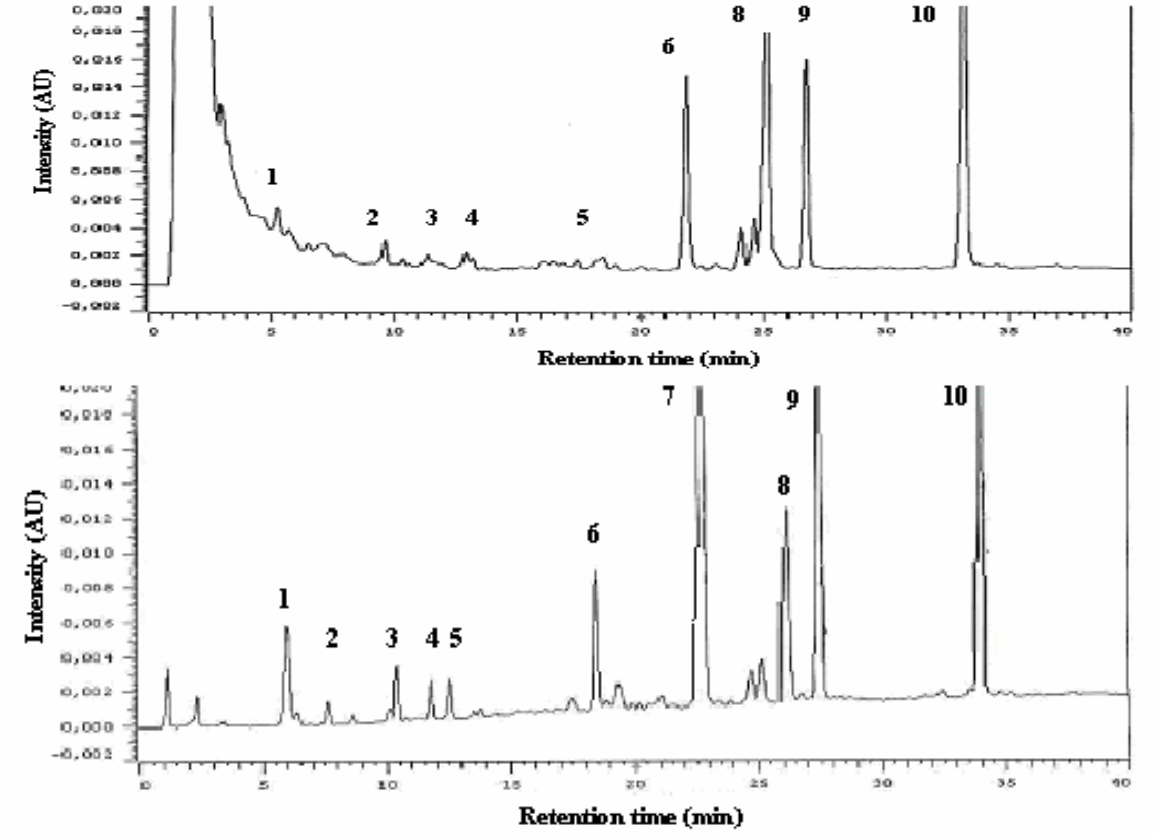
4.2.2 Individual glucosinolates in plants and exudates

Elicitors influenced differently the classes of glucosinolates. As it is possible to see from the Figure 4.9, glucosinolate profile was influenced on 10^{th} day after elicitor application.
Especially strong were altered indole glucosinolates (glucobrassicin, 4-methoxy-glucobrassicin and neoglucobrassicin by SA and MJ, while 4-hydroxy-glucobrassicin by MJ). Aromatic glucosinolate gluconasturtiin was strongly influenced by SA and to lesser extend by MJ. Some aliphatic glucosinolates (e.g. progoitrin and glucoalyssin) were slightly increased by MJ.
The main class of glucosinolates in leaves, primary roots, and exudates of untreated plants was aliphatic, while the smallest was aromatic. However, after application of SA and MJ to roots, the content of the three of glucosinolate classes were assessed in all parts of plants and their exudates.
On 30^{th} day aliphatic, aromatic, and indole glucosinolates for H leaves composed 67 : 0 : 33 % (Table 4.20). Application of SA as well as MJ decreased the part of aliphatic, while increased the part of indole glucosinolates in leaves. Moreover, elicitor application induced the appearance of aromatic gluconasturtiin in leaves of hydroponically grown plants.
The part of aliphatic glucosinolates in secondary roots was much smaller as in leaves, while H secondary roots had 25 % of aromatic glucosinolates (which were not observed in leaves) and 48 % of indole glucosinolates. As found in leaves, treatment with SA and MJ decreased the part of aliphatic glucosinolates in secondary roots, but MJ increased the part of aromatic, while SA of indole glucosinolates.
Elicitor application decreased the part of aliphatic glucosinolates in primary roots to 43 % for SA and 50 % for MJ as compared to 76 % for non-elicited treatment. However, in contrast to HSA_0 and HMJ_0 leaves as well as secondary roots the part of indole glucosinolates was not increased, but the part of aliphatic glucosinolates reached 42 and 39 %, respectively, while for H it was only 8% of total glucosinolates.
Composition of glucosinolate classes in exudates differed between systems. Aliphatic glucosinolates made the majority in exudates from H and AS, while for AD indole glucosinolates (Table 4.20, Attachment, Tables 4.20a and 4.20b). Application of SA in hydroponic increased the part of indole glucosinolates at the cost of aliphatic, while did not influence on aromatic. For $ADSA_0$ exudates the part of aliphatic glucosinolates decreased, while aromatic and indole increased. For $ASSA_0$ exudates the part of aliphatic glucosinolates decreased, aromatic glucosinolates increased, while indole did not change as compared to non-treated system. Application of MJ in H and AD caused essential increment of indole glucosinolate part in exudates, decreased aliphatic, while did not influence on aromatic glucosinolates. In HMJ_0 exudates the composition of aliphatic, aromatic, and indole glucosinolates was 13 : 24 : 63 % and in AD 18 : 26 : 57 %.

a)



b)



a) H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; b) HSA_0, - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; c) HMJ_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + methyl jasmonate applied on the beginning

1 – progoitrin, 2 – gluconapoleiferin, 3 – glucoalyssin, 4 – gluconapin, 5 - 4-hydroxy-glucobrassicin, 6 – glucobrassicanapin, 7 – glucobrassicin, 8 – gluconasturtiin, 9 - 4-methoxy-glucobrassicin, 10 – neoglucobrassicin

Figure 4.9. HPLC profile of glucosinolates recovered from exudates of turnip in hydroponic systems during the first 10 days after elicitor application

Table 4.20. Elicitor influence on composition of aliphatic, aromatic, and indole glucosinolates in plants and exudates of turnip in hydroponic on 30th day

	Treatment	Content / composition	Aliphatic	Aromatic	Indole
Leaves	H	mg plant^{-1}	$2.6^{c} \pm 0.1$	ND	$1.3^{a} \pm 0.1$
		%	67	0	33
	HSA_0	mg plant^{-1}	$1.8^{a} \pm 0.1$	$0.4^{a} \pm 0.2$	$1.9^{b} \pm 0.1$
		%	44	10	46
	HMJ_0	mg plant^{-1}	$2.2^{b} \pm 0.2$	$0.6^{a} \pm 0.2$	$2.8^{c} \pm 0.2$
		%	39	11	50
Secondary roots	H	mg plant^{-1}	$1.3^{a} \pm 0.2$	$1.2^{a} \pm 0.4$	$2.3^{a} \pm 0.2$
		%	27	25	48
	HSA_0	mg plant^{-1}	$1.5^{a} \pm 0.3$	$2.3^{b} \pm 0.4$	$6.3^{c} \pm 0.5$
			15	23	62
	HMJ_0	mg plant^{-1}	$1.1^{a} \pm 0.3$	$2.8^{b} \pm 0.3$	$3.9^{b} \pm 0.2$
		%	14	36	50
Primary roots	H	mg plant^{-1}	$6.8^{c} \pm 0.2$	$0.7^{a} \pm 0.2$	$1.4^{a} \pm 0.2$
		%	76	8	16
	HSA_0	mg plant^{-1}	$3.5^{a} \pm 0.4$	$3.4^{b} \pm 0.3$	$1.2^{a} \pm 0.2$
		%	43	42	15
	HMJ_0	mg plant^{-1}	$4.9^{b} \pm 0.4$	$3.8^{b} \pm 0.4$	$1.1^{a} \pm 0.3$
		%	50	39	11
Exudates	H	mg plant^{-1}	$2.1^{b} \pm 0.3$*	$1.2^{a} \pm 0.3$*	$1.6^{a} \pm 0.3$*
		%	43	24	33
	HSA_0	mg plant^{-1}	$1.2^{a} \pm 0.1$*	$1.8^{ab} \pm 0.4$*	$5.0^{b} \pm 0.5$*
		%	15	23	63
	HMJ_0	mg plant^{-1}	$1.1^{a} \pm 0.1$*	$2.1^{a} \pm 0.2$*	$5.6^{b} \pm 0.5$*
		%	13	24	64

H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; HSA_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; HMJ_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + methyl jasmonate applied on the beginning. The differences are compared for each treatment. Values followed by the same letter are not significantly different. * Sum of glucosinolates exuded during 30 days

In general, both of elicitors raised essentially the part of indole glucosinolates (especially SA) and increased slightly the part of aromatic glucosinolates. This could be explained by the ability of glucosinolate classes to be induced. Tryptophan-derived indole glucosinolates are more responsive to induction than aliphatic glucosinolates. Mikkelsen *et al*. (2000) pointed out that different indole glucosinolate methoxylating enzymes are influenced by SA and MJ, whereas aliphatic glucosinolates appear to be primarily genetically and not environmentally controlled. Furthermore, Zeng *et al*. (2003) reported that aromatic and indole glucosinolates are responsible for interaction between *Brassicaceae* and fungi in the contrast to aliphatic glucosinolates, and when the applied elicitors cause the defense reaction of plants, the synthesis of these two classes of glucosinolates is stimulated.

4.2.2.1 Aliphatic glucosinolates in plants and exudates

Aliphatic glucosinolates were the major class of glucosinolates for turnip for leaves and primary roots, and exudates in all systems (Table 4.21, Attachment, Tables 4.21 a and 4.21b). During the first half of experiment the major part of aliphatic glucosinolates was measured in leaves of treated as well as untreated plants, but on 30th day it took 24-27% of the total aliphatic content in plants. Until 25th day the application of elicitors did not essentially influence on the content of aliphatic glucosinolates in leaves as it also has been shown in the experiments of Zeng *et al.* (2003). The significant decrease of aliphatic glucosinolate content in leaves under SA and MJ treatment was measured on 30th day: HMJ_0 leaves had 75 % of the H glucosinolate content and $ADMJ_0$ leaves 70 % (Table 4.21, Attachment, Tables 4.21a). At this time aliphatic glucosinolate content for HSA_0 leaves became 70 % of that for H, the same was measured for AS and AD. We suspect that elicitors decrease the expression of CYP79F1, because its overexpression in *Arabidopsis thaliana* leads to increases in short-chain glucosinolate levels (Tantikanjana *et al*., 2004).

Table 4.21. Elicitor influence on aliphatic glucosinolate content in plants and exudates of turnip from hydroponic (mg plant^{-1})

Date	Treatment	Leaves	Secondary roots	Primary roots	Exudates
10 days	H	$0.8^{a} \pm 0.1$	$0.1^{a} \pm 0.0$		$0.5^{c} \pm 0.1$
	HSA_0	$0.8^{a} \pm 0.1$	$0.2^{b} \pm 0.0$		$0.1^{a} \pm 0.0$
	HMJ_0	$1.2^{b} \pm 0.2$	$0.1^{a} \pm 0.0$		$0.3^{b} \pm 0.1$
15 days	H	$1.2^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.7^{b} \pm 0.1$	$0.4^{a} \pm 0.1$
	HSA_0	$1.2^{a} \pm 0.1$	$0.3^{b} \pm 0.1$	$0.5^{a} \pm 0.1$	$0.5^{a} \pm 0.1$
	HMJ_0	$1.4^{a} \pm 0.3$	$0.3^{b} \pm 0.1$	$0.6^{ab} \pm 0.0$	$0.3^{a} \pm 0.1$
20 days	H	$1.6^{ab} \pm 0.2$	$0.5^{a} \pm 0.1$	$1.6^{b} \pm 0.1$	$0.3^{a} \pm 0.1$
	HSA_0	$1.4^{a} \pm 0.1$	$0.8^{b} \pm 0.1$	$0.9^{a} \pm 0.1$	$0.1^{a} \pm 0.1$
	HMJ_0	$1.8^{b} \pm 0.3$	$0.7^{ab} \pm 0.1$	$0.7^{a} \pm 0.1$	$0.3^{a} \pm 0.1$
25 days	H	$1.6^{a} \pm 0.0$	$0.9^{a} \pm 0.2$	$2.3^{a} \pm 0.4$	$0.4^{b} \pm 0.1$
	HSA_0	$1.7^{a} \pm 0.2$	$1.1^{a} \pm 0.2$	$1.5^{a} \pm 0.3$	$0.2^{ab} \pm 0.1$
	HMJ_0	$2.0^{a} \pm 0.1$	$0.9^{a} \pm 0.1$	$1.5^{a} \pm 0.3$	$0.2^{a} \pm 0.0$
30 days	H	$2.6^{c} \pm 0.1$	$1.3^{a} \pm 0.2$	$6.8^{c} \pm 0.2$	$0.5^{b} \pm 0.1$
	HSA_0	$1.8^{a} \pm 0.1$	$1.5^{a} \pm 0.3$	$3.5^{a} \pm 0.4$	$0.2^{a} \pm 0.1$
	HMJ_0	$2.2^{b} \pm 0.2$	$1.1^{a} \pm 0.3$	$4.9^{b} \pm 0.4$	$0.1^{a} \pm 0.1$
Total	H				$2.1^{b} \pm 0.3$*
	HSA_0				$1.2^{a} \pm 0.1$*
	HMJ_0				$1.1^{a} \pm 0.1$*

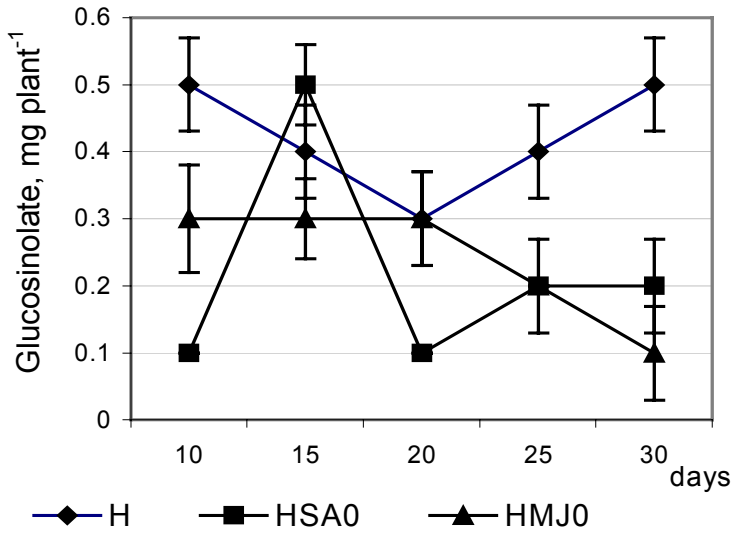
H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; HSA_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; HMJ_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + methyl jasmonate applied on the beginning. The differences are compared for each treatment. Values followed by the same letter are not significantly different. *Sum of glucosinolates exuded during 30 days

On 30th day in turnip primary roots the aliphatic glucosinolate content was higher as in other parts (64 % of total aliphatic glucosinolates in H plants). For H primary roots it reached 6.8 mg plant^{-1}, while for leaves 2.6, and for secondary roots 1.3 mg plant^{-1} (Table 4.21). The same distribution of aliphatic glucosinolates was in AD (Attachment, Table 4.21b). In primary roots the significant difference between the aliphatic glucosinolate content in treated with elicitors as well as untreated variants was measured at the end of experiment for all systems (Table 4.21, Attachment, Tables 4.21a and 4.21b). Application of both elicitors decreased the content of aliphatic glucosinolates in primary roots. On 30th day aliphatic glucosinolate content in HSA_0 primary roots was 3.5 mg plant^{-1} (2-folds lower as for H). For $ADSA_0$ and $ASSA_0$ the difference was smaller: aliphatic glucosinolate content in primary roots of SA treated plants was 1.6 and 1.4-fold lower as for AD and AS, respectively. MJ decreased the content of aliphatic glucosinolates in primary roots on 30th day to 4.9 mg plant^{-1} for HMJ_0 (1.4-fold lower as for H) and for $ADSA_0$ 3.9 mg plant^{-1} (1.5-fold lower as for AD).

In the contrast to leaves and primary roots, application of SA increased aliphatic glucosinolate content in secondary roots of all three systems during the whole experiment (Table 4.21, Attachment, Tables 4.21a and 4.21b). On 30th day aliphatic glucosinolate content for HSA_0 secondary roots with 1.5 mg plant^{-1} was 13 % higher as for H. MJ increased the aliphatic glucosinolate content in secondary roots during the whole time of plant growth for AD and until the 25th day for H.

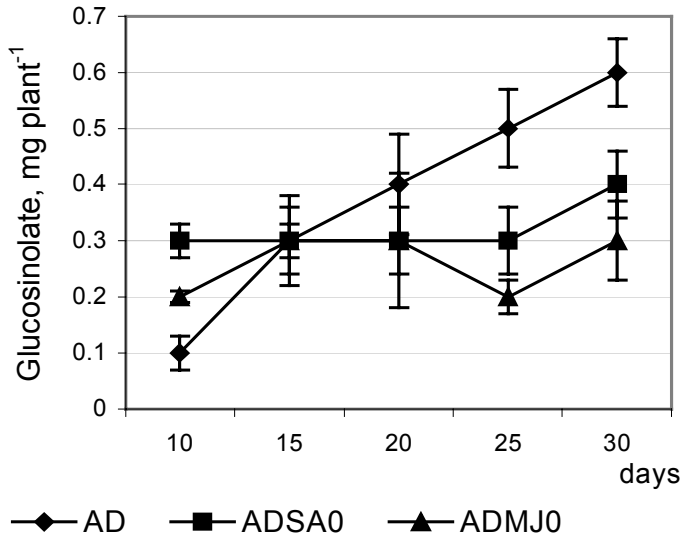
This could be explained by the fact that the highest expression level of CYP79F1 genes, participating in conversion of short-chain methionine derivatives (di- and trihomo-methionine) into aliphatic aldoximes (Mikkelsen *et al*., 2000), was observed in roots and normalized in leaves of *Arabidopsis thaliana* (Reintanz *et al*., 2001 and Glombitza *et al*., 2004).

Increase in the content of aliphatic glucosinolates in secondary roots and decreases in leaves and primary roots of turnip for both elicitor treatments could be also substituted by the independent regulation of glucosinolate syntheses in different plant organs (Tantikanjana *et al.*, 2004).

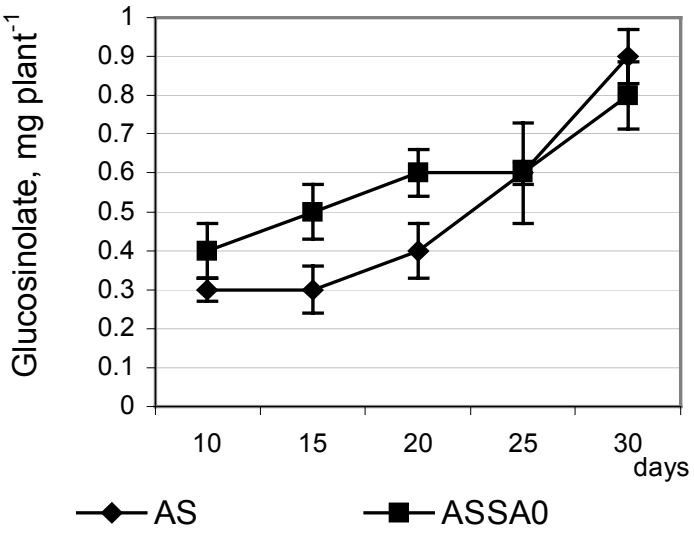


H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; HSA_0 - two times concentrated Hoagland solution + two times increased sulfur + salicylic acid; HMJ_0 - two times concentrated Hoagland solution + two times increased sulfur + methyl jasmonate applied on the beginning

Van Dam *et al*. (2003) explained on the example with *Brassica oleracea*, that stimulated by elicitor application aliphatic glucosinolates in roots, particularly glucobrassicanapin, gluconapin, progoitrin, and gluconapoleiferin (which are also typical for secondary roots turnip, as we have found), yield isothiocyanates. They are generally recognized as potent antimicrobial and antifungal compounds as well as deterrents of a wild rage ogeneralist and specialist herbivores.



AD – aeroponic with defensor, two times concentrated Hoagland solution + two times increased sulfur; $ADSA_0$ - two times concentrated Hoagland solution + two times increased sulfur + salicylic acid; $ADMJ_0$ - two times concentrated Hoagland solution + two times increased sulfur + methyl jasmonate applied on the beginning



AS – aeroponic with sprayer, two times concentrated Hoagland solution + two times increased sulfur; $ADSA_0$ - two times concentrated Hoagland solution + two times increased sulfur + salicylic acid

Figure 4.10. Elicitors influence on the kinetic of aliphatic glucosinolates in exudates of turnip

Aliphatic glucosinolates was the major class for H and AS exudates, while for AD indole glucosinolates made the majority. H plants exuded 2.1 mg $plant^{-1}$ of aliphatic glucosinolates during 30 days, AD 1.9, and AS 2.4 mg $plant^{-1}$ (Figure 4.10).

The amount of exuded aliphatic glucosinolates increased for H from 0.3 mg $plant^{-1}$ on 10^{th} day to 0.5 mg $plant^{-1}$ on 30^{th} day.

For both aeroponics was measured constant increase of aliphatic glucosinolates in exudates from 0.1 to 0.6 mg $plant^{-1}$ for AD and from 0.3 to 0.9 mg $plant^{-1}$ for AD (Figure 4.10).

Elicitors decreased the content of aliphatic glucosinolates in exudates for all systems. For HSA_0 the content of exuded aliphatic glucosinolates during 30 days was 1.2 mg $plant^{-1}$ (1.7-fold lower as for H), for $ADSA_0$ 1.5 mg $plant^{-1}$ (1.3-fold lower as for AD), but for $ASSA_0$ 2.7 mg $plant^{-1}$ (1.1-fold more then for AS). Decrease of aliphatic glucosinolates content in exuded was more essential under the influence of MJ. For HMJ_0 their content during the 30 days reached 1.1 mg $plant^{-1}$ (1.9-fold lower as for H) and in $ADMJ_0$ 1.3 mg $plant^{-1}$ (1.5-fold lower as for AD). Stronger effect of MJ, especially on the beginning of the experiment, could be partially explained by suppressing of secondary root growth.

Individual aliphatic glucosinolates

The previous experiment showed that turnip had a restricted profile comprising butenyl and pentenyl glucosinolates (gluconapin and glucobrassicanapin) and their hydroxylated homologues (progoitrin and gluconapoleiferin) (Table 4.22). Their content differed between the treatments. Elicitors matched two of four aliphatic glucosinolates: progoitrin and gluconapin.

Table 4.22. Elicitor influence on individual aliphatic glucosinolates content in plants and exudates of turnip from hydroponic on 30th day (mg plant^{-1})

Gluco-sinolates	Treat-ment	Content, Part	Leaves	Secondary roots	Primary roots	Exudates
Gluconapin	H	mg plant^{-1}	$0.7^{b} \pm 0.2$	$0.2^{a} \pm 0.1$	$1.7^{b} \pm 0.3$	$0.4^{b} \pm 0.0$*
		%	27	8	65	
	HSA_0	mg plant^{-1}	$0.3^{a} \pm 0.2$	$0.2^{a} \pm 0.1$	$0.6^{a} \pm 0.6$	$0.1^{a} \pm 0.1$*
		%	27	18	55	
	HMJ_0	mg plant^{-1}	$0.5^{b} \pm 0.2$	$0.1^{a} \pm 0.1$	$1.1^{a} \pm 0.2$	$0.2^{a} \pm 0.0$*
		%	29	6	65	
Progoitrin	H	mg plant^{-1}	$0.9^{b} \pm 0.0$	$0.6^{b} \pm 0.2$	$2.8^{b} \pm 0.2$	$0.6^{b} \pm 0.1$*
		%	21	14	65	
	HSA_0	mg plant^{-1}	$0.5^{ab} \pm 0.3$	$0.5^{ab} \pm 0.4$	$1.2^{a} \pm 0.4$	$0.4^{a} \pm 0.0$*
		%	23	23	55	
	HMJ_0	mg plant^{-1}	$0.4^{a} \pm 0.0$	$0.2^{a} \pm 0.2$	$1.4^{a} \pm 0.4$	$0.6^{b} \pm 0.1$*
		%	24	10	67	
Glucobras-sicanapin	H	mg plant^{-1}	$0.2^{a} \pm 0.1$	$0.1^{a} \pm 0.1$	$1.1^{a} \pm 0.1$	$0.4^{b} \pm 0.0$*
		%	14	7	79	
	HSA_0	mg plant^{-1}	$0.3^{a} \pm 0.1$	$0.2^{a} \pm 0.2$	$0.8^{a} \pm 0.4$	$0.1^{a} \pm 0.1$*
		%	23	15	62	
	HMJ_0	mg plant^{-1}	$0.6^{b} \pm 0.4$	$0.5^{a} \pm 0.1$	$1.0^{a} \pm 0.1$	$0.1^{a} \pm 0.1$*
		%	29	24	48	
Gluconapo-leiferin	H	mg plant^{-1}	$0.6^{a} \pm 0.2$	$0.3^{a} \pm 0.2$	$1.0^{b} \pm 0.1$	$0.5^{b} \pm 0.1$*
		%	32	16	53	
	HSA_0	mg plant^{-1}	$0.7^{a} \pm 0.1$	$0.5^{a} \pm 0.4$	$0.6^{a} \pm 0.2$	$0.3^{ab} \pm 0.2$*
		%	39	28	33	
	HMJ_0	mg plant^{-1}	$0.7^{a} \pm 0.6$	$0.3^{a} \pm 0.1$	$1.2^{b} \pm 0.5$	$0.1^{a} \pm 0.1$*
		%	29	21	50	

H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; HSA_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; HMJ_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + methyl jasmonate applied on the beginning. The differences are compared for each treatment. Values followed by the same letter are not significantly different. *Sum of glucosinolates exuded during 30 days

In general, elicitor application caused the increase in total aliphatic glucosinolates, as it was already shown, by the decrease in each individual aliphatic glucosinolate.
On 30th day of the experiment gluconapin and progoitrin content in leaves was increased by both of elicitors, more by MJ as by SA (Table 4.22). The same decrease was measured for progoitrin in secondary roots. SA caused stronger effect on both glucosinolates in primary roots: 65 - 67 % of gluconapin and progoitrin in plants was located in H and HMJ_0 primary roots, while 55 % in HSA_0 primary roots.

Table 4.23. Elicitor influence on progoitrin content in plants and exudates of turnip from hydroponic (mg plant^{-1})

Days	Treatments	Leaves	Secondary roots	Primary roots	Exudates
10	H	$0.3^{a} \pm 0.0$	ND	-	$0.1^{a} \pm 0.0$
	HSA_0	$0.6^{b} \pm 0.1$	ND	-	$0.1^{a} \pm 0.0$
	HMJ_0	$0.6^{b} \pm 0.1$	$0.1^{a} \pm 0.0$	-	$0.2^{a} \pm 0.1$
15	H	$0.5^{a} \pm 0.0$	ND	$0.1^{a} \pm 0.1$	$0.1^{a} \pm 0.0$
	HSA_0	$0.8^{b} \pm 0.2$	ND	$0.1^{a} \pm 0.0$	$0.2^{a} \pm 0.1$
	HMJ_0	$0.7^{b} \pm 0.1$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.2^{a} \pm 0.1$
20	H	$0.7^{b} \pm 0.0$	$0.3^{b} \pm 0.0$	$0.4^{a} \pm 0.2$	$0.1^{a} \pm 0.1$
	HSA_0	$0.7^{ab} \pm 0.3$	$0.3^{ab} \pm 0.1$	$0.2^{a} \pm 0.1$	$0.1^{a} \pm 0.1$
	HMJ_0	$0.5^{a} \pm 0.1$	$0.2^{a} \pm 0.0$	$0.2^{a} \pm 0.0$	$0.1^{a} \pm 0.0$
25	H	$0.7^{b} \pm 0.2$	$0.6^{b} \pm 0.1$	$0.8^{b} \pm 0.2$	$0.2^{a} \pm 0.1$
	HSA_0	$0.8^{c} \pm 0.1$	$0.4^{ab} \pm 0.1$	$0.3^{a} \pm 0.1$	$0.1^{a} \pm 0.1$
	HMJ_0	$0.4^{a} \pm 0.0$	$0.4^{a} \pm 0.0$	$0.3^{a} \pm 0.0$	$0.1^{a} \pm 0.0$
30	H	$0.9^{b} \pm 0.2$	$0.6^{b} \pm 0.1$	$2.8^{b} \pm 0.3$	$0.2^{a} \pm 0.1$
	HSA_0	$0.5^{a} \pm 0.1$	$0.5^{b} \pm 0.1$	$1.2^{a} \pm 0.1$	ND
	HMJ_0	$0.4^{a} \pm 0.0$	$0.2^{a} \pm 0.0$	$1.4^{a} \pm 0.1$	ND

H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; HSA_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; HMJ_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + methyl jasmonate applied on the beginning. ND- not determined. The differences are compared for each treatment. Values followed by the same letter are not significantly different.

In contrast to gluconapin and progoitrin, glucobrassicanapin and gluconapoleiferin content was increased in leaves and secondary roots after elicitor application; however, the total content of these glucosinolates in all plant organs decreased. This was caused by their decrease in primary roots, where they were mostly contended. H primary roots had 79 % of all glucobrassicanapin in plants and 53 % of all gluconapoleiferin. The part of two glucosinolates decrease under SA treatment to 62 and 33 %, and under MJ treatment to 48 and 50 %, respectively. Also Mikkelsen *et al*. (2003) and Kliebenstein *et al*. (2005) reported that SA and MJ could induce aliphatic glucosinolate synthesis. The different reaction on individual glucosinolate profiles synthesis after elicitor application in leaves, secondary, and primary roots could be explained by the different ecological importance of individual glucosinolates, as their structure is closely related to its potency as defense to phytophages (Van Dam *et al*., 2003).

Table 4.24. Elicitor influence on gluconapin content in plants and exudates of turnip from hydroponic (mg plant^{-1})

Days	Treatments	Leaves	Secondary roots	Primary roots	Exudates
10	H	$0.4^{b} \pm 0.0$	$0.1^{a} \pm 0.0$	-	$0.2^{b} \pm 0.0$
	HSA_0	$0.2^{a} \pm 0.0$	$0.2^{a} \pm 0.1$	-	$0.1^{a} \pm 0.0$
	HMJ_0	$0.3^{ab} \pm 0.1$	$0.1^{a} \pm 0.0$	-	$0.1^{a} \pm 0.0$
15	H	$0.5^{a} \pm 0.1$	$0.1^{a} \pm 0.0$	$0.2^{a} \pm 0.1$	$0.2^{a} \pm 0.1$
	HSA_0	$0.4^{a} \pm 0.0$	$0.2^{a} \pm 0.1$	$0.1^{a} \pm 0.0$	$0.2^{a} \pm 0.1$
	HMJ_0	$0.4^{a} \pm 0.1$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.2^{a} \pm 0.1$
20	H	$0.5^{a} \pm 0.2$	$0.1^{a} \pm 0.1$	$0.5^{b} \pm 0.1$	$0.1^{a} \pm 0.1$
	HSA_0	$0.3^{a} \pm 0.1$	$0.2^{ab} \pm 0.1$	$0.3^{ab} \pm 0.1$	ND
	HMJ_0	$0.5^{a} \pm 0.1$	$0.3^{b} \pm 0.0$	$0.2^{a} \pm 0.0$	ND
25	H	$0.5^{a} \pm 0.2$	$0.2^{a} \pm 0.1$	$0.7^{b} \pm 0.1$	ND
	HSA_0	$0.3^{a} \pm 0.1$	$0.3^{a} \pm 0.1$	$0.3^{a} \pm 0.1$	ND
	HMJ_0	$0.5^{a} \pm 0.1$	$0.3^{a} \pm 0.0$	$0.3^{a} \pm 0.0$	ND
30	H	$0.7^{b} \pm 0.1$	$0.2^{b} \pm 0.0$	$1.7^{c} \pm 0.3$	ND
	HSA_0	$0.3^{a} \pm 0.1$	$0.2^{b} \pm 0.0$	$0.6^{a} \pm 0.2$	ND
	HMJ_0	$0.5^{ab} \pm 0.2$	$0.1^{a} \pm 0.0$	$1.1^{b} \pm 0.2$	ND

H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; HSA_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; HMJ_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + methyl jasmonate applied on the beginning. ND- not determined. The differences are compared for each treatment. Values followed by the same letter are not significantly different.

Progoitrin was the most abundant aliphatic glucosinolate identified in turnip. On the beginning of plant growth, the major part of progoitrin was concentrated in leaves, but to the end of vegetation up to 70 % of progoitrin was measured in primary roots of plants. On 30th day its content in H plants reached 0.7 mg plant^{-1} for leaves, 0.2 mg plant^{-1} for secondary and 1.7 mg plant^{-1} for primary roots. In exudates its content varied between 0.1 and 0.2 mg plant^{-1} for each 5 days (Table 4.23). Application of elicitors decreased the progoitrin content for HSA_0 and HMJ_0 leaves to 0.5 and 0.4 mg plant^{-1} (2.3 and 2-folds lower as for H), and for secondary roots to 0.5 and 0.2 mg plant^{-1} (1.2 and 3-folds lower as for H). Neither in HSA_0 nor in HMJ_0 exudates progoitrin was presented between 25th-30th days. The total progoitrin content for H exudates was the same as for HMJ_0 (0.6 mg plant^{-1}), while for HSA_0 it decreased to 0.4 mg plant^{-1}. For AD exudates it reached 0.9 mg plant^{-1} and was decreased in $ADMJ_0$ to 0.4 mg plant^{-1}, while in $ADSA_0$ to 0.6 mg plant^{-1} (Attachment, Table 4.22a).

In addition to progoitrin, decrease of **gluconapin** under the influence of elicitors was also observed. Its content for H primary roots on 30th day reached 1.7 mg plant^{-1}, while for HSA_0 2.8 and for HMJ_0 1.5 fold lower (Table 4.24). For AD primary roots it reached 1.7 mg plant^{-1}, while for $ADSA_0$ 2.8, and for $ADMJ_0$ 8.5 fold lower (Attachment, Tables 4.22a). In H leaves gluconapin content during 30th days increased from 0.4 until 0.7 mg plant^{-1}, nearly did not change for HSA_0 leaves, and slightly increased for HMJ_0 leaves. Also for secondary roots the content of this glucosinolate practically did not change during the experiment for all treatments. Its concentration for H exudates collected during 30 days reached 0.6 mg plant^{-1} (Table 4.22), for AD 0.5 mg plant^{-1}, and for AS 0.9 mg plant^{-1} (Table 4.22, Attachment, Tables 4.22a and 4.22b).

However, the gluconapin in exudates of non-treated plants was found until the 20th day, while in exudates of treated with both elicitor plants until 15th day of experiment. It might be connected with decrease of gluconapin content in secondary roots at the end of experiment.
Until 15th day no **gluconapoleiferin** was measured in H leaves, but until the end of experiment its content reached 0.6 mg plant^{-1} (Table 4.25). Traces of this glucosinolate were found also in secondary roots in the first half of vegetation, but until 30th day its content for H became 0.3 mg plant^{-1}. In contrast to progoitrin and gluconapin treatment with SA and MJ did not cause the decrease in gluconapoleiferin. By the influence of SA its content increased in secondary roots, while under MJ in primary roots. Probably increase of glucosinolate content in secondary roots of HSA_0 was caused by its previous increase in primary roots and then transportation into the secondary roots and exudates.

Table 4.25. Elicitor influence on gluconapoleiferin content in plants and exudates of turnip from hydroponic (mg plant^{-1})

Days	Treatments	Leaves	Secondary roots	Primary roots	Exudates
10	H	ND	$0.1^{a} \pm 0.1$	-	$0.1^{a} \pm 0.1$
	HSA_0	ND	$0.1^{a} \pm 0.1$	-	ND
	HMJ_0	ND	$0.1^{a} \pm 0.0$	-	ND
15	H	$0.1^{a} \pm 0.1$	$0.1^{a} \pm 0.1$	$0.2^{ab} \pm 0.1$	$0.1^{a} \pm 0.0$
	HSA_0	ND	$0.1^{a} \pm 0.1$	$0.2^{a} \pm 0.0$	ND
	HMJ_0	ND	$0.1^{a} \pm 0.0$	$0.4^{b} \pm 0.1$	ND
20	H	$0.3^{a} \pm 0.1$	$0.1^{a} \pm 0.1$	$0.2^{a} \pm 0.1$	$0.1^{a} \pm 0.0$
	HSA_0	$0.3^{a} \pm 0.0$	$0.2^{a} \pm 0.1$	$0.2^{a} \pm 0.1$	$0.1^{a} \pm 0.1$
	HMJ_0	$0.4^{a} \pm 0.1$	ND	$0.2^{a} \pm 0.0$	$0.1^{a} \pm 0.0$
25	H	$0.3^{a} \pm 0.0$	$0.2^{a} \pm 0.1$	$0.2^{a} \pm 0.1$	$0.1^{a} \pm 0.0$
	HSA_0	$0.3^{a} \pm 0.0$	$0.4^{a} \pm 0.1$	$0.2^{a} \pm 0.1$	$0.1^{a} \pm 0.1$
	HMJ_0	$0.4^{a} \pm 0.1$	ND	$0.3^{a} \pm 0.0$	ND
30	H	$0.6^{a} \pm 0.1$	$0.3^{a} \pm 0.0$	$1.0^{b} \pm 0.3$	$0.1^{a} \pm 0.0$
	HSA_0	$0.7^{a} \pm 0.2$	$0.5^{a} \pm 0.2$	$0.6^{a} \pm 0.2$	$0.1^{a} \pm 0.0$
	HMJ_0	$0.7^{a} \pm 0.0$	$0.3^{a} \pm 0.1$	$1.2^{b} \pm 0.2$	ND

H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; HSA_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; HMJ_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + methyl jasmonate applied on the beginning. ND- not determined. The differences are compared for each treatment. Values followed by the same letter are not significantly different.

On the beginning of the experiment **glucobrassicanapin** was found only in H and HMJ_0, but not in HSA_0 leaves (Table 4.26). Until 20th day no glucobrassicanapin was found in secondary roots for all treatments, and in exudates it appeared first on 25th day for H and on 30th for HSA_0 and HMJ_0. MJ application increased the content of glucobrassicanapin in secondary roots and leaves to 1.0 and 0.6 mg plant^{-1} (3 and 5 folds more as for H), respectively. Also Loivamaki *et al*. (2004) reported, that MJ does not influence aliphatic glucosinolates in leaves of oilseed rape, accept for gluconapoleiferin.

Table 4.26. Elicitor influence on glucobrassicanapin content in plants and exudates of turnip from hydroponic (mg plant^{-1})

Days	Treatments	Leaves	Secondary roots	Primary roots	Exudates
10	H	$0.1^{a} \pm 0.1$	ND	-	ND
	HSA_0	ND	ND	-	ND
	HMJ_0	$0.3^{a} \pm 0.1$	ND	-	ND
15	H	$0.1^{a} \pm 0.0$	ND	$0.2^{a} \pm 0.0$	ND
	HSA_0	ND	ND	$0.2^{a} \pm 0.0$	ND
	HMJ_0	$0.4^{b} \pm 0.1$	ND	$0.1^{a} \pm 0.1$	ND
20	H	$0.2^{a} \pm 0.1$	ND	$0.4^{b} \pm 0.1$	ND
	HSA_0	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.1$	$0.2^{a} \pm 0.1$	ND
	HMJ_0	$0.6^{b} \pm 0.2$	$0.2^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	ND
25	H	$0.2^{a} \pm 0.1$	$0.1^{a} \pm 0.1$	$0.7^{a} \pm 0.2$	$0.1^{a} \pm 0.0$
	HSA_0	$0.3^{a} \pm 0.2$	$0.1^{a} \pm 0.1$	$0.8^{a} \pm 0.1$	ND
	HMJ_0	$0.7^{b} \pm 0.1$	$0.2^{a} \pm 0.1$	$0.7^{a} \pm 0.2$	ND
30	H	$0.2^{a} \pm 0.1$	$0.1^{a} \pm 0.0$	$1.1^{a} \pm 0.3$	$0.2^{a} \pm 0.1$
	HSA_0	$0.3^{a} \pm 0.0$	$0.2^{a} \pm 0.1$	$0.8^{a} \pm 0.2$	$0.1^{a} \pm 0.0$
	HMJ_0	$0.6^{b} \pm 0.2$	$0.5^{b} \pm 0.1$	$1.0^{a} \pm 0.2$	$0.1^{a} \pm 0.0$

H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; HSA_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; HMJ_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + methyl jasmonate applied on the beginning. ND- not determined. The differences are compared for each treatment. Values followed by the same letter are not significantly different.

The ratio of butenyl to pentenyl glucosinolates differed between plant organs: for H leaves it was 1.9, for secondary roots 1, for primary roots 1.2, and for exudates 1.1 (Table 4.27). By both of elicitor application, it decreased in for all plant parts as well as by SA in exudates. SA application led to 2.4 and MJ 3.2-folds decrease of butenyl to pentenyl glucosinolates ratio in H leaves, while in primary roots it became 1.5 and 1.7-fold lower, respectively. In secondary roots as well as in exudates MJ did not influence the ratio, while SA decreased it slightly. The changes of the ratio butenyl to pentenyl glucosinolates in leaves and primary roots under elicitor influence could be explained by changing the expression of *Gls-elong* genes in plants under elicitor influence. On the same time, decrease of this ratio in exudates after SA application proves the selectivity of exudative process (Reintanz *et al*., 2001).

Alkenyl glucosinolates gluconapin and glucobrassicanapin undergo hydroxylation and conversion to hydroxyalkenyl glucosinolates progoitrin and gluconapoleiferin. The ratio of hydroxyalkenyl to alkenyl glucosinolates for H leaves, primary roots, and exudates was about 3-fold lower as for H secondary roots (Table 4.27). Treatment with SA increased this ratio for leaves and exudates, but decreased for secondary roots. It looked like SA regulated the ratio to bring it to equal range. In HSA_0 secondary roots and exudates it became nearly the same: 3.3 and 3.5, respectively. MJ application decreased the ratio of hydroxyalkenyl to alkenyl glucosinolates 2.5-folds in leaves and 3-folds in secondary roots, however increased in primary roots and exudates.

Table 4.27. Elicitor influence on correlation between the subclasses of aliphatic glucosinolates in plants and exudates of turnip from hydroponic

butenyls : pentenyls**					
Treatment	Correlation / content	Leaves	Secondary roots	Primary roots	Exudates
H	n : 1	1.9 : 1	1 : 1	1.2 : 1	1.1 : 1
	mg plant^{-1}	1.5 : 0.8	0.7 : 0.7	4.5 : 3.9	1.0 : 0.9*
HSA_0	n : 1	0.8 : 1	0.9 : 1	0.9 : 1	1 : 1
	mg plant^{-1}	0.8 : 1.0	0.6 : 0.7	1.8 : 2.0	0.5 : 0.5*
HMJ_0	n : 1	0.6 : 1	1 : 1	0.7 : 1	1.1 : 1
	mg plant^{-1}	1.1 : 2.0	0.3 : 0.3	1.7 : 2.4	0.8 : 0.7*
Hydroxyalkenyls : alkenyls***					
H	n : 1	1.6 : 1	4.5 : 1	1.4 : 1	1.4 : 1
	mg plant^{-1}	1.4 : 0.9	0.9 : 0.2	3.8 : 2.8	1.1 : 0.8*
HSA_0	n : 1	2.0 : 1	3.3 : 1	1.3 : 1	3.5 : 1
	mg plant^{-1}	1.2 : 0.6	1.0 : 0.3	1.8 : 1.4	0.7 : 0.2*
HMJ_0	n : 1	0.7 : 1	1.5 : 1	2.0 : 1	2.3 : 1
	mg plant^{-1}	1.3 : 1.8	0.3 : 0.2	2.6 : 1.3	0.7 : 0.3*

H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; HSA_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning. The differences are compared for each treatment. Values followed by the same letter are not significantly different.
*Sum of glucosinolates exuded during 30 days; **butenyls - gluconapin + progoitrin; pentenyls – glucobrassicanapin + gluconapoleiferin; ***hydroxyalkenyls - progoitrin + gluconapoleiferin, alkenyls - glucobrassicanapin + gluconapin.

Magrath *et al.* (1994) explained that the hydroxylation of pentenyl glucosinolates is proportional to the hydroxylation of bytenyl glucosinolates and is regulated by the same *Gls-oh* genes. Probably, the elicitors influenced downregulation of *Gls-oh* genes differently in leaves, secondary and primary roots.

4.2.2.2 Aromatic glucosinolate (gluconasturtiin)

The content of gluconasturtiin in leaves varied essentially during the plant growth. For H leaves it increased from 0.1 mg plant^{-1} on 10th day to 0.2 on 20th, then disappeared to the end of the experiment. Also in AD and AS leaves gluconasturtiin was extremely reduced until the end of plant growth (Table 4.28, Attachment, Tables 4.28a and 4.28b).
Application of SA markedly increased the content of gluconasturtiin in leaves, especially during the first half of the experiment. On 10th day the content of glucosinolate in HSA_0 leaves became 3-folds higher then for H (0.3 mg plant^{-1}), in 5 days 5-folds higher (0.5 mg plant^{-1}), on 20th day it decreased, but to the end of the experiment gradually increased until 0.4 mg plant^{-1}. The same essential increment until 15th day was also measured for $ADSA_0$ (0.4 mg plant^{-1}, while for AD 0.1 mg plant^{-1}), the next 5 days decrease, and then gradually increased until the end of experiment. Probably the firs peak on 15th day is the response of plant on stress, simulated by SA application, and the second, slow increment is forming of plant

resistance against this factor. This supposes could be supported by the observation of Kiddle *et al*. (1994) that the response to elicitor influence is the only one event.
The increase in aromatic glucosinolates after SA and MJ application might be explained by SA (Wielanek and Urbanek, 1999) and MJ (Mikkelsen *et al*., 2003) induction of CYP79A2 that converts phenylalanine to aromatic aldoxime.

Table 4.28. Elicitor influence on aromatic glucosinolate content in plants and exudates of turnip from hydroponic (mg plant^{-1})

Date	Treatment	Leaves	Secondary roots	Primary roots	Exudates
10 days	H	$0.1^{a} \pm 0.1$	$0.2^{a} \pm 0.0$		$0.1^{a} \pm 0.0$
	HSA_0	$0.3^{b} \pm 0.0$	$0.5^{b} \pm 0.1$		$0.3^{b} \pm 0.0$
	HMJ_0	$0.1^{a} \pm 0.1$	$0.3^{ab} \pm 0.1$		$0.5^{c} \pm 0.0$
15 days	H	$0.1^{a} \pm 0.1$	$0.3^{a} \pm 0.1$	$1.6^{b} \pm 0.1$	$0.1^{a} \pm 0.0$
	HSA_0	$0.5^{b} \pm 0.1$	$0.6^{b} \pm 0.1$	$1.5^{b} \pm 0.1$	$0.3^{b} \pm 0.1$
	HMJ_0	$0.1^{a} \pm 0.1$	$0.5^{b} \pm 0.1$	$0.8^{a} \pm 0.0$	$0.3^{b} \pm 0.1$
20 days	H	$0.2^{a} \pm 0.2$	$0.9^{a} \pm 0.2$	$1.9^{b} \pm 0.1$	$0.2^{a} \pm 0.1$
	HSA_0	$0.2^{a} \pm 0.1$	$1.6^{b} \pm 0.1$	$2.0^{b} \pm 0.1$	$0.4^{ab} \pm 0.1$
	HMJ_0	$0.2^{a} \pm 0.2$	$1.2^{ab} \pm 0.2$	$0.7^{a} \pm 0.1$	$0.5^{b} \pm 0.1$
25 days	H	$0.1^{a} \pm 0.1$	$1.3^{a} \pm 0.2$	$2.2^{ab} \pm 0.4$	$0.3^{ab} \pm 0.1$
	HSA_0	$0.3^{a} \pm 0.2$	$2.6^{c} \pm 0.3$	$3.5^{b} \pm 0.2$	$0.4^{b} \pm 0.1$
	HMJ_0	$0.3^{a} \pm 0.1$	$1.9^{b} \pm 0.1$	$2.3^{a} \pm 0.3$	$0.3^{a} \pm 0.0$
30 days	H	ND	$1.2^{a} \pm 0.4$	$0.7^{a} \pm 0.2$	$0.4^{a} \pm 0.1$
	HSA_0	$0.4^{a} \pm 0.2$	$2.3^{b} \pm 0.4$	$3.4^{b} \pm 0.3$	$0.4^{a} \pm 0.1$
	HMJ_0	$0.6^{a} \pm 0.2$	$2.8^{b} \pm 0.3$	$3.8^{b} \pm 0.4$	$0.4^{a} \pm 0.1$
Total	H				$1.2^{a} \pm 0.3$*
	HSA_0				$1.8^{ab} \pm 0.4$*
	HMJ_0				$2.1^{a} \pm 0.2$*

H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; HSA_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; HMJ_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + methyl jasmonate applied on the beginning. The differences are compared for each treatment. Values followed by the same letter are not significantly different. ND- not determined. *Sum of glucosinolates exuded during 30 days

It is possible to conclude that SA prevented the disappearance of metabolites as in leaves of plants from hydroponic. Treatment with MJ oppositely did not change the gluconasturtiin content in leaves in the first half of the experiment as compared to untreated variants. But since 25th day the content of glucosinolate for HMJ_0 leaves became 0.3 mg plant^{-1} (3-folds more as for H) and for $ADMJ_0$ 0.4 mg plant^{-1} (4-folds more as for AD); on 30th day it reached 0.6 and 0.3 mg plant^{-1}, respectively (Table 4.28, Attachment, Tables 4.28a and 4.28b) .
For H and AS the content of gluconasturtiin in secondary roots of turnip increased gradually to 1.2 and 1.5 mg plant^{-1} on 30th day. For AD it increased until 1.3 mg plant^{-1} on 25th day, and then decreased to 0.8 mg plant^{-1} on 30th day.
Application of both of elicitors increased the content of gluconasturtiin in secondary roots. Already 10 days after the treatment, the differences in gluconasturiin content were measured. For HSA_0 it reached 0.5 and for HMJ_0 0.3 mg plant^{-1}, while for H 0.1 mg plant^{-1}. The fastest

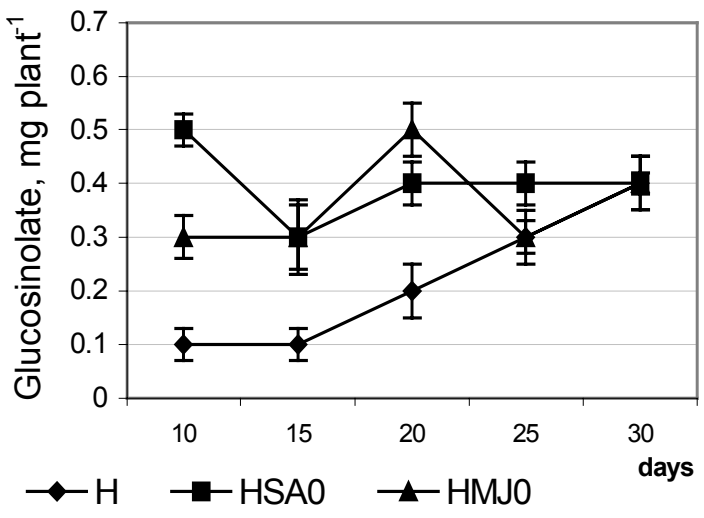
enhancement of gluconasturiin content in secondary roots of SA treated plants was observed between 15th-20th days: for HSA_0 by 1.0 mg plant^{-1} (from 0.6 until 1.6), for $ADSA_0$ by 1.1, and for $ASSA_0$ by 0.5 mg plant^{-1}. On 30th day the HSA_0 secondary roots 2-folds more gluconasturtiin then H, $ADSA_0$ 2.7-folds more then AD, and $ASSA_0$ 1.7-fold more then AS.

For MJ treatment the content of gluconasturtiin in secondary roots increased progressively with plant growth. At the end of experiment HMJ_0 secondary roots had 2.8 mg plant^{-1} of gluconasturtiin (2.3-folds more then H) and $ADSA_0$ 1.9 mg plant^{-1} (2.4-folds more then AD).

The content of gluconasturtiin in H primary roots until 25th day gradually increased until 2.2 mg plant^{-1}, but then decreased to 0.7 mg plant^{-1}. In aeroponics this decrease was not measured. This could be explained by the fact that the plants from hydroponic developed faster as these in aeroponics (see 4.2.3).

Treatment with SA increased the content of gluconasturtiin in primary roots right after the application. This effect kept until the end of experiment, when the HSA_0 primary roots accumulated 3.4 mg plant^{-1} (4.8-folds more then H). Until 20th day MJ treatment caused the decrease of gluconasturtiin content in primary roots: for HMJ_0 on 15th day it achieved 0.8 and on 20th day 0.7 mg plant^{-1} (respectively 0.7 and 1.2.mg plant^{-1} lower as for H), and on 30th day it reached 3.8 mg plant^{-1} (0.4 mg plant^{-1} higher as in H). The decrease of glucosinolate content in primary roots under MJ treatment could be explained by the suppression of plant growth and development under the influence of elicitor (see 4.2.3).

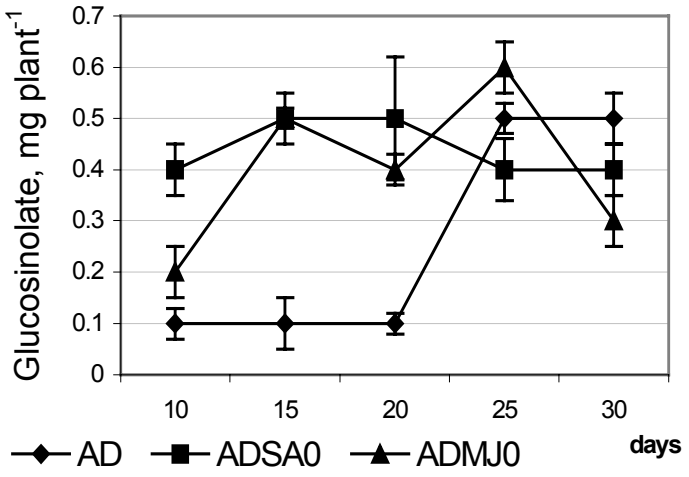
CYP79B2 converts tryptophan to aromatic aldoxime and has the highest expression level in leaves, but not in roots (Glombitza *et al.*, 2004). Increases of aromatic glucosinolate in leaves for MJ treatment as compared to the non-treated variant and the absence of an effect after SA application could be explained by the influence that MJ on CYP79B2 in contrast to SA (Wasternack and Parthier, 1997; Mikkelsen *et al*., 2000).



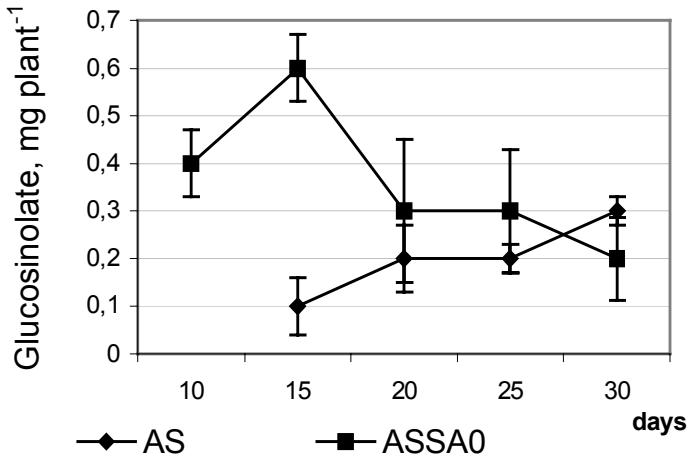
H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; HSA_0 - two times concentrated Hoagland solution + two times increased sulfur + salicylic acid; HMJ_0 - two times concentrated Hoagland solution + two times increased sulfur + methyl jasmonate applied on the beginning

The content of gluconasturtiin gradually increased in exudates of all non-elicited treatments: from 0.1 mg plant^{-1} for H, AD, and AS on 10th day to 0.4, 0.5, and 0.3 mg plant^{-1} on 30th day, respectively (Figure 4.11).

SA and MJ increased the content of aromatic glucosinolate right after the application, and the maximal effect was observed on 20th day in hydroponics (0.5 mg plant^{-1} of gluconasturtiin for HMJ_0 and 0.4 mg plant^{-1} for HSA_0), and on 15th day in aeroponics (0.5 mg plant^{-1} of gluconasturtiin for $ADMJ_0$, and 0.5 and 0.6 mg plant^{-1} for $ADSA_0$ and $ASSA_0$).



AD – aeroponic with defensor, two times concentrated Hoagland solution + two times increased sulfur; $ADSA_0$ - two times concentrated Hoagland solution + two times increased sulfur + salicylic acid; $ADMJ_0$ - two times concentrated Hoagland solution + two times increased sulfur + methyl jasmonate applied on the beginning



AS – aeroponic with sprayer, two times concentrated Hoagland solution + two times increased sulfur; $ADSA_0$ - two times concentrated Hoagland solution + two times increased sulfur + salicylic acid

Figure 4.11. Elicitor influence on kinetic of aromatic glucosinolates in exudates of turnip

However, the increment of gluconasturtiin content under the elicitor influence was diminished with time and at the end of experiment it became the same (for HSA_0 and HMJ_0) or lower (for $ADSA_0$, $ASSA_0$, and $ADMJ_0$) value as for untreated variants.

In general, SA increased the amount of exuded gluconasturtiin for HSA_0 to 1.5-fold more then for H (1.8 mg $plant^{-1}$), for $ADSA_0$ as well as for $ASSA_0$1.4-fold more then for AD and AS (3.7 and 2.5 mg $plant^{-1}$, respectively) (Table 4.28, Attachment, Table 4.28a and 4.28b).

MJ application led to enhance the gluconasturtiin content for HMJ_0 exudates to 2.1 mg $plant^{-1}$ (1.8-fold more then for H) and for $ADSA_0$ to 4.2 mg $plant^{-1}$ (1.6-fold more then for AD (Table 4.28, Attachment, Table 4.28a and 4.28b).

Wielanek and Urbanek (1999) also reported about increase the yield of aromatic glucosinolates by about 40-70% under the influence of MJ. In *Brassica napus* biosynthesis of gluconasturtiin is specifically induced by salicylic acid (SA), a signaling compound involved in many plant physiological processes (Kiddle *et al.*, 1994).

Gluconasturtiin showed the greatest increase in concentration, with only minor increases in other glucosinolates in developing leaves (Kiddle *et al*., 1994).

However, Doughty *et al*. (1995) found, that in *Brassica napus* MJ induced only indole glucosinolate synthesis, whereas aliphatic and aromatic glucosinolates were unaffected. The induction of gluconasturtiin content in turnip and its exudates may be related to the fact that

this is the only aromatic glucosinolate produced by this plant, similar as it was explained by Wielanek and Urbanek (1999) for *Tropaeolus majus* and glucotropaeolin.

4.2.2.3 Indole glucosinolates in plants and exudates

In this experiment treatment with both of elicitors increased the content of indole glucosinolates in all plant parts, in plants from all systems and this effect continued until the end of experiment.
The content of indole glucosinolates in H leaves was 0.6 mg plant^{-1} on 10th day and increased progressive until 1.3 mg plant^{-1} on 30th day. For both aeroponics it grew gradually until 1.4 and 1.6 mg plant^{-1} (Table 4.29, Attachment, Tables 4.29a and 4.29b).
Treatment with MJ and to lesser extend SA increased markedly indole glucosinolate content in leaves in all three systems. On 10th day HSA_0 and HMJ_0 leaves had 2.7 and 2.5-folds more indole glucosinolate then H leaves. On 15th day it became for HSA_0 and HMJ_0 leaves 2 and 9.3-folds more then for H. On 20th day the difference became 2.1-folds for HSA_0 and 4.7-folds for HMJ_0 as compared to H. The effect of glucosinolate enhance under the elicitor application decreased to the end of the experiment. On 25th days HSA_0 leaves had 1.8-fold more glucosinolates then for H, and on 30th day 1.5-fold more, while HMJ_0 leaves 3.0 and 2.1-folds more then for H.

Table 4.29. Elicitor influence on indole glucosinolate content in plants and exudates of turnip from hydroponic (mg plant^{-1})

Date	Treatment	Leaves	Secondary roots	Primary roots	exudates
10 days	H	$0.6^{a} \pm 0.1$	$0.2^{a} \pm 0.0$		$0.2^{a} \pm 0.0$
	HSA_0	$1.6^{b} \pm 0.1$	$0.6^{b} \pm 0.1$		$1.2^{b} \pm 0.0$
	HMJ_0	$1.5^{b} \pm 0.2$	$0.7^{b} \pm 0.1$		$1.3^{b} \pm 0.1$
15 days	H	$0.3^{a} \pm 0.0$	$0.4^{a} \pm 0.1$	$0.8^{c} \pm 0.0$	$0.2^{a} \pm 0.1$
	HSA_0	$1.7^{b} \pm 0.1$	$0.7^{b} \pm 0.2$	$0.6^{b} \pm 0.1$	$0.9^{b} \pm 0.1$
	HMJ_0	$2.8^{c} \pm 0.3$	$0.9^{b} \pm 0.2$	$0.1^{a} \pm 0.0$	$1.0^{b} \pm 0.0$
20 days	H	$0.7^{a} \pm 0.1$	$1.8^{a} \pm 0.1$	$1.3^{b} \pm 0.1$	$0.3^{a} \pm 0.0$
	HSA_0	$1.5^{b} \pm 0.1$	$2.4^{b} \pm 0.1$	$1.0^{a} \pm 0.1$	$1.2^{c} \pm 0.0$
	HMJ_0	$3.3^{c} \pm 0.3$	$3.5^{c} \pm 0.1$	$0.9^{a} \pm 0.0$	$0.8^{b} \pm 0.1$
25 days	H	$1.1^{a} \pm 0.0$	$2.2^{a} \pm 0.1$	$1.9^{a} \pm 0.3$	$0.4^{a} \pm 0.1$
	HSA_0	$2.0^{b} \pm 0.1$	$3.6^{b} \pm 0.2$	$1.8^{a} \pm 0.2$	$0.8^{b} \pm 0.1$
	HMJ_0	$3.3^{c} \pm 0.1$	$4.7^{c} \pm 0.1$	$1.6^{a} \pm 0.2$	$0.6^{ab} \pm 0.1$
30 days	H	$1.3^{a} \pm 0.1$	$2.3^{a} \pm 0.2$	$1.4^{a} \pm 0.2$	$0.5^{a} \pm 0.1$
	HSA_0	$1.9^{b} \pm 0.1$	$6.3^{c} \pm 0.5$	$1.2^{a} \pm 0.2$	$0.8^{b} \pm 0.0$
	HMJ_0	$2.8^{c} \pm 0.2$	$3.9^{b} \pm 0.2$	$1.1^{a} \pm 0.3$	$0.8^{b} \pm 0.1$
Total	H				$1.6^{a} \pm 0.3$*
	HSA_0				$5.0^{b} \pm 0.5$*
	HMJ_0				$5.6^{b} \pm 0.5$*

H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; HSA_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; HMJ_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + methyl jasmonate applied on the beginning. The differences are compared for each treatment. Values followed by the same letter are not significantly different. *Sum of glucosinolates exuded during 30 days

Mikkelsen *et al.* (2000) pointed out that tryptophan-derived indole glucosinolates are more responsive to induction than aliphatic glucosinolates. Different indole glucosinolates methoxylating enzymes are influenced by SA and MJ, whereas aliphatic glucosinolates appear to be primarily genetically and not environmentally controlled. The accumulation of indole glucosinolates following JA treatment and infection with certain pathogens may be regulated at the transcriptional level. It has been shown by Mikkelsen *et al*. (2001) on the example of *Arabidopsis thaliana*, Bodnaryk (1994) on the example of *Brassica napus and Brassica juncea* that MJ affected indole, but not aliphatic or aromatic glucosinolates, however it was mentioned, that the influence of elicitors on individual indole glucosinolates differ between the plants.

Kiddle *et al.* (1994) also showed that treatment of plants with SA and JA resulted in increased amounts of glucosinolates, although differences in the response were observed between the two treatments, JA induced mainly indole glucosinolates in leaves, whereas SA induced indole glucosinolates also in roots of *Brassica nigra* and *Brassica oleracea*.

During the experiment the content of indole glucosinolates in H secondary roots increased from 0.2 mg plant^{-1} on 10th day until 2.3 mg plant^{-1} on 30th day. In general, until 25th day MJ increased the content of indole glucosinolates more essential as SA in secondary roots of plants from hydroponic as well as aeroponic with defensor. On 20th day HMJ_0 secondary roots contented 4.7 mg plant^{-1} of indole glucosinolates, while HSA_0 3.6 mg plant^{-1} (4.8 and 2.1-folds more as H secondary roots). However, during the last 5 days of the experiment this changed: HSA_0 secondary roots accumulated 6.3 mg plant^{-1} of indole glucosinolates, while in HMJ_0 it decreased until 3.9 mg plant^{-1}. Indole glucosinolate content in $ADSA_0$ secondary roots reached 7.0 mg plant^{-1} and in $ADMJ_0$ secondary roots 4.6 mg plant^{-1}(2.2 and 1.5-fold more then for AD). Decrease of indole glucosinolate content on 30th day in secondary roots after MJ application and absence of this effect after SA treatment is probably possible to explain by longer but slower reaction of plants on SA treatment as on those caused by MJ or by more intensive exudation for MJ as for SA treated plants.

The obtained data for the induction by MJ indole glucosinolate accumulation are in agreement with these of Kiddle *et al.* (1994) and Mikkelsen *et al.* (2003). However, in our experiments, SA stimulated indole glucosinolates in secondary roots more than MJ.

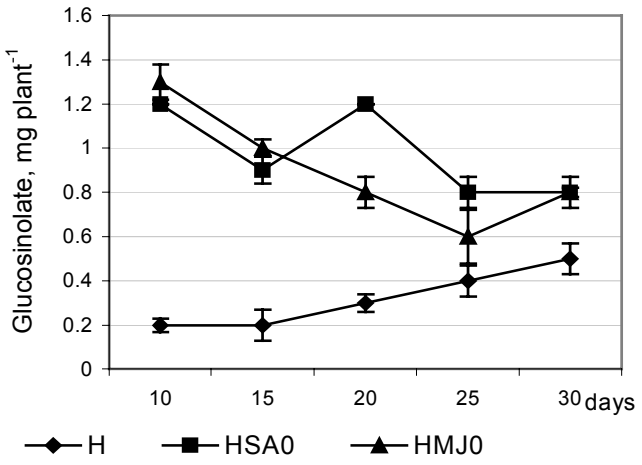
The dynamics of indole glucosinolates in primary roots of turnip from H and those exposed to elicitor treatment had the same tendencies. Glucosinolate content in all systems increased until the 25th day of the experiment and then decreased during the last 5 days for H, but not for AS and AD.

On 15th day after elicitor application the content of indole glucosinolates in H primary roots was 0.8, while in HSA_0 0.6, and HMJ_0 0.1 mg plant^{-1}. The content of glucosinolates reached the maximum on 25th day and for H was 1.9 mg plant^{-1}, for HSA_0 1.8, and for HMJ_0 1.6 mg plant^{-1}. On 30th day it decreased to 1.4, 1.2, and 1.1 mg plant^{-1} for H, HSA_0 and HMJ_0, respectively.

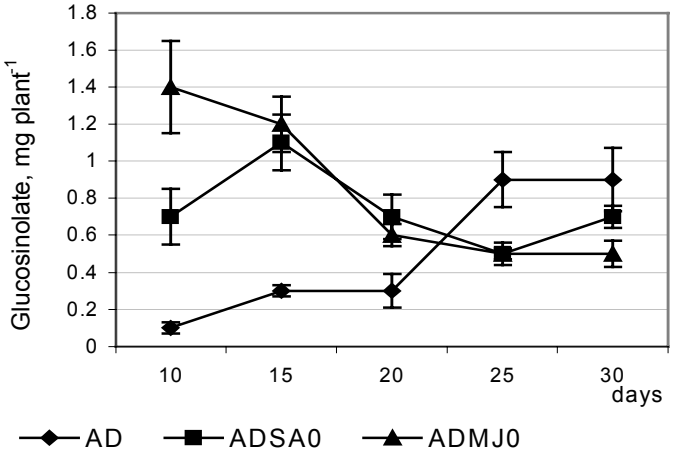
Glombitza *et al.* (2003) explained the stimulation of indole glucosinolates under SA and MJ influence by increase expression ratios of genes, responsible for converting indole aldoximes to indolethiohydroximates. CYP83B1 can also be expressed equally in leaves and roots of plants. This explains the increase in indole glucosinolates in all plant parts and exudates after MJ application as well as in secondary and primary roots and exudates after SA application.

Indole glucosinolates have been shown to accumulate systemically in plants treated with JA (Doughty *et al*., 1995). This suggests a role for indole glucosinolates in wound response as was found by Brader *et al.* (2001). This group showed that MJ triggered induction of the tryptophan biosynthesis pathway and tryptophan-oxidizing genes involved in tryptophan

biosynthesis pathway which resulted in increased indole glucosinolate content. The accumulation of indole glucosinolates following JA and MJ treatments as well as infection with certain pathogens could be regulated at the transcriptional level (Mikkelsen *et al*., 2003). Two cytochrome P-450 monooxygenases that are encoded by the tryptophan-metabolizing genes CYP79B2 and CYP79B3 are responsible for the specific conversion of tryptophan to indole-3-acetaldoxime (Halkier and Du, 1997). CYP79B2 and CYP79B3, which regulate indole glucosinolate synthesis, are induced by MJ treatment, and CYP79B2 is also induced by wounding as well as infection with *Pseudomonas syringae* (Brader *et al.,* 2001).



H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; HSA_0 - two times concentrated Hoagland solution + two times increased sulfur + salicylic acid; HMJ_0 - two times concentrated Hoagland solution + two times increased sulfur + methyl jasmonate applied on the beginning



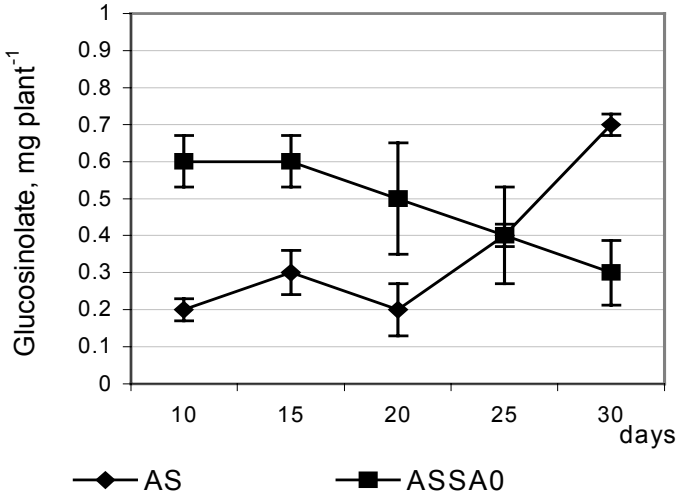
AD – aeroponic with defensor, two times concentrated Hoagland solution + two times increased sulfur; $ADSA_0$ - two times concentrated Hoagland solution + two times increased sulfur + salicylic acid; $ADMJ_0$ - two times concentrated Hoagland solution + two times increased sulfur + methyl jasmonate applied on the beginning

Zeng *et al.* (2003) reported that aromatic and indole glucosinolates are responsible for interaction between *Brassicacea*e and fungi in the contrast to aliphatic glucosinolates and hence SA and MJ mimicked fungi and herbivores attacks resulted in increase of indole glucosinolates.

Indole glucosinolate content in H exudates increased from 0.2 mg $plant^{-1}$ on 10^{th} day until 0.5 mg $plant^{-1}$ on 30^{th} day, while for AD it reached 0.9 mg $plant^{-1}$ and for AS 0.7 mg $plant^{-1}$ (Figure 4.12).

After the application of both elicitor the content of indole glucosinolates increased essentially and on 10^{th} day it reached 1.2 for HSA_0 and 1.3 mg $plant^{-1}$ for HMJ_0 (6 and 6.5-folds more then for H). At this time for $ADSA_0$ it reached 0.7 and for $ADMJ_0$ 1.4 mg $plant^{-1}$ (7 and 14-folds more then for AD). Then the intensity of rhizosecretion of indole glucosinolates decreased. This is in agreement with data received by Bodnaryk *et al.* (1994) and Doughty *et al.* (1995).

The maximal amount of indole glucosinolates was exuded by MJ treated H and AD plants on 10^{th} day. However, SA treatment caused maximal increase of indole glucosinolates in H exudates on 20^{th} and in AD as well as AS exudates on 15^{th} day after treatment.



AS – aeroponic with sprayer, two times concentrated Hoagland solution + two times increased sulfur; $ADSA_0$ - two times concentrated Hoagland solution + two times increased sulfur + salicylic acid

Figure 4.12. Influence of elicitors on kinetic of indole glucosinolates in exudates of turnip

On 30th day in the content of indole glucosinolates became 0.8 mg plant^{-1} in HSA_0 and HMJ_0, which was still more then for H (1.6-fold). However, plants from $ADSA_0$ and $ADMJ_0$ exuded 0.7 and 0.5 mg plant^{-1} of indole glucosinolates, which was 1.8 and 1.3-fold lower as for AD. $ASSA_0$ and $ADMJ_0$ plants exuded 0.7 and 0.5 mg plant^{-1} of indole glucosinolates (1.8 and 1.3-fold lower as for AD).

In general, during 30 days H plants exuded 1.6 mg plant^{-1} of indole glucosinolates, HSA_0 5.0 mg plant^{-1} (2.6-folds more), and HMJ_0 5.9 mg plant^{-1} (3.1-folds more) (Table 4.29).

Individual indole glucosinolates

Elicitors influenced differently on individual indole glucosinolate. SA increased the content of glucobrassicin, 4-methoxy-glucobrassicin, and neoglucobrassicin in plants more essential than MJ.

In this experiment both of elicitors increasedthe content of **glucobrassicin** in plants and exudates of turnip (Table 4.30). On the beginning of the experiment it was measured only in H leaves, but not in roots or exudates. Already 10 days after application SA and MJ increased its content in leaves 5 and 7-folds, respectively. However, this difference was reduced from the 25th day, and on 30th day glucobrassicin content in SA treated leaves was the same as for H leaves. Glucobrassicin content in secondary roots started to increase under elicitor treatment on 20th day of the experiment. SA stimulated mostly increase of glucobrassicin in secondary roots (7-folds more as compared to H), while MJ caused 3-folds increase. Both of elicitors did not influence glucobrassicin content in primary roots. As it is possible to conclude, both elicitors caused similar increase of glucobrassicin content in plants, but SA mostly in secondary roots, while MJ in leaves. We could suppose that this could be connected more with glucosinolate transport from leaves to roots as also with differences in induction of glucobrassicin synthesis. On 10th day no glucobrassicin was measured for H exudates, but 0.3 mg plant^{-1} appeared for HSA_0 and 0.2 mg plant^{-1} for HMJ_0. Later there was no clear difference between treated and non-treated variants.

The influence of elicitors on individual indole glucosinolates differ between the plants. Bodnaryk (1994) represented the results that treatment of the cotyledons of *Brassica napus* with JA caused 6-fold increase of glucobrassicin, but caused no change of 4-hydroxy-glucobrassicin. The same treatment of *Brassica rapa* caused no change in the concentration of glucobrassicin, but increased nine fold 4-hydroxy-glucobrassicin. Treatment of *Brassica*

juncea caused the concentration of both of these indole glucosinolates to increase by two fold. treatment of cotyledons of *Brassica napus* with SA had no detectable effect on glucobrassicin content (Bodnaryk,1994). This observation is in agreement with our data for turnip leaves, but not for other plant parts.

Table 4.30. Kinetic of elicitor influence on glucobrassicin content in plants and exudates of turnip from hydroponic (mg plant^{-1})

Days	Treatments	Leaves	Secondary roots	Primary roots	Exudates
10	H	$0.1^{a} \pm 0.0$	ND	-	ND
	HSA_0	$0.5^{b} \pm 0.1$	$0.2^{a} \pm 0.0$	-	$0.3^{a} \pm 0.1$
	HMJ_0	$0.7^{b} \pm 0.2$	$0.2^{a} \pm 0.1$	-	$0.2^{a} \pm 0.0$
15	H	$0.2^{a} \pm 0.0$	$0.2^{a} \pm 0.1$	$0.2^{a} \pm 0.0$	$0.2^{a} \pm 0.1$
	HSA_0	$0.4^{b} \pm 0.1$	$0.2^{a} \pm 0.0$	$0.2^{a} \pm 0.1$	$0.2^{a} \pm 0.0$
	HMJ_0	$0.8^{b} \pm 0.3$	$0.2^{a} \pm 0.1$	ND	$0.1^{a} \pm 0.1$
20	H	$0.2^{a} \pm 0.1$	$0.3^{a} \pm 0.1$	$0.3^{a} \pm 0.1$	$0.1^{a} \pm 0.1$
	HSA_0	$0.4^{b} \pm 0.0$	$0.8^{b} \pm 0.1$	$0.3^{a} \pm 0.1$	$0.1^{a} \pm 0.0$
	HMJ_0	$1.2^{c} \pm 0.2$	$1.0^{c} \pm 0.0$	$0.3^{a} \pm 0.0$	$0.1^{a} \pm 0.0$
25	H	$0.3^{a} \pm 0.1$	$0.2^{a} \pm 0.1$	$0.4^{a} \pm 0.2$	$0.1^{a} \pm 0.0$
	HSA_0	$0.4^{ab} \pm 0.2$	$1.5^{c} \pm 0.1$	$0.3^{a} \pm 0.1$	$0.1^{a} \pm 0.1$
	HMJ_0	$0.7^{b} \pm 0.1$	$1.1^{b} \pm 0.1$	$0.5^{a} \pm 0.2$	$0.1^{a} \pm 0.0$
30	H	$0.1^{a} \pm 0.1$	$0.3^{a} \pm 0.0$	$0.4^{a} \pm 0.2$	$0.1^{a} \pm 0.1$
	HSA_0	$0.1^{a} \pm 0.0$	$2.2^{c} \pm 0.4$	$0.3^{a} \pm 0.1$	$0.2^{a} \pm 0.0$
	HMJ_0	$0.5^{b} \pm 0.2$	$0.9^{b} \pm 0.3$	$0.5^{a} \pm 0.1$	$0.1^{a} \pm 0.0$
Total	H				$0.5^{a} \pm 0.2$
	HSA_0				$0.9^{a} \pm 0.3$
	HMJ_0				$0.6^{a} \pm 0.1$

H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; HSA_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; HMJ_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + methyl jasmonate applied on the beginning. The differences are compared for each treatment. Values followed by the same letter are not significantly different. ND- not determined. *Sum of glucosinolates exuded during 30 days.

Kliebestein *et al.* (2002) reported, that MJ induced elevation of glucobrassicin level for *Arabidopsis thaliana*, while this glucosinolate was not influenced by SA.

N**eoglucobrassicin** was the most predominant indole glucosinolate. It was also the most influenced by the two elicitors in secondary roots and exudates, but not in primary roots (Table 4.30). On 30th day in aeroponics production of neoglucobrassicin under the influence of SA and MJ was two times as high as for H (Attachment, Tables 4.30a and 4.30b).

Neoglucobrassicin content in leaves was strongly induced by the both of elicitors until the 25th day and became 3 and 6 fold higher for HSA_0 and HMJ_0, respectively. The next 5 days it increased for H by 0.3 mg plant^{-1}, while for HMJ_0 it decreased by 0.8 mg plant^{-1}.

Table 4.31. Elicitor influence on neoglucobrassicin content in plants and exudates of turnip from hydroponic (mg plant^{-1})

Days	Treatments	Leaves	Secondary roots	Primary roots	Exudates
10	H	$0.3^{a} \pm 0.1$	$0.2^{a} \pm 0.1$	-	$0.1^{a} \pm 0.1$
	HSA_0	$0.7^{b} \pm 0.2$	$0.4^{b} \pm 0.0$	-	$0.6^{b} \pm 0.2$
	HMJ_0	$0.5^{ab} \pm 0.1$	$0.5^{b} \pm 0.1$	-	$0.4^{b} \pm 0.1$
15	H	$0.3^{a} \pm 0.1$	$0.2^{a} \pm 0.1$	$0.4^{a} \pm 0.1$	$0.1^{a} \pm 0.1$
	HSA_0	$0.6^{b} \pm 0.1$	$0.5^{b} \pm 0.1$	$0.3^{a} \pm 0.0$	$0.8^{c} \pm 0.2$
	HMJ_0	$1.1^{c} \pm 0.3$	$0.7^{b} \pm 0.1$	ND	$0.5^{b} \pm 0.0$
20	H	$0.3^{a} \pm 0.1$	$1.0^{a} \pm 0.1$	$0.4^{a} \pm 0.2$	$0.2^{a} \pm 0.0$
	HSA_0	$0.8^{b} \pm 0.2$	$1.2^{b} \pm 0.2$	$0.4^{a} \pm 0.1$	$0.9^{c} \pm 0.2$
	HMJ_0	$1.4^{c} \pm 0.3$	$2.2^{c} \pm 0.4$	$0.2^{a} \pm 0.1$	$0.4^{b} \pm 0.1$
25	H	$0.3^{a} \pm 0.1$	$1.6^{a} \pm 0.2$	$0.6^{b} \pm 0.1$	$0.1^{a} \pm 0.0$
	HSA_0	$0.9^{b} \pm 0.2$	$1.6^{a} \pm 0.3$	$0.4^{ab} \pm 0.1$	$0.6^{b} \pm 0.2$
	HMJ_0	$1.8^{c} \pm 0.4$	$2.8^{b} \pm 0.4$	$0.3^{a} \pm 0.1$	$0.5^{b} \pm 0.2$
30	H	$0.5^{a} \pm 0.1$	$1.7^{a} \pm 0.2$	$0.7^{b} \pm 0.1$	$0.2^{a} \pm 0.1$
	HSA_0	$0.8^{ab} \pm 0.2$	$2.3^{ab} \pm 0.4$	$0.4^{a} \pm 0.1$	$0.4^{b} \pm 0.0$
	HMJ_0	$1.0^{b} \pm 0.2$	$2.6^{b} \pm 0.3$	$0.4^{a} \pm 0.0$	$0.5^{b} \pm 0.1$
Total	H				$0.7^{a} \pm 0.2$
	HSA_0				$3.0^{c} \pm 0.3$
	HMJ_0				$2.3^{b} \pm 0.2$

H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; HSA_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; HMJ_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + methyl jasmonate applied on the beginning. The differences are compared for each treatment. Values followed by the same letter are not significantly different. ND- not determined. *Sum of glucosinolates exuded during 30 days.

Mikkelsen *et al*. (2003) found, that treatment with JA induce the methoxylation in 1 position of glucobrassicin and lead to accumulation of neoglucobrassicin, while this induction was repressed by 1-aminocyclopropane-1-carboxilate (ethylene precursor), which influencing was similar to isonicotinic acid, a functional homologue of SA. This can explain especially high increase of neoglucobrassicin content under MJ influence and lower increase of this glucosinolate under the SA treatment.

From 20th day the highest content of neoglucobrassicin in plant parts was measured for secondary roots. H leaves on 30th day had 0.5 mg plant^{-1} of neoglucobrassicin, primary roots 0.7 and secondary roots 1.7 mg plant^{-1} (Table 4.31). In HSA_0 and HMJ_0 secondary roots its content increased to 2.3 and 2.6 mg plant^{-1} on 30th day, which was 1.3 and 1.5 times more as for H. But the highest content of neoglucobrassicin after MJ application was measured for leaves and secondary roots on 25th day 1.8 and 2.8 mg plant^{-1} (0.8 and 0.2 mg plant^{-1} more as 5 days later. This may be connected with transporting of neoglucobrassicin forom leaves into secondary roots and then exuding. SA application led to increase the neoglucobrassicin content in leaves, however it did not cause that strong increase of neoglucobrassicin in secondary roots until 25th day of the experiment. This glucosinolate was mostly exuded: 0.7 mg plant^{-1} during 30 days for H plants.

During first 10 days of the experiment **4-hydroxy-glucobrassicin** was measured only in leaves of treated as well as untreated with elicitor plants (Table 4.32). During the next 5 days small quantities of this glucosinolate have been also found in primary roots, moreover the simulative effect of SA and MJ was to observe for leaves: 3 and 4.5 fold more as for H leaves, respectively.

Table 4.32. Elicitor influence on 4-hydroxy-glucobrassicin content in plants and exudates of turnip from hydroponic (mg plant^{-1})

Days	Treatments	Leaves	Secondary roots	Primary roots	Exudates
10	H	$0.2^{ab} \pm 0.1$	ND	-	ND
	HSA_0	$0.4^{b} \pm 0.1$	ND	-	ND
	HMJ_0	$0.2^{a} \pm 0.0$	ND	-	ND
15	H	$0.2^{a} \pm 0.0$	ND	$0.2^{a} \pm 0.0$	ND
	HSA_0	$0.6^{b} \pm 0.1$	ND	$0.1^{ab} \pm 0.1$	ND
	HMJ_0	$0.9^{c} \pm 0.3$	ND	$0.1^{a} \pm 0.0$	ND
20	H	$0.2^{a} \pm 0.1$	$0.2^{ab} \pm 0.1$	$0.3^{b} \pm 0.0$	ND
	HSA_0	$0.4^{b} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.3^{ab} \pm 0.1$	ND
	HMJ_0	$0.5^{b} \pm 0.2$	$0.2^{b} \pm 0.0$	$0.2^{a} \pm 0.0$	ND
25	H	$0.3^{a} \pm 0.1$	$0.1^{a} \pm 0.1$	$0.4^{a} \pm 0.2$	ND
	HSA_0	$0.4^{ab} \pm 0.2$	$0.3^{b} \pm 0.0$	$0.4^{a} \pm 0.1$	$0.1^{a} \pm 0.0$
	HMJ_0	$0.7^{b} \pm 0.1$	$0.3^{ab} \pm 0.1$	$0.6^{a} \pm 0.2$	ND
30	H	$0.7^{a} \pm 0.1$	$0.1^{a} \pm 0.0$	$0.4^{a} \pm 0.0$	$0.1^{a} \pm 0.1$
	HSA_0	$0.9^{ab} \pm 0.3$	$0.7^{b} \pm 0.1$	$0.5^{a} \pm 0.1$	$0.1^{a} \pm 0.0$
	HMJ_0	$1.2^{b} \pm 0.3$	$0.1^{a} \pm 0.0$	$0.4^{a} \pm 0.1$	$0.1^{a} \pm 0.0$
Total	H				$0.1^{a} \pm 0.0$
	HSA_0				$0.2^{a} \pm 0.1$
	HMJ_0				$0.1^{a} \pm 0.0$

H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; HSA_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; HMJ_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + methyl jasmonate applied on the beginning. The differences are compared for each treatment. Values followed by the same letter are not significantly different. ND- not determined. *Sum of glucosinolates exuded during 30 days.

On 30th day MJ induced the content of 4-hydroxy-glucobrassicin in leaves, which is also in accordance with Loivamaki *et al*. (2004), however in secondary and primary roots its content was not influenced. In the contrast to MJ, SA caused smaller increase in 4-hydroxy-glucobrassicin content in leaves, but stronger in secondary root and primary roots, however it may be explained by the transportation of glucosinolate from leaves into other parts. Plants from all systems exuded traces of 4-hydroxy-glucobrassicin and only during the 5 last days of the experiment.

No **4-methoxy-glucobrassicin** was detected in all parts of plants until 20th day of experiment (Table 4.33). The next 5 days it was measured in roots and exudates, but not in leaves. The effect of SA became obvious on 30th day, when it caused 5.5 folds increase of glucosinolate in secondary roots. This could be caused by the induction of glucosinolate synthesis in secondary roots, but not by its transportation into secondary roots, because its synthesis was not matched in other plant parts. Both of elicitors induced the synthesis of 4-methoxy-

glucobrassicin in leaves; however, its content there reached only 0.1 mg plant^{-1}. MJ influenced to much lesser extend as SA on 4-methoxy-glucobrassicin content in all parts of plants.
Doughty *et al*. (1995) determined that JA and MJ at small doses induced large, sustained, systemic increases (up to 20-fold) in the concentration of specific indole glucosinolates in cotyledons and leaves of *Brassica napus* (glucobrassicin) and *Brassica rapa* (1-methoxy-glucobrassicin) which comprised 90 % of the total glucosinolates in treated leaves. Also Mikkelsen *at al*. (2003) have shown that treatment with 2,6-dichloronicotinic acid (functional homologue of SA) resulted in accumulation of 4-methoxy-glucobrassicin, and this induction was repressed by MJ.

Table 4.33. Elicitor influence on 4-methoxy-glucobrassicin content in plants and exudates of turnip from hydroponic (mg plant^{-1})

Days	Treatments	Leaves	Secondary roots	Primary roots	Exudates
10	H	ND	ND	-	ND
	HSA_0	ND	ND	-	ND
	HMJ_0	ND	ND	-	ND
15	H	ND	ND	ND	ND
	HSA_0	ND	ND	ND	ND
	HMJ_0	ND	ND	ND	ND
20	H	ND	$0.2^{ab} \pm 0.1$	$0.2^{a} \pm 0.1$	ND
	HSA_0	ND	$0.3^{b} \pm 0.0$	$0.2^{a} \pm 0.1$	$0.2^{a} \pm 0.1$
	HMJ_0	ND	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.2^{a} \pm 0.1$
25	H	ND	$0.2^{a} \pm 0.1$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$
	HSA_0	ND	$0.2^{a} \pm 0.1$	$0.2^{a} \pm 0.1$	$0.1^{a} \pm 0.0$
	HMJ_0	ND	$0.3^{a} \pm 0.1$	$0.1^{a} \pm 0.0$	$0.2^{a} \pm 0.0$
30	H	ND	$0.2^{a} \pm 0.0$	ND	$0.1^{a} \pm 0.1$
	HSA_0	$0.1^{a} \pm 0.0$	$1.1^{c} \pm 0.1$	$0.3^{b} \pm 0.1$	$0.1^{a} \pm 0.0$
	HMJ_0	$0.1^{a} \pm 0.0$	$0.3^{b} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.1$
Total	H				$0.2^{a} \pm 0.1$
	HSA_0				$0.4^{a} \pm 0.1$
	HMJ_0				$0.5^{a} \pm 0.2$

H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; HSA_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; HMJ_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + methyl jasmonate applied on the beginning. The differences are compared for each treatment. Values followed by the same letter are not significantly different. ND- not determined. *Sum of glucosinolates exuded during 30 days.

The ratio of aliphatic to indole glucosinolates differed between parts of plant and changed under elicitor treatment as well as with plant growth (Table 4.34). On 10th day the highest ratio was measured for leaves: 1.3 for H, while for HSA_0 and HMJ_0 leaves it was 2.6 and 1.5-fold lower, respectively. For non-treated leaves up to 15th day it increased strongly until 4, and gradually decreased to the end of experiment, but for HSA_0 as well as HMJ_0 leaves the ratio of aliphatic to indole glucosinolates was nearly the same during the whole experiment. Also Loivamaki *et al.* (2004) reported that MJ does not influence aliphatic glucosinolates in contrast to indole in leaves of *Arabidopsis thaliana*.

Table 4.34. Elicitor influence on correlation between the aliphatic and indole glucosinolates in plants of turnip from hydroponic on 30th day

Days	Treatment	Content / correlation	Leaves	Secondary roots	Primary Roots	Exudates
10	H	mg plant^{-1}	0.8 : 0.6	0.1 : 0.2		0.5 : 0.2
		n : 1	1.3 : 1	0.5 : 1		2.5 : 1
	HSA_0	mg plant^{-1}	0.8 : 1.6	0.2 : 0.6		0.1 : 1.2
		n : 1	0.5 : 1	0.3 : 1		0.1 : 1
	HMJ_0	mg plant^{-1}	1.2 : 1.5	0.1 : 0.7		0.3 : 1.3
		n : 1	0.8 : 1	0.1 : 1		0.4 : 1
15	H	mg plant^{-1}	1.2 : 0.3	0.1 : 0.4	0.7 : 0.8	0.4 : 0.2
		n : 1	4 : 1	0.2 : 1	0.9 : 1	0.5 : 1
	HSA_0	mg plant^{-1}	1.2 : 1.7	0.3 : 0.7	0.5 : 0.6	0.5 : 0.9
		n : 1	0.7 : 1	0.4 : 1	0.9 : 1	0.5 : 1
	HMJ_0	mg plant^{-1}	1.4 : 2.8	0.3 : 0.9	0.6 : 0.1	0.3 : 1.0
		n : 1	0.5 : 1	0.3 : 1	6 : 1	0.3 : 1
20	H	mg plant^{-1}	1.6 : 0.7	0.5 : 1.8	1.6 : 1.3	0.3 : 0.3
		n : 1	2.3 : 1	0.3 : 1	1.2 : 1	1 : 1
	HSA_0	mg plant^{-1}	1.4 : 1.5	0.8 : 2.4	0.9 : 1.0	0.1 : 1.2
		n : 1	1 : 1	0.3 : 1	0.9 : 1	0.1 : 1
	HMJ_0	mg plant^{-1}	1.8 : 3.3	0.7 : 3.5	0.7 : 0.9	0.3 : 0.8
		n : 1	0.5 : 1	0.2 : 1	0.8 : 1	0.4 : 1
25	H	mg plant^{-1}	1.6 : 1.1	0.9 : 2.2	2.3 : 1.9	0.4 : 0.4
		n : 1	1.3 : 1	0.2 : 1	1.2 : 1	1 : 1
	HSA_0	mg plant^{-1}	1.7 : 2.0	1.1 : 3.6	1.5 : 1.8	0.2 : 0.8
		n : 1	0.8 : 1	0.3 : 1	0.8 : 1	0.2 : 1
	HMJ_0	mg plant^{-1}	2.0 : 3.3	0.9 : 4.7	1.5 : 1.6	0.2 : 0.6
		n : 1	0.6 : 1	0.5 : 1	0.9 : 1	0.3 : 1
30	H	mg plant^{-1}	2.6 : 1.1	1.3 : 2.3	6.8 : 1.4	0.5 : 0.9
		n : 1	1.8 : 1	0.6 : 1	4.9 : 1	1 : 1
	HSA_0	mg plant^{-1}	1.8 : 2.0	1.5 : 6.3	3.5 : 1.2	0.2 : 0.8
		n : 1	0.9 : 1	0.2 : 1	0.3 : 1	0.2 : 1
	HMJ_0	mg plant^{-1}	2.2 : 2.8	1.1 : 3.9	4.9 : 1.1	0.1 : 0.8
		n : 1	0.8 : 1	0.3 : 1	4.8 : 1	0.1 : 1
Total	H	mg plant^{-1}				2.1 : 1.6*
		n : 1				1.3 : 1
	HSA_0	mg plant^{-1}				1.2 : 5.0*
		n : 1				0.2 : 1
	HMJ_0	mg plant^{-1}				1.1 : 5.6*
		n : 1				0.2 : 1

H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; HSA_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; HMJ_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + methyl jasmonate applied on the beginning.
The differences are compared for each treatment. Values followed by the same letter are not significantly different.

This ratio was much lower for secondary roots and kept nearly on the same level for all treatments during the whole experiment. For primary roots from H and HMJ_0 it increased rapidly until 4.9 and 4.8 on 30^{th} day, but this increase was not measured for HSA_0 primary roots. In exudates of non-treated plants the ratio aliphatic : indole glucosinolates decreased from 2.5 on 10^{th} day to 1.0 on 30^{th} day.
Interesting, that in exudates of treated with SA and MJ plants it decreased as compared to H. On 10^{th} day it reached for HSA_0 0.1, and for HMJ_0 0.4, varied between 0.1 and 0.5 during the plant growth and on 30^{th} day became 0.2 for HSA_0 and 0.1 for HMJ_0. This means, treated with elicitors plants exuded much more indole glucosinolates as aliphatic. The ratio of aliphatic to indole glucosinolates exuded during 30 days in H was 2.1 (plants exuded 2.1-folds more aliphatic glucosinolates as indole), but for HSA_0 as well as for HMJ_0 it became 0.2, which means it was exuded 5-folds more indole glucosinolates as aliphatic.
During the whole experiment the ratio of aliphatic to indole glucosinolates for H exudates was always higher as for secondary roots, while for HSA_0 exudates it was lower or the same as for their secondary roots. For HMJ_0 exudates it was higher until the 20^{th} day of the experiment and then became lower as for their secondary roots.
According to Charron *et al.* (2004), the indole glucosinolate concentration is inversely related to aliphatic glucosinolate concentration. The inverse relationship results from a shift in the amino acid composition which acts as precursors for the different classes of glucosinolates.
Complex interactions are observed between different glucosinolate biosynthetic pathways (Chen *et al.*, 2003). Disruption of CYP79F1 gene, entcoding cytochrome P450, participating in the conversion of homo- to pentahomo-methionine to aldoximes, leads to decreasing the level of aliphatic glucosinolates biosynthesis, while increases level of indole glucosinolates (Kliebenstein *et al*., 2005). The other common point in synthesis of different classes of glucosinolates is that CYP83A1 participating conversion of aliphatic aldoxymes to thiohydroxymates also metabolizes the aromatic and indole aldoximes with low affinity (Bak and Feyereisen, 2001).

4.2.3 Fresh weight of plants and glucosinolate concentration

Fresh weight of leaves of exposed to SA treatment plants was not obviously changed during the first 10 days for treated as compared to untreated plants (Table 4.35, Attachment, Tables 4.35a and 4.35b). At the end of experiment the fresh weight of leaves of SA treated plants did not significantly differ of these from H as well as AS and AD.
Application of MJ resulted in suppressing of fresh weight as compared to untreated variants. At the beginning of experiment the influence of elicitor was much more essential as later. On 10^{th} day fresh weight of HMJ_0 leaves was 4.3 g $plant^{-1}$, for H it reached 8.6 g. For $ADMJ_0$ leaves fresh weight was 4.9 g, while for AD 8.8 g. On 30^{th} day fresh weight of H leaves was 24.5 g, and for HMJ_0 22.9 g; for AD 29.7 g, and for $ADMJ_0$ 26.3 g.
Furmanova (1997) wrote that MJ in concentration 100 μM completely stopped the growth of *Taxus media* tissue culture for 7 days.
In general, the highest concentration of glucosinolates was measured for primary roots, where it reached for 30-days-old plants (on the example of H) 1.4 mg $plant^{-1}$, which was 4.5 fold more as for secondary roots and 7 fold more as for leaves.
Also the concentration of glucosinolates per g fresh weight for leaves of SA treated plants was nearly the same as for the plants without treatment. On 10^{th} day for H leaves it was 0.2 mg g^{-1}, while for HSA_0 0.3 mg g^{-1}. On 30^{th} day it was for HSA_0 0.1 mg g^{-1}, while for H 0.1 mg g^{-1}.

Table 4.35. Elicitor influence on fresh weight and glucosinolate concentration in plants of turnip from hydroponic

Date	Treatment	Fresh weight, g/ concentration of glucosinolates, mg g^{-1}	Leaves	Roots		Leaf : root index
				secondary	primary	
10 days	H	g	$8.6^{b} \pm 0.9$	$2.8^{b} \pm 1.0$	ND	3.1
		mg g^{-1}	0.2	0.1		
	HSA_0	g	$8.8^{b} \pm 0.8$	$4.9^{c} \pm 0.6$	ND	1.8
		mg g^{-1}	0.3	0.3		
	HMJ_0	g	$4.3^{a} \pm 0.4$	$0.9^{a} \pm 0.3$	ND	4.6
		mg g^{-1}	0.7	1.1		
15 days	H	g	$11.7^{b} \pm 1.4$	$7.6^{b} \pm 1.5$	$3.1^{b} \pm 0.4$	1.1
		mg g^{-1}	0.1	0.1	1.0	
	HSA_0	g	$13.9^{b} \pm 1.8$	$10.4^{c} \pm 0.7$	$2.3^{b} \pm 0.5$	1.1
		mg g^{-1}	0.2	0.2	1.2	
	HMJ_0	g	$4.3^{a} \pm 1.9$	$5.3^{a} \pm 0.5$	$1.0^{a} \pm 0.2$	0.7
		mg g^{-1}	1.0	0.3	1.5	
20 days	H	g	$20.3^{b} \pm 2.2$	$8.1^{a} \pm 1.8$	$4.6^{c} \pm 0.4$	1.4
		mg g^{-1}	0.1	0.4	1.0	
	HSA_0	g	$22.4^{b} \pm 1.9$	$12.9^{b} \pm 0.5$	$3.5^{b} \pm 0.2$	1.5
		mg g^{-1}	0.1	0.4	1.1	
	HMJ_0	g	$14.8^{a} \pm 0.5$	$7.3^{a} \pm 1.2$	$1.8^{a} \pm 0.2$	1.6
		mg g^{-1}	0.4	0.7	1.3	
25 days	H	g	$22.4^{b} \pm 0.4$	$9.7^{a} \pm 1.2$	$5.4^{c} \pm 0.5$	1.5
		mg g^{-1}	0.1	0.5	1.2	
	HSA_0	g	$24.6^{c} \pm 1.0$	$16.3^{c} \pm 1.8$	$4.2^{b} \pm 0.3$	1.5
		mg g^{-1}	0.2	0.4	1.6	
	HMJ_0	g	$18.2^{a} \pm 0.9$	$12.6^{b} \pm 1.0$	$2.3^{a} \pm 0.3$	1.2
		mg g^{-1}	0.3	0.6	2.3	
30 days	H	g	$24.5^{a} \pm 1.8$	$15.0^{a} \pm 2.7$	$6.4^{b} \pm 0.7$	1.1
		mg g^{-1}	0.2	0.3	1.4	
	HSA_0	g	$27.4^{b} \pm 0.8$	$18.6^{a} \pm 1.9$	$5.8^{b} \pm 0.5$	1.2
		mg g^{-1}	0.1	0.5	1.4	
	HMJ_0	g	$22.9^{a} \pm 1.5$	$15.1^{a} \pm 2.7$	$4.6^{a} \pm 0.5$	1.2
		mg g^{-1}	0.2	0.5	2.2	

H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; HSA_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; HMJ_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + methyl jasmonate applied on the beginning.
The differences are compared for each treatment. Values followed by the same letter are not significantly different.

However, the concentration of glucosinolates for MJ treated leaves was on the beginning of the experiment much higher as for SA treated or untreated. On 10^{th} day it reached 0.7 mg g^{-1} (3.5 fold more as for H), on 15^{th} day it increased to 1.0 mg g^{-1} (10 fold more as for H), but the next 5 days it started to decrease and on 30^{th} day reached the H level. This can be explained

by stimulation of glucosinolate synthesis, but on the same time by suppressing of fresh weight growth. Elicitors also influenced on fresh weight of secondary and primary roots. SA caused slight raising of fresh weight of roots (Table 4.35, Attachment, Tables 4.35a and 4.35b). On 5^{th} day fresh weight of H secondary roots was 2.8 g plant^{-1}, while for HSA_0 it reached 4.9 g plant^{-1} (1.7 fold more as for H). On 30^{th} day fresh weight of H secondary roots was 15.0 g and $HSA_0$18.6 g plant^{-1}; for AD it reached 21.1 and for $ADSA_0$ 24.0 g plant^{-1}; while for AS 21.9 and for $ASSA_0$ 26.1 g plant^{-1}. SA slightly decreased fresh weight of primary roots for all systems. Increase of secondary root fresh weight under SA influence could be explained by elicitor influence auxin status of plants, which stimulate secondary root development, particularly to appearance of greater number of cluster roots (Skene , 2001).

The difference in fresh weight of H roots after MJ application was even more essential as for leaves: on 10^{th} day the fresh weight of roots achieved 2.9 g in H, while for HMJ_0 only 0.9 g; 3.0 g for AD and 0.9 g for $ADMJ_0$. The final weight of roots was nearly the same for treated and non-treated samples: 21.4 g for H and 19.8 g for HMJ_0; 26.8 g for AD and 24.1 g for $ADMJ_0$.

Also the concentration of glucosinolates for secondary roots of SA treated plants was on 10^{th} day 0.3 mg g^{-1}, which was 3 fold more as for H, it increased sustainable and on 30^{th} day reached its maximum with 0.5 mg g^{-1} (1.7 fold more as for H). The same on 30^{th} day was also the concentration of glucosinolates for HMJ_0 secondary roots, 0.5 mg plant^{-1}, but it oppositely to H and HSA_0 from 1.1 mg g^{-1} on 10^{th} day. This can be explained by the fact, that MJ suppressed secondary root growth stronger as leaves, but the intensity of glucosinolate synthesis was not matched.

Also Berger *et al*. (1996) wrote that MJ suppressed root growth and inhibits IAA-stimulated coleoptiles elongation, which was explained by Grellmann and Mullet (1997) by blocking incorporation of glucose into cell wall polysaccharides. However, Wielanek and Urbanek (1999) reported about increase of hairy root biomass growth under the influence of MJ.

Plants exposed to SA and MJ treatment decreased in fresh weight of primary roots as compared to the untreated plants. On 15^{th} day fresh weight of H primary roots was 3.1 g, of HSA_0 2.3 g, and of HMJ_0 1.0 g. At the end of the experiment H primary roots weighted 6.4 g, which was 0.6 g and 1.8 g more as for HSA_0 and HMJ_0, respectively. Rossato *et al*. (2002) reported that growth of *Brassica napus* was strongly reduced by MJ, especially the root growth was reduced by 70 % as compared to that for non-treated plants. Moreover, they wrote that nitrate and potassium rot uptake as well as photosynthesis, chlorophyll content and protein amount of Rubisco have been reduced under the influence of MJ. Halkier *et al.* (2005) explained the decrease in root biomass growth under MJ influence by the influencing of elicitor on the expression of gene entconding γ-glutamatcysteinsynthase, which is responsible for cell division in roots meristem and consequently on root biomass growth.

The maximal increase of glucosinolate concentration in primary roots after SA treatment was on 25^{th} day: it reached 1.6 mg g^{-1}, while for H it was 1.2 mg g^{-1}. However, on 30^{th} day it was the same as for H. Treated with MJ plants had constantly increased concentration of glucosinolates in primary roots: on 15^{th} day it was 1.5 mg g^{-1} (1.5 mg plant^{-1} more as for H), and on 30^{th} day reached 2.2 mg plant^{-1} (1.6 fold more as for H).

Plants, treated with SA and MJ had on 10^{th} day after treatment nearly the same content of glucosinolates, but the fresh weight of HMJ_0 leaves was two times lower as for HSA_0 and the fresh weight of roots 5 folds lower. It is possible to assume that if MJ cause the same increase in production of defense compound, it became not economical for plants because it deprives growth of too many resources, and this could be the reason of decrease of plant growth.

Summary

The response of plants on root treatment with elicitors was inducible, which means that the root induction by SA and MJ has an impact on glucosinolate content in plant organs as well as in exudates of turnip. The effect of elicitors covered also leaves, not only the treated secondary roots, but to the lesser extend formed 10 days after the treatment primary roots.
Although SA and MJ induced systemic changes, the effect has been greater at the site of treatment, namely in secondary roots, then in other plant parts.
Chemical diversity and content of exudates could be dramatically enhanced by elicitation process. Plant response to the elicitor application occurred mostly during the first days after treatment, then followed gradual decline. This could be explained by the immediate defense reaction of plants, caused by the addition of elicitor, which diminish with the time.
Not all classes of glucosinolates responded equally to elicitor treatment.
Application of SA as well as MJ decreased the part of aliphatic, while increased the part of indole glucosinolates in leaves. Moreover, elicitor application induced the appearance of aromatic gluconasturtiin in leaves of hydroponically grown plants. Elicitor application decreased the part of aliphatic glucosinolates in primary roots. Both of elicitors raised essentially the part of indole glucosinolates (especially SA) and increased slightly the part of aromatic glucosinolates. Different effects of elicitors on the individual glucosinolates could be explained by biological functions of those in defense reactions caused by interaction between plant-herbivores or plant-microorganisms (Margath *et al*., 1994).
Aliphatic glucosinolates were the major class of glucosinolates in leaves, primary roots, and exudates in all systems. Both elicitors decreased aliphatic glucosinolates, especially in primary roots and exudates. In the contrast to leaves and primary roots, application of SA increased aliphatic glucosinolate content in secondary roots of all three systems during the whole experiment. This could be explained by the fact that the highest expression level of CYP79F1 genes, participating in conversion of short-chain methionine derivatives into aliphatic aldoximes, is reported to be in plant roots (Glombitsa *et al*., 2004).
Aliphatic glucosinolates was the major class for H and AS exudates, while for AD indole glucosinolates made the majority. Elicitors decreased the content of aliphatic glucosinolates in exudates for all systems, which in case with MJ had stronger effect as with SA, especially on the beginning of the experiment. This could be partially explained by suppressing of secondary root growth under MJ influence.
Elicitors matched two of four aliphatic glucosinolates: progoitrin and gluconapin. In contrast to them, glucobrassicanapin and gluconapoleiferin content was increased in leaves and secondary roots after elicitor application. This resulted in decrease of butenyl to pentenyl ratio under SA and MJ influence for leaves of treated plants (2.4 and 3.2 folds as compared to H) and for primary roots (1.5 and 1.7-folds), but not for secondary roots and exudates.
Application of elicitors, especially SA, markedly increased the content of gluconasturtiin in leaves and secondary roots. SA (Wielanek and Urbanek, 1999) and MJ (Mikkelsen et al., 2003) induction of CYP79A2 that converts phenylalanine to aromatic aldoxime might explain the increase in aromatic glucosinolates after SA and MJ application.
Treatment with both of elicitors increased the content of indole glucosinolates in all plant parts. Treatment with MJ and SA increased markedly indole glucosinolate content in leaves in all three systems: on 10^{th} day they had 2.5 and 2.7-folds more indole glucosinolate then H leaves, on 15^{th} day it became 9.3 and 2-folds more then for H, respectively.
Until 25^{th} day MJ increased the content of indole glucosinolates more essential as SA in secondary roots of plants. On 20^{th} day HMJ_0 secondary roots contented 4.8 and HSA_0 and

2.1-folds more indole glucosinolate as H secondary roots. However, during the last 5 days of the experiment this changed: HSA_0 secondary roots accumulated 6.3 mg plant^{-1} of indole glucosinolates, while in HMJ_0 it decreased until 3.9 mg plant^{-1}. Decrease of indole glucosinolate content on 30th day in secondary roots after MJ application and absence of this effect after SA treatment is probably possible to explain by longer but slower reaction of plants on SA treatment as on those caused by MJ or by more intensive exudation for MJ as for SA treated plants.

After the application of both elicitor the content of indole glucosinolates increased essentially right after the treatment and on 10th day it became for HSA_0 and HMJ_0 6 and 6.5-folds more then for H. On 30th day in the content of indole glucosinolates in HSA_0 and HMJ_0 was still more then for H (1.6-fold). However, plants from $ADSA_0$ and $ADMJ_0$ exuded 1.8 and 1.3-fold loweresser amount of indole glucosinolates as AD. In general, during 30 days H plants exuded 1.6 mg plant^{-1} of indole glucosinolates, HSA_0 5.0 mg plant^{-1} (2.6-folds more), and HMJ_0 5.9 mg plant^{-1} (3.1-folds more).

Elicitors influenced differently on individual indole glucosinolate. SA increased the content of glucobrassicin, 4-methoxy-glucobrassicin, and neoglucobrassicin in plants more essential than MJ. Neoglucobrassicin was the most predominant indole glucosinolate and also the most influenced.

The ratio of aliphatic to indole glucosinolates differed between parts of plant and changed under elicitor treatment as well as with plant growth. Interesting, that in exudates of treated with SA and MJ plants it decreased as compared to H. Treated with elicitor plants exuded much more indole glucosinolates as aliphatic. Elicitor-induced rhizosecretion is based on *de novo* synthesis of secondary metabolites and not on elicitor-induced leakage from root tissues.

Fresh weight of leaves of SA treated plants did not significantly differ of these from H. Secondary root fresh weight increased under SA influence, which could be explained by elicitor influence auxin status of plants, which stimulate secondary root development, particularly to appearance of greater number of cluster roots (Skene, 2001). The concentration of glucosinolates per g fresh weight for leaves of SA treated plants was nearly the same as for the plants without treatment.

Application of MJ resulted in suppressing of fresh weight. Right after treatment the influence of elicitor was much more essential as later: on 10th day fresh weight of HMJ_0 leaves and secondary roots was 2 and 3.2-fold lower as for H. However, the glucosinolate concentration for MJ treated leaves and secondary roots was on the beginning of the experiment 10 and 3-folds higher as for H, but then started to decrease and on 30th day reached H level. This could be explained by stimulation of glucosinolate synthesis by MJ, but on the same time by suppressing of plant growth. This was explained by Grellmann and Mullet (1997) by blocking incorporation of glucose into cell wall polysaccharides and Halkier *et al. (*2005) by the influencing on the expression of gene entconding γ-glutamatcysteinsynthase, which is responsible for cell division in roots meristem.

Consequently, plants, treated with SA and MJ had on 10th day after treatment nearly the same content of glucosinolates, but the fresh weight of HMJ_0 plants was much lower and SA treated or control. It is possible to assume that if MJ cause the same increase in production of defense compound, it became not economical for plants because it deprives growth of too many resources, and this could be the reason of decrease of plant growth.

4.3 Influence of salicylic acid, applied at the different stages of plant development on glucosinolate production in leaves, roots, and root exudates of turnip

It has been shown in previous experiments, salicylic acid (SA) applied on the beginning of the plant growth influenced essentially on glucosinolate content in turnip plants and exudates. The aim of this experiment was to estimate the time-depended development of phenotypic plasticity of turnip plants under the SA influence and to find out the stage of experiment, at which the application of SA would make the greatest influence on glucosinolate content. For this purpose, SA has been applied on different stages of plant growth.
We suppose, two different resistances involved in plant answer on SA application were observed in the experiment: Systemic Acquired Resistance (SAR) (Kliebestein *et al*., 2002) and Age-related Resistance (ARR) (Kus *et al*., 2002). Different responses occur to the same pathogen at different stages of plant development as it has been shown on the example of *Arabidopsis thaliana*.

4.3.1 Total glucosinolate content in plants and exudates

The distribution of glucosinolates in plant organs has been changed under the SA influence.
The induction of glucosinolate syntheses in leaves after SA application was measured already directly on 10^{th} day after the elicitor application. HSA_0 leaves contented 2.7 mg $plant^{-1}$ glucosinolates (1.2 mg $plant^{-1}$ more as H), on 20^{th} day HSA_{15} leaves had 5.2 mg $plant^{-1}$ (2.8 mg $plant^{-1}$ more as H), on 25^{th} day HSA_{20} leaves had 5.0 mg $plant^{-1}$ (2.2 mg $plant^{-1}$ more as H), and on 30^{th} day HSA_{20} leaves contented 7.0 mg $plant^{-1}$ (3.1 mg $plant^{-1}$ more as H) (Table 4.36). The effect of elicitor treatment in leaves was maximal after the treatment and then decreased with the time. For HSA_{15} leaves total glucosinolate content increased from 5^{th} until 15^{th} day after treatment by 1.0 mg $plant^{-1}$, while for H leaves at this time by 1.5 mg $plant^{-1}$.
Agrawal and Karban (1999) by study the plant defense processes reported that the induction of these processes depend on plant developmental stage and may change with plant age.
It is well known that although glucosinolate main role in plants is protective, their synthesis occurs during the course of normal growth and development (i.e. in the absence of pathogen or herbivore). According to Gatehouse (2002) phytochemicals are accumulated and stored, so that when attacked, the plant is already provided with the mean to deter or kill the herbivore or pathogens. This provides the biosynthetic function for substances that had previously been considered wasteful. These defence mechanism can be described as static or constitutive (as we could observe for non-treated plants) or active, induced mechanism (which was induced by SA application). The both responses may result in the production of the same defensive compounds, as glucosinolates, but differ in the kinetics of their production. We could assume that the kinetics of static and induced accumulation of glucosinolates was different for turnip plants; moreover, the proceeding of induced accumulation of glucosinolates depended on stage of plant development. The products of static and induced mechanisms, in this case individual glucosinolates of non-elicited and elicited plants are also different.
Induction of glucosinolate synthesis in leaves after root treatment with SA approve that plant defense response are activated locally, but also may occur in distal unaffected tissues, leading to SAR, which according to Leon *et al.* (2001) is long lasting and effective against the broad spectrum of virulent and avirulent pathogens. And SA has been identified as a positive component playing an essential role in the SAR transduction pathway. This explains the fact

that reaction of plants on SA application to roots is observed also in other plant parts. After elicitor application the increase of glucosinolate synthesis in leaves was more intensive as for the late-treated plants. On 30th day the content of glucosinolates for HSA_0 leaves increased to the level for H leaves, but in leaves from later treatments the simulative effect was still to observe. Rauth (2002) showed that in cultures exposed to SA treatment, myrosinase activity increased by about 50 % above control. Decrease of total glucosinolate content on 30th day after SA application for HSA_0 may be explained by enhance myrosinase activity under elicitor influence and consequently later destruction of glucosinolates in leaves.

Table 4.36. Influence of salicylic acid applied on different stages of plant growth on total glucosinolate content in plants and exudates of turnip (mg plant^{-1})

Date	Treatment	Leaves	Secondary roots	Primary roots	Exudates
10 days	H	$1.5^{a} \pm 0.2$	$0.4^{a} \pm 0.0$		$0.7^{a} \pm 0.2$
	HSA_0	$2.7^{b} \pm 0.3$	$1.3^{b} \pm 0.1$		$1.5^{b} \pm 0.0$
15 days	H	$1.7^{a} \pm 0.1$	$0.8^{a} \pm 0.1$	$3.0^{b} \pm 0.2$	$0.8^{a} \pm 0.1$
	HSA_0	$3.4^{b} \pm 0.2$	$1.6^{b} \pm 0.2$	$2.7^{b} \pm 0.3$	$1.8^{b} \pm 0.3$
20 days	H	$2.4^{a} \pm 0.4$	$3.2^{a} \pm 0.3$	$4.8^{c} \pm 0.3$	$0.9^{a} \pm 0.1$
	HSA_0	$3.1^{b} \pm 0.2$	$4.8^{b} \pm 0.3$	$3.9^{a} \pm 0.2$	$1.9^{b} \pm 0.3$
	HSA_{15}	$5.2^{c} \pm 0.8$	$6.2^{c} \pm 0.7$	$4.3^{a} \pm 0.5$	$2.6^{c} \pm 0.2$
25 days	H	$2.8^{a} \pm 0.1$	$4.4^{a} \pm 0.4$	$6.4^{a} \pm 1.1$	$1.2^{a} \pm 0.1$
	HSA_0	$4.0^{b} \pm 0.4$	$7.3^{b} \pm 0.7$	$6.8^{a} \pm 0.7$	$1.4^{b} \pm 0.1$
	HSA_{15}	$5.4^{c} \pm 0.7$	$8.8^{b} \pm 1.1$	$5.2^{a} \pm 0.9$	$2.4^{c} \pm 0.3$
	HSA_{20}	$5.0^{bc} \pm 0.8$	$7.3^{b} \pm 1.0$	$5.6^{a} \pm 0.7$	$2.3^{c} \pm 0.4$
30 days	H	$3.9^{a} \pm 0.3$	$4.9^{a} \pm 0.8$	$9.0^{b} \pm 0.6$	$1.2^{a} \pm 0.2$
	HSA_0	$4.1^{b} \pm 0.4$	$10.2^{c} \pm 2.1$	$8.0^{ab} \pm 1.0$	$1.3^{a} \pm 0.1$
	HSA_{15}	$6.3^{c} \pm 0.9$	$9.7^{c} \pm 0.7$	$7.7^{a} \pm 0.9$	$2.1^{b} \pm 0.3$
	HSA_{20}	$6.6^{c} \pm 0.8$	$8.2^{b} \pm 0.5$	$6.7^{a} \pm 0.6$	$2.3^{bc} \pm 0.4$
	HSA_{25}	$7.0^{c} \pm 0.5$	$9.0^{bc} \pm 0.3$	$6.9^{a} \pm 0.7$	$2.8^{c} \pm 0.3$
Total	H				$4.6^{a} \pm 0.3$*
	HSA_0				$7.8^{c} \pm 0.7$*
	HSA_{15}				$8.6^{c} \pm 0.3$*
	HSA_{20}				$7.1^{bc} \pm 0.5$*
	HSA_{25}				$6.5^{b} \pm 0.5$*

H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; HSA_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; HSA_{15} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 15th day; HSA_{20} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 20th day; HSA_{25} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 25th day. The differences are compared for each treatment. Values followed by the same letter are not significantly different. *Sum of glucosinolates exuded during 30 days

Also Kiddle *et al*. (1994) wrote that applying SA to soil drench to oilseed rape plants increased the concentration of glucosinolates in their leaves and the intensity of this induction depended on the age of leaf.

Kus *et al.* (2002) reported that the positive correlation observed between the productions of defense-associated compounds in older plants suggests that the various forms of ARR may be controlled developmentally. Physiological and developmental modifications leading to expression of ARR involve accumulation of defense compounds, particularly antimicrobial substances inhibiting the pathogen development, in plant tissues (Hugot *et al*., 2004). According to Kus *et al.* (2002) the ARR response has been observed in *Arabidopsis thaliana* to a different patovar *Pseudomona syringae*, as demonstrated by a 100-fold reduction in bacteria growth in mature plants compared with young plants.

The continuity of the effect of elicitor application on glucosinolate level in secondary roots led to the highest accumulation of glucosinolates in plants treated on earlier stages of development. HSA_0 secondary roots on 30^{th} day had 10.2 mg $plant^{-1}$ of glucosinolates, HSA_{15} 9.7, HSA_{20} 8.2, and HSA_{25} 9.0 mg $plant^{-1}$ (Table 4.21).

Glucosinolate content in primary roots of plants was not essentially influenced by SA application even when treatment was provided in time, when primary roots have already been formed. The small decrease of total glucosinolate content was measured as compared to the control, which is the result of slowing down the plant development under the influence of SA and, consequently, later development of primary roots (see 4.3.3).

As we can see, SA treatment caused systemic induction of glucosinolate accumulation in plants. Treatment of secondary roots led to increment of glucosinolate content in leaves.

Wallsgrove *et al.* (2003) pointed that it appears to be a correlation between the speeds and extend of such induction and resistance to pathogens and glucosinolate response could be a good marker for the resistance.

Glucosinolate content in exudates in exposed to SA treatment systems increased during the first 5-10 days after the application and afterwards decreased. This decrease was possible to measure for HSA_0 20 days and HSA_{15} 15 days after elicitor application (Figure 4.13).

On 10^{th} day HSA_0 plants exuded 1.5 mg $plant^{-1}$ of glucosinolates (0.8 mg $plant^{-1}$ more as H plants), between 15^{th}-20^{th} day HSA_{15} plants exuded 2.6 mg $plant^{-1}$ (1.5 mg $plant^{-1}$ more as H), from 20^{th} to 25^{th} day HSA_{20} plants exuded 2.3 mg $plant^{-1}$ (1.1 mg $plant^{-1}$ more), and between 25^{th}-30^{th} day HSA_{25} plants exuded 2.8 mg $plant^{-1}$ (1.6 mg $plant^{-1}$ more as H) (Table 4.36). The most intensive was the exudation 5 days after the elicitor application, and then it started to decrease (Figures 4.13 and 4.14).

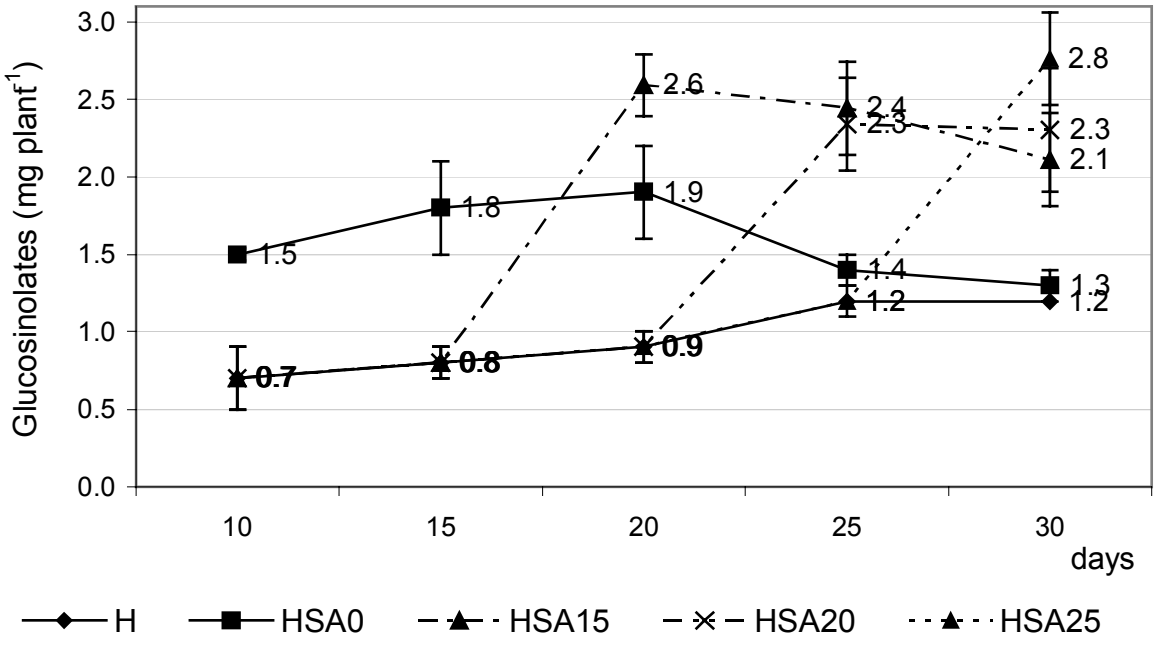
The highest increase of glucosinolates in exudates right after treatment was measured for HSA_{25} plants, but the total yield of glucosinolates in exudates from this system was lower as from HSA_0, HSA_{15}, and HSA_{25}, because plants were only 5 days in system after the elicitor application. The lowest yield of glucosinolates in exudates during 30 days was in H with 4.8 mg $plant^{-1}$, 6.4 mg $plant^{-1}$ was recovered from HSA_{25}, while from HSA_{15} it reached 8.6 mg $plant^{-1}$.

Increase of exuded glucosinolates may happen due to the release them as plant defense substances triggered in natural conditions by pathogens, which influence is mimicked by elicitor. It is known that plant reaction on pathogen attack as well as on elicitor application diminish with the time (Bennett and Wallsgrove, 1994).

Also Kus *et al.* (2002) reported that SA stimulates the production and secretion of antibacterial compounds, and its accumulation contributes to the antibacterial activity in plants displaying ARR.

Decrease of glucosinolates in exudates in 10-20 days after treatment could be explained by the hypothesis, suggested by Hugot *el al.* (2004) toward the mechanism of ARR. At the early step of infection, the host plants recognize some pathosecreted molecules (in our experiment, elicitor SA) that coordinate activation of defense reactions. Such defense reaction in our experiment is exuding of glucosinolates into growing medium. At the later stages of plant

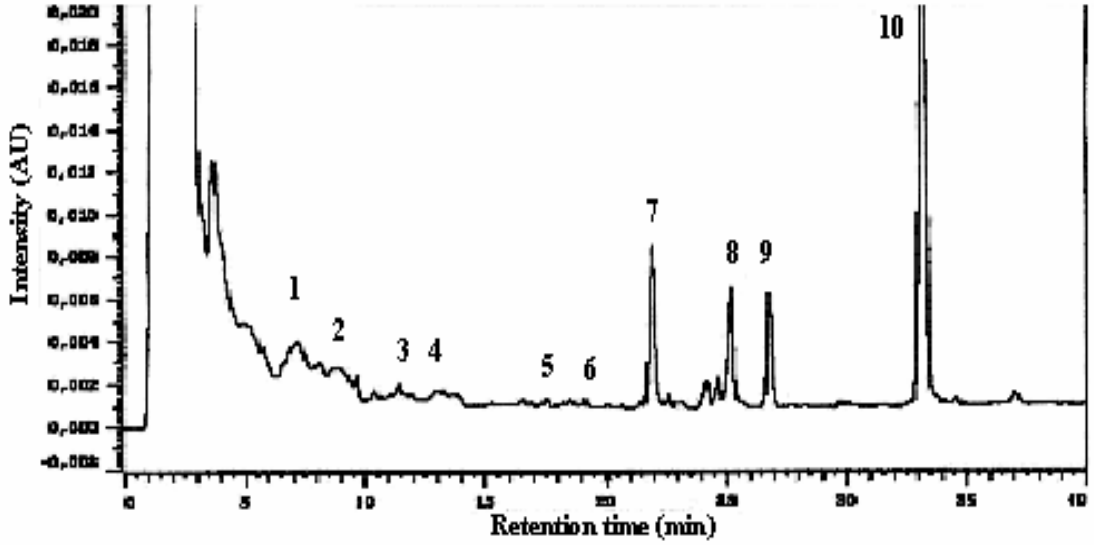
growth, a number of host-secreted molecules accumulate in the extracellular space and contribute to the control of invasion of pathogens. This can explain the observed in our experiment decrease in exudation of glucosinolates as well as their increase in plant tissues. Hugot *et al.* (2004) suggested that synthesis and secretion of defense-related substances are a critical part of the establishment to resistance. We can say that this establishment process takes from 10 to 20 days in plants of turnip, because during this time occurs the maximal accumulation of glucosinolates in plant tissues as well as most intensive exuding of glucosinolates into growing surrounding. And if the task is to receive the glucosinolates from exudates, it should be done until 20th day after treatment with elicitor, but if the source of glucosinolates should be plant tissues that it is more reasonable to collect the glucosinolates 30 days after the treatment.
According to Roshchina and Roshchina (1993) the plant age at elicitor application influence the metabolite profile in exudates, it depends on root enzymatic activity and recycling of glucosinolates by the root system.



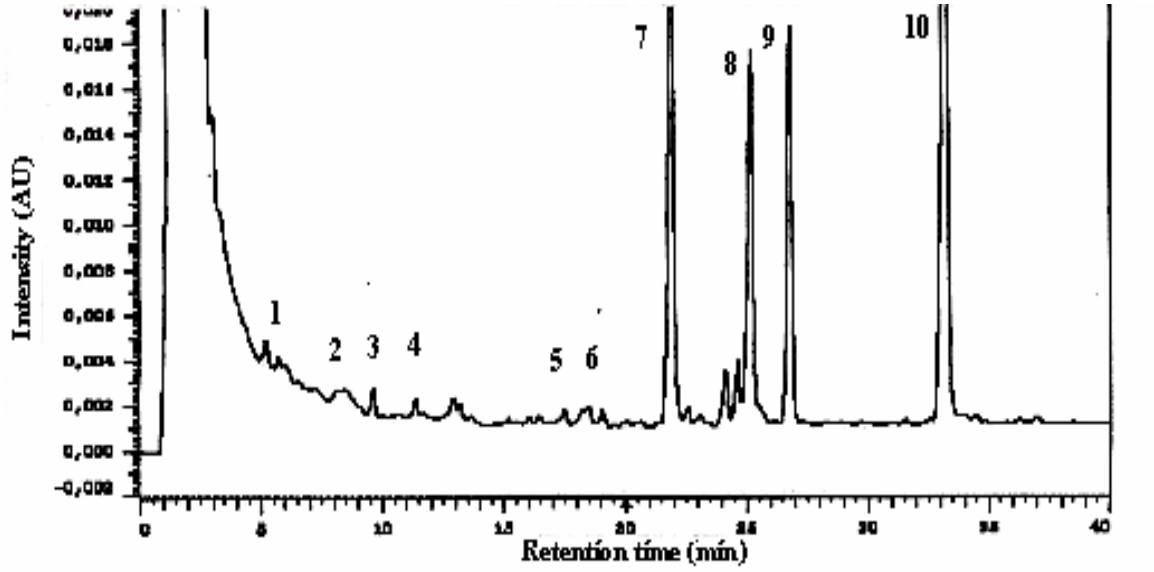
H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; HSA_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; HSA_{15} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 15th day; HSA_{20} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 20th day; HSA_{25} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 25th day.

Figure 4.13. Influence of salicylic acid applied on different stages of plant growth on kinetic of total glucosinolates in exudates of turnip grown in hydroponic

a)



b)



a). H - hydroponic with two times concentrated Hoagland solution and two times increased concentration of sulfur, b) HSA_{15} - hydroponic with two times concentrated Hoagland solution and two times increased concentration of sulfur and salicylic acid applied on 15th day of experiment.
1 – progoitrin, 2 – gluconapoleiferin, 3 – glucoalyssin, 4 – gluconapin, 5 - 4-hydroxy-glucobrassicin, 6 – glucobrassicanapin, 7 – glucobrassicin, 8 – gluconasturtiin, 9 - 4-methoxy-glucobrassicin, 10 – neoglucobrassicin

Figure 4.14. HPLC profile of glucosinolates recovered from root exudates of H and HSA_{15} turnip plants on 20th day

4.3.2 Individual glucosinolates in plants and exudates

The part of indole, aliphatic, and aromatic glucosinolates in plants and their exudates has been also changed under the influence of elicitor (Table 4.37). The ability to be induced depended on the time of SA application.
The comprising of aliphatic, aromatic, and indole glucosinolates in H leaves on 30th day of the experiment was 67 : 0 : 33 %, respectively. SA application on all stages led to decrease the part of aliphatic, increase the part of indole, and induced the synthesis of aromatic

glucosinolates. As it was mentioned, the part of aliphatic glucosinolates reached in leaves of H plants 67 %, but the later SA was applied, the lower was the part of aliphatic glucosinolates in leaves: for HSA_0 it reached 44 %, while for HSA_{25} 24 %. This means, the reaction of leaves on SA root treatment caused first the increase of part of indole glucosinolates, which was reduce with the time in post-treatment period. Moreover, SA application induced synthesis of aromatic glucosinolates in leaves. On 30^{th} day the biggest part of indole glucosinolates was measured for HSA_{20} leaves, which could be explained as that the maximal response in leaves on SA treatment, expressed in increasing the part of indole glucosinolates, occurred 10 days after plant treatment with elicitor.
For leaves and primary roots the part of aromatic glucosinolates increased for SA treatment, while in secondary roots it did not changed after elicitor application on all stages. The part of indole glucosinolates increased, mostly for HSA_0 and HSA_{25}. This could be explained by the fact that the indole glucosinolate synthesis is activated mostly on 5^{th} day after treatment. The increase on 25^{th} day could be connected with activation of glucosinolate synthesis in secondary roots as well as with transportation of indole glucosinolates from leaves and primary roots, which is more possible, because in these organs the part of indole glucosinolates for HSA_{25} increased as compared to treatments on earlier stages.
In primary roots the effect of SA application was admitted mostly for HSA_0 and HSA_{15} treatments. The part of aliphatic glucosinolates for HSA_0 was decreased 1.8 fold and for HSA_{15} 2.9 fold as compared to H. For HSA_0 the part of aromatic glucosinolates increased 5 fold and for HSA_{15} 2.8 fold The part of aliphatic glucosinolates in exudates was the highest for H (43 : 24 : 33 % of aliphatic, aromatic and indole glucosinolates), while the lowest was measured for HSA_0 (15 : 23 : 62 %). Plants treated on later stages exuded glucosinolates nearly in the same proportion: 34-37 % of aliphatic, 26-24 % of aromatic and 39-41 % of indole glucosinolates.
In general, application of SA increased the part of indole glucosinolates in plants and exudates and aromatic in plants. Also Mikkelsen *et al.* (2000) told that tryptophan-derived indole glucosinolates are more responsive to induction than aliphatic glucosinolates. They also assumed that different indole glucosinolates methoxylating enzymes are influenced by SA, whereas aliphatic glucosinolates appear to be primarily genetically and not environmentally controlled.

4.3.2.1 Aliphatic glucosinolates in plants and exudates

The content of aliphatic glucosinolates changed after the application of elicitor, but the response was not identical in plants, treated with SA at the different time of experiment.
The decrease of aliphatic glucosinolate content in leaves under the SA influence was measured only on 30^{th} day of experiment. At this time the aliphatic glucosinolate content in HSA_0 as well as in HSA_{15} leaves was 1.8 mg $plant^{-1}$ (1.4-time lower as in H leaves), for HSA_{20} it reached 2.0 mg $plant^{-1}$, and the lowest content was measured for HSA_{25} leaves 1.7 mg $plant^{-1}$ (Table 4.38).
In primary roots of turnip the aliphatic glucosinolate content was higher as in other parts. For H primary roots on 30^{th} day it reached 6.8 mg $plant^{-1}$ (while for leaves 2.6 and for secondary roots 1.3 mg $plant^{-1}$) (Table 4.38). This could be explained by the fact that the highest expression level of CYP79F1 genes, participating in conversion of methionine derivatives into aliphatic aldoximes was observed in roots and normalized in leaves of *Arabidopsis thaliana* (Glombitza *et al*., 2004).

Table 4.37. Influence of salicylic acid applied on different stages of plant growth on composition of aliphatic, aromatic, and indole glucosinolates in plants and exudates of turnip on 30th day

Object	Treatment	Content / part	Aliphatic	Aromatic	Indole
Leaves	H	mg plant^{-1}	$2.6^{b} \pm 0.1$	ND	$1.3^{a} \pm 0.1$
		%	67	0	33
	HSA_0	mg plant^{-1}	$1.8^{a} \pm 0.1$	$0.4^{a} \pm 0.2$	$1.9^{b} \pm 0.1$
		%	44	10	46
	HSA_{15}	mg plant^{-1}	$1.8^{a} \pm 0.3$	$1.3^{c} \pm 0.2$	$3.2^{c} \pm 0.1$
		%	29	21	51
	HSA_{20}	mg plant^{-1}	$2.0^{a} \pm 0.2$	$0.9^{b} \pm 0.1$	$3.8^{c} \pm 0.5$
		%	30	13	57
	HSA_{25}	mg plant^{-1}	$1.7^{a} \pm 0.1$	$2.0^{d} \pm 0.2$	$3.3^{c} \pm 0.4$
		%	24	29	47
Secondary roots	H	mg plant^{-1}	$1.3^{a} \pm 0.2$	$1.2^{a} \pm 0.4$	$2.3^{a} \pm 0.2$
		%	27	25	48
	HSA_0	mg plant^{-1}	$1.5^{ab} \pm 0.3$	$2.3^{b} \pm 0.4$	$6.3^{d} \pm 0.5$
		%	15	23	62
	HSA_{15}	mg plant^{-1}	$1.8^{bc} \pm 0.2$	$2.9^{c} \pm 0.1$	$5.0^{c} \pm 0.2$
		%	19	30	52
	HSA_{20}	mg plant^{-1}	$2.1^{c} \pm 0.3$	$2.3^{b} \pm 0.3$	$3.8^{b} \pm 0.3$
		%	26	28	46
	HSA_{25}	mg plant^{-1}	$1.7^{b} \pm 0.0$	$1.8^{a} \pm 0.2$	$5.1^{c} \pm 0.3$
		%	20	21	59
Primary roots	H	mg plant^{-1}	$6.8^{d} \pm 0.2$	$0.7^{a} \pm 0.2$	$1.4^{a} \pm 0.2$
		%	76	8	16
	HSA_0	mg plant^{-1}	$3.5^{b} \pm 0.4$	$3.4^{d} \pm 0.3$	$1.2^{a} \pm 0.2$
		%	43	42	15
	HSA_{15}	mg plant^{-1}	$2.0^{a} \pm 0.0$	$2.3^{c} \pm 0.2$	$3.4^{c} \pm 0.4$
		%	26	30	44
	HSA_{20}	mg plant^{-1}	$3.7^{b} \pm 0.1$	$1.3^{b} \pm 0.0$	$1.7^{b} \pm 0.0$
		%	55	19	25
	HSA_{25}	mg plant^{-1}	$5.1^{c} \pm 0.3$	$0.9^{a} \pm 0.1$	$1.4^{a} \pm 0.2$
		%	69	12	19
Exudates	H	mg plant^{-1}	$2.1^{b} \pm 0.3$*	$1.2^{a} \pm 0.3$*	$1.6^{a} \pm 0.3$*
		%	43	24	33
	HSA_0	mg plant^{-1}	$1.2^{a} \pm 0.1$*	$1.8^{abc} \pm 0.4$*	$5.0^{d} \pm 0.5$*
		%	15	23	63
	HSA_{15}	mg plant^{-1}	$2.9^{c} \pm 0.3$	$2.2^{c} \pm 0.3$	$3.5^{c} \pm 0.2$*
		%	34	26	41
	HSA_{20}	mg plant^{-1}	$2.6^{bc} \pm 0.4$	$1.7^{b} \pm 0.1$	$2.7^{b} \pm 0.3$*
		%	37	24	39
	HSA_{25}	mg plant^{-1}	$2.3^{b} \pm 0.2$	$1.5^{ab} \pm 0.1$	$2.4^{b} \pm 0.1$*
		%	37	25	38

H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; HSA_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; HSA_{15} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 15th day; HSA_{20} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 20th day; HSA_{25} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 25th day. The differences are compared for each treatment. Values followed by the same letter are not significantly different. *Sum of glucosinolates exuded during 30 days

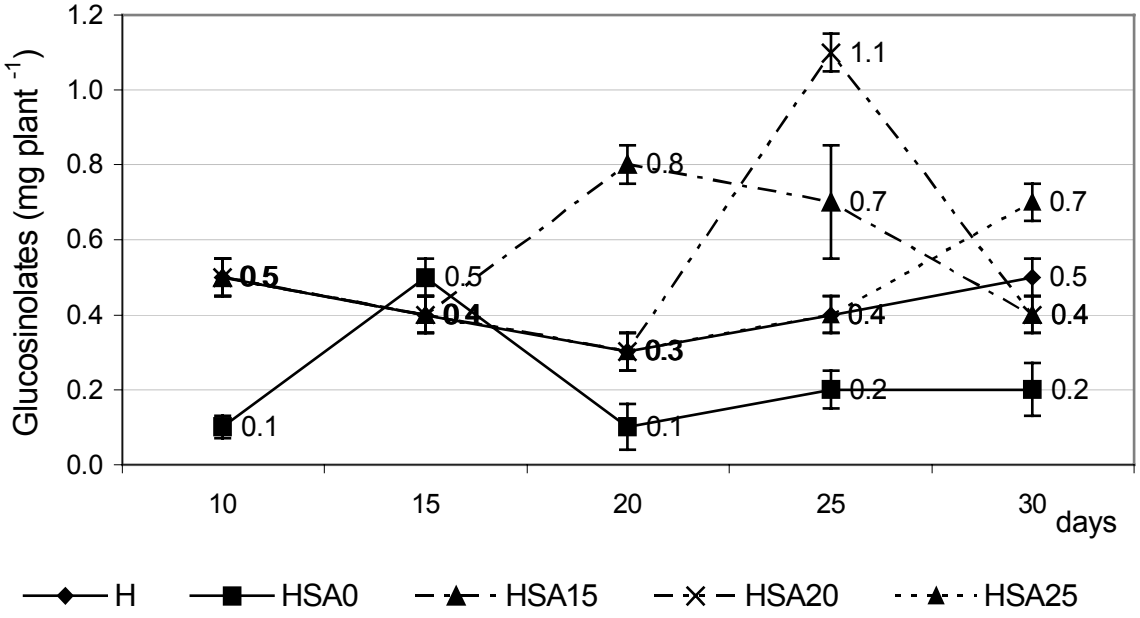
Most essential SA influenced on aliphatic glucosinolate content in primary roots. Already on 25th day their content was decreased in all treated systems, but the next 5 days this effect became more obvious. On 30th day aliphatic glucosinolate content in H primary roots reached 6.8 mg plant^{-1}, while in HSA_{15} only 2.0 mg plant^{-1}. HSA_0 and HSA_{20} primary roots had 3.5 and 3.7 mg plant^{-1} of aliphatic glucosinolates, and HSA_{25} 5.1 mg plant^{-1} (Table 4.38).
In contrast to leaves and primary roots, application of SA increased aliphatic glucosinolate content in secondary roots of all systems. On 30th day aliphatic glucosinolate content in H secondary roots was 1.3 mg plant^{-1}, while in HSA_{20} it was the highest one with 2.1 mg plant^{-1} (Table 4.38). Increase of the content of aliphatic glucosinolates in secondary roots and decrease in leaves and primary roots of turnip could be caused by the independent regulation of glucosinolate syntheses in different plant organs (Tantikanjana *et al*., 2004).

Table 4.38. Influence of salicylic acid applied on different stages of plant growth on aliphatic glucosinolate content in plants and exudates of turnip from hydroponic (mg plant^{-1})

Date	Treatment	Leaves	Secondary roots	Primary roots	Exudates
10 days	H	$0.8^{a} \pm 0.1$	$0.1^{a} \pm 0.0$		$0.5^{b} \pm 0.1$
	HSA_0	$0.8^{a} \pm 0.1$	$0.2^{b} \pm 0.0$		$0.1^{a} \pm 0.0$
15 days	H	$1.2^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.7^{a} \pm 0.1$	$0.4^{a} \pm 0.1$
	HSA_0	$1.2^{a} \pm 0.1$	$0.3^{b} \pm 0.1$	$0.5^{a} \pm 0.1$	$0.5^{a} \pm 0.1$
20 days	H	$1.6^{a} \pm 0.2$	$0.5^{a} \pm 0.1$	$1.6^{b} \pm 0.1$	$0.3^{a} \pm 0.1$
	HSA_0	$1.4^{a} \pm 0.1$	$0.8^{b} \pm 0.1$	$0.9^{a} \pm 0.1$	$0.1^{a} \pm 0.1$
	HSA_{15}	$1.3^{a} \pm 0.3$	$1.0^{b} \pm 0.2$	$1.5^{b} \pm 0.2$	$0.8^{b} \pm 0.1$
25 days	H	$1.6^{a} \pm 0.0$	$0.9^{a} \pm 0.2$	$2.3^{b} \pm 0.4$	$0.4^{ab} \pm 0.1$
	HSA_0	$1.7^{a} \pm 0.2$	$1.1^{ab} \pm 0.2$	$1.5^{a} \pm 0.3$	$0.2^{a} \pm 0.1$
	HSA_{15}	$1.6^{a} \pm 0.0$	$1.8^{b} \pm 0.3$	$2.3^{b} \pm 0.4$	$0.7^{b} \pm 0.2$
	HSA_{20}	$1.5^{a} \pm 0.3$	$1.5^{b} \pm 0.2$	$1.5^{a} \pm 0.3$	$1.1^{b} \pm 0.1$
30 days	H	$2.6^{b} \pm 0.1$	$1.3^{a} \pm 0.2$	$6.8^{d} \pm 0.2$	$0.5^{bc} \pm 0.1$
	HSA_0	$1.8^{a} \pm 0.1$	$1.5^{ab} \pm 0.3$	$3.5^{b} \pm 0.4$	$0.2^{a} \pm 0.1$
	HSA_{15}	$1.8^{a} \pm 0.3$	$1.8^{bc} \pm 0.2$	$2.0^{a} \pm 0.0$	$0.4^{b} \pm 0.0$
	HSA_{20}	$2.0^{a} \pm 0.2$	$2.1^{c} \pm 0.3$	$3.7^{b} \pm 0.1$	$0.4^{ab} \pm 0.1$
	HSA_{25}	$1.7^{a} \pm 0.1$	$1.7^{b} \pm 0.0$	$5.1^{c} \pm 0.3$	$0.7^{c} \pm 0.1$
Total	H				$2.1^{b} \pm 0.3$*
	HSA_0				$1.2^{a} \pm 0.1$*
	HSA_{15}				$2.9^{c} \pm 0.3$*
	HSA_{20}				$2.6^{bc} \pm 0.4$*
	HSA_{25}				$2.3^{b} \pm 0.2$*

H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; HSA_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; HSA_{15} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 15th day; HSA_{20} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 20th day; HSA_{25} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 25th day. The differences are compared for each treatment. Values followed by the same letter are not significantly different. *Sum of glucosinolates exuded during 30 days

Aliphatic was the major class of glucosinolates in H exudates. Plants from H exuded 2.1 mg plant^{-1} of glucosinolates during 30 days. In all systems the content of aliphatic glucosinolates exuded during 30 days increased (by 0.2 mg plant^{-1} for HSA_{25} until 0.7 mg plant^{-1} for HSA_{15}), except for HSA_{20}, where it reached 1.1 mg plant^{-1}.
Interestingly is that 5 days after SA treatment the content of aliphatic glucosinolates in exudates was much higher as for system without treatment, but then it decreased. For example, on 20th day HSA_{15} exudates had 0.8 mg plant^{-1}of aliphatic glucosinolates (0.5 mg plant^{-1} more as for H), on 25th day HSA_{15} plants exuded 0.7 mg plant^{-1} (0.3 mg plant^{-1} more as for H), and on 30th day they had 0.4 mg plant^{-1} (0.1 mg plant^{-1} lower as for H) (Table 4.38, Figure 4.16).



H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; HSA_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; HSA_{15} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 15th day; HSA_{20} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 20th day; HSA_{25} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 25th day.

Figure 4.15. Influence of salicylic acid applied on different stages of plant growth on kinetic of aliphatic glucosinolates in exudates

Individual aliphatic glucosinolates

In all treatments, except HSA_{15}, the biggest part of progoitrin was concentrated in primary roots. However, in HSA_{15} the progoitrin content in leaves, secondary root, and exudates was higher as in all other treatments. It may be explained by transport of progoitrin from primary roots into the secondary roots and exudates. During 30 days H plants exuded 0.6 mg plant^{-1} of progoitrin, while for HSA_0 it decreased to 0.4 mg plant^{-1}. HSA_{15} plants exuded the highest quantity of progoitrin 0.9 mg plant^{-1}, while for the later applications it decreased and for HSA_{20} became 0.5 mg plant^{-1} lower as for H.

Table 4.39. Influence of salicylic acid applied on different stages of plant growth on individual aliphatic glucosinolates content in plants and exudates of turnip from hydroponic on 30th day (mg plant^{-1})

Gluco-sinolates	Treat-ment	Content / pa	Leaves	Secondary root	Primary roots	Exudates
Progoitrin	H	mg plant^{-1}	$0.9^{a} \pm 0.2$	$0.6^{ab} \pm 0.1$	$2.8^{e} \pm 0.3$	$0.6^{b} \pm 0.1$*
		%	21	14	65	
	HSA_0	mg plant^{-1}	$0.5^{a} \pm 0.1$	$0.5^{a} \pm 0.1$	$1.2^{c} \pm 0.1$	$0.4^{a} \pm 0.0$*
		%	23	23	55	
	HSA_{15}	mg plant^{-1}	$0.6^{a} \pm 0.2$	$0.8^{b} \pm 0.1$	$0.6^{a} \pm 0.0$	$1.0^{c} \pm 0.2$*
		%	30	40	30	
	HSA_{20}	mg plant^{-1}	$0.7^{a} \pm 0.1$	$0.7^{b} \pm 0.0$	$0.9^{b} \pm 0.0$	$0.8^{bc} \pm 0.1$*
		%	30	30	39	
	HSA_{25}	mg plant^{-1}	$0.6^{a} \pm 0.0$	$0.7^{ab} \pm 0.1$	$1.9^{d} \pm 0.1$	$0.5^{ab} \pm 0.1$*
		%	19	22	59	
Gluconapin	H	mg plant^{-1}	$0.7^{b} \pm 0.1$	$0.2^{a} \pm 0.0$	$1.7^{c} \pm 0.3$	$0.4^{b} \pm 0.0$*
		%	27	8	65	
	HSA_0	mg plant^{-1}	$0.3^{a} \pm 0.1$	$0.2^{a} \pm 0.0$	$0.6^{a} \pm 0.2$	$0.2^{a} \pm 0.1$*
		%	27	18	55	
	HSA_{15}	mg plant^{-1}	$0.4^{a} \pm 0.0$	$0.4^{b} \pm 0.1$	$0.6^{a} \pm 0.1$	$0.6^{c} \pm 0.1$*
		%	29	29	43	
	HSA_{20}	mg plant^{-1}	$0.6^{b} \pm 0.1$	$0.4^{b} \pm 0.0$	$1.1^{b} \pm 0.1$	$0.6^{bc} \pm 0.2$*
		%	29	19	52	
	HSA_{25}	mg plant^{-1}	$0.4^{a} \pm 0.0$	$0.4^{b} \pm 0.0$	$1.3^{b} \pm 0.1$	$0.5^{bc} \pm 0.1$*
		%	19	19	62	
Gluconapo-leiferin	H	mg plant^{-1}	$0.6^{ab} \pm 0.1$	$0.3^{a} \pm 0.0$	$1.0^{b} \pm 0.3$	$0.5^{a} \pm 0.1$*
		%	32	16	53	
	HSA_0	mg plant^{-1}	$0.7^{ab} \pm 0.2$	$0.5^{abc} \pm 0.2$	$0.6^{ab} \pm 0.2$	$0.3^{a} \pm 0.2$*
		%	39	28	33	
	HSA_{15}	mg plant^{-1}	$0.6^{ab} \pm 0.1$	$0.4^{b} \pm 0.0$	$0.4^{a} \pm 0.0$	$0.6^{a} \pm 0.2$*
		%	43	29	29	
	HSA_{20}	mg plant^{-1}	$0.5^{a} \pm 0.0$	$0.6^{c} \pm 0.1$	$0.8^{b} \pm 0.1$	$0.7^{a} \pm 0.2$*
		%	26	32	42	
	HSA_{25}	mg plant^{-1}	$0.6^{b} \pm 0.0$	$0.5^{c} \pm 0.0$	$0.9^{a} \pm 0.1$	$0.6^{a} \pm 0.1$*
		%	30	25	45	
Glucobras-sicanapin	H	mg plant^{-1}	$0.2^{a} \pm 0.1$	$0.1^{a} \pm 0.0$	$1.1^{a} \pm 0.3$	$0.4^{b} \pm 0.0$*
		%	14	7	79	
	HSA_0	mg plant^{-1}	$0.3^{a} \pm 0.0$	$0.2^{a} \pm 0.1$	$0.8^{a} \pm 0.2$	$0.2^{a} \pm 0.1$*
		%	23	15	62	
	HSA_{15}	mg plant^{-1}	$0.3^{b} \pm 0.0$	$0.3^{b} \pm 0.0$	$0.5^{a} \pm 0.0$	$0.5^{c} \pm 0.0$*
		%	14	27	45	
	HSA_{20}	mg plant^{-1}	$0.2^{b} \pm 0.1$	$0.4^{b} \pm 0.0$	$0.9^{a} \pm 0.1$	$0.6^{cd} \pm 0.1$*
		%	23	15	62	
	HSA_{25}	mg plant^{-1}	$0.2^{b} \pm 0.0$	$0.1^{b} \pm 0.0$	$1.0^{a} \pm 0.1$	$0.6^{d} \pm 0.0$*
		%	27	27	45	

H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; HSA_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; HSA_{15} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 15th day; HSA_{20} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 20th day; HSA_{25} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 25th day. The differences are compared for each treatment. Values followed by the same letter are not significantly different. *Sum of glucosinolates exuded during 30 days

As it was already shown in previous experiments, progoitrin was the major aliphatic glucosinolate in turnip. On the beginning of plant growth its major part was concentrated in leaves, but to the end of vegetation up to 65 % of progoitrin was measured in primary roots of H plants. However, treatment with SA reduced it, especially strong for HSA_{15} primary roots, which had 0.6 mg plant^{-1} of progoitrin (4.6-fold lower as for H) (Tables 4.39 and 4.40). On 30th day the decreased of progoitrin content in primary roots under the SA influence was distinctly seen: for HSA_0 1.2 mg plant^{-1} (2.3 fold lower as for H), for HSA_{15} 0.6 mg plant^{-1} (4.6 fold lower), for HSA_{20} 0.9 mg plant^{-1} (3.1 fold lower), and for HSA_{25} 1.9 mg plant^{-1} (1.5 fold lower as for H).

Table 4.40. Influence of salicylic acid applied on different stages of plant growth on progoitrin content in plants and exudates of turnip from hydroponic (mg plant^{-1})

Days	Treatments	Leaves	Secondary roots	Primary roots	Exudates
10	H	$0.3^{a} \pm 0.0$	ND	-	$0.1^{a} \pm 0.0$
	HSA_0	$0.6^{b} \pm 0.1$	ND	-	$0.1^{a} \pm 0.0$
15	H	$0.5^{a} \pm 0.0$	ND	$0.1^{a} \pm 0.1$	$0.1^{a} \pm 0.0$
	HSA_0	$0.8^{b} \pm 0.2$	ND	$0.1^{a} \pm 0.0$	$0.2^{a} \pm 0.1$
20	H	$0.7^{a} \pm 0.0$	$0.3^{a} \pm 0.0$	$0.4^{a} \pm 0.2$	$0.1^{a} \pm 0.1$
	HSA_0	$0.7^{a} \pm 0.3$	$0.3^{a} \pm 0.1$	$0.2^{a} \pm 0.1$	$0.1^{a} \pm 0.1$
	HSA_{15}	$0.8^{a} \pm 0.1$	$0.3^{a} \pm 0.0$	$0.4^{a} \pm 0.1$	$0.3^{a} \pm 0.1$
25	H	$0.7^{a} \pm 0.2$	$0.6^{a} \pm 0.1$	$0.8^{c} \pm 0.2$	$0.2^{ab} \pm 0.1$
	HSA_0	$0.8^{a} \pm 0.1$	$0.4^{a} \pm 0.1$	$0.3^{a} \pm 0.1$	$0.1^{a} \pm 0.1$
	HSA_{15}	$0.8^{a} \pm 0.2$	$0.5^{a} \pm 0.0$	$0.5^{b} \pm 0.0$	$0.2^{a} \pm 0.0$
	HSA_{20}	$0.7^{a} \pm 0.1$	$0.6^{a} \pm 0.1$	$0.3^{a} \pm 0.0$	$0.4^{b} \pm 0.1$
30	H	$0.9^{a} \pm 0.2$	$0.6^{ab} \pm 0.1$	$2.8^{e} \pm 0.3$	$0.2^{ab} \pm 0.1$
	HSA_0	$0.5^{a} \pm 0.1$	$0.5^{a} \pm 0.1$	$1.2^{c} \pm 0.1$	ND
	HSA_{15}	$0.6^{a} \pm 0.2$	$0.8^{b} \pm 0.1$	$0.6^{a} \pm 0.0$	$0.2^{a} \pm 0.0$
	HSA_{20}	$0.7^{a} \pm 0.1$	$0.7^{b} \pm 0.0$	$0.9^{b} \pm 0.0$	$0.2^{ab} \pm 0.1$
	HSA_{25}	$0.6^{a} \pm 0.0$	$0.7^{ab} \pm 0.1$	$1.9^{d} \pm 0.1$	$0.4^{b} \pm 0.1$

H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; HSA_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; HSA_{15} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 15th day; HSA_{20} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 20th day; HSA_{25} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 25th day. The differences are compared for each treatment. Values followed by the same letter are not significantly different. ND- not determined.

The decrease of progoitrin content for leaves was also measured for SA treatments, but after 15 days of elicitor application. However, HSA_{15} plants had the biggest part of progoitrin in their secondary roots 40 %, while for H it reached 14 %, which corresponded 0.8 and 0.6 mg plant^{-1}. It could be explained by the transferring of progoitrin from primary into secondary roots 15-20 days after SA treatment. This can be substituted by the fact that HSA_0 primary roots had also lower content of this glucosinolate on 20th day of the experiment (0.1 mg plant^{-1}, while at that time H as well as HSA_{15} primary roots had 0.4 mg plant^{-1} of progoitin).

SA application on the beginning of the experiment caused the increase in progoitrin exudation until the 30th day for HSA_0, however, for HSA_{15}, HSA_{20}, and HSA_{25} on 5th day after the elicitor application the increase in progoitrin content in exudates has been measured.
Application of SA on different stages caused decrease in **gluconapin** content, particularly in leaves and primary roots, however its content in secondary roots increased (Table 4.41). For example, on 25th day H leaves had 0.5 mg plant^{-1} and primary roots 0.7 mg plant^{-1} of gluconapin, which was higher as for HSA_0, but the secondary roots of HSA_0 plants had more of gluconapin as H secondary roots. On 30th day H leaves and primary roots had 0.7 and 1.7 mg plant^{-1} of gluconapin, which was 0.3 and 1.1 mg plant^{-1} more as for HSA_{15}, but the secondary roots of HSA_{15} plants had 0.4 mg plant^{-1} of gluconapin, which is 0.2 mg plant^{-1} more as H secondary roots. The most expressed difference was observed 15-25 days after elicitor application.

Table 4.41. Influence of salicylic acid applied on different stages of plant growth on gluconapin content in plants and exudates of turnip from hydroponic (mg plant^{-1})

Days	Treatments	Leaves	Secondary roots	Primary roots	Exudates
10	H	$0.4^{b} \pm 0.0$	$0.1^{a} \pm 0.0$	-	$0.2^{b} \pm 0.0$
	HSA_0	$0.2^{a} \pm 0.0$	$0.2^{a} \pm 0.1$	-	$0.1^{a} \pm 0.0$
15	H	$0.5^{a} \pm 0.1$	$0.1^{a} \pm 0.0$	$0.2^{a} \pm 0.1$	$0.2^{a} \pm 0.1$
	HSA_0	$0.4^{a} \pm 0.0$	$0.2^{a} \pm 0.1$	$0.1^{a} \pm 0.0$	$0.2^{a} \pm 0.1$
20	H	$0.5^{b} \pm 0.2$	$0.1^{a} \pm 0.1$	$0.5^{a} \pm 0.1$	$0.1^{a} \pm 0.1$
	HSA_0	$0.3^{ab} \pm 0.1$	$0.2^{ab} \pm 0.1$	$0.3^{a} \pm 0.1$	ND
	HSA_{15}	$0.2^{a} \pm 0.0$	$0.3^{b} \pm 0.0$	$0.4^{a} \pm 0.0$	$0.2^{a} \pm 0.0$
25	H	$0.5^{ab} \pm 0.2$	$0.2^{a} \pm 0.1$	$0.7^{c} \pm 0.1$	ND
	HSA_0	$0.3^{a} \pm 0.1$	$0.3^{ab} \pm 0.1$	$0.3^{a} \pm 0.1$	ND
	HSA_{15}	$0.4^{ab} \pm 0.1$	$0.4^{b} \pm 0.0$	$0.5^{b} \pm 0.0$	$0.1^{a} \pm 0.0$
	HSA_{20}	$0.5^{b} \pm 0.0$	$0.4^{ab} \pm 0.1$	$0.7^{c} \pm 0.1$	$0.3^{b} \pm 0.0$
30	H	$0.7^{b} \pm 0.1$	$0.2^{a} \pm 0.0$	$1.7^{c} \pm 0.3$	ND
	HSA_0	$0.3^{a} \pm 0.1$	$0.2^{a} \pm 0.0$	$0.6^{a} \pm 0.2$	ND
	HSA_{15}	$0.4^{a} \pm 0.0$	$0.4^{b} \pm 0.1$	$0.6^{a} \pm 0.1$	ND
	HSA_{20}	$0.6^{b} \pm 0.1$	$0.4^{b} \pm 0.0$	$1.1^{b} \pm 0.1$	ND
	HSA_{25}	$0.4^{a} \pm 0.0$	$0.4^{b} \pm 0.0$	$1.3^{b} \pm 0.1$	ND

H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; HSA_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; HSA_{15} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 15th day; HSA_{20} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 20th day; HSA_{25} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 25th day. The differences are compared for each treatment. Values followed by the same letter are not significantly different. ND- not determined.

Right after the treatment with SA plants exuded more gluconapin as non treated plants. For example, between 15th and 20th day HSA_{15} plants exudated 0.2 mg plant^{-1} of glucosinolate, while H 0.1 mg plant^{-1}, and on 25th day HSA_{20} plants exuded 0.3 mg plant^{-1}, while no gluconapin was determined in H exudates.

Table 4.42. Influence of salicylic acid applied on different stages of plant growth on gluconapoleiferin content in plants and exudates of turnip from hydroponic (mg plant^{-1})

Days	Treatments	Leaves	Secondary roots	Primary roots	Exudates
10	H	ND	$0.1^{a} \pm 0.1$	-	$0.1^{a} \pm 0.1$
	HSA_0	ND	$0.1^{a} \pm 0.1$	-	ND
15	H	$0.1^{a} \pm 0.1$	$0.1^{a} \pm 0.1$	$0.2^{a} \pm 0.1$	$0.1^{a} \pm 0.0$
	HSA_0	ND	$0.1^{a} \pm 0.1$	$0.2^{a} \pm 0.0$	ND
20	H	$0.3^{ab} \pm 0.1$	$0.1^{a} \pm 0.1$	$0.2^{a} \pm 0.1$	$0.1^{a} \pm 0.0$
	HSA_0	$0.3^{b} \pm 0.0$	$0.2^{a} \pm 0.1$	$0.2^{a} \pm 0.1$	$0.1^{a} \pm 0.1$
	HSA_{15}	$0.2^{a} \pm 0.0$	$0.2^{a} \pm 0.0$	$0.2^{a} \pm 0.0$	ND
25	H	$0.3^{b} \pm 0.0$	$0.2^{a} \pm 0.1$	$0.2^{a} \pm 0.1$	$0.1^{a} \pm 0.0$
	HSA_0	$0.3^{b} \pm 0.0$	$0.4^{a} \pm 0.1$	$0.2^{a} \pm 0.1$	$0.1^{ab} \pm 0.1$
	HSA_{15}	$0.3^{b} \pm 0.0$	$0.4^{a} \pm 0.0$	$0.4^{a} \pm 0.1$	$0.2^{b} \pm 0.0$
	HSA_{20}	$0.2^{a} \pm 0.0$	$0.4^{a} \pm 0.1$	$0.3^{a} \pm 0.0$	$0.1^{a} \pm 0.0$
30	H	$0.6^{ab} \pm 0.1$	$0.3^{a} \pm 0.0$	$1.0^{b} \pm 0.3$	$0.1^{a} \pm 0.0$
	HSA_0	$0.7^{ab} \pm 0.2$	$0.5^{abc} \pm 0.2$	$0.6^{ab} \pm 0.2$	$0.1^{a} \pm 0.0$
	HSA_{15}	$0.6^{ab} \pm 0.1$	$0.4^{b} \pm 0.0$	$0.4^{a} \pm 0.0$	$0.2^{b} \pm 0.0$
	HSA_{20}	$0.5^{a} \pm 0.0$	$0.6^{c} \pm 0.1$	$0.8^{b} \pm 0.1$	$0.1^{a} \pm 0.0$
	HSA_{25}	$0.6^{b} \pm 0.0$	$0.5^{c} \pm 0.0$	$0.9^{a} \pm 0.1$	$0.1^{a} \pm 0.0$

H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; HSA_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; HSA_{15} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 15th day; HSA_{20} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 20th day; HSA_{25} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 25th day. The differences are compared for each treatment. Values followed by the same letter are not significantly different. ND- not determined.

Until 20th day no **gluconapoleiferin** was measured in H leaves. Treatment with SA_0 led to appearing of this glucosinolate in leaves on 20th day (Table 4.42), but 25 days after treatment it was increased especially in leaves in contrast to progoitrin and gluconapin.

Also **glucobrassicanapin** until 20th day was found only in H, but not in HSA_0 leaves (Table 4.43). Also application of SA on 15th day led to decrease of this glucosinolate in leaves; however, in HSA_{15} as well as HSA_{20} secondary roots glucobrassicanapin was higher as in H secondary roots. The transporting of glucosinolate from leaves into secondary roots could cause this.

The ratio of butenyl to pentenyl glucosinolates was higher for all parts of H plants, as for these treated with SA (Table 4.44). This ratio was nearly the same for H leaves, secondary and primary roots (2, 2, and 2.1, respectively). Earlier treated plants had lower ratio of butenyl to pentenyl glucosinolates. For example, in HSA_0 leaves it became 0.8, while in HSA_{15} leaves 1.1, in HSA_{20} 1.9, but for HSA_{20} leaves it was lower as for HSA_{20} and reached 1.3. However, HSA_{20} secondary and primary roots had higher ratio of butenyl to pentenyl glucosinolates as other treated with SA plants.

As it was mentioned in previous chapter, decrease of the ratio of butenyl to pentenyl glucosinolates could be explained by changing the expression of *Gls-elong* genes (Giamoustaris and Mithen, 1996) under the influence of SA and depending of this expression on ARR.

Table 4.43. Influence of salicylic acid applied on different stages of plant growth on glucobrassicanapin content in plants and exudates of turnip from hydroponic (mg plant^{-1})

Days	Treatments	Leaves	Secondary roots	Primary roots	Exudates
10	H	$0.1^{a} \pm 0.1$	ND	-	ND
	HSA_0	ND	ND	-	ND
15	H	$0.1^{a} \pm 0.0$	ND	$0.2^{a} \pm 0.0$	ND
	HSA_0	ND	ND	$0.2^{a} \pm 0.0$	ND
20	H	$0.2^{a} \pm 0.1$	ND	$0.4^{b} \pm 0.1$	ND
	HSA_0	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.1$	$0.2^{a} \pm 0.1$	ND
	HSA_{15}	$0.1^{b} \pm 0.0$	$0.2^{a} \pm 0.0$	$0.5^{a} \pm 0.0$	$0.1^{a} \pm 0.0$
25	H	$0.2^{a} \pm 0.1$	$0.1^{a} \pm 0.1$	$0.7^{a} \pm 0.2$	$0.1^{a} \pm 0.0$
	HSA_0	$0.3^{a} \pm 0.2$	$0.1^{a} \pm 0.1$	$0.8^{a} \pm 0.1$	ND
	HSA_{15}	$0.1^{b} \pm 0.0$	$0.2^{b} \pm 0.0$	$0.9^{a} \pm 0.1$	$0.2^{a} \pm 0.0$
	HSA_{20}	$0.2^{b} \pm 0.0$	$0.2^{a} \pm 0.1$	$0.5^{a} \pm 0.0$	$0.2^{a} \pm 0.0$
30	H	$0.2^{a} \pm 0.1$	$0.1^{a} \pm 0.0$	$1.1^{a} \pm 0.3$	$0.2^{a} \pm 0.1$
	HSA_0	$0.3^{a} \pm 0.0$	$0.2^{a} \pm 0.1$	$0.8^{a} \pm 0.2$	$0.1^{a} \pm 0.0$
	HSA_{15}	$0.3^{b} \pm 0.0$	$0.3^{b} \pm 0.0$	$0.5^{a} \pm 0.0$	$0.1^{a} \pm 0.0$
	HSA_{20}	$0.2^{b} \pm 0.1$	$0.4^{b} \pm 0.0$	$0.9^{a} \pm 0.1$	$0.1^{a} \pm 0.0$
	HSA_{25}	$0.2^{b} \pm 0.0$	$0.1^{b} \pm 0.0$	$1.0^{a} \pm 0.1$	$0.2^{a} \pm 0.0$

H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; HSA_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; HSA_{15} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 15th day; HSA_{20} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 20th day; HSA_{25} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 25th day. The differences are compared for each treatment. Values followed by the same letter are not significantly different. ND- not determined.

In exudates this ratio decreased only for HSA_{25} as compared to H, was slightly influenced for HSA_0 and HSA_{20} and in contrast to plants decreased for HSA_{15} exudates. Increase of butenyl to pentenyl ratio in plants and decrease in exudates after SA application is possible to explain by selective SA influence on exudation of glucosinolates: preferably exuded were gluconapin and progoitrin. This may be connected with the role of these individual glucosinolates in defense reaction on pathogens, whose invasion is mimicked by SA (Lee *et al*., 1995).

The ratio of hydroxyalkenyl to alkenyl glucosinolates for plants and exudates also changed under the influence of SA applied on all stages. For H and HSA_{15} leaves it reached 1.7 and was increased for HSA_0 and HSA_{20}, the ratio in primary roots correlated with that in leaves. In secondary roots and exudates (except these in HSA_{15}) the ratio of hydroxyalkenyl to alkenyl glucosinolates decreased under SA influence. Hydroxylation of pentenyl and bytenyl glucosinolates was regulated by the same *Gls-oh* genes (Magrath *et al.*, 1994). Probably, SA influence on down-regulation of *Gls-oh* genes is dynamical process and change during the post-treatment period.

Table 4.44. Influence of salicylic acid applied on different stages of plant growth on correlation between the subclasses of aliphatic glucosinolates in plants and exudates of turnip

Butenyls : pentenyls**					
Treatment	Correlation/ content	Leaves	Secondary roots	Primary roots	Exudates
H	n : 1	1.9 : 1	1 : 1	1.2 : 1	1.1 : 1
	mg plant^{-1}	1.5 : 0.8	0.7 : 0.7	4.5 : 3.9	1.0 : 0.9*
HSA_0	n : 1	0.8 : 1	0.9 : 1	0.9 : 1	1 : 1
	mg plant^{-1}	0.8 : 1.0	0.6 : 0.7	1.8 : 2.0	0.5 : 0.5*
HSA_{15}	n : 1	1.1 : 1	1.7 : 1	1.3 : 1	1.5 : 1
	mg plant^{-1}	1.0 : 0.9	1.2 : 0.7	1.2 : 0.9	1.6 :1.1*
HSA_{20}	n : 1	1.9 : 1	1.1 : 1	1.2 : 1	1.1 : 1
	mg plant^{-1}	1.3 : 0.7	1.1 : 1.0	2.0 : 1.7	1.4 : 1.3*
HSA_{25}	n : 1	1.3 : 1	1.8 : 1	1.1 : 1	0.8 : 1
	mg plant^{-1}	1.0 : 0.8	1.1 : 0.6	3.2 : 1.9	1.0 : 1.2*
Hydroxyalkenyls : alkenyls***					
H	n : 1	1.6 : 1	4.5 : 1	1.4 : 1	1.4 : 1
	mg plant^{-1}	1.4 : 0.9	0.9 : 0.2	3.8 : 2.8	1.1 : 0.8*
HSA_0	n : 1	2.0 : 1	3.3 : 1	1.3 : 1	3.5 : 1
	mg plant^{-1}	1.2 : 0.6	1.0 : 0.3	1.8 : 1.4	0.7 : 0.2*
HSA_{15}	n : 1	1.7 : 1	1.7 : 1	0.9 : 1	1.5 : 1
	mg plant^{-1}	1.2 : 0.9	1.2 : 0.7	1.0 : 1.1	1.6 :1.1*
HSA_{20}	n : 1	1.9 : 1	1.6 : 1	0.9 : 1	1.3 : 1
	mg plant^{-1}	1.5 : 0.7	1.3 : 1.0	1.7 : 2.0	1.5 : 1.2*
HSA_{25}	n : 1	1.3 : 1	2.4 : 1	1.2 : 1	0.8 : 1
	mg plant^{-1}	2.0 : 0.8	1.2 : 0.6	2.8 : 2.3	1.0 : 1.1*

* butenyls - gluconapin + progoitrin; pentenyls – glucobrassicanapin + gluconapoleiferin.
**hydroxyalkenyls - progoitrin + gluconapoleiferin, alkenyls - glucobrassicanapin + gluconapin.
H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; HSA_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; HSA_{15} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 15th day; HSA_{20} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 20th day; HSA_{25} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 25th day. The differences are compared for each treatment. Values followed by the same letter are not significantly different. *Sum of glucosinolates exuded during 30 days.

4.3.2.2 Aromatic glucosinolate (gluconasturtiin)

The content of aromatic gluconasturtiin in leaves was much lower as the content of aliphatic or indole glucosinolates and on 30th day it disappeared. Treatment with SA prevented the disappearance of glucosinolate: for HSA_0 leaves it became 0.4 mg plant^{-1} on 30th day, while the increment was much more essential in leaves of plants, treated on 15th, 20th or 25th days (Table 4.45). Already 5 days after treatment HSA_{15} leaves had 1.0 mg plant^{-1} of gluconasturtiin and on 30th day it reached its maximum with 1.3 mg plant^{-1}. The highest content was in HSA_{20} leaves on 10th after treatment day: 2.0 mg plant^{-1}. Probably it could continue to increase if the plant growth in system would be prolonged. It seems that the

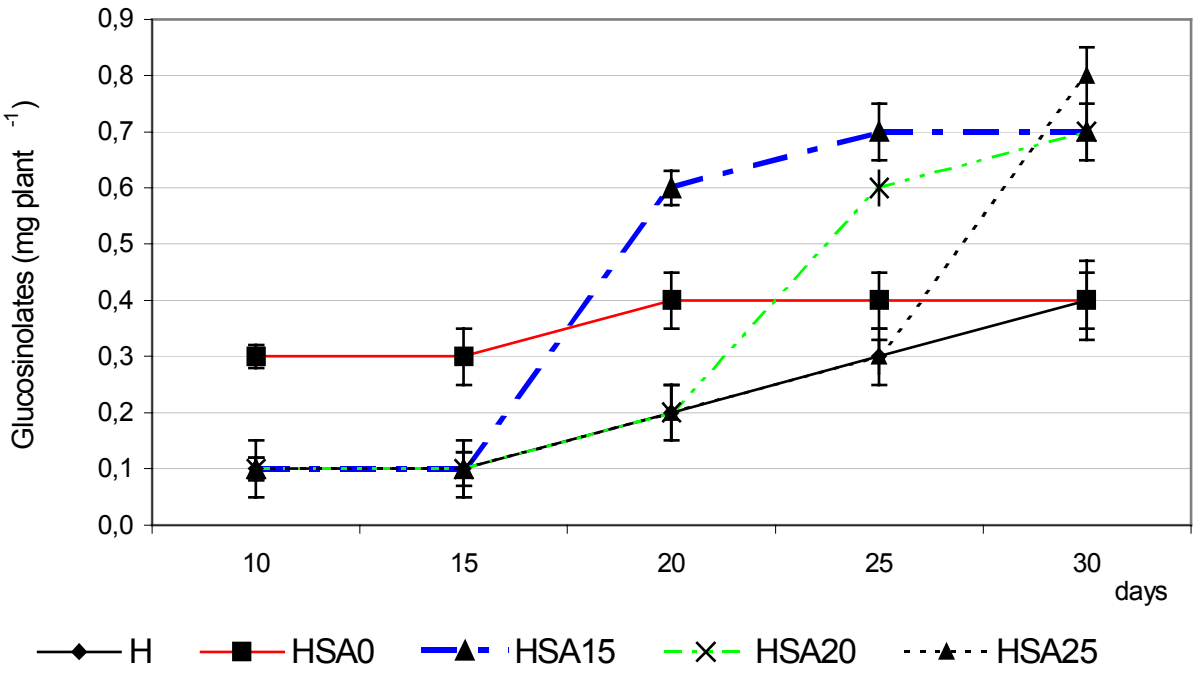
induction of aromatic glucosinolate synthesis after SA application is maximally expressed 15-25 days after the treatment.
Increase of aromatic glucosinolates content after SA application could be explained by induction of genes, involved in the conversion of phenylalanine to aromatic aldoximes, particularly *CYP79A2*. It is equally expressed in leaves and roots (Mikkelsen *et al*., 2003), as well as content of gluconasturiin increased in all parts of plants.
The changes in gluconasturtiin content in secondary roots under the SA treatments corresponded to these in other plant parts. For H it increased gradually to 1.2 mg plant^{-1} on 30th day. The highest content of gluconasturtiin for HSA_0 secondary roots was 2.6 mg plant^{-1} on 25th day, on 30th it reduced to 2.3 mg plant^{-1}, while for HSA_{20} it increased at this time from 1.5 to 2.3 mg plant^{-1}. The maximal gluconasturtiin content was for HSA_{15} on 30th day 2.9 mg plant^{-1}.

Table 4.45. Influence of salicylic acid on gluconasturtiin content in plants and exudates of turnip from hydroponic (mg plant^{-1})

Date	Treatment	Leaves	Secondary roots	Primary roots	Exudates
10 days	H	$0.1^{a} \pm 0.1$	$0.2^{a} \pm 0.0$		$0.1^{a} \pm 0.0$
	HSA_0	$0.3^{b} \pm 0.0$	$0.5^{b} \pm 0.1$		$0.3^{b} \pm 0.0$
15 days	H	$0.1^{a} \pm 0.1$	$0.3^{a} \pm 0.1$	$1.6^{a} \pm 0.1$	$0.1^{a} \pm 0.0$
	HSA_0	$0.5^{b} \pm 0.1$	$0.6^{b} \pm 0.1$	$1.5^{a} \pm 0.1$	$0.3^{b} \pm 0.1$
20 days	H	$0.2^{a} \pm 0.2$	$0.9^{a} \pm 0.2$	$1.9^{a} \pm 0.1$	$0.2^{a} \pm 0.1$
	HSA_0	$0.2^{a} \pm 0.1$	$1.6^{c} \pm 0.1$	$2.0^{a} \pm 0.1$	$0.4^{a} \pm 0.1$
	HSA_{15}	$1.0^{a} \pm 0.0$	$1.3^{b} \pm 0.1$	$1.8^{a} \pm 0.1$	$0.6^{b} \pm 0.0$
25 days	H	$0.1^{a} \pm 0.1$	$1.3^{a} \pm 0.2$	$2.2^{a} \pm 0.4$	$0.3^{a} \pm 0.1$
	HSA_0	$0.3^{a} \pm 0.2$	$2.6^{c} \pm 0.3$	$3.5^{b} \pm 0.2$	$0.4^{a} \pm 0.1$
	HSA_{15}	$0.6^{b} \pm 0.0$	$1.7^{b} \pm 0.1$	$2.4^{a} \pm 0.2$	$0.7^{b} \pm 0.1$
	HSA_{20}	$0.1^{a} \pm 0.0$	$1.5^{ab} \pm 0.1$	$2.2^{a} \pm 0.1$	$0.6^{b} \pm 0.0$
30 days	H	ND	$1.2^{a} \pm 0.4$	$0.7^{a} \pm 0.2$	$0.4^{a} \pm 0.1$
	HSA_0	$0.4^{a} \pm 0.2$	$2.3^{b} \pm 0.4$	$3.4^{d} \pm 0.3$	$0.4^{a} \pm 0.1$
	HSA_{15}	$1.3^{c} \pm 0.2$	$2.9^{c} \pm 0.1$	$2.3^{c} \pm 0.2$	$0.7^{b} \pm 0.1$
	HSA_{20}	$0.9^{b} \pm 0.1$	$2.3^{b} \pm 0.3$	$1.3^{b} \pm 0.0$	$0.7^{b} \pm 0.2$
	HSA_{25}	$2.0^{d} \pm 0.2$	$1.8^{a} \pm 0.2$	$0.9^{a} \pm 0.1$	$0.8^{b} \pm 0.1$
Total	H				$1.2^{a} \pm 0.3$*
	HSA_0				$1.8^{abc} \pm 0.4$*
	HSA_{15}				$2.2^{c} \pm 0.3$*
	HSA_{20}				$1.7^{b} \pm 0.1$*
	HSA_{25}				$1.5^{ab} \pm 0.1$*

H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; HSA_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; HSA_{15} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 15th day; HSA_{20} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 20th day; HSA_{25} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 25th day. The differences are compared for each treatment. Values followed by the same letter are not significantly different. *Sum of glucosinolates exuded during 30 days.

The content of gluconasturtiin for H primary roots was maximal on 25th day with 2.2 mg plant^{-1}, but the next 5 days decreased rapidly until 0.7 mg plant^{-1}. The most essential increase of glucosinolate in HSA_{15} primary roots was on 25th day, it reached 3.5 mg plant^{-1}.
It is also important to admit, that in primary roots of HSA_{20} and HSA_{25} plants the reducing of gluconasturtiin content was also measured, as for the primary roots of H plants.
Gluconasturtiin content in H exudates gradually increased from 0.1 mg plant^{-1} on 10th day to 0.4 mg plant^{-1} on 30th day (Table 4.26). Application of SA_0 increased the content of aromatic glucosinolate in exudates and the maximal effect was measured on 20th day (0.5 mg plant^{-1}), than it stayed on the same level to the end of experiment. Also for HSA_{15} the increment of gluconasturtiin was measured already 5 days after treatment and also became stable until 30th day. The same tendency was measured also for HSA_{20}.
In general, SA increased the total amount of exuded during 30 days gluconasturtiin for HSA_0 to 1.8 mg plant^{-1} (1.5-time more as for H), for HSA_{15} to 2.2 mg plant^{-1} (2-times more), for HSA_{20} to 1.7 mg plant^{-1} (1.5-time more), and for HSA_{15} to 1.5 mg plant^{-1} (1.4-time more) (Table 4.17).



H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; HSA_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; HSA_{15} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 15th day; HSA_{20} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 20th day; HSA_{25} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 25th day.

Figure 4.16. Influence of salicylic acid on kinetic of aromatic glucosinolate content in exudates of turnip from hydroponic

4.3.2.3 Indole glucosinolates in plants and exudates

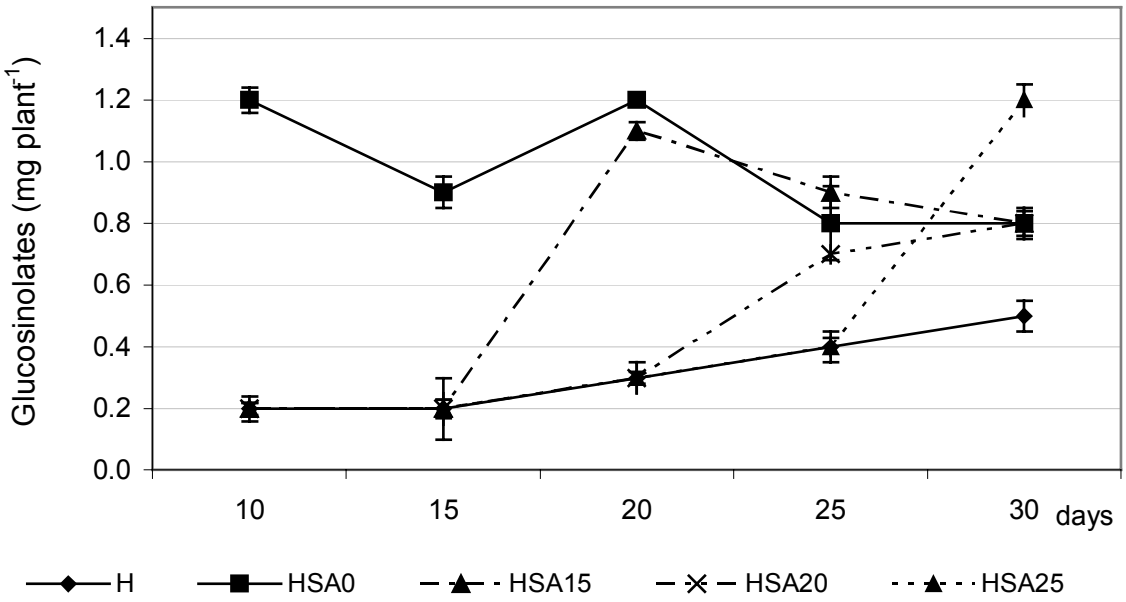
Treatment with SA increased the content of indole glucosinolates in leaves from all systems and this effect continued until the end of experiment. The content of indole glucosinolates in H leaves increased from 0.6 mg plant^{-1} on 10th day until 1.3 mg plant^{-1} on 30th day (Table 4.46). 5 days after treatment HSA_{15} leaves contented 2.9 mg plant^{-1} of indole glucosinolates (4.1-times more as for H) and on 30th day it reached 3.2 mg plant^{-1} (2.5-times more as for H). The highest increase of indole glucosinolates was measured for HSA_{20}: on 30th day it reached 3.8 mg plant^{-1} (2.9-times more as for H), which means that maximal answer in leaves on SA treatment happened on 5-10th day after elicitor application.

Table 4.46. Influence of salicylic acid applied on different stages of plant growth on indole glucosinolate content in plants and exudates of turnip from hydroponic (mg plant^{-1})

	Treatment	Leaves	Secondary roots	Primary roots	Exudates
10 days	H	$0.6^{a} \pm 0.1$	$0.2^{a} \pm 0.0$		$0.2^{a} \pm 0.0$
	HSA_0	$1.6^{b} \pm 0.1$	$0.6^{b} \pm 0.1$		$1.2^{b} \pm 0.0$
15 days	H	$0.3^{a} \pm 0.0$	$0.4^{a} \pm 0.1$	$0.8^{a} \pm 0.0$	$0.2^{a} \pm 0.1$
	HSA_0	$1.7^{b} \pm 0.1$	$0.7^{a} \pm 0.2$	$0.6^{a} \pm 0.1$	$0.9^{b} \pm 0.1$
20 days	H	$0.7^{a} \pm 0.1$	$1.8^{a} \pm 0.1$	$1.3^{b} \pm 0.1$	$0.3^{a} \pm 0.0$
	HSA_0	$1.5^{b} \pm 0.1$	$2.4^{b} \pm 0.1$	$1.0^{a} \pm 0.1$	$1.2^{b} \pm 0.0$
	HSA_{15}	$2.0^{c} \pm 0.4$	$3.6^{c} \pm 0.5$	$1.0^{b} \pm 0.0$	$1.1^{b} \pm 0.1$
25 days	H	$1.1^{a} \pm 0.0$	$2.2^{a} \pm 0.1$	$1.9^{a} \pm 0.3$	$0.4^{a} \pm 0.1$
	HSA_0	$2.0^{b} \pm 0.1$	$3.6^{b} \pm 0.2$	$1.8^{a} \pm 0.2$	$0.8^{b} \pm 0.1$
	HSA_{15}	$2.4^{b} \pm 0.3$	$3.6^{d} \pm 0.7$	$2.1^{a} \pm 0.1$	$0.9^{b} \pm 0.1$
	HSA_{20}	$2.0^{b} \pm 0.1$	$3.1^{c} \pm 0.2$	$1.9^{a} \pm 0.2$	$0.7^{b} \pm 0.1$
30 days	H	$1.3^{a} \pm 0.1$	$2.3^{a} \pm 0.2$	$1.4^{a} \pm 0.2$	$0.5^{a} \pm 0.1$
	HSA_0	$1.9^{b} \pm 0.1$	$6.3^{d} \pm 0.5$	$1.2^{a} \pm 0.2$	$0.8^{b} \pm 0.0$
	HSA_{15}	$2.6^{c} \pm 0.1$	$5.0^{c} \pm 0.2$	$3.1^{c} \pm 0.4$	$0.8^{b} \pm 0.1$
	HSA_{20}	$3.8^{c} \pm 0.5$	$3.8^{b} \pm 0.3$	$1.7^{b} \pm 0.0$	$0.8^{b} \pm 0.1$
	HSA_{25}	$3.3^{c} \pm 0.4$	$5.1^{c} \pm 0.3$	$1.4^{a} \pm 0.2$	$1.2^{c} \pm 0.1$
Total	H				$1.6^{a} \pm 0.3$*
	HSA_0				$5.0^{d} \pm 0.5$*
	HSA_{15}				$3.5^{c} \pm 0.2$*
	HSA_{20}				$2.7^{b} \pm 0.3$*
	HSA_{25}				$2.4^{b} \pm 0.1$*

H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; HSA_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; HSA_{15} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 15th day; HSA_{20} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 20th day; HSA_{25} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 25th day. The differences are compared for each treatment. Values followed by the same letter are not significantly different. *Sum of glucosinolates exuded during 30 days

The content of indole glucosinolates in secondary roots was higher as in primary roots and leaves. During the experiment in H secondary roots it increased from 0.2 mg plant^{-1} on 10th day until 2.3 mg plant^{-1} on 30th day (Table 4.46). On 30th day HSA_0 secondary roots contented 6.3 mg plant^{-1} of indole glucosinolates, which was 1.3 mg plant^{-1} more as for HSA_{15}, 2.5 mg plant^{-1} more as for HSA_{20}, and 1.2 mg plant^{-1} more as for HSA_{25}. The highest accumulation of indole glucosinolates in secondary roots was measured 30 days after SA application.
Indole glucosinolate content was not strongly increased in primary roots after SA treatments. Only in HSA_{15} it was measured the enhancement of indole glucosinolates until 3.4 mg plant^{-1}, which was 2.4 times more as for H (Table 4.46). *CYP83B1* responsible for converting indole aldoximes to indolethiohydroximates is regulated by SA and is expressed equally in leaves and roots of plants Glombitza *et al*. (2003). This explains the increase of indole glucosinolates in secondary and primary roots and exudates after SA application.
Indole glucosinolate content in exudates from H gradually increased from 0.2 mg plant^{-1} on 10th day until 0.5 mg plant^{-1} on 30th day (Figure 4.18). After SA_0 application the content of indole glucosinolates increased essentially and on 10th day it reached 1.2 mg plant^{-1} (6-times more as for H). Interesting, that in contrast to aliphatic and aromatic, the content of exuded indole glucosinolates for HSA_0 did not became lower as for H on 30th day and was 0.8 mg plant^{-1}. On 5th day after the treatment in HSA_{15} exudates indole glucosinolate content reached 1.1 mg plant^{-1} (3.7 times more as for H) and kept on the same level until 30th day. The highest content of indole glucosinolates in exudates on 30th day was for HSA_{20} and HSA_{25}.



H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; HSA_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; HSA_{15} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 15th day; HSA_{20} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 20th day; HSA_{25} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 25th day.

Figure 4.17. Influence of salicylic acid applied on different stages of plant growth on the kinetic of indole glucosinolates in exudates of turnip grown in hydroponic

Individual indole glucosinolates

Glucobrassicin content in leaves increased under SA treatment: on 10th day HSA_0 leaves accumulated 5 mg plant^{-1} of glucosinolate, which was 5 fold more as for leaves of untreated plants, but later for HSA_0 leaves it decreased to the end of the experiment nearly to the control level. Maximal increase of glucobrassicin in leaves was measured for HSA_{20} and HSA_{25} leaves on 30th day 0.9 and 0.8 mg plant^{-1} (9 and 8-folds more as for H, respectively) (Table 4.47). After SA treatment, glucobrassicin content in secondary roots increased gradually during the experiment and reached its maximum on 30th day for all treatments: 2.2 mg plant^{-1} for HSA_0 (7.3 fold more as for H), 1.4 mg plant^{-1} for HSA_{15} (4.7 fold more), 1.1 mg plant^{-1} for HSA_{20} (3.7 fold more), and 1.3 mg plant^{-1} for HSA_{25} (4.3 fold more).

Table 4.47. Influence of salicylic acid applied on different stages of plant growth on glucobrassicin content in plants and exudates of turnip from hydroponic (mg plant^{-1})

Days	Treatments	Leaves	Secondary roots	Primary roots	Exudates
10	H	$0.1^{a} \pm 0.0$	ND	-	ND
	HSA_0	$0.5^{b} \pm 0.1$	$0.2^{a} \pm 0.0$	-	$0.3^{a} \pm 0.1$
15	H	$0.2^{a} \pm 0.0$	$0.2^{a} \pm 0.1$	$0.2^{a} \pm 0.0$	$0.2^{a} \pm 0.1$
	HSA_0	$0.4^{b} \pm 0.1$	$0.2^{a} \pm 0.0$	$0.2^{a} \pm 0.1$	$0.2^{a} \pm 0.0$
20	H	$0.2^{a} \pm 0.1$	$0.3^{a} \pm 0.1$	$0.3^{a} \pm 0.1$	$0.1^{a} \pm 0.1$
	HSA_0	$0.4^{b} \pm 0.0$	$0.8^{b} \pm 0.1$	$0.3^{a} \pm 0.1$	$0.1^{a} \pm 0.0$
	HSA_{15}	$0.5^{c} \pm 0.0$	$0.9^{b} \pm 0.1$	$0.2^{a} \pm 0.0$	$0.3^{a} \pm 0.0$
25	H	$0.3^{a} \pm 0.1$	$0.2^{a} \pm 0.1$	$0.4^{a} \pm 0.2$	$0.1^{a} \pm 0.0$
	HSA_0	$0.4^{a} \pm 0.2$	$1.5^{d} \pm 0.1$	$0.3^{a} \pm 0.1$	$0.1^{a} \pm 0.1$
	HSA_{15}	$0.4^{a} \pm 0.1$	$1.1^{c} \pm 0.1$	$0.3^{a} \pm 0.0$	$0.3^{b} \pm 0.0$
	HSA_{20}	$0.8^{b} \pm 0.1$	$0.5^{b} \pm 0.0$	$0.3^{a} \pm 0.1$	$0.3^{b} \pm 0.0$
30	H	$0.1^{a} \pm 0.1$	$0.3^{a} \pm 0.0$	$0.4^{a} \pm 0.2$	$0.1^{ab} \pm 0.1$
	HSA_0	$0.1^{a} \pm 0.0$	$2.2^{d} \pm 0.4$	$0.3^{a} \pm 0.1$	$0.2^{b} \pm 0.0$
	HSA_{15}	$0.5^{b} \pm 0.1$	$1.4^{c} \pm 0.1$	$0.8^{b} \pm 0.1$	$0.1^{a} \pm 0.0$
	HSA_{20}	$0.9^{c} \pm 0.1$	$1.1^{b} \pm 0.1$	$0.3^{a} \pm 0.0$	$0.1^{a} \pm 0.0$
	HSA_{25}	$0.8^{bc} \pm 0.2$	$1.3^{bc} \pm 0.3$	$0.3^{a} \pm 0.1$	$0.2^{ab} \pm 0.1$
Total	H				$0.4^{a} \pm 0.1$*
	HSA_0				$0.7^{ab} \pm 0.2$*
	HSA_{15}				$0.8^{b} \pm 0.1$*
	HSA_{20}				$0.8^{b} \pm 0.2$*
	HSA_{25}				$0.5^{a} \pm 0.2$*

H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; HSA_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; HSA_{15} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 15th day; HSA_{20} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 20th day; HSA_{25} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 25th day. The differences are compared for each treatment. Values followed by the same letter are not significantly different. ND- not determined. * Sum of glucosinolates exuded during 30 days

It was not influenced in HSA_0 primary roots as compared to H, in HSA_{15} primary roots it started to increase on 25th day of the experiment and reached 0.8 mg plant^{-1} on 30th day. However, HSA_{15} was the only treatment, were the increase of glucobrassicin was observed for primary roots. The maximum of glucobrassicin was exuded into solution right after treatment with elicitor, and then its content in exudates decreased until the control level. On 10th day no glucobrassicin was measured for H exudates, while for HSA_0 it was 0.3 mg plant^{-1}, but on 20th day in both systems plants exuded 0.1 mg plant^{-1} of glucobrassicin. On 20th day HSA_{15} plants exuded 0.3 mg plant^{-1} of this glucosinolate, while on 30th day it reduced to 0.1. Also HSA_{20} plants exude on 25th day 0.3 mg plant^{-1} of glucobrassicin and on 30th day it was measured the decrease of this glucosinolate in exudates until 0.2.
At all stages of this experiment as well as for previous experiments the majority of indole glucosinolates in plants exudates was represented by neoglucobrassicin (Table 4.48).

Table 4.48. Influence of salicylic acid applied on different stages of plant growth on neoglucobrassicin content in plants and exudates of turnip from hydroponic (mg plant^{-1})

Days	Treatments	Leaves	Secondary roots	Primary roots	Exudates
10	H	$0.3^{a} \pm 0.1$	$0.2^{a} \pm 0.1$	-	$0.1^{a} \pm 0.1$
	HSA_0	$0.7^{b} \pm 0.2$	$0.4^{b} \pm 0.0$	-	$0.6^{b} \pm 0.2$
15	H	$0.3^{a} \pm 0.1$	$0.2^{a} \pm 0.1$	$0.4^{a} \pm 0.1$	$0.1^{a} \pm 0.1$
	HSA_0	$0.6^{b} \pm 0.1$	$0.5^{b} \pm 0.1$	$0.3^{a} \pm 0.0$	$0.8^{c} \pm 0.2$
20	H	$0.3^{a} \pm 0.1$	$1.0^{a} \pm 0.1$	$0.4^{a} \pm 0.2$	$0.2^{a} \pm 0.0$
	HSA_0	$0.8^{b} \pm 0.2$	$1.2^{b} \pm 0.2$	$0.4^{a} \pm 0.1$	$0.9^{b} \pm 0.2$
	HSA_{15}	$1.0^{b} \pm 0.1$	$1.9^{c} \pm 0.2$	$0.2^{a} \pm 0.1$	$0.6^{b} \pm 0.1$
25	H	$0.3^{a} \pm 0.1$	$1.6^{a} \pm 0.2$	$0.6^{b} \pm 0.1$	$0.1^{a} \pm 0.0$
	HSA_0	$0.9^{b} \pm 0.2$	$1.6^{a} \pm 0.3$	$0.4^{ab} \pm 0.1$	$0.6^{b} \pm 0.2$
	HSA_{15}	$1.1^{c} \pm 0.1$	$1.7^{c} \pm 0.1$	$0.6^{a} \pm 0.0$	$0.6^{b} \pm 0.1$
	HSA_{20}	$0.8^{b} \pm 0.1$	$1.4^{c} \pm 0.2$	$0.6^{a} \pm 0.1$	$0.3^{b} \pm 0.0$
30	H	$0.5^{a} \pm 0.1$	$1.7^{b} \pm 0.2$	$0.7^{b} \pm 0.1$	$0.2^{a} \pm 0.1$
	HSA_0	$0.8^{ab} \pm 0.2$	$2.3^{c} \pm 0.4$	$0.4^{a} \pm 0.1$	$0.4^{b} \pm 0.0$
	HSA_{15}	$1.4^{b} \pm 0.2$	$2.1^{c} \pm 0.1$	$1.0^{c} \pm 0.1$	$0.4^{ab} \pm 0.1$
	HSA_{20}	$1.1^{b} \pm 0.1$	$1.3^{a} \pm 0.1$	$0.6^{b} \pm 0.1$	$0.4^{b} \pm 0.0$
	HSA_{15}	$0.8^{ab} \pm 0.1$	$1.4^{ab} \pm 0.2$	$0.4^{a} \pm 0.0$	$0.3^{ab} \pm 0.0$
Total	H				$0.7^{a} \pm 0.1$*
	HSA_0				$3.4^{d} \pm 0.4$*
	HSA_{15}				$1.8^{c} \pm 0.2$*
	HSA_{20}				$1.1^{b} \pm 0.2$*
	HSA_{15}				$0.8^{ab} \pm 0.2$*

H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; HSA_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; HSA_{15} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 15th day; HSA_{20} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 20th day; HSA_{25} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 25th day. The differences are compared for each treatment. Values followed by the same letter are not significantly different. ND- not determined. * Sum of glucosinolates exuded during 30 days

Its content was strongly influenced in leaves after SA treatment. On 10th day HSA_0 leaves had 0.7 mg plant^{-1} of glucosinolate (2.5 fold more as for H), which decreased until 0.9 mg plant^{-1} on 25th day (3 fold more as for H) and decreased to the end of the experiment. Probably the maximal accumulation of neoglucobrassicin in leaves occurs in leaves 25 days after SA application, because the decrease of this glucosinolate in leaves of plants from later treatments was not observed: on 30th day they reached their maximum for HSA_{15} 1.4 mg plant^{-1}, while for HSA_0 it became 1.1 mg plant^{-1}, and for HSA_{25} 0.8 mg plant^{-1}.

In secondary roots the increase of neoglucobrassicin content was measured after the treatment, and then it decreased, which probably may be explained by very intensive exudation of this glucosinolate. In HSA_0 secondary roots until 20th day its content was higher as for the secondary roots of non treated plants, then it reached the same level, but the next 5 days its content was 0.6 mg plant^{-1} higher as for H, which could be explained as slowing down of its exuding and transporting from leaves and secondary roots (where its content decreased at this time). Also HSA_{15} secondary roots became the increase in neoglucobrassicin on 20th day of the experiment until 1.9 mg plant^{-1}, which the next 5 days was decreased to 1.7 mg plant^{-1} and on 30th day again increased. Neoglucobrassicin was the glucosinolate, which exudation was more as others stimulated by SA. Already 10 days after the treatment HSA_0 plants exuded 0.6 mg plant^{-1} of neoglucobrassicin (6 fold more as H plants), the intensity of exudation reached maximum on 20th day and the decrease to 0.4 mg plant^{-1} (2 fold more as for H). For HSA_{15} the maximum of exuded neoglucobrassicin was measured 10 days after treatment (0.6 mg plant^{-1}). The later plants were treated with SA, the smaller was the peak of neoglucobrassicin exudation: HSA_{20} plants on 25th day exuded 0.3 mg plant^{-1} of glucosinolate (3 fold more as H plants). The highest content of neoglucobrassicin exuded during 30 days was received in HSA_0 3.4 mg plant^{-1}, while in HSA_{15} 1.8 mg plant^{-1}, in HSA_{20} 1.1 mg plant^{-1}, and in HSA_{25} 0.8 mg plant^{-1}, which was nearly the same as for the control.

During first 10 days of the experiment **4-hydroxy-glucobrassicin** was measured only in leaves of treated as well as untreated with elicitor plants (Table 4.49).

On 20th day the simulative effect of SA application on 4-hydroxy-glucobrassicin was measured for HSA_0 and HSA_{15} leaves. However, the highest increase of 4-hydroxy-glucobrassicin was admitted for HSA_{20} and HSA_{25} leaves, which probably could be connected with changes of induction of glucosinolate synthesis in leaves during the plant growth or with transportation of glucosinolate form leaves, because HSA_{15} leaves had lower content of 4-hydroxy-glucobrassicin on 30th day as H leaves, but in primary roots it was higher as for all other treatments. Application of SA on 20th and 25th day caused the strong increase of this glucosinolate in leaves, and for HSA_{25} in secondary roots, and probably this caused the increase of 4-hydroxy-glucobrassicin in exudates only for this system (Table 4.50). In general, non treated plants exuded 0.1 mg plant^{-1} during the whole experiment, and it was only during the last 5 days, the maximal content was in HSA_{25} exudates 0.3 mg plant^{-1}, which was also exuded at the end of the experiment.

Very small content of **4-methoxy-glucobrassicin** was detected in leaves and primary roots of untreated plants although SA_{15} application led to accumulation of 0.6 and 0.7 mg plant^{-1} (Table 4.50).

Table 4.49. Influence of salicylic acid applied on different stages of plant growth on 4-hydroxy-glucobrassicin content in plants and exudates of turnip from hydroponic (mg plant^{-1})

Days	Treatments	Leaves	Secondary roots	Primary roots	Exudates
10	H	$0.2^{ab} \pm 0.1$	ND	-	ND
	HSA_0	$0.4^{b} \pm 0.1$	ND	-	ND
15	H	$0.2^{a} \pm 0.0$	ND	$0.2^{a} \pm 0.0$	ND
	HSA_0	$0.6^{b} \pm 0.1$	ND	$0.1^{ab} \pm 0.1$	ND
20	H	$0.2^{a} \pm 0.1$	$0.2^{a} \pm 0.1$	$0.3^{a} \pm 0.0$	ND
	HSA_0	$0.4^{b} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.3^{a} \pm 0.1$	ND
	HSA_{15}	$0.5^{b} \pm 0.2$	$0.4^{b} \pm 0.0$	$0.3^{a} \pm 0.0$	ND
25	H	$0.5^{a} \pm 0.1$	$0.1^{a} \pm 0.1$	$0.4^{a} \pm 0.2$	ND
	HSA_0	$0.7^{ab} \pm 0.2$	$0.3^{b} \pm 0.0$	$0.4^{a} \pm 0.1$	$0.1^{a} \pm 0.0$
	HSA_{15}	$0.6^{a} \pm 0.0$	$0.4^{c} \pm 0.0$	$0.7^{b} \pm 0.1$	$0.1^{a} \pm 0.0$
	HSA_{20}	$0.9^{b} \pm 0.1$	$0.8^{d} \pm 0.1$	$0.3^{a} \pm 0.0$	$0.1^{a} \pm 0.0$
30	H	$0.7^{a} \pm 0.1$	$0.1^{a} \pm 0.0$	$0.6^{b} \pm 0.0$	$0.1^{a} \pm 0.1$
	HSA_0	$0.9^{ab} \pm 0.3$	$0.7^{b} \pm 0.1$	$0.4^{a} \pm 0.1$	$0.1^{a} \pm 0.0$
	HSA_{15}	$0.6^{a} \pm 0.2$	$0.6^{b} \pm 0.0$	$1.0^{c} \pm 0.1$	$0.1^{a} \pm 0.0$
	HSA_{20}	$1.3^{b} \pm 0.2$	$0.6^{b} \pm 0.1$	$0.4^{a} \pm 0.1$	$0.1^{a} \pm 0.0$
	HSA_{25}	$1.3^{b} \pm 0.1$	$1.4^{c} \pm 0.1$	$0.3^{a} \pm 0.1$	$0.3^{b} \pm 0.1$
Total	H				$0.1^{a} \pm 0.1$*
	HSA_0				$0.2^{a} \pm 0.0$*
	HSA_{15}				$0.2^{a} \pm 0.0$*
	HSA_{20}				$0.2^{a} \pm 0.0$*
	HSA_{25}				$0.3^{b} \pm 0.0$*

H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; HSA_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; HSA_{15} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 15th day; HSA_{20} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 20th day; HSA_{25} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 25th day. The differences are compared for each treatment. Values followed by the same letter are not significantly different. ND-not determined. * Sum of glucosinolates exuded during 30 days

No 4-methoxy-glucobrassicin was detected in all parts of plants from 20th day of experiment, but later the glucosinolate appeared in all plant parts which could be explained by the induction of 4-methoxy-glucobrassicin synthesis on later stages of plant growth or by its conversion from glucobrassicin (Halkier, 2005). The next 5 days it was detected in roots and exudates of all plants, but only in leaves after SA treatment. In leaves of non treated plants 4-methoxy-glucobrassicin was not measured. The effect of SA became obvious on 30th day, when it caused increase of glucosinolate in secondary roots for HSA_0 (5.5 fold more as for H), for HSA_{15} (3.5 fold more), HSA_{20} (0.4 fold more), and HSA_{25} (5 fold more).

Table 4.50. Influence of salicylic acid applied on different stages of plant growth on 4-methoxy-glucobrassicin content in plants and exudates of turnip from hydroponic (mg plant^{-1})

Days	Treatments	Leaves	Secondary roots	Primary roots	Exudates
10	H	ND	ND	-	ND
	HSA_0	ND	ND	-	ND
15	H	ND	ND	ND	ND
	HSA_0	ND	ND	ND	ND
20	H	ND	$0.2^{a} \pm 0.1$	$0.2^{a} \pm 0.1$	ND
	HSA_0	ND	$0.3^{a} \pm 0.0$	$0.2^{a} \pm 0.1$	$0.2^{a} \pm 0.1$
	HSA_{15}	ND	$0.4^{b} \pm 0.0$	$0.3^{a} \pm 0.0$	$0.3^{a} \pm 0.1$
25	H	ND	$0.2^{a} \pm 0.1$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$
	HSA_0	ND	$0.2^{a} \pm 0.1$	$0.2^{ab} \pm 0.1$	$0.1^{a} \pm 0.0$
	HSA_{15}	$0.3^{a} \pm 0.0$	$0.4^{ab} \pm 0.1$	$0.3^{b} \pm 0.1$	$0.2^{b} \pm 0.0$
	HSA_{20}	$0.3^{a} \pm 0.1$	$0.4^{b} \pm 0.0$	$0.2^{ab} \pm 0.1$	$0.4^{c} \pm 0.1$
30	H	ND	$0.2^{a} \pm 0.0$	ND	$0.1^{ab} \pm 0.1$
	HSA_0	$0.1^{a} \pm 0.0$	$1.1^{c} \pm 0.1$	$0.3^{a} \pm 0.1$	$0.1^{a} \pm 0.0$
	HSA_{15}	$0.6^{b} \pm 0.1$	$0.9^{bc} \pm 0.2$	$0.7^{b} \pm 0.0$	$0.1^{ab} \pm 0.1$
	HSA_{20}	$0.5^{b} \pm 0.0$	$0.8^{b} \pm 0.0$	$0.4^{a} \pm 0.0$	$0.2^{b} \pm 0.0$
	HSA_{25}	$0.4^{b} \pm 0.1$	$1.0^{bc} \pm 0.2$	$0.4^{a} \pm 0.1$	$0.4^{c} \pm 0.1$
Total	H				$0.2^{a} \pm 0.1$*
	HSA_0				$0.5^{b} \pm 0.1$*
	HSA_{15}				$0.6^{b} \pm 0.0$*
	HSA_{20}				$0.6^{b} \pm 0.1$*
	HSA_{25}				$0.5^{b} \pm 0.1$*

H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; HSA_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; HSA_{15} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 15th day; HSA_{20} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 20th day; HSA_{25} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 25th day. The differences are compared for each treatment. Values followed by the same letter are not significantly different. ND-not determined. * Sum of glucosinolates exuded during 30 days.

The lower increase of 4-methoxy-glucobrassicin in leaves of HSA_{15} as in HSA_0 and HSA_{25} can be explain by the strongest increase of this glucosinolate in leaves and primary roots 0.6 and 0.7 mg plant^{-1}, which was respectively 6 and 2.5 fold more as for H. This could be explained by the faster transportation of 4-methoxy-glucobrassicin into secondary roots for HSA_0 and HSA_{25}, as for HSA_{15} and HSA_{20}.

The highest **ratio of aliphatic to indole glucosinolates** was measured on 10th day for leaves: for H1H it reached 1.3, then it increase to 2.3 on 20th day and then decreased to the end of experiment (Table 4.51). Treatment with SA at all stages caused the decrease of the ratio of aliphatic to indole glucosinolates in leaves. This meant, threatment with SA caused more intensive synthesis of inole as aliphatic glucosinolates in leaves.

Table 4.51. Elicitor influence on correlation between the aliphatic and indole glucosinolates in plants of turnip from hydroponic on 30th day

Days	Treatment	Content / correlation	Leaves	Secondary roots	Primary Roots	Exudates
10	H	mg plant^{-1}	0.8 : 0.6	0.1 : 0.2		0.5 : 0.2
		n : 1	1.3 : 1	0.5 : 1		2.5 : 1
	HSA_0	mg plant^{-1}	0.8 : 1.6	0.2 : 0.6		0.1 : 1.2
		n : 1	0.5 : 1	0.3 : 1		0.1 : 1
15	H	mg plant^{-1}	1.2 : 0.3	0.1 : 0.4	0.7 : 0.8	0.4 : 0.2
		n : 1	4 : 1	0.2 : 1	0.9 : 1	0.5 : 1
	HSA_0	mg plant^{-1}	1.2 : 1.7	0.3 : 0.7	0.5 : 0.6	0.5 : 0.9
		n : 1	0.7 : 1	0.4 : 1	0.9 : 1	0.5 : 1
20	H	mg plant^{-1}	1.6 : 0.7	0.5 : 1.8	1.6 : 1.3	0.3 : 0.3
		n : 1	2.3 : 1	0.3 : 1	1.2 : 1	1 : 1
	HSA_0	mg plant^{-1}	1.4 : 1.5	0.8 : 2.4	0.9 : 1.0	0.1 : 1.2
		n : 1	1 : 1	0.3 : 1	0.9 : 1	0.1 : 1
	HSA_{15}	mg plant^{-1}	1.3 : 0.4	1.0 : 3.6	1.5 : 1.0	0.2 : 1.1
		n : 1	3.2 : 1	0.3 : 1	1.5 : 1	0.2 : 1
25	H	mg plant^{-1}	1.6 : 1.1	0.9 : 2.2	2.3 : 1.9	0.4 : 0.4
		n : 1	1.3 : 1	0.2 : 1	1.2 : 1	1 : 1
	HSA_0	mg plant^{-1}	1.7 : 2.0	1.1 : 3.6	1.5 : 1.8	0.2 : 0.8
		n : 1	0.8 : 1	0.3 : 1	0.8 : 1	0.2 : 1
	HSA_{15}	mg plant^{-1}	1.6 : 2.4	1.8 : 3.6	2.3 : 2.1	0.7 : 0.9
		n : 1	0.7 : 1	0.5 : 1	1.2 : 1	0.8 : 1
	HSA_{20}	mg plant^{-1}	1.5 : 2.0	1.5 : 3.1	1.5 : 1.9	1.1 : 0.7
		n : 1	0.7 : 1	0.5 : 1	0.8 : 1	1.6 : 1
30	H	mg plant^{-1}	2.6 : 1.1	1.3 : 2.3	6.8 : 1.4	0.5 : 0.9
		n : 1	1.8 : 1	0.6 : 1	4.9 : 1	1 : 1
	HSA_0	mg plant^{-1}	1.8 : 2.0	1.5 : 6.3	3.5 : 1.2	0.2 : 0.8
		n : 1	0.9 : 1	0.2 : 1	0.3 : 1	0.2 : 1
	HSA_{15}	mg plant^{-1}	1.8 : 2.6	1.5 : 5.0	3.5 : 3.1	0.2 : 0.8
		n : 1	0.7 : 1	0.3 : 1	1.1 : 1	0.2 : 1
	HSA_{20}	mg plant^{-1}	2.0 : 3.8	2.1 : 3.8	3.7 : 1.7	0.4 : 0.8
		n : 1	0.6 : 1	0.6 : 1	2.2 : 1	0.5 : 1
	HSA_{25}	mg plant^{-1}	1.7 : 3.3	1.7 : 5.1	5.1 : 1.4	0.7 : 1.2
		n : 1	0.5 : 1	0.3 : 1	3.6 : 1	0.6 : 1
Total	H	mg plant^{-1}				2.1 : 1.6*
		n : 1				1.3 : 1
	HSA_0	mg plant^{-1}				1.2 : 5.0*
		n : 1				0.2 : 1
	HSA_{15}	mg plant^{-1}				2.9 : 3.5*
		n : 1				0.8 : 1
	HSA_{20}	mg plant^{-1}				2.6 : 2.7*
		n : 1				1 : 1
	HSA_{25}	mg plant^{-1}				2.3 : 2.4*
		n : 1				1 : 1

H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; HSA_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; HMJ_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + methyl jasmonate applied on the beginning. The differences are compared for each treatment. Values followed by the same letter are not significantly different. *Sum of glucosinolates exuded during 30 days

This ratio was much lower for secondary roots as for other plant parts. However, treatment with SA caused slight increase of this ratio as well as it was measured for exudates. The later the plants were treated with the elicitor, the higher was the ratio of aliphatic to indole glucosinolates in secondary and primary roots as well as in exudates. This may be explained by different reactions of plants on stress factor on the different growth stages. Younger plants reacted on SA treatment with more intensive changes in profile of individual glucosinolates as the elder plants. Moreover, the profile of non-treated plants also changes during the plant growth and this causes the different reaction of plants.
According to Charron *et al.* (2004), the indole glucosinolate concentration is inversely related to aliphatic glucosinolate concentration. The inverse relationship results from a shift in the amino acid composition which acts as precursors for the different classes of glucosinolates.
Chen *et al.*, (2003) explained the complexity of formation the ratio of aliphatic to indole glucosinolates as interactions between different glucosinolate biosynthetic pathways. Disruption of CYP79F1 gene, entcoding cytochrome P450, participating in the conversion of homo- to pentahomo-methionine to aldoximes, leads to decreasing the level of aliphatic glucosinolates biosynthesis, while increases level of indole glucosinolates (Kliebenstein *et al*., 2005). The other common point in synthesis of different classes of glucosinolates is that CYP83A1 participating conversion of aliphatic aldoxymes to thiohydroxymates also metabolizes the aromatic and indole aldoximes with low affinity (Bak and Feyereisen, 2001).

4.2.3 Fresh weight of plants and glucosinolate concentration

Application of SA on different stages did not cause significant differences in fresh weight of shoots and roots of plants. The highest difference was measured between H and HSA_0 for shoots (24.5 and 27.4 g $plant^{-1}$) as well as for primary roots (6.4 and 5.8 g $plant^{-1}$) (Table 4.52).

Table 4.52. Influence of salicylic acid applied on different stages of plant growth on fresh weight (g) and glucosinolate concentration (mg g^{-1}) in turnip plants from hydroponic

Treat-ment	Fresh weight, g/ concentration of glucosinolates, mg g^{-1}	Shoots	Roots		Shoot : root index
			secondary	primary	
H	g	$24.5^{a} \pm 1.8$	$15.0^{a} \pm 2.7$	$6.4^{a} \pm 0.7$	1.1
	mg g^{-1}	0.2	0.3	1.4	
HSA_0	g	$27.4^{b} \pm 0.8$	$18.6^{a} \pm 1.9$	$5.8^{a} \pm 0.5$	1.2
	mg g^{-1}	0.1	0.5	1.4	
HSA_{15}	g	$26.6^{ab} \pm 2.2$	$18.7^{a} \pm 1.8$	$6.0^{a} \pm 1.2$	1.2
	mg g^{-1}	0.2	0.5	1.3	
HSA_{20}	g	$27.0^{ab} \pm 2.0$	$15.3^{a} \pm 1.3$	$6.6^{a} \pm 0.9$	1.3
	mg g^{-1}	0.2	0.5	1.0	
HSA_{25}	g	$24.4^{ab} \pm 3.3$	$15.8^{a} \pm 2.2$	$6.0^{a} \pm 0.7$	1.1
	mg g^{-1}	0.3	0.6	1.1	

H - hydroponic, two times concentrated Hoagland solution + two times increased sulfur; HSA_0 - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; HSA_{15} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 15^{th} day; HSA_{20} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 20^{th} day; HSA_{25} - hydroponic, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on 25^{th} day. The differences are compared for each treatment. Values followed by the same letter are not significantly different.

Also Sudha and Ravishankar (2003) reported about the marginally higher growth of plants and cell cultures, treated with SA. Thelen et *al*. (2005) on the example of *Centarea maculosa* treatment with SA showed the simulation of compensatory root growth.
Increase in concentration of glucosinolates was measured for HSA_{15} leaves 0.3 mg g^{-1}, while for other treatments it was 0.2 mg g^{-1}; accept of HSA_0 with 0.1 mg g^{-1}. This also support the fact that SA applied on the beginning of plant growth stimulate it, but applied on later stages did not cause the increase of growth and in this case is to observe the effect of glucosinolate dilution under the biomass accumulation.
For secondary roots application of SA caused until 2 fold the increase in glucosinolate concentration: for H secondary roots it reached 0.3 mg g^{-1}, while for HSA_0 0.6 mg $plant^{-1}$, which means, that SA influence much more on the intensity of glucosinolate synthesis, as on fresh weight accumulation, especially for secondary roots. The opposite was measured for primary roots, where glucosinolate concentration as well as primary root fresh weight decreased under SA application: it was 1.4 mg g^{-1} for H primary roots and 1.0 mg g^{-1} for $HSA_{15.}$ This proves the fact that SA can slow down the development of plants without suppression in biomass accumulation.

Summary

This experiment has shown that the highest glucosinolate accumulation occurred in plants and exudates of turnip when SA was applied on 15^{th} day, when the plant growth was most intensive.
The kinetics of static and induced accumulation of glucosinolates was different for turnip plants; moreover, the proceeding of induced accumulation of glucosinolates depended on stage of plant development.
SA treatment caused systemic induction of glucosinolate accumulation in plants. Treatment of secondary roots led to enchnce of glucosinolate content in leaves.
Glucosinolate content in exudates in exposed to SA treatment systems increased during the first 5-10 days after the application and afterwards decreased. This decrease was possible to measure for HSA_0 20 days and HSA_{15} 15 days after elicitor application
The highest increase of glucosinolates in exudates right after treatment was measured for HSA_{25} plants, but the total yield of glucosinolates in exudates from this system was lower as from $HSA_{0,}$ HSA_{15}, and HSA_{25}, because plants were only 5 days in system after the elicitor application. Probably in the future could be possible to slow down the plant growth and prolong the period of their growing in systems (by application of phytohormones, *ect*.). The lowest yield of glucosinolates in exudates during 30 days was in H with 4.8 mg $plant^{-1}$, 6.4 mg $plant^{-1}$ was recovered from $HSA_{25,}$ while from HSA_{15} it reached 8.6 mg $plant^{-1}$.
On the later stages of post-treatment period it was measured the decrease in exudation of glucosinolates but their increase in plant tissues. The synthesis and secretion of defense-related substances are a critical part of the establishment to resistance, conasequently we can assume that this establishment process takes from 10 to 20 days in plants of turnip, because during this time occurs the maximal accumulation of glucosinolates in plant tissues as well as most intensive exuding of glucosinolates into growing medium. And if the task is to receive the glucosinolates from exudates, it should be done until 20^{th} day after treatment with elicitor, but if the source of glucosinolates should be plant tissues that it is more reasonable to collect the glucosinolates 30 days after the treatment.

In contrast to leaves and primary roots, application of SA increased aliphatic glucosinolate content in secondary roots of all systems, which could be explained by the independent regulation of glucosinolate syntheses in different plant organs. Interestingly is that 5 days after SA treatment the content of aliphatic glucosinolates in exudates was much higher as for system without treatment, but then it became lower.

SA caused the selective exudation, because after its application the ratio of butenyl to pentenyl glucosinolates increased in plants, but decreased in exudates: preferably exuded were gluconapin and progoitrin.

It seems that the induction of aromatic glucosinolate synthesis after SA application is maximally expressed 15-25 days after the treatment.

Treatment with SA increased the content of indole glucosinolates in leaves from all systems and this effect continued until the end of experiment mostly by costs of glucobrassicin and neoglucobrassicin.

Application of SA on different stages did not cause significant differences in fresh weight of shoots and roots of plants. SA can slow down the development of plants without suppression in biomass accumulation.

Different responses occurred for the same elicitor applied on different stages of turnip plant development.

5. CONCLUSIONS

The represented studies concern the development of the technology for turnip growth in soil-free systems with the purpose to recover glucosinolates from plants and their exudates. The main approaches consist in the enhancement of glucosinolate content in plant tissues as well as intensification of glucosinolate exudation from roots.

The key conclusions, received in the course of the investigations can be summarised as following.

1. Turnip can be cultivated as soil-free culture for the production of glucosinolates from plants and their exudates. The type of growing system influences on glucosinolate synthesis, plant growth, particularly that of the secondary (fine) roots, and intensity of exudation. The optimal system for turnip growth under the Hoagland solution is AD and to the lesser extends H and AS. However, after the modification of nutrient supply H shows to be more optimal according to the aims of the researches.

2. Nutrient supply and type of system influence on individual and consequently total glucosinolate content in plants as well as exudates of turnip. Moreover, it differs between parts of plants and changes during plant growth. Secondary roots have higher concentration of glucosinolates as other plant parts, which means, they could be used as a rich source of glucosinolates.

a) Increase of Hoagland solution concentration as well as sulfur supply leads to enhance of total glucosinolate content, particularly aliphatic glucosinolates in plants, mostly by costs of secondary roots.

b) Sulfur application leads to disappearing of aromatic glucosinolate gluconasturtiin, while for H2H its content as well as that of indole glucosinolates increases.

c) H2H intensifies the exudation: the amount of exuded during 30 days glucosinolates reaches for this system 48% of that in secondary roots, while for H1H it reaches 18% and for H2H2S 39%. This can be explained by 2H stimulation of root growth and correspondingly increase of secondary root growth.

d) Rhizosecretion of glucosinolates is selective process and not leaching, which could be explained by ecological role of exuded glucosinolates. Plants exude preferably aliphatic glucosinolates on the beginning of their growth, while later morstly indole glucosinolates. In addition, plants excrete more intensively butenyl as pentenyl glucosinolates and hydroxyalkenyl as alkenyl.

e) Increased sulfur application leads to enhance of aliphatic glucosinolates in exudates, while under the influence 2H the excretion of indole glucosinolates is more intensive.

f) Slowing down the intensity of glucosinolate exudation for plants after 30 days of growth in systems could be explained by altering of roots, and expediently by decrease of their metabolic activity.

3. Plant treatment with elicitors, particularly SA and MJ, leads to increase of glucosinolates in plants and exudates.

a) The response of plants on root treatment with elicitors is inducible. The root induction by SA and MJ has an impact on glucosinolate content also in leaves and primary roots of turnip as well as in exudates. However, the kinetics of induced accumulation of glucosinolates differs between plant parts, the effect of elicitor application is greater at the site of treatment, namely in secondary roots.

b) Not all classes of glucosinolates respond equally to elicitor treatment. This could be explained by the independent regulation of glucosinolate syntheses in different plant organs. Application of MJ decreases aliphatic, while increases indole glucosinolates in all plant parts and prevents the disappearance of aromatic gluconasturtiin in leaves, which can be explained by synthesis intensification or by slowing down the intensity of glucosinolate transport to the roots. Application of SA increases aliphatic glucosinolate content in secondary roots, and indole glucosinolates in all plant parts. Different effects of elicitors on the individual glucosinolates could be exounded by the defense mechanisms, they are involved in.

c) SA induces secondary root growth, which could be explained by elicitor influence auxin status of plants. However, the concentration of glucosinolates for SA treated plants is nearly the same as for the plants without treatment.

d) Application of MJ results in suppressing of plant growth. Right after the treatment the fresh weight of HMJ_0 leaves and secondary roots is 2 and 3.2-fold lower as for H, however, the glucosinolate concentration for MJ treated leaves and secondary roots is 10 and 3-folds higher as that for H. This could be explained by stimulation of glucosinolate synthesis by MJ, but on the same time by suppressing of plant and especially secondary root growth, as it was explained by Hansen and Halkier (2005) by the influencing on the expression of gene entconding γ-glutamatcysteinsynthase, responsible for cell division in roots meristem. It is possible to assume that if MJ causes the same increase in production of defense compound, it become not economical for plants to grow intensively, because it deprives too many resources, and this could be the reason of decrease of plant growth.

e) Chemical diversity and content of exudates could be dramatically enhanced by elicitation process. Both of elicitors stimulate rhizosecretion of glucosinolates, especially during the first days after treatment, and then the effect declines gradually. In the course of post-treatment period, the intensity of glucosinolate exudation decreases, but their content increases in plant tissues. According to Hugot *et al*. (2004) the balance between the synthesis and secretion of defense-related substances is a critical point of the establishment of plant resistance. For turnip plants it takes 10-20 days, because from this time the glucosinolate content in plant tissues starts to increase strongly, while the intensity of exudation decreases.

f) Elicitor-induced rhizosecretion is based on *de novo* synthesis of secondary metabolites and not on leakage from root tissues. This is substituted by the fact that the profile of glucosinolates in exudates differs of those in plants. Elicitors decrease the content of aliphatic glucosinolates in exudates for all systems, which in case with MJ has stronger effect as with SA. After the application of both of elicitors, the content of indole glucosinolates increases essentially right after the treatment: on 10^{th} day for HSA_0 and HMJ_0 it exceeds 6 and 6.5-folds of that for H. In general, during 30 days H plants exude 1.6 mg $plant^{-1}$ of indole glucosinolates, HSA_0 5.0 mg $plant^{-1}$ (2.6-folds more), and HMJ_0 5.9 mg $plant^{-1}$ (3.1-folds more).

4. Plant response on SA treatment changes during the growth period. Inductivity of glucosinolate accumulation depends on stage of plant development.
a) The highest total glucosinolate accumulation occurs in plants and exudates of turnip when SA is applied on 15th day, in the period of the most intensive plant growth. During 30 days H plants exude 4.8 mg plant^{-1} of glucosinolates, HSA_{25} 6.4 mg plant^{-1}, while HSA_{15} 8.6 mg plant^{-1}.
b) The induction of glucosinolate synthesis in plants after SA application is maximally expressed 10-20 days after the treatment. However, glucosinolate content in exudates increases during the first 5-10 days after the application and afterwards decreases. Therefore, if the task is to receive the glucosinolates from exudates, it should be done until the 20th day after the treatment with elicitor, but if the source of glucosinolates is plant tissues, it is more reasonable to collect the phytochemicals after the 20th day after the treatment.
c) The highest increase of glucosinolates in exudates right after the treatment is measured for HSA_{25} plants, but the total yield of glucosinolates is lower that from earlier treated plants, which is connected with short post-treatment period of plant growth in system. Probably, it could be possible to slow down the plant growth intensity and hence prolong the period of their growing in systems, which can prolong the time for exudates collecting.

This study could contribute to the understanding of the biology of the root exudation process and to the development of approaches for designing of novel strategies for the production and isolation of glucosinolates. In addition, the knowledge of the regulation of glucosinolate synthesis in plants as well as their rhizosecretion would be a major advance in the production of low-cost nutraceuticals.

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7. ATTACHMENT

7.3 Supplementary tables

Table 4.1a. Influence of plant nutrition on total glucosinolate content in plants and exudates of turnip from aeroponic with defensor (mg plant^{-1})

Date	Treatment	Leaves	Secondary roots	Primary roots	Exudates
10 days	AD1H	$1.5^{a} \pm 0.2$	$0.6^{a} \pm 0.0$	ND	$0.2^{a} \pm 0.0$
	AD2H	$1.8^{ab} \pm 0.3$	$0.7^{ab} \pm 0.1$	ND	$0.2^{a} \pm 0.1$
	AD2H2S	$2.0^{b} \pm 0.1$	$1.0^{b} \pm 0.2$	ND	$0.3^{a} \pm 0.0$
20 days	AD1H	$2.2^{a} \pm 0.4$	$1.6^{a} \pm 0.2$	$2.3^{a} \pm 0.2$	$0.8^{a} \pm 0.1$
	AD2H	$3.4^{b} \pm 0.3$	$3.8^{c} \pm 0.1$	$2.6^{a} \pm 0.3$	$1.7^{b} \pm 0.3$
	AD2H2S	$2.5^{a} \pm 0.1$	$3.3^{b} \pm 0.4$	$5.0^{b} \pm 0.4$	$1.5^{b} \pm 0.3$
30 days	AD1H	$3.6^{a} \pm 0.3$	$2.8^{a} \pm 0.2$	$4.9^{a} \pm 0.6$	$1.2^{a} \pm 0.3$
	AD2H	$3.6^{a} \pm 0.7$	$4.5^{b} \pm 0.8$	$7.0^{b} \pm 0.4$	$2.0^{a} \pm 0.6$
	AD2H2S	$3.9^{a} \pm 0.8$	$5.6^{b} \pm 0.7$	$10.2^{c} \pm 0.9$	$4.0^{c} \pm 0.3$
Total	AD1H				$2.2^{a} \pm 0.4$*
	AD2H				$3.6^{b} \pm 0.2$*
	AD2H2S				$5.9^{c} \pm 0.6$*

AD1H - aeroponic with defensor with Hoagland solution, AD2H - aeroponic with defensor with two time concentrated Hoagland solution, AD2H2S - aeroponic with defensor with two times concentrated Hoagland solution and two times increased concentration of sulfur. The differences are compared for each treatment. Values followed by the same letter are not significantly different. *Sum of glucosinolates exuded during 30 days

Table 4.1b. Influence of plant nutrition on total glucosinolate content in plants and exudates of turnip from aeroponic with sprayers (mg plant^{-1})

Date	Treatment	Leaves	Secondary roots	Primary roots	Exudates
10 days	AS1H	$0.7^{a} \pm 0.2$	$1.0^{a} \pm 0.2$	ND	$0.2^{a} \pm 0.1$
	AS2H	$0.9^{a} \pm 0.0$	$0.9^{a} \pm 0.0$	ND	$0.2^{a} \pm 0.1$
	AS2H2S	$2.1^{b} \pm 0.2$	$1.5^{b} \pm 0.0$	ND	$0.4^{a} \pm 0.2$
20 days	AS1H	$2.5^{a} \pm 0.4$	$1.4^{a} \pm 0.2$	$3.0^{a} \pm 0.3$	$1.0^{a} \pm 0.3$
	AS2H	$3.1^{a} \pm 0.3$	$2.1^{ab} \pm 0.6$	$5.0^{b} \pm 0.8$	$1.4^{a} \pm 0.2$
	AS2H2S	$2.7^{a} \pm 0.5$	$3.0^{b} \pm 0.7$	$6.4^{b} \pm 0.6$	$1.4^{a} \pm 0.4$
30 days	AS1H	$4.9^{a} \pm 0.7$	$2.4^{a} \pm 0.2$	$8.3^{a} \pm 0.4$	$1.2^{a} \pm 0.0$
	AS2H	$4.6^{a} \pm 0.3$	$5.0^{b} \pm 0.2$	$9.6^{b} \pm 0.6$	$1.6^{b} \pm 0.3$
	AS2H2S	$3.9^{a} \pm 0.4$	$7.1^{c} \pm 0.3$	$10.7^{c} \pm 0.2$	$3.2^{c} \pm 0.2$
Total	AS1H				$2.4^{a} \pm 0.4$*
	AS2H				$3.1^{a} \pm 0.3$*
	AS2H2S				$5.0^{b} \pm 0.4$*

AS1H - aeroponic with sprayers with Hoagland solution, AS2H - aeroponic with sprayers with two times concentrated Hoagland solution, AS2H2S - aeroponic with sprayers with two times concentrated Hoagland solution and two times increased concentration of sulfur. The differences are compared for each treatment. Values followed by the same letter are not significantly different. *Sum of glucosinolates exuded during 30 days

Table 4.3a. Influence of plant nutrition on composition of aliphatic, aromatic, and indole glucosinolates in plants and exudates of turnip from aeroponic with defensor on 30th day

Object	Treatment		Aliphatic	Aromatic	Indole
Leaves	AD1H	mg plant^{-1}	1.6^{b} ± 0.4	0.6^{b} ± 0.1	1.3^{a} ± 0.1
		%	46	17	37
	AD2H	mg plant^{-1}	1.4^{b} ± 0.2	0.8^{b} ± 0.3	1.4^{a} ± 0.1
		%	39	22	39
	AD2H2S	mg plant^{-1}	2.4^{c} ± 0.3	0.1^{a} ± 0.0	1.4^{a} ± 0.3
		%	62	3	36
Secondary roots	AD1H	mg plant^{-1}	0.8^{a} ± 0.1	0.7^{a} ± 0.1	1.3^{a} ± 0.3
		%	29	25	46
	AD2H	mg plant^{-1}	0.8^{a} ± 0.1	1.0^{b} ± 0.1	2.7^{b} ± 0.3
		%	18	22	60
	AD2H2S	mg plant^{-1}	0.9^{a} ± 0.2	0.8^{ab} ± 0.2	3.1^{b} ± 0.3
		%	19	17	65
Primary roots	AD1H	mg plant^{-1}	2.6^{a} ± 0.2	1.3^{a} ± 0.3	1.0^{a} ± 0.0
		%	53	27	20
	AD2H	mg plant^{-1}	3.7^{b} ± 0.3	1.6^{a} ± 0.3	1.8^{b} ± 0.3
		%	52	23	25
	AD2H2S	mg plant^{-1}	5.7^{c} ± 0.4	2.2^{b} ± 0.1	2.9^{c} ± 0.2
		%	53	20	27
Exudates	AD1H	mg plant^{-1}	0.9^{a} ± 0.2*	0.6^{a} ± 0.2*	0.6^{a} ± 0.1*
		%	43	29	29
	AD2H	mg plant^{-1}	1.4^{b} ± 0.2*	1.1^{ab} ± 0.3*	1.2^{b} ± 0.1*
		%	38	30	32
	AD2H2S	mg plant^{-1}	1.9^{b} ± 0.5*	1.3^{b} ± 0.1*	2.6^{c} ± 0.6*
		%	33	22	45

AD1H - aeroponic with defensor with Hoagland solution, AD2H - aeroponic with defensor with two time concentrated Hoagland solution, AD2H2S - aeroponic with defensor with two times concentrated Hoagland solution and two times increased concentration of sulfur. The differences are compared for each treatment. Values followed by the same letter are not significantly different. *Sum of glucosinolates exuded during 30 days. *Sum of glucosinolates exuded during 30 days

Table 4.3b. Influence of plant nutrition on composition of aliphatic, aromatic, and indole glucosinolates in plants and exudates of turnip from aeroponic with sprayers on 30th day

Object	Treatment		Aliphatic	Aromatic	Indole
Leaves	AS1H	mg plant^{-1}	2.6^{b} ± 0.2	0.7^{b} ± 0.2	1.6^{a} ± 0.1
		%	53	14	33
	AS2H	mg plant^{-1}	2.1^{a} ± 0.2	0.6^{b} ± 0.2	1.9^{a} ± 0.1
		%	46	13	41
	AS2H2S	mg plant^{-1}	2.6^{b} ± 0.4	0.1^{a} ± 0.0	1.6^{a} ± 0.1
		%	60	2	37
Secondary roots	AS1H	mg plant^{-1}	0.7^{a} ± 0.1	0.7^{a} ± 0.2	1.0^{a} ± 0.2
		%	29	29	42
	AS2H	mg plant^{-1}	0.8^{a} ± 0.1	1.6^{a} ± 0.2	2.6^{b} ± 0.5
		%	16	32	52
	AS2H2S	mg plant^{-1}	0.8^{a} ± 0.1	1.5^{a} ± 0.2	2.5^{b} ± 0.5
		%	17	31	52
Primary roots	AS1H	mg plant^{-1}	3.7^{a} ± 0.7	2.6^{a} ± 0.2	2.0^{a} ± 0.4
		%	45	31	24
	AS2H	mg plant^{-1}	4.0^{a} ± 0.3	2.9^{a} ± 0.2	2.7^{ab} ± 0.7
		%	42	30	28
	AS2H2S	mg plant^{-1}	5.2^{b} ± 0.9	3.7^{b} ± 0.3	3.5^{a} ± 0.1
		%	42	30	28
Exudates	AS1H	mg plant^{-1}	1.0^{a} ± 0.3*	1.0^{a} ± 0.2*	1.0^{a} ± 0.2*
		%	33	33	33
	AS2H	mg plant^{-1}	1.0^{a} ± 0.2*	1.0^{a} ± 0.3*	1.0^{a} ± 0.2*
		%	33	33	33
	AS2H2S	mg plant^{-1}	2.5^{b} ± 0.1*	0.8^{a} ± 0.1*	1.8^{b} ± 0.4*
		%	49	16	35

AS1H - aeroponic with sprayers with Hoagland solution, AS2H - aeroponic with sprayers with two times concentrated Hoagland solution, AS2H2S - aeroponic with sprayers with two times concentrated Hoagland solution and two times increased concentration of sulfur. The differences are compared for each treatment. Values followed by the same letter are not significantly different. *Sum of glucosinolates exuded during 30 days

Table 4.4a. Influence of plant nutrition on aliphatic glucosinolate content in plants and exudates of turnip from aeroponic with defensor (mg plant^{-1})

Date	Treatment	Leaves	Secondary roots	Primary roots	Exudates
10 days	AD1H	$1.0^{b} \pm 0.0$	$0.2^{a} \pm 0.1$	ND	$0.1^{a} \pm 0.1$
	AD2H	$0.9^{b} \pm 0.2$	$0.2^{a} \pm 0.1$	ND	$0.1^{a} \pm 0.0$
	AD2H2S	$1.2^{b} \pm 0.2$	$0.2^{a} \pm 0.1$	ND	$0.1^{a} \pm 0.0$
20 days	AD1H	$0.9^{b} \pm 0.1$	$0.7^{b} \pm 0.1$	$1.3^{ab} \pm 0.2$	$0.3^{a} \pm 0.1$
	AD2H	$1.3^{b} \pm 0.3$	$0.4^{a} \pm 0.1$	$1.1^{a} \pm 0.2$	$0.8^{b} \pm 0.0$
	AD2H2S	$1.9^{a} \pm 0.3$	$0.5^{ab} \pm 0.2$	$1.7^{b} \pm 0.3$	$0.7^{b} \pm 0.2$
30 days	AD1H	$1.6^{b} \pm 0.4$	$0.8^{a} \pm 0.1$	$2.6^{a} \pm 0.2$	$0.5^{a} \pm 0.1$
	AD2H	$1.4^{b} \pm 0.2$	$0.8^{a} \pm 0.1$	$3.7^{b} \pm 0.3$	$0.5^{b} \pm 0.0$
	AD2H2S	$2.4^{c} \pm 0.3$	$0.9^{a} \pm 0.2$	$5.7^{c} \pm 0.4$	$1.1^{c} \pm 0.1$
Total	AD1H				$0.9^{a} \pm 0.2$*
	AD2H				$1.4^{b} \pm 0.2$*
	AD2H2S				$1.9^{b} \pm 0.5$*

AD1H - aeroponic with defensor with Hoagland solution, AD2H - aeroponic with defensor with two time concentrated Hoagland solution, AD2H2S - aeroponic with defensor with two times concentrated Hoagland solution and two times increased concentration of sulfur. The differences are compared for each treatment. Values followed by the same letter are not significantly different.. *Sum of glucosinolates exuded during 30 days

Table 4.4b. Influence of plant nutrition on aliphatic glucosinolate content in plants and exudates of turnip from aeroponic with sprayers (mg plant^{-1})

Date	Treatment	Leaves	Secondary roots	Primary roots	Exudates
10 days	AS1H	$0.4^{a} \pm 0.0$	$0.5^{b} \pm 0.0$	ND	$0.1^{a} \pm 0.0$
	AS2H	$0.4^{a} \pm 0.0$	$0.3^{a} \pm 0.0$	ND	$0.1^{a} \pm 0.1$
	AS2H2S	$1.2^{b} \pm 0.2$	$0.3^{a} \pm 0.0$	ND	$0.3^{b} \pm 0.0$
20 days	AS1H	$1.0^{a} \pm 0.2$	$0.5^{a} \pm 0.1$	$1.2^{a} \pm 0.2$	$0.5^{a} \pm 0.1$
	AS2H	$1.1^{a} \pm 0.3$	$0.5^{a} \pm 0.2$	$2.2^{a} \pm 0.1$	$0.8^{a} \pm 0.1$
	AS2H2S	$1.9^{b} \pm 0.2$	$0.5^{a} \pm 0.1$	$1.9^{b} \pm 0.4$	$0.7^{a} \pm 0.1$
30 days	AS1H	$2.6^{b} \pm 0.2$	$0.7^{a} \pm 0.1$	$3.7^{a} \pm 0.7$	$0.6^{a} \pm 0.1$
	AS2H	$2.1^{a} \pm 0.2$	$0.8^{a} \pm 0.1$	$4.0^{a} \pm 0.3$	$0.5^{a} \pm 0.2$
	AS2H2S	$2.6^{b} \pm 0.4$	$0.8^{a} \pm 0.1$	$5.2^{b} \pm 0.9$	$1.5^{a} \pm 0.1$
Total	AS1H				$1.0^{a} \pm 0.3$*
	AS2H				$1.0^{a} \pm 0.2$*
	AS2H2S				$2.5^{b} \pm 0.1$*

AS1H - aeroponic with sprayers with Hoagland solution, AS2H - aeroponic with sprayers with two times concentrated Hoagland solution, AS2H2S - aeroponic with sprayers with two times concentrated Hoagland solution and two times increased concentration of sulfur. The differences are compared for each treatment. Values followed by the same letter are not significantly different. *Sum of glucosinolates exuded during 30 days

Table 4.5a. Influence of plant nutrition on individual aliphatic glucosinolates content in plants and exudates of turnip from aeroponic with defensor on 30th day (mg plant^{-1})

Gluco-sinolates	Treat-ment		Leaves	Secondary roots	Primary roots	Exudates
Gluconapin	AD1H	mg plant^{-1}	0.4^{a} ± 0.1	0.1^{a} ± 0.0	0.7^{a} ± 0.1	0.2^{a} ± 0.0*
		%	33	8	58	
	AD2H	mg plant^{-1}	0.5^{ab} ± 0.1	0.2^{ab} ± 0.1	0.9^{a} ± 0.1	0.4^{b} ± 0.0*
		%	31	13	56	
	AD2H2S	mg plant^{-1}	0.7^{b} ± 0.1	0.3^{b} ± 0.1	1.9^{b} ± 0.2	0.6^{c} ± 0.1*
		%	24	10	66	
Progoitrin	AD1H	mg plant^{-1}	0.6^{a} ± 0.0	0.4^{ab} ± 0.1	0.9^{a} ± 0.2	0.4^{a} ± 0.1*
		%	32	21	47	
	AD2H	mg plant^{-1}	0.6^{a} ± 0.1	0.3^{a} ± 0.0	1.7^{b} ± 0.3	0.5^{ab} ± 0.2*
		%	23	12	65	
	AD2H2S	mg plant^{-1}	1.0^{b} ± 0.2	0.4^{b} ± 0.0	2.2^{b} ± 0.4	0.8^{b} ± 0.2*
		%	28	11	61	
Glucobras-sicanapin	AD1H	mg plant^{-1}	0.3^{b} ± 0.0	0.1^{a} ± 0.1	0.4^{a} ± 0.1	0.2^{a} ± 0.0*
		%	38	13	50	
	AD2H	mg plant^{-1}	0.2^{a} ± 0.0	0.1^{a} ± 0.1	0.5^{a} ± 0.1	0.2^{ab} ± 0.1*
		%	25	13	63	
	AD2H2S	mg plant^{-1}	0.3^{ab} ± 0.1	0.1^{a} ± 0.1	0.8^{b} ± 0.1	0.3^{b} ± 0.1*
		%	25	8	67	
Gluconapo-leiferin	AD1H	mg plant^{-1}	0.3^{b} ± 0.0	0.2^{b} ± 0.0	0.6^{a} ± 0.1	0.1^{a} ± 0.0*
		%	27	18	55	
	AD2H	mg plant^{-1}	0.1^{a} ± 0.0	0.1^{a} ± 0.0	0.6^{a} ± 0.2	0.3^{b} ± 0.0*
		%	13	13	75	
	AD2H2S	mg plant^{-1}	0.6^{c} ± 0.2	0.2^{ab} ± 0.1	0.8^{b} ± 0.1	0.2^{ab} ± 0.1*
		%	38	13	50	

AD1H - aeroponic with defensor with Hoagland solution, AD2H - aeroponic with defensor with two time concentrated Hoagland solution, AD2H2S - aeroponic with defensor with two times concentrated Hoagland solution and two times increased concentration of sulfur. The differences are compared for each treatment. Values followed by the same letter are not significantly different. *Sum of glucosinolates exuded during 30 days

Table 4.5b. Influence of plant nutrition on individual aliphatic glucosinolates content in plants and exudates of turnip from aeroponic with sprayers on 30th day (mg plant^{-1})

Gluco-sinolates	Treat-ment		Leaves	Secondary roots	Primary roots	Exudates
Gluconapin	AS1H	mg plant^{-1}	0.6^{a} ± 0.0	0.3^{b} ± 0.0	0.9^{a} ± 0.2	0.1^{a} ± 0.0*
		%	33	17	50	
	AS2H	mg plant^{-1}	0.6^{a} ± 0.1	0.2^{a} ± 0.0	1.0^{a} ± 0.2	0.2^{b} ± 0.0*
		%	33	11	56	
	AS2H2S	mg plant^{-1}	0.6^{a} ± 0.1	0.2^{ab} ± 0.1	1.7^{b} ± 0.2	0.9^{c} ± 0.1*
		%	24	8	68	
Progoitrin	AS1H	mg plant^{-1}	1.1^{a} ± 0.2	0.2^{a} ± 0.0	1.3^{a} ± 0.2	0.4^{a} ± 0.1*
		%	42	8	50	
	AS2H	mg plant^{-1}	0.9^{a} ± 0.2	0.4^{b} ± 0.1	1.8^{b} ± 0.2	0.4^{a} ± 0.1*
		%	29	13	58	
	AS2H2S	mg plant^{-1}	1.2^{a} ± 0.2	0.4^{b} ± 0.1	2.0^{b} ± 0.2	1.0^{b} ± 0.2*
		%	33	11	56	
Glucobras-sicanapin	AS1H	mg plant^{-1}	0.5^{b} ± 0.1	0.1^{a} ± 0.0	0.6^{a} ± 0.1	0.2^{a} ± 0.0*
		%	38	8	54	
	AS2H	mg plant^{-1}	0.2^{a} ± 0.0	0.1^{a} ± 0.0	0.7^{a} ± 0.1	0.2^{a} ± 0.0*
		%	20	10	70	
	AS2H2S	mg plant^{-1}	0.4^{b} ± 0.1	0.2^{a} ± 0.1	0.6^{a} ± 0.1	0.3^{a} ± 0.1*
		%	33	17	50	
Gluconapo-leiferin	AS1H	mg plant^{-1}	0.4^{a} ± 0.1	0.2^{b} ± 0.0	0.7^{a} ± 0.2	0.1^{a} ± 0.0*
		%	31	15	54	
	AS2H	mg plant^{-1}	0.4^{a} ± 0.1	0.1^{a} ± 0.0	0.5^{a} ± 0.1	0.2^{b} ± 0.0*
		%	40	10	50	
	AS2H2S	mg plant^{-1}	0.5^{a} ± 0.2	0.1^{a} ± 0.0	0.7^{a} ± 0.1	0.2^{ab} ± 0.1*
		%	38	8	54	

AS1H - aeroponic with sprayers with Hoagland solution, AS2H - aeroponic with sprayers with two times concentrated Hoagland solution, AS2H2S - aeroponic with sprayers with two times concentrated Hoagland solution and two times increased concentration of sulfur. The differences are compared for each treatment. Values followed by the same letter are not significantly different. * Sum of glucosinolates exuded during 30 days

Table 4.8a. Individual indole glucosinolates in plants and exudates from aeroponic with defensor on 30th day (mg plant^{-1})

Glucosinolates	Treatment	Leaves	Secondary roots	Primary roots	Exudates
Glucobrassicin	AD1H	0.4^{a} ± 0.0	0.1^{a}± 0.0	0.2^{a} ± 0.0	0.2^{a} ± 0.1
	AD2H	0.5^{a} ± 0.1	0.8^{b}± 0.2	0.6^{b} ± 0.1	0.4^{b} ± 0.0
	AD2H2S	0.9^{b} ± 0.1	1.1^{b}± 0.2	0.8^{b} ± 0.1	0.8^{c} ± 0.3
4-hydroxy-glucobrassicin	AD1H	0.2^{a} ± 0.1	0.4^{a}± 0.1	0.2^{a} ± 0.0	0.2^{ab} ± 0.1
	AD2H	0.6^{b} ± 0.1	0.8^{b}± 0.2	0.4^{b} ± 0.1	0.3^{b} ± 0.0
	AD2H2S	0.5^{b} ± 0.0	0.3^{a} ± 0.1	0.5^{b} ± 0.1	0.1^{a} ± 0.1
4-methoxy-glucobrassicin	AD1H	0.4^{b} ± 0.0	0.1^{ab}± 0.1	0.2^{a} ± 0.0	0.1^{a} ± 0.0
	AD2H	0.2^{a} ± 0.1	0.1^{a}± 0.0	0.2^{a} ± 0.0	0.2^{b} ± 0.0
	AD2H2S	0.1^{a} ± 0.0	0.2^{b} ± 0.0	0.6^{b} ± 0.2	0.5^{c} ± 0.1
Neoglucobrassicin	AD1H	0.3^{b} ± 0.1	0.7^{a}± 0.2	0.4^{a} ± 0.1	0.1^{a} ± 0.1
	AD2H	0.1^{ab} ± 0.1	1.0^{ab}± 0.2	0.5^{a} ± 0.2	0.3^{b} ± 0.0
	AD2H2S	0.1^{a} ± 0.0	1.2^{b} ± 0.2	1.4^{b} ± 0.2	1.2^{c} ± 0.2

AD1H - aeroponic with defensor with Hoagland solution, AD2H - aeroponic with defensor with two times concentrated Hoagland solution, AD2H2S - aeroponic with defensor with two times concentrated Hoagland solution and two times increased concentration of sulfur. The differences are compared for each treatment. Values followed by the same letter are not significantly different.

Table 4.8b. Individual indole glucosinolates in plants and exudates from aeroponic with sprayers on 30th day (mg plant^{-1})

Glucosinolates	Treatment	Leaves	Secondary roots	Primary roots	Exudates
Glucobrassicin	AD1H	0.4^{a} ± 0.1	0.3^{a}± 0.0	0.6^{a}± 0.1	0.3^{b} ± 0.0
	AD2H	0.6^{a} ± 0.2	0.6^{b}± 0.1	0.8^{a}± 0.1	0.1^{a} ± 0.0
	AD2H2S	0.6^{a} ± 0.1	0.7^{b}± 0.1	0.9^{a}± 0.2	0.4^{b} ± 0.2
4-hydroxy-glucobrassicin	AD1H	0.5^{a} ± 0.1	0.3^{a}± 0.1	0.5^{a}± 0.0	0.2^{a} ± 0.1
	AD2H	0.7^{a} ± 0.2	0.8^{b}± 0.2	0.7^{b}± 0.2	0.2^{a} ± 0.2
	AD2H2S	0.7^{a} ± 0.2	0.4^{a} ± 0.1	0.5^{ab} ± 0.2	0.1^{a} ± 0.2
4-methoxy-glucobrassicin	AD1H	0.2^{ab} ± 0.1	0.3^{a}± 0.0	0.4^{a}± 0.0	0.2^{a} ± 0.2
	AD2H	0.2^{b} ± 0.0	0.5^{b}± 0.1	0.7^{b}± 0.2	0.2^{a} ± 0.1
	AD2H2S	0.1^{a} ± 0.0	0.5^{ab} ± 0.2	0.9^{b} ± 0.3	0.5^{a} ± 0.2
Neoglucobrassicin	AD1H	0.4^{a} ± 0.2	0.4^{a}± 0.1	0.5^{a}± 0.2	0.4^{a} ± 0.2
	AD2H	0.4^{a} ± 0.1	0.7^{ab}± 0.2	0.5^{a}± 0.1	0.5^{a} ± 0.1
	AD2H2S	0.4^{a} ± 0.1	0.9^{b} ± 0.2	1.3^{b} ± 0.2	0.9^{b} ± 0.2

AS1H - aeroponic with sprayers with Hoagland solution, AS2H - aeroponic with sprayers with two times concentrated Hoagland solution, AS2H2S - aeroponic with sprayers with two times concentrated Hoagland solution and two times increased concentration of sulfur. The differences are compared for each treatment. Values followed by the same letter are not significantly different.

Table 4.11a. Influence of plant nutrition on gluconasturtiin content in plants and exudates of turnip from aeroponic with defensor (mg plant^{-1})

Date	Treatment	Leaves	Secondary roots	Primary roots	Exudates
10 days	AD1H	$0.2^{a} \pm 0.0$	$0.2^{a} \pm 0.0$	ND	$0.1^{a} \pm 0.0$
	AD2H	$0.3^{b} \pm 0.0$	$0.3^{b} \pm 0.0$	ND	$0.1^{a} \pm 0.0$
	AD2H2S	$0.2^{ab} \pm 0.2$	$0.2^{ab} \pm 0.1$	ND	$0.1^{a} \pm 0.1$
20 days	AD1H	$0.5^{a} \pm 0.1$	$0.2^{a} \pm 0.0$	$0.6^{a} \pm 0.0$	$0.2^{a} \pm 0.0$
	AD2H	$0.9^{b} \pm 0.0$	$1.2^{b} \pm 0.4$	$1.0^{b} \pm 0.0$	$0.3^{a} \pm 0.1$
	AD2H2S	$0.2^{a} \pm 0.0$	$0.9^{b} \pm 0.2$	$1.6^{c} \pm 0.2$	$0.2^{a} \pm 0.0$
30 days	AD1H	$0.6^{b} \pm 0.1$	$0.7^{a} \pm 0.1$	$1.3^{a} \pm 0.3$	$0.3^{a} \pm 0.1$
	AD2H	$0.8^{b} \pm 0.3$	$1.0^{b} \pm 0.1$	$1.6^{a} \pm 0.3$	$0.7^{b} \pm 0.2$
	AD2H2S	$0.1^{a} \pm 0.0$	$0.8^{ab} \pm 0.2$	$2.2^{b} \pm 0.1$	$1.0^{b} \pm 0.1$
Total	AD1H				$0.6^{a} \pm 0.2$*
	AD2H				$1.1^{ab} \pm 0.3$*
	AD2H2S				$1.3^{b} \pm 0.1$*

AD1H - aeroponic with defensor with Hoagland solution, AD2H - aeroponic with defensor with two time concentrated Hoagland solution, AD2H2S - aeroponic with defensor with two times concentrated Hoagland solution and two times increased concentration of sulfur. The differences are compared for each treatment. Values followed by the same letter are not significantly different. ND – not determined. *Sum of glucosinolates exuded during 30 days

Table 4.11b. Influence of plant nutrition on gluconasturtiin content in plants and exudates of turnip from aeroponic with sprayers (mg plant^{-1})

Date	Treatment	Leaves	Secondary roots	Primary roots	Exudates
10 days	AS1H	$0.1^{a} \pm 0.0$	$0.3^{a} \pm 0.2$	ND	ND
	AS2H	$0.2^{ab} \pm 0.1$	$0.3^{a} \pm 0.2$	ND	$0.1^{a} \pm 0.0$
	AS2H2S	$0.2^{b} \pm 0.0$	$0.7^{a} \pm 0.1$	ND	ND
20 days	AS1H	$0.7^{ab} \pm 0.3$	$0.5^{a} \pm 0.2$	$1.1^{a} \pm 0.2$	$0.2^{a} \pm 0.0$
	AS2H	$0.8^{b} \pm 0.1$	$0.7^{a} \pm 0.2$	$1.7^{b} \pm 0.2$	$0.3^{ab} \pm 0.1$
	AS2H2S	$0.4^{a} \pm 0.0$	$1.3^{a} \pm 0.2$	$2.2^{c} \pm 0.2$	$0.3^{b} \pm 0.0$
30 days	AS1H	$0.7^{b} \pm 0.2$	$0.7^{a} \pm 0.2$	$2.6^{a} \pm 0.2$	$0.2^{a} \pm 0.1$
	AS2H	$0.6^{b} \pm 0.2$	$1.6^{a} \pm 0.2$	$2.9^{a} \pm 0.2$	$0.4^{ab} \pm 0.2$
	AS2H2S	$0.1^{a} \pm 0.0$	$1.5^{a} \pm 0.2$	$3.7^{b} \pm 0.3$	$0.5^{b} \pm 0.0$
Total	AS1H				$1.0^{a} \pm 0.2$*
	AS2H				$1.0^{a} \pm 0.3$*
	AS2H2S				$0.8^{a} \pm 0.1$*

AS1H - aeroponic with sprayers with Hoagland solution, AS2H - aeroponic with sprayers with two times concentrated Hoagland solution, AS2H2S - aeroponic with sprayers with two times concentrated Hoagland solution and two times increased concentration of sulfur. The differences are compared for each treatment. Values followed by the same letter are not significantly different. ND – not determined. *Sum of glucosinolates exuded during 30 days

Table 4.12a. Influence of plant nutrition on indole glucosinolate content in plants and exudates of turnip from aeroponic with defensor (mg plant^{-1})

Date	Treatment	Leaves	Secondary roots	Primary roots	Exudates
10 days	AD1H	$0.3^{a} \pm 0.1$	$0.2^{a} \pm 0.0$	ND	ND
	AD2H	$0.6^{b} \pm 0.1$	$0.3^{a} \pm 0.1$	ND	ND
	AD2H2S	$0.2^{a} \pm 0.1$	$1.0^{b} \pm 0.2$	ND	$0.1^{a} \pm 0.0$
20 days	AD1H	$0.8^{a} \pm 0.1$	$0.7^{a} \pm 0.3$	$0.4^{a} \pm 0.0$	$0.2^{a} \pm 0.1$
	AD2H	$1.2^{b} \pm 0.1$	$2.2^{b} \pm 0.3$	$0.5^{a} \pm 0.2$	$0.3^{a} \pm 0.1$
	AD2H2S	$0.9^{a} \pm 0.1$	$2.4^{b} \pm 0.3$	$1.3^{b} \pm 0.0$	$0.6^{b} \pm 0.1$
30 days	AD1H	$1.3^{a} \pm 0.1$	$1.3^{a} \pm 0.3$	$1.0^{a} \pm 0.0$	$0.4^{a} \pm 0.1$
	AD2H	$1.4^{a} \pm 0.1$	$2.7^{b} \pm 0.3$	$1.8^{b} \pm 0.3$	$0.9^{b} \pm 0.1$
	AD2H2S	$1.4^{a} \pm 0.3$	$3.1^{b} \pm 0.3$	$2.9^{c} \pm 0.2$	$1.8^{c} \pm 0.3$
Total	AD1H				$0.6^{a} \pm 0.1$*
	AD2H				$1.2^{b} \pm 0.1$*
	AD2H2S				$2.6^{c} \pm 0.6$*

AD1H - aeroponic with defensor with Hoagland solution, AD2H - aeroponic with defensor with two time concentrated Hoagland solution, AD2H2S - aeroponic with defensor with two times concentrated Hoagland solution and two times increased concentration of sulfur. The differences are compared for each treatment. Values followed by the same letter are not significantly different. ND – not determined. *Sum of glucosinolates exuded during 30 days

Table 4.12a. Influence of plant nutrition on indole glucosinolate content in plants and exudates of turnip from aeroponic with sprayers (mg plant^{-1})

Date	Treatment	Leaves	Secondary roots	Primary roots	Exudates
10 days	AS1H	$0.2^{a} \pm 0.1$	$0.3^{a} \pm 0.0$	ND	ND
	AS2H	$0.2^{a} \pm 0.1$	$0.3^{a} \pm 0.0$	ND	ND
	AS2H2S	$0.3^{a} \pm 0.0$	$1.0^{b} \pm 0.2$	ND	$0.2^{a} \pm 0.0$
20 days	AS1H	$0.8^{a} \pm 0.1$	$0.5^{a} \pm 0.0$	$0.6^{a} \pm 0.1$	$0.3^{a} \pm 0.1$
	AS2H	$1.2^{a} \pm 0.1$	$0.9^{b} \pm 0.1$	$1.1^{b} \pm 0.0$	$0.2^{a} \pm 0.1$
	AS2H2S	$0.8^{a} \pm 0.1$	$2.4^{c} \pm 0.1$	$0.8^{a} \pm 0.1$	$0.5^{a} \pm 0.2$
30 days	AS1H	$1.6^{a} \pm 0.1$	$1.0^{a} \pm 0.2$	$2.0^{a} \pm 0.4$	$0.4^{a} \pm 0.1$
	AS2H	$1.9^{a} \pm 0.1$	$2.6^{b} \pm 0.5$	$2.7^{ab} \pm 0.7$	$0.7^{ab} \pm 0.2$
	AS2H2S	$1.6^{a} \pm 0.1$	$2.5^{b} \pm 0.5$	$3.5^{a} \pm 0.1$	$1.1^{b} \pm 0.3$
Total	AS1H				$1.0^{a} \pm 0.2$*
	AS2H				$1.0^{a} \pm 0.2$*
	AS2H2S				$1.8^{b} \pm 0.4$*

AS1H - aeroponic with sprayers with Hoagland solution, AS2H - aeroponic with sprayers with two times concentrated Hoagland solution, AS2H2S - aeroponic with sprayers with two times concentrated Hoagland solution and two times increased concentration of sulfur. The differences are compared for each treatment. Values followed by the same letter are not significantly different. ND – not determined. *Sum of glucosinolates exuded during 30 days

Table 4.18a. Influence of plant nutrition on on fresh weight (g) and glucosinolate concentration (mg g^{-1}) in turnip plants from aeroponic with defensor

Time	Treat-ment	Fresh weight, g/ concentration of glucosinolates, mg g^{-1}	Leaves	Roots		Leaf : root index
				secondary	primary	
10 days	AD1H	g	$5.7^{b} \pm 0.7$	$1.3^{c} \pm 0.4$		4.4
		mg g^{-1}	0.2	0.2		
	AD2H	g	$5.4^{b} \pm 0.7$	$1.8^{c} \pm 0.4$		3.0
		mg g^{-1}	0.2	0.4		
	AD2H2S	g	$8.8^{b} \pm 0.5$	$2.8^{c} \pm 0.0$		3.1
		mg g^{-1}	0.2	0.1		
20 days	AD1H	g	$17.2^{b} \pm 1.4$	$8.6^{c} \pm 0.3$	$2.4^{a} \pm 0.9$	2.0
		mg g^{-1}	0.3	0.9	0.4	
	AD2H	g	$17.9^{b} \pm 1.3$	$9.6^{c} \pm 1.0$	$2.3^{a} \pm 0.9$	1.9
		mg g^{-1}	0.2	1	0.6	
	AD2H2S	g	$24.5^{b} \pm 1.6$	$12.4^{b} \pm 1.3$	$1.6^{a} \pm 0.1$	2.0
		mg g^{-1}	0.1	1	0.4	
30 days	AD1H	g	$20.2^{b} \pm 0.8$	$12.7^{c} \pm 1.9$	$4.4^{a} \pm 0.3$	1.6
		mg g^{-1}	0.2	1.1	0.2	
	AD2H	g	$27.1^{b} \pm 1.3$	$18.3^{c} \pm 2.8$	$5.2^{a} \pm 0.2$	1.5
		mg g^{-1}	0.2	1.2	0.2	
	AD2H2S	g	$29.7^{b} \pm 1.2$	$21.1^{a} \pm 2.1$	$5.7^{b} \pm 0.1$	1.3
		mg g^{-1}	0.2	1.4	0.3	

AD1H - aeroponic with defensor with Hoagland solution, AD2H - aeroponic with defensor with two time concentrated Hoagland solution, AD2H2S - aeroponic with defensor with two times concentrated Hoagland solution and two times increased concentration of sulfur. The differences are compared for each treatment. Values followed by the same letter are not significantly different.

Table 4.18b. Influence of plant nutrition on on fresh weight (g) and glucosinolate concentration (mg g^{-1}) in turnip plants from aeroponic with sprayers

Time	Treat-ment	Fresh weight, g/ concentration of glucosinolates, mg g^{-1}	Leaves	Roots		Leaf : root index
				secondary	primary	
10 days	AS1H	g	13.2[a] ± 0.9	4.7[a] ± 0.2		2.8
		mg g^{-1}	0.2	0.2		
	AS2H	g	13.8[a] ± 0.7	3.3[a] ± 0.4		4.1
		mg g^{-1}	0.2	0.4		
	AS2H2S	g	13.7[a] ± 1.3	3.0[a] ± 0.4		4.3
		mg g^{-1}	0.2	0.1		
20 days	AS1H	g	18.9[a] ± 0.8	6.1[a] ± 0.7	3.6[a] ± 0.2	3.1
		mg g^{-1}	0.3	0.9	0.4	
	AS2H	g	20.4[a] ± 1.9	9.0[a] ± 0.4	3.3[a] ± 0.2	2.3
		mg g^{-1}	0.2	1	0.6	
	AS2H2S	g	27.7[a] ± 2.8	13.5[a] ± 0.7	4.2[a] ± 0.8	2.0
		mg g^{-1}	0.1	1	0.4	
30 days	AS1H	g	32.9[a] ± 3.4	14.4[a] ± 0.6	7.6[a] ± 0.4	2.3
		mg g^{-1}	0.2	1.1	0.2	
	AS2H	g	31.0[a] ± 1.7	18.7[a] ± 0.3	7.0[a] ± 0.3	1.6
		mg g^{-1}	0.2	1.2	0.2	
	AS2H2S	g	36.6[a] ± 2.8	21.9[a] ± 1.1	9.2[a] ± 0.3	1.6
		mg g^{-1}	0.2	1.4	0.3	

AS1H - aeroponic with sprayers with Hoagland solution, AS2H - aeroponic with sprayers with two times concentrated Hoagland solution, AS2H2S - aeroponic with sprayers with two times concentrated Hoagland solution and two times increased concentration of sulfur. The differences are compared for each treatment. Values followed by the same letter are not significantly different.

Table 4.19a. Elicitor influence on total glucosinolate content in plants and exudates of turnip from aeroponic with defensor (mg plant^{-1})

Date	Treatment	Leaves	Secondary roots	Primary roots	Exudates
10 days	AD	$2.0^{a} \pm 0.1$	$1.0^{a} \pm 0.2$		$0.3^{a} \pm 0.0$
	$ADSA_0$	$3.0^{b} \pm 0.2$	$2.0^{b} \pm 0.4$		$1.4^{b} \pm 0.2$
	$ADMJ_0$	$3.6^{b} \pm 0.7$	$2.5^{b} \pm 0.2$		$1.8^{b} \pm 0.2$
15 days	AD	$2.1^{a} \pm 0.5$	$1.8^{a} \pm 0.3$	$3.0^{b} \pm 0.3$	$0.7^{a} \pm 0.2$
	$ADSA_0$	$3.3^{b} \pm 0.4$	$3.8^{b} \pm 0.6$	$3.8^{b} \pm 0.8$	$1.9^{b} \pm 0.1$
	$ADMJ_0$	$6.0^{b} \pm 0.5$	$5.5^{b} \pm 1.2$	$2.0^{a} \pm 0.1$	$2.0^{b} \pm 0.3$
20 days	AD	$2.5^{a} \pm 0.1$	$3.4^{a} \pm 0.4$	$5.0^{c} \pm 0.4$	$0.8^{a} \pm 0.1$
	$ADSA_0$	$3.7^{b} \pm 0.6$	$6.4^{b} \pm 0.5$	$4.3^{b} \pm 0.1$	$1.5^{b} \pm 0.1$
	$ADMJ_0$	$6.2^{c} \pm 0.2$	$7.4^{c} \pm 0.3$	$2.6^{a} \pm 0.1$	$1.3^{c} \pm 0.2$
25 days	AD	$3.0^{a} \pm 0.1$	$5.0^{a} \pm 0.3$	$8.4^{ab} \pm 0.7$	$1.9^{b} \pm 0.2$
	$ADSA_0$	$4.6^{b} \pm 0.5$	$8.8^{b} \pm 0.9$	$6.8^{b} \pm 0.3$	$1.1^{a} \pm 0.3$
	$ADMJ_0$	$6.8^{b} \pm 0.4$	$8.3^{b} \pm 0.7$	$7.5^{a} \pm 0.5$	$1.2^{a} \pm 0.4$
30 days	AD	$3.9^{a} \pm 0.8$	$5.6^{a} \pm 0.7$	$10.2^{a} \pm 0.9$	$2.1^{c} \pm 0.3$
	$ADSA_0$	$4.6^{c} \pm 0.4$	$10.8^{c} \pm 1.3$	$8.6^{a} \pm 0.7$	$1.5^{b} \pm 0.1$
	$ADMJ_0$	$5.9^{b} \pm 0.4$	$7.6^{b} \pm 0.4$	$9.0^{a} \pm 1.1$	$1.0^{a} \pm 0.1$
Total	AD				$5.9^{a} \pm 0.5$*
	$ADSA_0$				$7.4^{b} \pm 0.4$*
	$ADMJ_0$				$7.3^{b} \pm 0.5$*

AD – aeroponic with defensor, two times concentrated Hoagland solution + two times increased sulfur; $ADSA_0$ - aeroponic with defensor, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; $ADMJ_0$ - aeroponic with defensor, two times concentrated Hoagland solution + two times increased sulfur + methyl jasmonate applied on the beginning. The differences are compared for each treatment. Values followed by the same letter are not significantly different. *Sum of glucosinolates exuded during 30 days

Table 4.19a. Elicitor influence on total glucosinolate content in plants and exudates of turnip from aeroponic with sprayers (mg plant^{-1})

Date	Treatment	Leaves	Secondary roots	Primary roots	Exudates
10 days	AS	$2.1^{a} \pm 0.2$	$1.5^{a} \pm 0.0$		$0.4^{a} \pm 0.2$
	$ASSA_0$	$2.8^{b} \pm 0.4$	$2.0^{b} \pm 0.2$		$1.4^{b} \pm 0.3$
15 days	AS	$2.1^{a} \pm 0.1$	$1.8^{a} \pm 0.1$	$5.5^{a} \pm 1.0$	$0.6^{a} \pm 0.3$
	$ASSA_0$	$4.2^{b} \pm 0.3$	$5.9^{b} \pm 0.6$	$4.0^{a} \pm 0.7$	$1.6^{b} \pm 0.2$
20 days	AS	$2.7^{a} \pm 0.5$	$3.0^{a} \pm 0.7$	$6.4^{b} \pm 0.6$	$0.8^{a} \pm 0.1$
	$ASSA_0$	$4.6^{b} \pm 0.6$	$7.1^{b} \pm 0.9$	$4.7^{a} \pm 0.6$	$1.3^{b} \pm 0.1$
25 days	AS	$3.4^{a} \pm 0.5$	$5.3^{a} \pm 0.6$	$8.7^{a} \pm 1.2$	$1.2^{a} \pm 0.1$
	$ASSA_0$	$4.6^{a} \pm 0.6$	$8.4^{b} \pm 0.4$	$6.8^{a} \pm 1.0$	$1.3^{a} \pm 0.1$
30 days	AS	$3.9^{a} \pm 0.4$	$7.1^{a} \pm 0.3$	$10.7^{b} \pm 0.2$	$2.0^{b} \pm 0.2$
	$ASSA_0$	$5.1^{b} \pm 0.4$	$10.8^{b} \pm 0.5$	$8.6^{a} \pm 0.7$	$1.3^{a} \pm 0.2$
Total	AS				$5.0^{a} \pm 0.4$*
	$ASSA_0$				$6.9^{b} \pm 0.4$*

AS – aeroponic with sprayers, two times concentrated Hoagland solution + two times increased sulfur; $ASSA_0$ - aeroponic with sprayers, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning. The differences are compared for each treatment. Values followed by the same letter are not significantly different.. *Sum of glucosinolates exuded during 30 days.

Table 4.20a. Elicitor influence on composition of aliphatic, aromatic, and indole glucosinolates in plants and exudates of turnip in aeroponic with defensor on 30th day

	Treatment		Aliphatic	Aromatic	Indole
Leaves	AD	mg plant^{-1}	2.6^{c} ± 0.1	ND	1.3^{a} ± 0.1
		%	67	0	33
	$ADSA_0$	mg plant^{-1}	1.8^{a} ± 0.1	0.4^{a} ± 0.2	1.9^{b} ± 0.1
		%	44	10	46
	$ADMJ_0$	mg plant^{-1}	2.2^{b} ± 0.2	0.6^{a} ± 0.2	2.8^{c} ± 0.2
		%	39	11	50
Secondary roots	AD	mg plant^{-1}	1.3^{a} ± 0.2	1.2^{a} ± 0.4	2.3^{a} ± 0.2
		%	27	25	48
	$ADSA_0$	mg plant^{-1}	1.5^{a} ± 0.3	2.3^{b} ± 0.4	6.3^{c} ± 0.5
			15	23	62
	$ADMJ_0$	mg plant^{-1}	1.1^{a} ± 0.3	2.8^{b} ± 0.3	3.9^{b} ± 0.2
		%	14	36	50
Primary roots	AD	mg plant^{-1}	6.8^{c} ± 0.2	0.7^{a} ± 0.2	1.4^{a} ± 0.2
		%	76	8	16
	$ADSA_0$	mg plant^{-1}	3.5^{a} ± 0.4	3.4^{b} ± 0.3	1.2^{a} ± 0.2
		%	43	42	15
	$ADMJ_0$	mg plant^{-1}	4.9^{b} ± 0.4	3.8^{b} ± 0.4	1.1^{a} ± 0.3
		%	50	39	11
Exudates	AD	mg plant^{-1}	1.9^{a} ± 0.5*	1.3^{a} ± 0.1*	2.6^{a} ± 0.6*
		%	33	22	45
	$ADSA_0$	mg plant^{-1}	1.5^{a} ± 0.4*	2.2^{b} ± 0.2*	3.7^{b} ± 0.1*
		%	20	30	50
	$ADMJ_0$	mg plant^{-1}	1.3^{a} ± 0.4*	1.9^{b} ± 0.4*	4.2^{b} ± 0.8*
		%	18	26	57

AD – aeroponic with defensor, two times concentrated Hoagland solution + two times increased sulfur; $ADSA_0$ - aeroponic with defensor, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; $ADMJ_0$ - aeroponic with defensor, two times concentrated Hoagland solution + two times increased sulfur + methyl jasmonate applied on the beginning. The differences are compared for each treatment. Values followed by the same letter are not significantly different. ND – not determined. *Sum of glucosinolates exuded during 30 days

Table 4.20b. Elicitor influence on composition of aliphatic, aromatic, and indole glucosinolates in plants and exudates of turnip in aeroponic with sprayers on 30th day

	Treatment		Aliphatic	Aromatic	Indole
Leaves	AS	mg plant^{-1}	2.6^{c} ± 0.1	ND	1.3^{a} ± 0.1
		%	67	0	33
	$ASSA_0$	mg plant^{-1}	1.8^{a} ± 0.1	0.4^{a} ± 0.2	1.9^{b} ± 0.1
		%	44	10	46
Secondary roots	AS	mg plant^{-1}	1.3^{a} ± 0.2	1.2^{a} ± 0.4	2.3^{a} ± 0.2
		%	27	25	48
	$ASSA_0$	mg plant^{-1}	1.5^{a} ± 0.3	2.3^{b} ± 0.4	6.3^{c} ± 0.5
			15	23	62
Primary roots	AS	mg plant^{-1}	6.8^{c} ± 0.2	0.7^{a} ± 0.2	1.4^{a} ± 0.2
		%	76	8	16
	$ASSA_0$	mg plant^{-1}	3.5^{a} ± 0.4	3.4^{b} ± 0.3	1.2^{a} ± 0.2
		%	43	42	15
Exudates	AS	mg plant^{-1}	2.4^{a} ± 0.1*	0.8^{b} ± 0.1*	1.8^{a} ± 0.2*
		%	48	16	36
	$ASSA_0$	mg plant^{-1}	2.7^{a} ± 0.2*	1.7^{b} ± 0.1*	2.5^{b} ± 0.2*
		%	39	25	36

AS – aeroponic with sprayers, two times concentrated Hoagland solution + two times increased sulfur; $ASSA_0$ - aeroponic with sprayers, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning. The differences are compared for each treatment. Values followed by the same letter are not significantly different. ND – not determined. *Sum of glucosinolates exuded during 30 days

Table 4.21a. Elicitor influence on aliphatic glucosinolate content in plants and exudates of turnip from aeroponic with defensor (mg plant^{-1})

Date	Treatment	Leaves	Secondary roots	Primary roots	Exudates
10 days	AD	$1.2^{b} \pm 0.2$	$0.2^{ab} \pm 0.1$		$0.1^{a} \pm 0.0$
	$ADSA_0$	$0.8^{a} \pm 0.1$	$0.2^{a} \pm 0.0$		$0.3^{c} \pm 0.0$
	$ADMJ_0$	$1.5^{b} \pm 0.3$	$0.4^{b} \pm 0.2$		$0.2^{b} \pm 0.0$
15 days	AD	$1.6^{a} \pm 0.4$	$0.4^{ab} \pm 0.1$	$1.0^{b} \pm 0.0$	$0.3^{a} \pm 0.0$
	$ADSA_0$	$1.6^{a} \pm 0.2$	$0.3^{a} \pm 0.1$	$1.0^{a} \pm 0.4$	$0.3^{a} \pm 0.1$
	$ADMJ_0$	$2.1^{a} \pm 0.4$	$0.7^{b} \pm 0.2$	$0.7^{ab} \pm 0.2$	$0.3^{a} \pm 0.1$
20 days	AD	$1.6^{a} \pm 0.3$	$0.5^{a} \pm 0.2$	$1.7^{b} \pm 0.2$	$0.4^{a} \pm 0.2$
	$ADSA_0$	$1.6^{a} \pm 0.2$	$0.8^{ab} \pm 0.1$	$0.9^{a} \pm 0.1$	$0.3^{a} \pm 0.1$
	$ADMJ_0$	$2.1^{a} \pm 0.4$	$0.9^{b} \pm 0.1$	$1.2^{a} \pm 0.3$	$0.3^{a} \pm 0.2$
25 days	AD	$1.8^{a} \pm 0.3$	$0.7^{a} \pm 0.2$	$3.5^{a} \pm 0.2$	$0.5^{b} \pm 0.2$
	$ADSA_0$	$1.7^{a} \pm 0.2$	$1.1^{a} \pm 0.1$	$3.2^{a} \pm 0.2$	$0.3^{ab} \pm 0.2$
	$ADMJ_0$	$2.0^{a} \pm 0.4$	$0.9^{ab} \pm 0.2$	$3.8^{a} \pm 0.3$	$0.2^{a} \pm 0.0$
30 days	AD	$2.7^{c} \pm 0.3$	$0.9^{a} \pm 0.2$	$5.7^{c} \pm 0.4$	$0.6^{a} \pm 0.1$
	$ADSA_0$	$1.9^{a} \pm 0.4$	$1.3^{b} \pm 0.1$	$3.5^{a} \pm 0.2$	$0.4^{a} \pm 0.2$
	$ADMJ_0$	$2.1^{ab} \pm 0.5$	$1.2^{ab} \pm 0.1$	$3.9^{b} \pm 0.2$	$0.3^{a} \pm 0.2$
Total	AD				$1.9^{a} \pm 0.5$*
	$ADSA_0$				$1.5^{a} \pm 0.4$*
	$ADMJ_0$				$1.3^{a} \pm 0.4$*

AD – aeroponic with defensor, two times concentrated Hoagland solution + two times increased sulfur; $ADSA_0$ - aeroponic with defensor, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; $ADMJ_0$ - aeroponic with defensor, two times concentrated Hoagland solution + two times increased sulfur + methyl jasmonate applied on the beginning. The differences are compared for each treatment. Values followed by the same letter are not significantly different. *Sum of glucosinolates exuded during 30 days

Table 4.21b. Elicitor influence on aliphatic glucosinolate content in plants and exudates of turnip from aeroponic with sprayers (mg plant^{-1})

Date	Treatment	Leaves	Secondary roots	Primary roots	Exudates
10 days	AS	$1.2^{b} \pm 0.2$	$0.3^{b} \pm 0.0$		$0.3^{a} \pm 0.0$
	$ASSA_0$	$0.6^{a} \pm 0.2$	$0.1^{a} \pm 0.1$		$0.4^{a} \pm 0.2$
15 days	AS	$1.6^{a} \pm 0.3$	$0.4^{a} \pm 0.2$	$1.0^{a} \pm 0.0$	$0.3^{a} \pm 0.1$
	$ASSA_0$	$1.4^{a} \pm 0.2$	$0.6^{a} \pm 0.1$	$1.0^{a} \pm 0.2$	$0.4^{a} \pm 0.1$
20 days	AS	$1.9^{b} \pm 0.2$	$0.5^{a} \pm 0.1$	$1.9^{a} \pm 0.4$	$0.4^{a} \pm 0.1$
	$ASSA_0$	$1.5^{a} \pm 0.1$	$0.8^{b} \pm 0.0$	$1.5^{a} \pm 0.5$	$0.5^{a} \pm 0.1$
25 days	AS	$2.1^{a} \pm 0.5$	$0.7^{a} \pm 0.2$	$3.8^{b} \pm 0.4$	$0.6^{a} \pm 0.0$
	$ASSA_0$	$1.7^{a} \pm 0.3$	$0.9^{a} \pm 0.2$	$1.9^{a} \pm 0.3$	$0.6^{a} \pm 0.2$
30 days	AS	$2.6^{b} \pm 0.4$	$0.8^{a} \pm 0.1$	$5.2^{b} \pm 0.9$	$0.9^{a} \pm 0.1$
	$ASSA_0$	$1.7^{a} \pm 0.3$	$1.1^{a} \pm 0.5$	$3.9^{a} \pm 0.3$	$0.8^{a} \pm 0.2$
Total	AS				$2.4^{a} \pm 0.1$*
	$ASSA_0$				$2.7^{a} \pm 0.2$*

AS – aeroponic with sprayers, two times concentrated Hoagland solution + two times increased sulfur; $ASSA_0$ - aeroponic with sprayers, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning. The differences are compared for each treatment. Values followed by the same letter are not significantly different. *Sum of glucosinolates exuded during 30 days

Table 4.22a. Elicitor influence on individual aliphatic glucosinolates content in plants and exudates of turnip from aeroponic with defensor on 30th day (mg plant^{-1})

Gluco-sinolates	Treat-ment		Leaves	Secondary roots	Primary roots	Exudates
Gluconapin	AD	mg plant^{-1}	0.6^{c} ± 0.1	0.1^{a} ± 0.0	1.7^{b} ± 0.3	0.5^{a} ± 0.2*
		%	25	4	71	
	$ADSA_0$	mg plant^{-1}	0.1^{a} ± 0.0	0.1^{a} ± 0.0	0.6^{b} ± 0.1	0.4^{a} ± 0.1*
		%	13	13	75	
	$ADMJ_0$	mg plant^{-1}	0.4^{b} ± 0.0	0.2^{b}± 0.0	0.2^{a} ± 0.1	0.4^{a} ± 0.1*
		%	50	25	25	
Progoitrin	AD	mg plant^{-1}	0.9^{a} ± 0.1	0.4^{a} ± 0.1	1.9^{a} ± 0.2	0.9^{b} ± 0.2*
		%	28	13	59	
	$ADSA_0$	mg plant^{-1}	0.8^{a} ± 0.2	0.4^{a} ± 0.1	1.8^{a} ± 0.1	0.6^{b} ± 0.1*
		%	27	13	60	
	$ADMJ_0$	mg plant^{-1}	0.7^{a} ± 0.1	0.4^{a} ± 0.0	1.5^{a} ± 0.2	0.4^{ab} ± 0.2*
		%	27	15	58	
Glucobras-sicanapin	AD	mg plant^{-1}	0.2^{b} ± 0.0	0.1^{a} ± 0.1	1.0^{a} ± 0.3	0.2^{a} ± 0.1*
		%	15	8	77	
	$ADSA_0$	mg plant^{-1}	0.2^{b} ± 0.0	0.5^{b} ± 0.1	0.7^{a} ± 0.2	0.2^{a} ± 0.0*
		%	14	36	50	
	$ADMJ_0$	mg plant^{-1}	0.1^{a} ± 0.0	0.1^{a} ± 0.0	0.8^{a} ± 0.2	0.2^{a} ± 0.1*
		%	10	10	80	
Gluconapo-leiferin	AD	mg plant^{-1}	1.0^{a} ± 0.2	0.3^{a} ± 0.1	1.0^{b} ± 0.2	0.3^{a} ± 0.1*
		%	43	13	43	
	$ADSA_0$	mg plant^{-1}	0.8^{a} ±0.2	0.3^{a} ± 0.1	0.4^{a} ± 0.1	0.3^{a} ± 0.0*
		%	53	20	27	
	$ADMJ_0$	mg plant^{-1}	1.0^{a} ± 0.2	0.4^{a} ± 0.2	1.4^{c} ± 0.1	0.3^{a} ± 0.1*
		%	36	14	50	

AD – aeroponic with defensor, two times concentrated Hoagland solution + two times increased sulfur; $ADSA_0$ - aeroponic with defensor, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; $ADMJ_0$ - aeroponic with defensor, two times concentrated Hoagland solution + two times increased sulfur + methyl jasmonate applied on the beginning. The differences are compared for each treatment. Values followed by the same letter are not significantly different. *Sum of glucosinolates exuded during 30 days

Table 4.22b. Elicitor influence on individual aliphatic glucosinolates content in plants and exudates of turnip from aeroponic with sprayers on 30th day (mg plant^{-1})

Gluco-sinolates	Treat-ment		Leaves	Secondary roots	Primary roots	Exudates
Gluconapin	AS	mg plant^{-1}	0.6^{b} ± 0.1	0.2^{a} ± 0.1	1.7^{a} ± 0.2	0.9^{a} ± 0.2*
		%	24	8	68	
	$ASSA_0$	mg plant^{-1}	0.3^{a} ± 0.0	0.5^{b} ± 0.1	1.4^{a} ± 0.3	1.3^{a} ± 0.3*
		%	14	23	64	
Progoitrin	AS	mg plant^{-1}	1.2^{b} ± 0.2	0.4^{a} ± 0.1	2.0^{b} ± 0.2	1.0^{a} ± 0.2*
		%	33	11	56	
	$ASSA_0$	mg plant^{-1}	0.6^{a} ± 0.1	0.3^{a} ± 0.2	1.2^{a} ± 0.2	0.8^{a} ± 0.2*
		%	29	14	57	
Glucobras-sicanapin	AS	mg plant^{-1}	0.4^{a} ± 0.1	0.2^{a} ± 0.1	0.6^{a} ± 0.1	0.3^{a} ± 0.1*
		%	33	17	50	
	$ASSA_0$	mg plant^{-1}	0.4^{a} ± 0.1	0.1^{a} ± 0.2	0.6^{a} ± 0.3	0.5^{a} ± 0.1*
		%	36	9	55	
Gluconapo-leiferin	AS	mg plant^{-1}	0.5^{a} ± 0.2	0.1^{a} ± 0.0	1.0^{a} ± 0.1	0.2^{a} ± 0.1*
		%	31	6	63	
	$ASSA_0$	mg plant^{-1}	0.4^{a} ± 0.1	0.2^{b} ± 0.0	0.7^{a} ± 0.1	0.2^{a} ± 0.0*
		%	31	15	54	

AS – aeroponic with sprayers, two times concentrated Hoagland solution + two times increased sulfur; $ASSA_0$ - aeroponic with sprayers, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning. The differences are compared for each treatment. Values followed by the same letter are not significantly different. *Sum of glucosinolates exuded during 30 days

Table 4.28a. Elicitor influence on aromatic glucosinolate content in plants and exudates of turnip from aeroponic with defensor (mg plant^{-1})

Date	Treatment	Leaves	Secondary roots	Primary roots	Exudates
10 days	AD	$0.2^{a} \pm 0.2$	$0.2^{a} \pm 0.1$		$0.1^{a} \pm 0.1$
	$ADSA_0$	$0.3^{a} \pm 0.1$	$0.5^{b} \pm 0.1$		$0.4^{b} \pm 0.1$
	$ADMJ_0$	$0.1^{a} \pm 0.1$	$0.3^{ab} \pm 0.1$		$0.2^{ab} \pm 0.1$
15 days	AD	$0.1^{a} \pm 0.1$	$0.3^{a} \pm 0.1$	$0.7^{b} \pm 0.1$	$0.1^{a} \pm 0.1$
	$ADSA_0$	$0.4^{b} \pm 0.1$	$0.6^{b} \pm 0.1$	$1.5^{b} \pm 0.2$	$0.5^{b} \pm 0.1$
	$ADMJ_0$	$0.3^{ab} \pm 0.2$	$1.1^{c} \pm 0.2$	$1.2^{a} \pm 0.2$	$0.5^{b} \pm 0.0$
20 days	AD	$0.2^{a} \pm 0.0$	$0.9^{a} \pm 0.2$	$1.6^{b} \pm 0.2$	$0.1^{a} \pm 0.0$
	$ADSA_0$	$0.3^{a} \pm 0.1$	$1.5^{b} \pm 0.3$	$2.0^{b} \pm 0.1$	$0.5^{b} \pm 0.2$
	$ADMJ_0$	$0.2^{a} \pm 0.0$	$1.4^{b} \pm 0.2$	$1.3^{a} \pm 0.1$	$0.4^{b} \pm 0.0$
25 days	AD	$0.2^{a} \pm 0.1$	$1.3^{a} \pm 0.0$	$2.2^{ab} \pm 0.4$	$0.5^{a} \pm 0.0$
	$ADSA_0$	$0.2^{ab} \pm 0.2$	$2.1^{b} \pm 0.4$	$2.6^{b} \pm 0.4$	$0.4^{a} \pm 0.1$
	$ADMJ_0$	$0.4^{b} \pm 0.1$	$1.9^{b} \pm 0.2$	$2.3^{a} \pm 0.2$	$0.6^{a} \pm 0.2$
30 days	AD	$0.1^{a} \pm 0.2$	$0.8^{a} \pm 0.2$	$2.2^{a} \pm 0.1$	$0.5^{a} \pm 0.1$
	$ADSA_0$	$0.3^{ab} \pm 0.0$	$2.2^{b} \pm 0.1$	$2.8^{b} \pm 0.1$	$0.4^{a} \pm 0.1$
	$ADMJ_0$	$0.5^{b} \pm 0.1$	$1.9^{b} \pm 0.2$	$2.6^{b} \pm 0.3$	$0.3^{a} \pm 0.1$
Total	AD				$1.3^{a} \pm 0.1$*
	$ADSA_0$				$2.2^{b} \pm 0.2$*
	$ADMJ_0$				$1.9^{b} \pm 0.4$*

AD – aeroponic with defensor, two times concentrated Hoagland solution + two times increased sulfur; $ADSA_0$ - aeroponic with defensor, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; $ADMJ_0$ - aeroponic with defensor, two times concentrated Hoagland solution + two times increased sulfur + methyl jasmonate applied on the beginning. The differences are compared for each treatment. Values followed by the same letter are not significantly different. *Sum of glucosinolates exuded during 30 days.

Table 4.28b. Elicitor influence on aromatic glucosinolate content in plants and exudates of turnip from aeroponic with sprayers (mg plant^{-1})

Date	Treatment	Leaves	Secondary roots	Primary roots	Exudates
10 days	AS	$0.2^{a} \pm 0.0$	$0.7^{a} \pm 0.1$		ND
	$ASSA_0$	$0.4^{b} \pm 0.0$	$1.2^{b} \pm 0.1$		$0.4^{a} \pm 0.1$
15 days	AS	$0.3^{a} \pm 0.2$	$0.9^{a} \pm 0.2$	$1.1^{a} \pm 0.1$	$0.1^{a} \pm 0.0$
	$ASSA_0$	$0.5^{b} \pm 0.1$	$1.9^{b} \pm 0.3$	$1.9^{b} \pm 0.0$	$0.6^{b} \pm 0.1$
20 days	AS	$0.4^{a} \pm 0.0$	$1.3^{a} \pm 0.2$	$2.2^{a} \pm 0.2$	$0.2^{a} \pm 0.0$
	$ASSA_0$	$0.5^{a} \pm 0.2$	$2.4^{b} \pm 0.2$	$2.3^{a} \pm 0.1$	$0.3^{a} \pm 0.1$
25 days	AS	$0.5^{a} \pm 0.2$	$1.5^{a} \pm 0.2$	$2.7^{a} \pm 0.2$	$0.2^{a} \pm 0.1$
	$ASSA_0$	$0.5^{a} \pm 0.1$	$2.5^{b} \pm 0.1$	$2.5^{a} \pm 0.2$	$0.3^{a} \pm 0.1$
30 days	AS	$0.1^{a} \pm 0.0$	$1.5^{a} \pm 0.2$	$3.7^{b} \pm 0.3$	$0.3^{a} \pm 0.0$
	$ASSA_0$	$0.4^{b} \pm 0.2$	$2.5^{b} \pm 0.2$	$2.8^{a} \pm 0.2$	$0.2^{a} \pm 0.0$
30 days	AS				$0.8^{b} \pm 0.1$*
	$ASSA_0$				$1.7^{b} \pm 0.1$*

AS – aeroponic with sprayers, two times concentrated Hoagland solution + two times increased sulfur; $ASSA_0$ - aeroponic with sprayers, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning. The differences are compared for each treatment. Values followed by the same letter are not significantly different. *Sum of glucosinolates exuded during 30 days.

Table 4.29a. Elicitor influence on indole glucosinolate content in plants and exudates of turnip from aeroponic with defensor (mg plant^{-1})

Date	Treatment	Leaves	Secondary roots	Primary roots	Exudates
10 days	AD	$0.2^{a} \pm 0.1$	$1.0^{a} \pm 0.2$		$0.1^{a} \pm 0.0$
	$ADSA_0$	$1.2^{b} \pm 0.3$	$2.0^{a} \pm 0.2$		$0.7^{b} \pm 0.2$
	$ADMJ_0$	$1.6^{b} \pm 0.2$	$1.6^{a} \pm 0.3$		$1.4^{c} \pm 0.3$
15 days	AD	$0.7^{a} \pm 0.3$	$1.3^{a} \pm 0.2$	$0.8^{c} \pm 0.1$	$0.3^{a} \pm 0.0$
	$ADSA_0$	$1.7^{b} \pm 0.2$	$2.8^{b} \pm 0.2$	$1.3^{b} \pm 0.2$	$1.1^{b} \pm 0.2$
	$ADMJ_0$	$3.2^{c} \pm 0.3$	$3.4^{b} \pm 0.7$	$0.9^{a} \pm 0.1$	$1.2^{b} \pm 0.2$
20 days	AD	$0.9^{a} \pm 0.1$	$2.4^{a} \pm 0.3$	$1.3^{b} \pm 0.0$	$0.3^{a} \pm 0.1$
	$ADSA_0$	$2.2^{b} \pm 0.4$	$3.0^{b} \pm 0.2$	$1.9^{a} \pm 0.1$	$0.7^{b} \pm 0.1$
	$ADMJ_0$	$3.2^{c} \pm 0.3$	$4.4^{c} \pm 0.1$	$1.1^{a} \pm 0.0$	$0.6^{b} \pm 0.1$
25 days	AD	$1.6^{a} \pm 0.3$	$3.0^{a} \pm 0.3$	$1.9^{a} \pm 0.1$	$0.9^{b} \pm 0.2$
	$ADSA_0$	$2.5^{b} \pm 0.3$	$4.6^{b} \pm 0.4$	$2.1^{a} \pm 0.2$	$0.5^{a} \pm 0.1$
	$ADMJ_0$	$3.5^{c} \pm 0.1$	$4.7^{b} \pm 0.3$	$1.6^{a} \pm 0.0$	$0.5^{a} \pm 0.1$
30 days	AD	$1.4^{a} \pm 0.3$	$3.1^{a} \pm 0.3$	$2.9^{a} \pm 0.2$	$0.9^{a} \pm 0.3$
	$ADSA_0$	$2.3^{b} \pm 0.5$	$7.0^{c} \pm 0.6$	$2.3^{a} \pm 0.1$	$0.7^{a} \pm 0.2$
	$ADMJ_0$	$3.2^{b} \pm 0.4$	$4.6^{b} \pm 0.3$	$2.6^{a} \pm 0.2$	$0.5^{a} \pm 0.2$
30 days	AD				$2.6^{a} \pm 0.6$*
	$ADSA_0$				$3.7^{b} \pm 0.1$*
	$ADMJ_0$				$4.2^{b} \pm 0.8$*

AD – aeroponic with defensor, two times concentrated Hoagland solution + two times increased sulfur; $ADSA_0$ - aeroponic with defensor, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; $ADMJ_0$ - aeroponic with defensor, two times concentrated Hoagland solution + two times increased sulfur + methyl jasmonate applied on the beginning. The differences are compared for each treatment. Values followed by the same letter are not significantly different. *Sum of glucosinolates exuded during 30 days

Table 4.29b. Elicitor influence on indole glucosinolate content in plants and exudates of turnip from aeroponic with sprayers (mg plant^{-1})

Date	Treatment	Leaves	Secondary roots	Primary roots	Exudates
10 days	AS	$0.3^{a} \pm 0.0$	$1.0^{a} \pm 0.2$		$0.2^{a} \pm 0.0$
	$ASSA_0$	$0.7^{b} \pm 0.2$	$1.9^{b} \pm 0.1$		$0.6^{b} \pm 0.1$
15 days	AS	$0.8^{a} \pm 0.3$	$2.1^{a} \pm 0.1$	$1.1^{a} \pm 0.1$	$0.3^{a} \pm 0.0$
	$ASSA_0$	$0.7^{a} \pm 0.0$	$3.8^{b} \pm 0.2$	$1.3^{a} \pm 0.2$	$0.6^{b} \pm 0.1$
20 days	AS	$0.8^{a} \pm 0.1$	$2.4^{a} \pm 0.1$	$0.8^{a} \pm 0.1$	$0.2^{a} \pm 0.1$
	$ASSA_0$	$1.9^{b} \pm 0.2$	$4.0^{b} \pm 0.3$	$1.5^{b} \pm 0.1$	$0.5^{a} \pm 0.2$
25 days	AS	$1.3^{a} \pm 0.2$	$3.0^{a} \pm 0.0$	$1.9^{a} \pm 0.2$	$0.4^{a} \pm 0.0$
	$ASSA_0$	$2.5^{b} \pm 0.2$	$5.0^{b} \pm 0.1$	$2.1^{a} \pm 0.2$	$0.4^{a} \pm 0.2$
30 days	AS	$1.6^{a} \pm 0.1$	$2.5^{a} \pm 0.5$	$3.5^{a} \pm 0.1$	$0.7^{b} \pm 0.0$
	$ASSA_0$	$2.3^{b} \pm 0.2$	$5.9^{b} \pm 3.3$	$2.6^{a} \pm 0.3$	$0.3^{a} \pm 0.1$
Total	AS				$1.8^{a} \pm 0.2$*
	$ASSA_0$				$2.5^{b} \pm 0.2$*

AS – aeroponic with sprayers, two times concentrated Hoagland solution + two times increased sulfur; $ASSA_0$ - aeroponic with sprayers, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning. The differences are compared for each treatment. Values followed by the same letter are not significantly different. *Sum of glucosinolates exuded during 30 days

Table 4.30a. Elicitor influence on individual indole glucosinolates in plants and exudates from aeroponic with defensor (mg plant^{-1})

Glucosinolates	Treatment	Leaves	Secondary roots	Primary roots	Exudates
Glucobrassicin	AD	$0.9^{b} \pm 0.1$	$1.1^{a} \pm 0.2$	$0.8^{a} \pm 0.1$	$0.8^{a} \pm 0.3$*
	$ADSA_0$	$0.9^{b} \pm 0.1$	$2.1^{b} \pm 0.4$	$0.7^{a} \pm 0.2$	$1.1^{ab} \pm 0.3$*
	$ADMJ_0$	$0.5^{a} \pm 0.0$	$1.2^{a} \pm 0.1$	$0.7^{a} \pm 0.0$	$1.5^{b} \pm 0.4$*
4-hydroxy-glucobrassicin	AD	$0.5^{a} \pm 0.0$	$0.3^{b} \pm 0.1$	$0.5^{a} \pm 0.1$	$0.1^{a} \pm 0.1$*
	$ADSA_0$	$0.3^{a} \pm 0.2$	$0.1^{ab} \pm 0.1$	$0.3^{ab} \pm 0.2$	$0.1^{a} \pm 0.0$*
	$ADMJ_0$	$1.0^{b} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.1$	$0.1^{a} \pm 0.1$*
4-methoxy-glucobrassicin	AD	$0.1^{a} \pm 0.0$	$0.5^{a} \pm 0.0$	$0.6^{b} \pm 0.2$	$0.5^{a} \pm 0.1$*
	$ADSA_0$	$0.2^{a} \pm 0.0$	$1.2^{b} \pm 0.1$	$0.4^{a} \pm 0.0$	$1.0^{b} \pm 0.3$*
	$ADMJ_0$	$0.1^{a} \pm 0.0$	$0.9^{b} \pm 0.2$	$0.3^{a} \pm 0.1$	$1.1^{b} \pm 0.3$*
Neoglucobrassicin	AD	$0.1^{a} \pm 0.0$	$1.2^{a} \pm 0.2$	$1.4^{a} \pm 0.2$	$1.2^{a} \pm 0.2$*
	$ADSA_0$	$0.8^{b} \pm 0.2$	$3.0^{b} \pm 0.4$	$1.4^{a} \pm 0.1$	$1.6^{b} \pm 0.1$*
	$ADMJ_0$	$0.7^{b} \pm 0.1$	$2.8^{b} \pm 0.5$	$1.9^{b} \pm 0.2$	$1.5^{ab} \pm 0.1$*

AD – aeroponic with defensor, two times concentrated Hoagland solution + two times increased sulfur; $ADSA_0$ - aeroponic with defensor, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; $ADMJ_0$ - aeroponic with defensor, two times concentrated Hoagland solution + two times increased sulfur + methyl jasmonate applied on the beginning. The differences are compared for each treatment. Values followed by the same letter are not significantly different. *Sum of glucosinolates exuded during 30 days

Table 4.17b. Elicitor influence on individual indole glucosinolates in plants and exudates from aeroponic with sprayers (mg plant^{-1})

Glucosinolates	Treatment	Leaves	Secondary roots	Primary roots	Exudates
Glucobrassicin	AS	$0.6^{b} \pm 0.1$	$0.7^{a} \pm 0.1$	$0.9^{a} \pm 0.2$	$0.4^{a} \pm 0.2$*
	$ASSA_0$	$0.5^{b} \pm 0.1$	$1.6^{b} \pm 0.2$	$0.7^{b} \pm 0.0$	$0.5^{ab} \pm 0.2$*
4-hydroxy-glucobrassicin	AS	$0.7^{a} \pm 0.2$	$0.4^{b} \pm 0.1$	$0.5^{b} \pm 0.2$	$0.1^{a} \pm 0.2$*
	$ASSA_0$	$0.9^{a} \pm 0.2$	$0.1^{ab} \pm 0.0$	$0.3^{ab} \pm 0.1$	$0.1^{a} \pm 0.1$*
4-methoxy-glucobrassicin	AS	$0.1^{a} \pm 0.0$	$0.5^{a} \pm 0.2$	$0.9^{a} \pm 0.3$	$0.5^{a} \pm 0.2$*
	$ASSA_0$	$0.2^{a} \pm 0.1$	$1.4^{b} \pm 0.2$	$0.4^{b} \pm 0.2$	$0.3^{b} \pm 0.1$*
Neoglucobrassicin	AS	$0.4^{a} \pm 0.1$	$0.9^{a} \pm 0.2$	$1.3^{a} \pm 0.2$	$0.9^{a} \pm 0.2$*
	$ASSA_0$	$0.7^{b} \pm 0.1$	$2.8^{b} \pm 0.2$	$2.0^{b} \pm 0.1$	$1.7^{b} \pm 0.2$*

AS – aeroponic with sprayers, two times concentrated Hoagland solution + two times increased sulfur; $ASSA_0$ - aeroponic with sprayers, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; $ASMJ_0$ - aeroponic with sprayer, two times concentrated Hoagland solution + two times increased sulfur + methyl jasmonate applied on the beginning. The differences are compared for each treatment. Values followed by the same letter are not significantly different. *Sum of glucosinolates exuded during 30 days

Table 4.35a. Elicitor influence on fresh weight and glucosinolate concentration in plants of turnip from aeroponic with defensor

Date	Treatment	Fresh weight, g/ concentration of glucosinolates, mg g^{-1}	Leaves	Roots		Leaf : root index
				secondary	primary	
10 days	AD	g	$8.8^{b} \pm 0.5$	$2.8^{c} \pm 0.0$		2.9
		mg g^{-1}	0.2	0.1		
	$ADSA_0$	g	$12.7^{c} \pm 0.5$	$2.2^{b} \pm 0.4$		5.9
		mg g^{-1}	0.3	0.3		
	$ADMJ_0$	g	$4.9^{a} \pm 1.2$	$0.9^{a} \pm 0.2$		5.6
		mg g^{-1}	0.7	1.1		
15 days	AD	g	$12.2^{b} \pm 1.2$	$10.8^{ab} \pm 1.2$	$1.5^{a} \pm 0.3$	0.8
		mg g^{-1}	0.1	0.1	1.0	
	$ADSA_0$	g	$12.7^{b} \pm 0.5$	$13.0^{b} \pm 1.0$	$1.5^{a} \pm 0.3$	0.9
		mg g^{-1}	0.2	0.2	1.2	
	$ADMJ_0$	g	$4.9^{a} \pm 1.2$	$5.2^{a} \pm 0.8$	$1.0^{a} \pm 0.2$	0.8
		mg g^{-1}	1.0	0.3	1.5	
20 days	AD	g	$24.5^{b} \pm 1.6$	$12.4^{b} \pm 1.3$	$1.6^{a} \pm 0.1$	1.7
		mg g^{-1}	0.1	0.4	1.0	
	$ADSA_0$	g	$28.9^{c} \pm 2.4$	$14.6^{b} \pm 1.1$	$1.4^{a} \pm 0.3$	1.8
		mg g^{-1}	0.1	0.4	1.1	
	$ADMJ_0$	g	$19.6^{a} \pm 2.2$	$9.3^{a} \pm 1.2$	$1.4^{a} \pm 0.3$	1.8
		mg g^{-1}	0.4	0.7	1.3	
25 days	AD	g	$27.3^{b} \pm 1.2$	$20.1^{a} \pm 2.9$	$2.9^{b} \pm 0.4$	1.2
		mg g^{-1}	0.1	0.5	1.2	
	$ADSA_0$	g	$30.2^{c} \pm 1.6$	$18.1^{a} \pm 2.9$	$2.7^{b} \pm 0.1$	1.5
		mg g^{-1}	0.2	0.4	1.6	
	$ADMJ_0$	g	$23.2^{a} \pm 1.6$	$16.7^{a} \pm 1.9$	$2.1^{a} \pm 0.2$	1.2
		mg g^{-1}	0.3	0.6	2.3	
30 days	AD	g	$29.7^{b} \pm 1.2$	$21.1^{a} \pm 2.1$	$5.7^{b} \pm 0.1$	1.1
		mg g^{-1}	0.2	0.3	1.4	
	$ADSA_0$	g	$29.4^{b} \pm 1.7$	$24.0^{a} \pm 2.6$	$5.9^{b} \pm 0.7$	1.0
		mg g^{-1}	0.1	0.5	1.4	
	$ADMJ_0$	g	$26.3^{a} \pm 1.1$	$19.9^{a} \pm 2.2$	$4.2^{a} \pm 0.2$	1.1
		mg g^{-1}	0.2	0.5	2.2	

AD – aeroponic with defensor, two times concentrated Hoagland solution + two times increased sulfur; $ADSA_0$ - aeroponic with defensor, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning; $ADMJ_0$ - aeroponic with defensor, two times concentrated Hoagland solution + two times increased sulfur + methyl jasmonate applied on the beginning. The differences are compared for each treatment. Values followed by the same letter are not significantly different.

Table 4.35a. Elicitor influence on fresh weight and glucosinolate concentration in plants of turnip from aeroponic with sprayers

Date	Treatment	Fresh weight, g/ concentration of glucosinolates, mg g^{-1}	Leaves	Roots		Leaf : root index
				secondary	primary	
10 days	AS	g	13.7[a] ± 1.3	3.0[a] ± 0.4		3.3
		mg g^{-1}	0.2	0.1		
	$ASSA_0$	g	14.3[a] ± 0.8	3.0[a] ± 0.4		3.5
		mg g^{-1}	0.3	0.3		
15 days	AS	g	17.0[a] ± 1.4	9.6[a] ± 2.0	3.0[a] ± 0.5	1.3
		mg g^{-1}	0.1	0.1	1.0	
	$ASSA_0$	g	18.0[a] ± 1.1	9.6[a] ± 1.9	3.0[a] ± 0.5	1.4
		mg g^{-1}	0.2	0.2	1.2	
20 days	AS	g	27.7[a] ± 2.8	13.5[a] ± 0.7	4.2[a] ± 0.8	1.6
		mg g^{-1}	0.1	0.4	1.0	
	$ASSA_0$	g	27.3[a] ± 1.4	14.7[a] ± 1.0	3.6[a] ± 0.6	1.5
		mg g^{-1}	0.1	0.4	1.1	
25 days	AS	g	30.8[a] ± 2.1	15.8[a] ± 0.6	7.6[a] ± 0.9	1.3
		mg g^{-1}	0.1	0.5	1.2	
	$ASSA_0$	g	31.8[a] ± 3.0	16.1[a] ± 2.1	6.2[a] ± 0.6	1.4
		mg g^{-1}	0.2	0.4	1.6	
30 days	AS	g	36.6[a] ± 2.8	21.9[a] ± 1.1	9.2[a] ± 0.3	1.2
		mg g^{-1}	0.2	0.3	1.4	
	$ASSA_0$	g	37.7[a] ± 2.7	26.1[b] ± 1.9	8.2[a] ± 0.8	1.1
		mg g^{-1}	0.1	0.5	1.4	

AS – aeroponic with sprayers, two times concentrated Hoagland solution + two times increased sulfur; $ASSA_0$ - aeroponic with sprayers, two times concentrated Hoagland solution + two times increased sulfur + salicylic acid applied on the beginning. The differences are compared for each treatment. Values followed by the same letter are not significantly different.

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