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Hypericin and Hyperforin Production in St. John's Wort in Vitro Culture: Influence of Saccharose, Polyethylene Glycol, Methyl Jasmonate, and *Agrobacterium tumefaciens*

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Influence of saccharose in the presence or absence of polyethylene glycol (PEG), methyl jasmonate, and an inactivated bacterial culture of *Agrobacterium tumefaciens* in cultivation medium on morphology of *Hypericum perforatum* L. and production of hypericin and hyperforin was studied under in vitro conditions. Production of hypericin and hyperforin was influenced by the presence of different concentrations of saccharose (10−30 g L^{-1}) in cultivation medium. Addition of PEG (1.25−5 g L^{-1}) in the presence of saccharose (10−30 g L^{-1}) increased production of hypericin and hyperforin in the *H. perforatum* in vitro culture. Synthesis of hypericin and hyperforin was unchanged or reduced for most of the experimental plants at higher contents of PEG (10 and 15 g L^{-1}). Concentrations of hypericin and hyperforin in the *H. perforatum* were on the order 10^0 and 10^3 μg g^{-1} of dry plant material, respectively. Production of hypericin and hyperforin was stimulated either in the presence of a chemical elicitor (methyl jasmonate) or an inactivated bacterial culture of *A. tumefaciens*. Morphological changes induced by the abovementioned substances were observed and described in detail. The obtained results will be applied in experimental botany and in the technology of *H. perforatum* cultivation for pharmaceutical applications.

KEYWORDS: Hypericin; hyperforin; saccharose; polyethylene glycol; methyl jasmonate; *Agrobacterium tumefaciens;* St. John's Wort; *Hypericum perforatum* L.

INTRODUCTION

Biotic and abiotic stressors are the principal limiting factors of plant growth. At present, high attention is focused on the problematic stress of plants, especially from the point of view of applicability of the knowledge obtained in agricultural and/or environmental research. Information on basic principles of biochemical reactions of plants to stress conditions is the first step to increase resistance of economically important plants against unwanted influence of environment on the basis of breeding selection and genetic modification. Different stress factors i.e., drought, extraordinary salinity of soils, presence of xenobiotics (agrochemicals, heavy metals, atmospheric pollutants, etc.), pathogens, or herbivores (*1*−*3*) can cause serious depression of plant growth and stimulation of defense mechanisms on the other hand.

Protective or detoxification compounds are usually synthesized by plants under stress conditions. Specific adaptations very typical for some taxonomic groups or individual plants also exist. The ability of the plants of genus *Hypericum* to synthesize natural insecticides in the case of attack of plants by pathogen or herbivore is a typical adaptation. *Hypericum perforatum*, *Hypericum crispum*, and *Hypericum hirsutum* belong to the most common examples of the genus *Hypericum* (*3*, *4*).

Defense mechanisms of a plant to a biotic stress are very complicated. Jasmonic acid (JA) probably plays an important role in the plant responses. The production of hypericin and hyperforin in the plants was stimulated by application of chemical (JA and MeJa) and biotic (*Colletotrichum gloeosporioides*) elicitors (*4*, *5*). Thus, the relation between the elevated production of hypericin and hyperforin and the defense responses of *H. perforatum* against pathogens was confirmed.

Many other substances with toxic or nontoxic properties also influenced synthesis of hypericin. Influence of heavy metals (*6*) and fluoride (*7*) on production of secondary metabolites of *H. perforatum* was studied. Many authors focused attention to a synthesis of bioactive compounds under different conditions of cultivation [i.e., dark conditions influenced (*5*) growth and synthesis of hypericin in *H. perforatum*] and also to their distribution in plants in different ontogenetic stages of plants having different activity of metabolism (*7*−*10*).

It was confirmed that hypericin and hyperforin are produced in organs of *H. perforatum* as an antimicrobial, antivirological,

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and antiherbivore agents. Production of hypericin, and also some other secondary metabolites, can be seriously influenced by addition of saccharides and/or by application of different amounts of plant hormones, i.e., 2,4-D, kinetin, and others. The problematic was mentioned in several papers (i.e., refs *11*, *12*).

Detailed information on the synthesis of the substances is important not only from the point of view of our knowledge of defense mechanism of plants against biotic stress but also from the biotechnological aspects (searching for new biocides and alternative procedures of biological protection of plants). Antidepressive, antitumor, and other biomedicinal properties induced interest in detailed studies of the principles of syntheses of naphtodianthrones and acylphloroglucinols due to frequent applications of *H. perforatum* in the pharmaceutical industry (*13*−*15*).

A well-known antidepressant, Psychotonin, based on the extracts from *H. perforatum*, is registered. Application of hypericin in the cure of viral diseases like HIV (*16*) is also discussed. Many further physiological effects connected to hypericin and hyperforin such as their photodynamic properties and others are described in literature (*16*−*19*). *H. perforatum* and other species of the genus *Hypericum* synthesize not only hypericin and hyperforin but they are also a rich source of flavonoids, especially of rutin, which could be very useful in protective mechanism of plants against biotic and abiotic stresses and elicitors by their synergic effect (*20*).

The aim of the present paper was to study the influence of saccharose in the presence or absence of polyethylene glycol, methyl jasmonate, and an inactivated bacterial culture of *Agrobacterium tumefaciens* in cultivation medium on the morphology of *H. perforatum* and production of hypericin and hyperforin under in vitro conditions.

MATERIALS AND METHODS

Plant Material. *H. perforatum* L. var. Topas plants were cultivated from seeds collected at the Center of Medicinal Plants of the Faculty of Medicine, Masaryk University in Brno in 2005. The seeds were washed with Jar detergent solution (Procter & Gamble−Rakona, Rakovník, Czech Republic) in test tubes for 60 min. The detergent solution was removed; the seeds were washed repeatedly with sterile distilled water and disinfected with 15% (v/v) Savo solution (content of sodium hypochlorite max. 5%, Bochemie, Bohumín, Czech Republic) for 20 min. The seeds were subsequently washed 10 times with sterile distilled water and applied (inoculated) on the cultivation medium for seedling (13 days). All the operations were done in an FATRAN LF aseptic box (Chirana, Brno, Czech Republic).

Cultivation Medium. The experiments used commercially available 50% Murashige−Skoog's (MSCM) cultivation medium (*21*) with concentration 2.151 g L^{-1} and addition of glycine (2 mg L^{-1}), *myo*-inositol (100 mg L^{-1}), nicotinic acid (0.5 mg L^{-1}), pyridoxine hydrochloride (0.5 mg L^{-1}), and thiamine hydrochloride (0.1 mg L^{-1}) that was solidified with Gelrite (3.0 g L^{-1}) (all from Duchefa, Haarlem, The Netherlands). The components of the cultivation medium were mixed and completely dissolved in the appropriate amount of water using an electromagnetic stirrer. The homogenized cultivation medium (75 mL) was transferred into an infusion bottle (0.5 L). The bottle was sealed with aluminum foil. The cultivation medium and all instruments were sterilized in autoclave (type AUT 26/2, Chirana, Brno, Czech Republic) at 121 °C and pressure 110 kPa for 20 min. All the operations needing sterile conditions were done in the FATRAN LF aseptic box (Chirana).

Cultivation Conditions. Small plants were picked into infusion bottles with the 50% MSCM (with 100% vitamins, 10 g L^{-1} saccharose, 75 μg L^{-1} giberellic acid, and 0.25% Gelrite) and grown up to 3−4 cm in a MIR Sanyo cultivation box (Sanyo Electric Co., Osaka, Japan) at 22 ± 4 °C and illumination/darkness period of 16/8 h (cultivation period 15 days at the light intensity 8000 Lx). Osram L36W/77 Fluora (Osram, München, Germany) lamps were used as a source of light with a maximum intensity of light at $\lambda = 400-500$ nm and 600−700 nm and with less intense maximum at $\lambda = 550$ nm. The lamps simulate natural sunlight with the simultaneous increase of the light intensity in the red and blue range. The plants were cultivated in sterile conditions using cultivation medium with addition of Gelrite or in a liquid medium using a cotton bridge in test tubes or infusion bottles.

Saccharose and PEG or MeJa Studies. A cultivation period of 21 days under the abovementioned conditions was applied for the plants using 50% MSCM (with 100% vitamins, 10 g L^{-1} saccharose, 75 μg L^{-1} giberellic acid, and 0.25% Gelrite) with addition of saccharose (p.a., Pliva-Lachema, Brno, Czech Republic) in concentrations of 10, 20, and 30 g L^{-1} in the presence or absence of polyethylene glycol 6000 (PEG, Fluka, Buchs, Switzerland) in concentrations of 1.25, 2.5, 5, 10, and 15 g L^{-1} or in the presence of 1 mg L^{-1} methyl jasmonate (Sigma Aldrich, St. Louis, MI).

A. tumefaciens. The bacterial culture was obtained from the Institute of Molecular Biology of the Czech Academy of Sciences (Prague, Czech Republic). A bacterial suspension at 3×10^8 of bacteria per milliliter of suspension (1° McF, controlled by densitometry) was prepared. Aliquots of the suspension were stored in a freeze box at −80 °C. *A. tumefaciens* was inoculated on Langley and Koda (LKM) medium (composition: 10 g L^{-1} saccharose, 8 g L^{-1} casein hydrolysate, 2 g L^{-1} yeast extract, 2 g L^{-1} KH_2PO_4, and 0.3 g L^{-1} $MgSO_4$) under sterile conditions after removing from the refridgerator, thawing, and cultivating in the dark in sterile glass vessels sealed with Al foil at 25 °C for 25 h using a Heidolph Instruments Rotamax 120 shaker (Heidolph Instruments, Kelheim/Donau, Germany).

After incubation, the diluted *A. tumefaciens* solution was transferred under sterile conditions into Petri dishes with LKM. The solution was incubated for 24 h at 25 °C in the dark to obtain the titer of bacterial solution of *A. tumefaciens* (titer 8×10^{11} bacterial per L) that was added to the experimental medium (50% MSCM; see above). The *A. tumefaciens* solution in the LKM used for the study of the influence of *A. tumefaciens* on *H. perforatum* was inactivated at 80 °C using a water bath for 40 min. The growth of *A. tumefaciens* and its rate were observed by microscopy; in addition, applied *A. tumefaciens* and its microbial activity were tested using experimental plants of *Arabidopsis thaliana* and also observed by microscopy (Olympus AX 70).

Sample Treatment. Photo documentation of all plants was done after complete cultivation (Olympus C-4040 ZOOM, Olympus, Tokyo, Japan). The complete shoot of all plants in a single infusion bottle were collected in three independent replicates for each experimental variant. The samples were weighed immediately to obtain fresh weight and inserted into plastic test tubes, frozen with liquid nitrogen, and stored in a freeze box at a constant temperature of −80 °C. All the samples were lyophilized in a Christ Alpha 1−2 B lyophilizer (Braun Biotech Int., Osterode, Germany) and homogenized in a ceramic ball-mill Vibrom 2S (Jebavý, Třebechovice, Czech Republic) under liquid nitrogen. Dry matter was determined gravimetrically using a MA 30 moisture analyzer (Sartorius, Goettingen, Germany).

Homogenized samples (20 mg, parts were analyzed in three independent replicates) were extracted with 40 mL of 80% (v/v) ethanol (temperature program 1, run temperature of the cooling/heating block 150 °C for 30 min, decrease the temperature of the cooling/heating block to 30 °C for 5 min; temperature program 2, run temperature of the cooling/heating block 150 °C for 30 min, decrease the temperature of a cooling/heating block to 30 °C for 5 min) in a modified Soxhlet apparatus "fex Ika Werke 50" (IKA-Werke, Staufen, Germany). The final extract was filtered through a nylon membrane filter (0.45 μm, 13 mm size, Alltech Associates, Deerfield, CA) before an injection into HPLC system.

Chemicals and HPLC Instrument. Acetonitrile (ACN) for HPLC and ammonium acetate ($AcNH_4$) were obtained from Merck (Darmstadt, Germany). Hypericin, hyperforin, and other chemicals of ACS purity (American Chemical Standard) were from Sigma Aldrich (St. Louis, MI). Stock solutions of hypericin (40 μg L^{-1}) and hyperforin (100 μg L^{-1}) in methanol used for optimization and calibration of the method were stored in the dark at 4 °C. All solutions used for HPLC analyses were filtered through disposable PVDF membrane filters (0.45 μm, 13 mm size, MetaChem, Torrance, CA) before injection into HPLC.

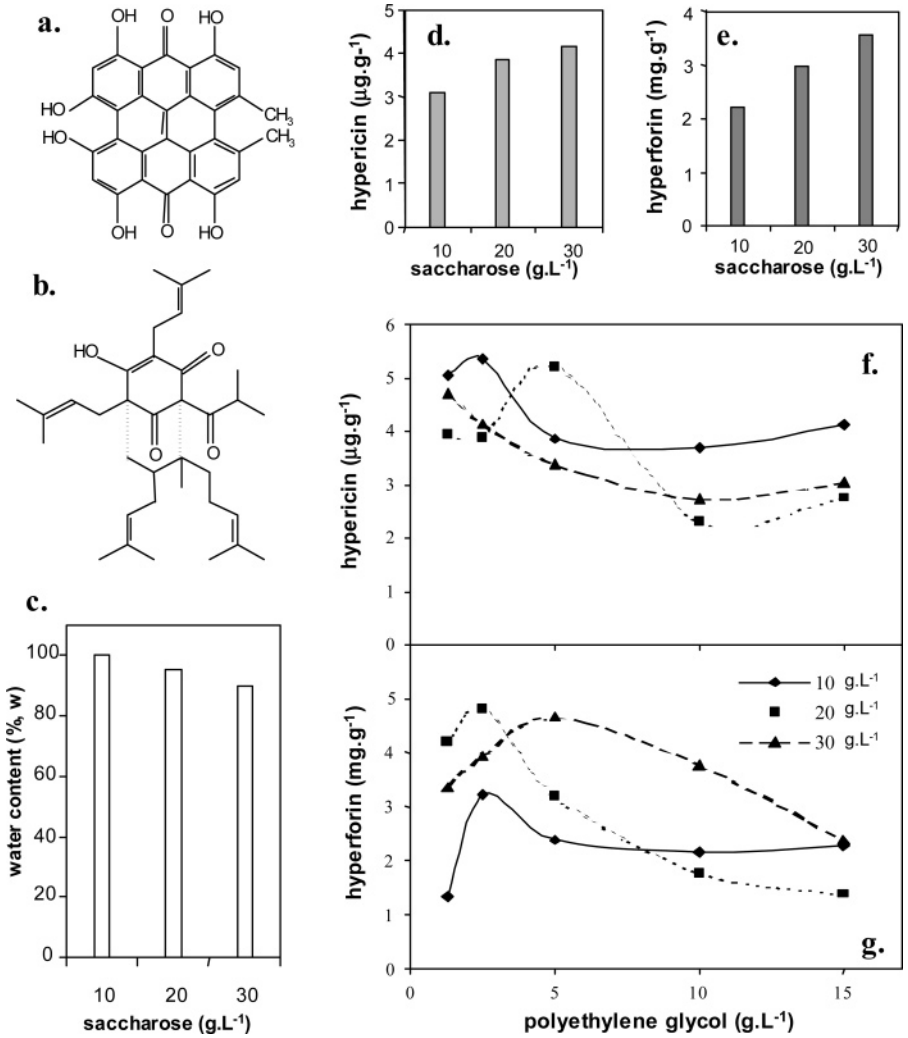

Figure 1. Structural formulas of hypericin and hyperforin (a, b). Influence of saccharose on water content (c) and production of hypericin (d) and hyperforin (e) in shoots of *H. perforatum*. Influence of polyethylene glycol (PEG) on production of hypericin (f) and hyperforin (g) at different concentrations of saccharose in cultivation medium. Cultivation period 21 days; $n = 3$; amount of hypericin and hyperforin per dry weight; for other details, see Materials and Methods.

An HP 1100 chromatographic system (Hewlett-Packard, Waldbronn, Germany) equipped with a degassing membrane module (G1322A), two pumps (G1312A), an automatic autosampler (G1313A), a thermostating column oven (G1316A), and UV−vis DAD detector (G1315A) was controlled with a ChemStation (Rev. A07.01) program. Hypericin and hyperforin were separated by a linear gradient elution on a reverse-phase Zorbax SB-CN (75 mm × 4,6 mm, particle size 3.5 μm, Agilent, Palo Alto, CA) column using a mobile phase consisting of acetonitrile and 10 mmol L^{-1} ammonium acetate [gradient (%, v/v) 0 min 50/50, 5 min 100/0, 8 min 100/0, 10 min 50/50]. Flow rate was 0.8 mL min^{-1} and the temperature of the column oven was set at 35 °C. Absorbance was measured at 292 nm. Retention times were 3.51 and 3.99 min for hypericin and hyperforin, respectively.

RESULTS AND DISCUSSION

Influence of Saccharose. The concentration of hypericin and hyperforin was determined after 21 days of cultivation of plants (shoots) in MSCM medium containing 10, 20, and 30 g L^{-1} saccharose (see Materials and Methods). The content of water decreased in plant organs (**Figure 1**c) and the concentrations of hypericin and hyperforin increased in dry matter with the increasing concentration of saccharose in the cultivation medium (**Figure 1d,e**). The concentration of hypericin in plant organs increased from 3 to 4 μg g^{-1} (**Figure 1d**), which is 3 orders of magnitude lower than the concentration of hyperforin (ca. 2000−3500 $\mu g \cdot g^{-1}$) in plants cultivated in MSCM containing saccharose at 10 and 30 g L^{-1} (see **Figure 1e**). A similar topic was studied using *H. perforatum* plants cultivated in a bioreactor. The influence of saccharose addition in the concentration range 15−60 g L^{-1} on production of hypericin, pseudohypericin, and hyperforin was studied. The concentration of hypericin was significantly unchanged when 15 and 30 g L^{-1} concentrations were applied. Serious reduction of production of not only hypericin but also hyperforin was observed (*22*) at a higher concentration of saccharose (60 g L^{-1}).

The effect of other saccharides on production ability of callus culture of *H. perforatum* was studied by Kirakosyan et al. (*23*), too. They studied the influence of mannan, β-glucan, and pectin. In addition to their observation, it was confirmed that the water stress induced in plants increased the synthesis of secondary metabolites (*24*). It was also found that the concentration of hypericin in a callus culture is lower than in integral plants (*25*, *26*). The contents of hypericin were even approximately the same in plants cultivated in vitro and those grown in field conditions (*25*). A remarkable increase of hypericin and pseudohypericin concentrations was observed under elicitation by mannan (*26*). The changes in hypericin synthese in the presence of saccharose could be indicated by (i) the presence of saccharose as a chemical species, (ii) the osmotic stress

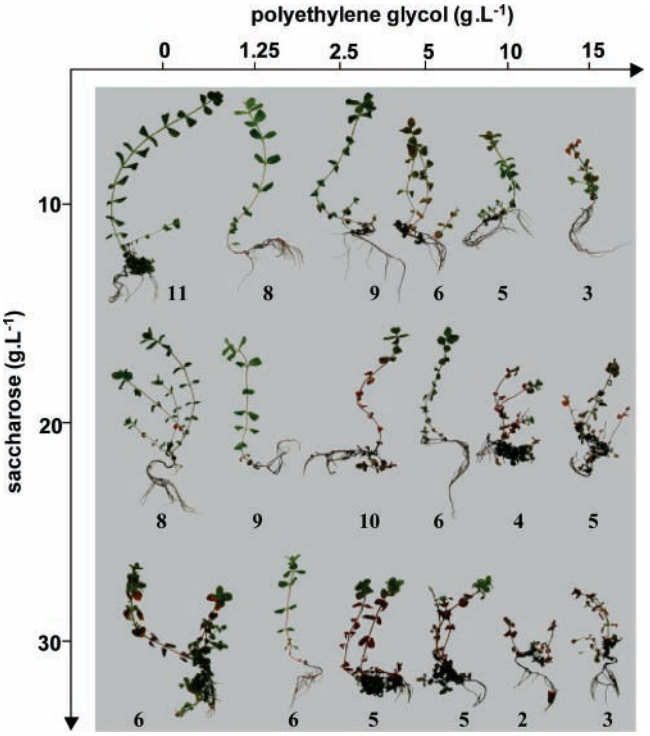

Figure 2. *H. perforatum* plants after 21 days of cultivation in MSCM containing 10, 20, and 30 g L^{-1} saccharose and different concentrations of PEG (0, 1.25, 2.5, 5, 10, 15 g L^{-1}). Numbers given in the figure correspond to the plant height in cm.

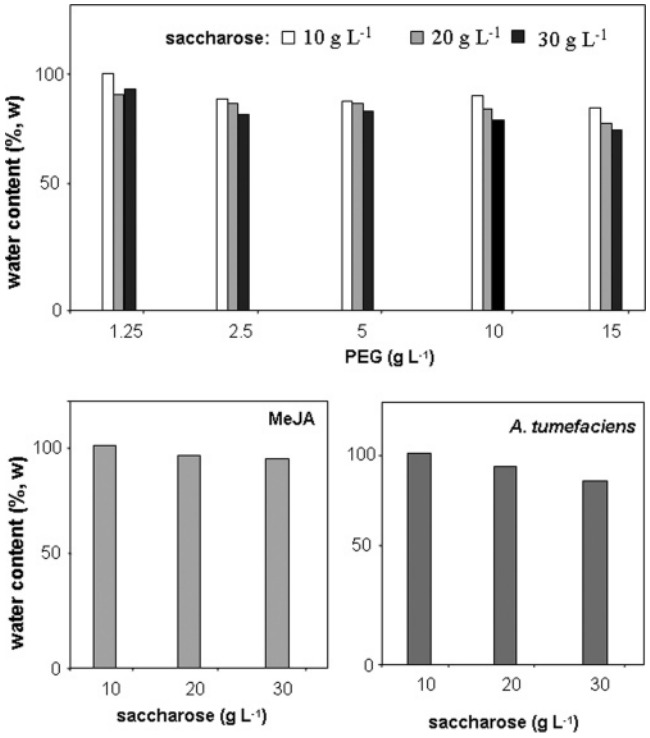

Figure 3. Content of water in *H. perforatum* samples.

induced by its presence in medium, and (iii) the elevated amounts of carbon present in the form of saccharose in cultivation medium.

From the comparison of the habitus and morphology of the plants cultivated in MSCM with different additions of saccharose it is evident that a serious reduction of growth, reduction in the number of leaf bases, shortening of internodials, appearance of reddish leaves and stems, and nonsignificant reduction of the area of leaves was observed with increasing concentration of saccharose (**Figure 2**). The influence of osmotic stress induced by the addition of saccharose and/or other osmotics into cultivation media was studied until now using callus cultures. It was shown that the presence of selected elicitors influenced morphogenesis of callus culture and formation of necrotic regions (*26*).

The first nonsignificant changes of the leaf color to red were observed at a concentration of saccharose of 20 g L^{-1}. The hardly observable changes started in the lowest parts of the plants. The stems were pink in the lowest parts. More evident changes were observed (red and in some cases dark red) at a concentration of saccharose of 30 g L^{-1} and they became evident also in higher parts (leaves, stems) of plants.

Accumulation of hypericin and other colorants (i.e., anthocyanins) in plant organs probably induced the changes in the color of leaves and stems. The secondary metabolites increase resistance of plants against stress conditions produced by the environment (synthesis of anthocyanins is promoted under osmotic stress). Water potential in leaves is probably also decreased; thus, uptake of water by roots is increased and loss of water by transpiration is reduced (*27*).

Influence of Saccharose and PEG. PEG is nonpenetrating osmoticum that was used for induction of water stress in many plants (*28*, *29*). This is due to the fact that the transportation of PEG across the cell membrane is restricted and thus its secondary toxic effect is eliminated because of its high molecular weight. The serious decrease of intake of water was observed due to the increased concentration (increased osmotic strength) in cultivation medium (*30*).

Addition of PEG (1.25, 2.5, 5, 10, 15 g L^{-1}) to the MSCM containing 10, 20, and 30 g L^{-1} saccharose dramatically influenced the production of hypericin and hyperforin mainly at the PEG concentrations 1.25 and 5 g L^{-1}. Maximum concentrations of hypericin and hyperforin depended on PEG concentration (see **Figure 1f,g**). The elevated production of hypericin and hyperforin can be induced by the addition of lower concentrations of PEG. The production of hypericin and hyperforin in *H. perforatum* is nonsignificantly changed or unchanged at higher PEG concentrations. Water content in shoots of *H. perforatum* was reduced with increasing concentration of PEG in cultivation medium similarly to saccharose alone (see **Figure 3**). Influence of PEG on synthesis of metabolites was studied in matured ridicules of cucumber (*31*). Induced osmotic stress activated synthesis of metabolites connected to metabolism of phospholipids.

The remarkable changes in habitus and morphology of *H. perforatum* were observed under increased insufficiency of water in plant organs and with increasing effects of osmotic stress induced by the presence of PEG (**Figure 2**). Generally, it can be concluded that the intensity of color change to red and the level of necrotization increased with increasing PEG concentration (apical growth and size of leaves are reduced). The effects are more pronounced at the higher concentrations of saccharose and constant PEG concentration. Generally, the intensity of color change to red leaves and stems appeared at lower concentrations of saccharose and PEG than necrosis of leaves and stems (the color changes in plant organs are primary effects followed by necrotization with increasing concentration of saccharose and PEG). Effects of osmotic stress similar to our experiments using *H. perforatum* were described also in the case of other plants. For example, the serious reduction of growth and

elongation of roots of *Arabidopsis thaliana* that were observed in the presence of PEG were similar to the effect of 100 nM NaCl (*30*).

Influence of Saccharose and MeJa. Effects of substances linked to initiation of defense reactions of *H. perforatum* against attack of pathogens on production of bioactive metabolites were studied in the last decades (*4*, *32*−*35*). The combined influence of saccharose (10, 20, 30 g L^{-1}) and MeJa on production of hypericin and hyperforin was studied. The influence of JA, MeJa, and/or salicylic acid and other chemical elicitors on production of hypericin and hyperforin was studied at present (*5*). The evident increase of production of hypericin in the presence of jasmonic acid and methyl jasmonate was observed.

The remarkable stimulation effects on production of hypericin and hyperforin was observed in the presence of 1 mg L^{-1} MeJa added into the MSCM containing 10, 20, and/or 30 g L^{-1} of saccharose in our experiments (**Figure 4a,b**). The content of water in organs of the plants stimulated by MeJa was reduced upon increasing the concentration of saccharose (**Figure 3**). Walker et al. (*5*) studied the stimulation of hypericin production in a cell suspension culture of *H. perforatum* cultivated in a medium containing salicylic and jasmonic acids. A remarkable increase of content of hypericin in dry matter was observed in the presence of jasmonic acid in contradiction to salicylic acid. The effect of elicitors was studied also using cells cultivated under normal light conditions and in darkness. The plants cultivated in dark produced approximately 2 times higher amounts of hypericin compare to those cultivated under standard conditions. The highest concentrations of hypericin were determined in the cell suspension culture cultivated in dark and in the leaves of plants cultivated in vitro. The effects of MeJa and salicylic acid on synthesis of hypericin, hyperforin, and derivatives of hypericin were studied using a *H. perforatum* plants (*4*). A serious increase of sytheses of hypericin and hyperforin and their derivatives was observed in the presence of both elicitors.

The presence of MeJa affected also the morphology and coloration of the cultivated plants. A red coloration of aerial parts of plants was observed in all the variants and it was more evident at the higher concentrations of saccharose. The root system of plants cultivated on MSCM containing saccharose and MeJa was reduced compared to a system of plants cultivated under the same conditions in the absence of MeJa (see **Figure 4c**). Also overall growth of the plants was retarded in the presence of MeJa.

A remarkable increase of concentration of hypericin (ca. 150%) in plants cultivated in medium containing 10 g L^{-1} saccharose and MeJa compared to the plants cultivated in the absence of MeJa was observed. The hypericin synthesis was less remarkable in the plants cultivated in medium containing 20 and 30 g L^{-1} of saccharose (**Figure 4a**). A more evident increase of hyperforin concentrations compare to hypericin concentrations was found for plants under the influence of MeJa. The highest increase of hyperforin concentration (280%) was observed in plants cultivated in medium containing 30 g L^{-1} saccharose (**Figure 4b**).

Influence of Saccharose and *A. tumefaciens.* As compared with the results obtained under application of MeJa, the increase of the concentrations of hypericin and hyperforin and even the reduction of growth of *H. perforatum* (see **Figure 4c**) was not so remarkable for plants cultivated in the presence of an inactivated bacterial culture of *A. tumefaciens*. *A. tumefaciens* is a Gram-negative bacteria infecting plants and forming tumors in plant organs. It is widely explored in gene engineering (*36*)

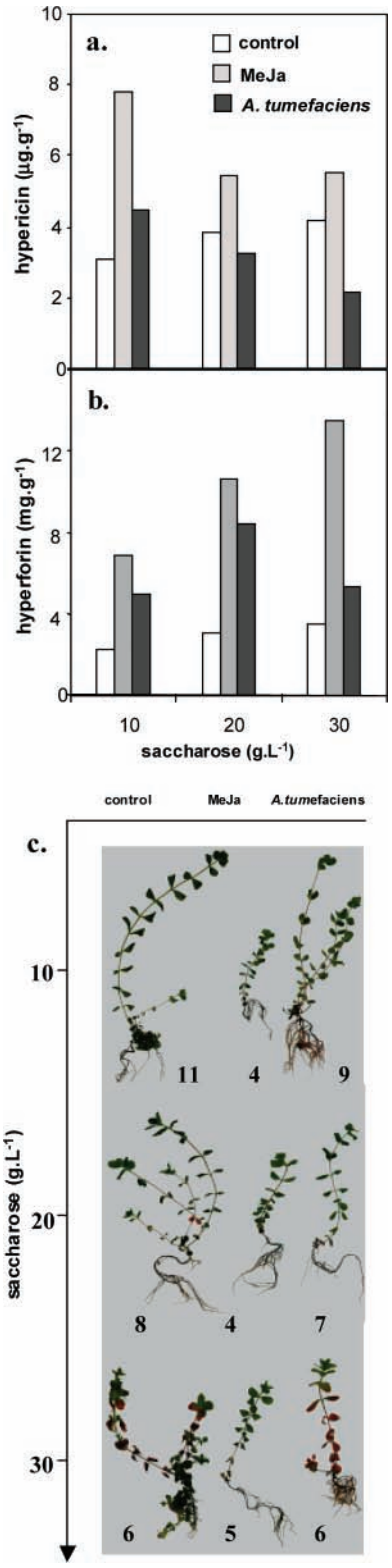

Figure 4. Influence of methyl jasmonate (MeJa) and inactivated bacterial culture *A. tumefaciens* on content of hypericin (a) and hyperforin (b) in shoots of *H. perforatum* after 21 days of cultivation in the presence of 10, 20, and 30 g L^{-1} saccharose in cultivation medium; photodocumentation is included (c). Numbers given in the **Figure 4c** correspond to the plant height in cm.

at present. An inactivated culture of *A. tumefaciens* was selected due to its well-known properties and behavior, simplicity of manipulation, well-known mechanisms of pathogenesis, availablility, and long-term experiences with its cultivation in our

department. Another reason for the selection was the fact that the influence of *A. tumefaciens* on production of secondary metabolites in plants was an insufficiently studied part of plant physiology or pathophysiology until now.

We were interested in comparing the effects of a nonspecific group of elicitors present in an inactivated culture of *A. tumefaciens* and the effects of individual elicitor (MeJa), the positive effect of which is well-known.

The negative influence of *A. tumefaciens* on the synthesis of hypericin depending on amount of saccharose in cultivation medium was observed. The synthesis of hypericin was decreased in plants cultivated in medium containing 20 and 30 g L^{-1} saccharose and *A. tumefaciens* compared to the plants cultivated in medium without *A. tumefaciens* (**Figure 3a**). The highest concentration of hyperforin was in the experiments conducted in medium with 20 g L^{-1} saccharose. An increase in the concentration of hyperforin in shoots of *H. perforatum* was observed after application of *A. tumefaciens* compared to the control plants (**Figure 4b**). Vinterhalter et al. (*37*) studied the influence of saccharose on biomass production and growth of hairy root culture of *H. perforatum* that was transformed by the *Agrobacterium rhizogenes* bacteria. A depression of biomass production of the plants cultivated under different concentrations of saccharose was observed (*37*). The influence of *Colletotrichum gloeosporioides* pathogen was also studied using culture of *H. perforatum*. Application of different titers of pathogen showed a positive stimulation of the syntheses of hypericin and partly also of hyperforin in the plants (*4*). The syntheses of the secondary metabolites were reduced at the elevated concentrations of the pathogen.

The reason for the synthesis of hypericin and hyperforin not being activated when *A. tumefaciens* was applied similarly to MeJa is probably the evidently more complicated and complex influence of the heat-inactivated pathogen on the growth and synthesis of bioactive metabolites compared to the application of a selected chemical elicitor. It can be concluded that the effect of MeJa and *A. tumefaciens* was predominantly related to the concentration of saccharose in the cultivation medium.

The influence of infection of *H. perforatum* by different pathogens and herbivores on production of bioactive metabolites (*4*, *32*−*35*) was studied. Similarly to our results, the effects on production of hypericin and hyperforin in the presence of pathogens and herbivores were contradictory; they were not positive in all cases. The effects of fungal cell wall extracts from *Phytophthora cinnamoni* on production of hypericin in a suspension culture of *H. perforatum* were studied (*5*), and no measurable increase of hypericin production against control plants was observed.

Conclusion. Our results confirmed that the variability of hypericin and hyperforin concentration depended on the concentration of saccharose with addition of PEG, MeJa, or inactivated bacterial culture of *A. tumefaciens* to which the *H. perforatum* plants were exposed in the course of cultivation. It was shown that the water and osmotic imbalance can seriously influence the synthesis of hypericin and hyperforin in *H. perforatum* plants. Saccharides are common constituents of cultivation medium for *H. perforatum*, i.e. concentrations of 20 and 30 g L^{-1} saccharose were applied for cultivation of *H. perforatum* (*4*) or *H. androsaemum* L. (*38*), respectively.

Several papers on the effect of jasmonic acid and MeJa on the synthesis of hypericin and other secondary metabolites have been published. The main reason to study the influence of a combination of an elevated concentration of sugar in the presence of PEG and other elicitors in the cultivation medium was the novelty of the study (until now it was not studied). Our results show that it is very difficult to compare results and effects of individual elicitors at different concentrations of saccharose and PEG in cultivation medium. We were also interested in the reaction of the plants induced by the changes in osmotic conditions (abiotic stress) in combination with applied elicitor, i.e., MeJa, which is connected with the reaction of a plant.

Our results confirmed the fact that comparison of determined values of hypericin and hyperforin concentrations obtained in experiments with different concentrations of saccharose in cultivation medium will be very difficult or even impossible. The results improved our knowledge of the behavior of *H. perforatum* under stress conditions. The results could be very helpful to experimental botany and the agrotechnology of production of *H. perforatum* for the pharmaceutical industry and traditional medicine.

ABBREVIATIONS USED

PEG, polyethylene glycol; JA, jasmonic acid; MeJa, methyl jasmonate; MSCM, Murashige−Skoog cultivation medium; LKM, Langley and Koda medium.

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