EFFECT OF ISOMERS OF HYDROXYBENZOIC ACID ON THE GROWTH AND METABOLISM OF *CHLORELLA VULGARIS* BEIJERINCK (CHLOROPHYCEAE)

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ABSTRACT

The isomers *o*-, *m*-, and *p*- of hydroxybenzoic acid (HBA) in the concentration range 10^{-6} - 10^{-4} M in the unicellular green alga *Chlorella vulgaris* (Chlorophyceae) display marked biological activity. The *o*-HBA isomer, commonly known as salicylic acid, in a concentration of 10^{-4} M exerted the most stimulating effect on the parameters analysed (the number of cells, dry mass, the content of chlorophylls *a* and *b*, carotenoids, soluble proteins and their secretion, monosaccharides, DNA and RNA) whereas *p*-HBA had weak stimulating properties. On the other hand, *m*-HBA had a weak inhibitory effect on the growth of *C. vulgaris* and all the biochemical parameters analysed in comparison with the control culture of algae devoid of HBA isomers.

KEY WORDS: *Chlorella vulgaris*, isomers *o*-, *m*- and *p*-hydroxybenzoic acid, growth, chlorophylls, carotenoids, DNA, RNA, proteins, monosaccharides.

INTRODUCTION

The phenolic acids commonly occurring in plants are usually hydroxyl derivatives of benzoic acid (C_6-C_1). Thanks to their specific physico-chemical properties, phenolic acids have a high and widely differentiated physiological-metabolic activity in plants, which is mainly inhibitory, rarely stimulatory or modifying. Apart from the phytohormones such as: abscisic acid, auxins, brassinosteroids, cytokinins, ethylene, gibberellins they are included among the main groups of endogenous regulators of plant growth and metabolism (Basyouni et al. 1964; Raskin 1992a, b; Pierpoint 1994; Czerpak and Bajguz 1998).

Apart from a direct effect on many biochemical and physiological processes in plants, some phenolic acids play the role of phytoncides or phytoalexins which, in many plant species, protect the plants from the invasion of viral, bacterial and fungal pathogens. Some of them, particularly *o*-hydroxyacids, readily chelate bivalent ions of metals, which contributes greatly to their cumulation in the soil and assimilation by the plants. Some of the phenolic acids, e.g. gallic and ellagic acids, readily enter into condensation reactions with each other forming compounds of the groups hydrolysing tannins which have a bacteriostatic effect by inhibiting the growth of numerous pathogens. A number of the phenolic acids play an important role in chemical interactions of the allelopathy type between the

higher plants, and also in their action on micro-organisms and animals. That is why phenolic acids derived from secretion or from decayed plants play an important part in the shaping of ecological and phytosociological relations particularly in land biocoenoses (Nitsch and Nitsch 1962; Aberg 1981).

Of the phenolic acids commonly occurring in vascular plants *o*-hydroxybenzoic acid (HBA), known as salicylic acid and has a specific effect on many physiological-metabolic processes in plants. Salicylic acid (SA) has a significant regulatory effect in numerous plant species on such as seed germination, growth of the root system and leaves, the biosynthesis of chlorophylls and proteins, the absorption and transport of bivalent ions by the cellular membrane, resistance to pathogens and certain stresses, particularly oxidizing stresses (Chen et al. 1993, 1995; Raskin 1995; Zhang and Klessig 1997; Xie et al. 1998; Xie and Chen 1999). In plants such as tobacco (*Nicotiana tabacum*) and even in algae of the *Chlamydomonas* genus, *o*-HBA is an endogenous chemical transmitter inducing an alternative oxidase. SA also stimulates cellular respiration at the level of glycolysis and the tricarboxylate cycle. In thermogenous plants, it induces a rise in temperature during the flowering period at the cost of lowering the ATP content, which is of great significance in the pollination of flowers by insects (Tomaszewski 1960, 1964; Malamy and Klessig 1992; Macheix 1996).

In plants SA also has a regulatory effect on H_2O_2 metabolism in pathological and stress states (Sanchez-Casas and Klessig 1994; Ruffer et al. 1995; Durner and Klessig 1996). Furthermore, SA induces the expression of certain genes responsible for defence and resistance mechanisms in plants protecting them from the invasion of pathogens resulting in, for example, the synthesis of PR proteins directly related to pathogenesis (Rao et al. 1997; Xie et al. 1998; Cronje and Bornman 1999).

SA has a stimulative effect on the processes of protein phosphorylation activating kinases, particularly MAP kinase (Zhang and Klessig 1997; Xie et al. 1998). In addition, SA delays the ageing process of plants mainly by inhibiting the synthesis of cellulase, some proteinases, ethylene, jasmonic acid and systemin. It has been found that SA, especially when interacting with IAA in germinating seeds stimulates the growth of roots, anthocyanin synthesis and the activity of nitrate reductase (Romani et al. 1962; Roustan et al. 1990; Vazquez and Mato 1991; Doares et al. 1995; Ferrarese et al. 1996; Reymond and Farmer 1998).

On the other hand, the studies of Raskin (1995) showed that *o*- and *p*-HBA applied in higher concentrations of the order of 10^{-3} M inhibit seed germination and the growth of some plants which may have a marked influence on the development of allelopathy. Pancheva and co-workers (1996, 1998) in experiments on barley (*Hordeum vulgare*) on applying 1 mM SA noted a weakening of the activity of rubisco, the key enzyme in the dark phase of photosynthesis, which resulted in a lower activity of this process.

Only a few authors (Pilet and Gaspar 1965; Pilet 1966) have reported that the *o*-, *m*- and *p*-HBA isomers applied in low concentrations 10^{-6} - 10^{-5} M have a slight stimulative effect, around 15%, on the growth of the epicotyl and stem of the lentil (*Lens culinaris*). The most stimulating isomer was *o*-HBA, *m*-HBA had a weaker effect while *p*-HBA had only a slight effect. On the other hand, in higher concentrations, 10^{-4} - 10^{-3} M HBA isomers have an inhibitory effect on the growth of plants together with increasing the activity of IAA oxidase. It was found that the *o*-HBA isomer had a negligible inhibitory effect on the above parameters, *m*-HBA had a slightly stronger effect whereas *p*-HBA had the greatest inhibitory effect.

From some later studies made on cucumbers, carrots and tobacco infected with pathogens (Klessig and Malamy 1994; Durner et al. 1997; Smith-Becker et al. 1998; Xie and Chen 1999) it was found that the *m*- and *p*-HBA isomers do not possess such a potent biological activity as that of *o*-HBA, that is salicylic acid. For example, the *m*- and *p*-HBA isomers did not stimulate synthesis of the resistance proteins PR, or induce thermogenesis and an alternative respiratory pathway (Rhoads and McIntosh 1962).

In view of the above, comparative studies of the biological activity of the *o*-, *m*- and *p*-HBA were carried out using the unicellular green alga, *Chlorella vulgaris*, as an experimental model.

MATERIALS AND METHODS

Plant material and growth conditions

C. vulgaris Beijerinck (Chlorophyceae) was grown under controlled condition at $25 \pm 0.5^\circ\text{C}$. Illumination was supplied during 16 h photoperiod (8 h dark period) by a bank

of fluorescent lights yielding a photon flux of $50 \mu\text{mol m}^{-2}\text{s}^{-1}$ at the surface of the tubes. Permanent synchronous growth was established according to the method of Pirson and Lorenzen (1966), in the conditions developed by Sayegh and Greppin (1973). The culture medium used was modified Knop's medium in the following nutritive solution: 0.5 g KNO_3 , 0.5 g $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 0.2 g KH_2PO_4 , 0.15 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.003 g H_3BO_3 , 0.002 g $\text{MnCl}_2 \cdot \text{H}_2\text{O}$, 0.0003 g NH_4VO_3 , 0.0002 g $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.0001 g $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 7\text{H}_2\text{O}$, 1 l distilled water. The pH of the medium was adjusted to 6.8 with 1 N NaOH.

HBA isomers

HBA isomers were purchased from Sigma (USA) and prepared as 10^{-4} M ethanolic stock solution and stored at -20°C . The appropriate amounts of HBA isomers stock solution for the strongest solution were transferred directly into culture medium and weaker solutions were prepared by serial dilution. Equal amount of ethanol was added to the controls. Figure 1 shows the structures of HBAs that were used in the experiment.

Fig. 1. Structures of HBA isomers used.

Analysis of Plant Material

Number of cells was determined by direct counting of cells in the growth medium using the Bürker chamber. To determine the dry mass, 10 ml algal cultures was filtered through preweighed glass microfiber filters (Whatman, Maidstone, UK) washed with distilled water to remove non-biological materials, such as mineral salt precipitates, dried overnight at 105°C for a few hours to a constant weight. The dry weight was expressed in g on 1 l of the algal culture (Lee and Low 1993).

For DNA and RNA determination, the algae were first collected by centrifugation (4500 rpm, 15 min, 20°C) of 25 ml culture samples and then the algal pellet was quickly frozen in liquid nitrogen, powdered with a mortar and pestle. Nucleic acids were isolated with the DNeasy and RNeasy Plant Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

Measurement of protein content was done by extracting the algal pellet overnight in 0.1 N NaOH at 4°C . Concentration of cellular protein and protein excreted to the medium were determined by the method of Lowry et al. (Lowry et al. 1951) with a protein kit calibrated with bovine serum albumin (Sigma; St. Louis, MO; USA) as the standard.

The determination of chlorophylls and total carotenoids content followed homogenisation of the algal fresh weight in methanol. The absorbance of the extract was measured

with a Shimadzu UV-Vis 1201 spectrophotometer at 470, 653 and 666 nm. The amounts of chlorophyll *a* and *b*, carotenoids present in the extract were calculated according to the equations of Wellburn (Wellburn 1994).

For sugar determination, the algae were first collected by centrifugation of 10 ml culture samples. Sugar concentration in the cell was determined using the Somogyi method (Somogyi 1954).

Replication and statistical analysis

Each treatment consisted of 5 replicates and each experiment was carried out at least twice at different times. Minitab statistical package was used to carry out a one-way ANOVA. Significance was determined using *t*-tests and LSD values based on the ANOVA data.

RESULTS

Among the *o*-, *m*- and *p*-HBA isomers, *o*-HBA was found to exert the greatest stimulative effect on the unicellular green alga, *C. vulgaris*, *p*-HBA had a slightly weaker effect, whereas *m*-HBA was found to have an inhibitory effect particularly between the 3rd and 9th day of the alga culture. The *o*- and *p*-HBA isomers displayed their greatest stimulative activity in a concentration of 10^{-4} M whereas the inhibitory effect of *m*-HBA was usually strongest in a concentration of 10^{-6} M (Figs 2-10).

Fig. 2. Effect of HBA isomers on the number of *Chlorella vulgaris* cells (expressed as 10^6 ml $^{-1}$, SE < 5%).

Fig. 3. Effect of HBA isomers on the content of dry mass of *Chlorella vulgaris* (expressed as g l $^{-1}$, SE < 5%).

Fig. 4. Effect of HBA isomers at the 6th day of cultivation on the content of DNA and RNA in the cell of *Chlorella vulgaris* (expressed as 10^{-8} μ g cell $^{-1}$, SE < 5%).

Fig. 5. Effect of HBA isomers on the content of protein in the cell of *Chlorella vulgaris* (expressed as 10^{-8} μ g cell $^{-1}$, SE < 5%).

Fig. 6. Effect of HBA isomers on the content of extracellular protein of *Chlorella vulgaris* (expressed as 10^{-8} μ g cell $^{-1}$, SE < 5%).

Under the influence of 10^{-4} M *o*-HBA between the 6th and 9th day of the *C. vulgaris* culture stimulation of the number of cells of between 147 and 154%, the content of dry mass 127-144%, monosaccharides 159-212%, chlorophyll *a* 147-156%, chlorophyll *b* 144-155%, carotenoids 144-167%, soluble proteins 127-132% and their secretion 132-137% and DNA 119-129% and RNA 112-116% in comparison with the control culture contents (taken to be 100%) was observed.

Under the influence of 10^{-4} M *p*-HBA, also between the 6th and 9th day of the alga culture, stimulation was

Fig. 7. Effect of HBA isomers on the content of chlorophyll *a* in the cell of *Chlorella vulgaris* (expressed as $10^{-8} \mu\text{g cell}^{-1}$, SE < 5%).

Fig. 8. Effect of HBA isomers on the content of chlorophyll *b* in the cell of *Chlorella vulgaris* (expressed as $10^{-8} \mu\text{g cell}^{-1}$, SE < 5%).

Fig. 9. Effect of HBA isomers on the content of carotenoids in the cell of *Chlorella vulgaris* (expressed as $10^{-8} \mu\text{g cell}^{-1}$, SE < 5%).

observed of the number of cells from 115 to 128%, the dry mass content from 112-117%, monosaccharides 122-140%, chlorophyll *a* 129-142%, chlorophyll *b* 128-135%, carotenoids 130-139%, soluble proteins 108-115% and their secretion 111-120%, DNA 105-114% and RNA 104% in relation to the control (100%).

On the other hand, the isomer *m*-HBA in a concentration range of 10^{-6} - 10^{-4} M had a slightly inhibitory effect on the growth and biochemical parameters analysed during the 9 days' culture of *C. vulgaris*. Under its influence, chiefly at a concentration of 10^{-6} M, a maximal fall in the number of cells of 83-87% occurred, of dry mass 67-88%, monosac-

Fig. 10. Effect of HBA isomers on the content of sugar in the cell of *Chlorella vulgaris* (expressed as $10^{-8} \mu\text{g cell}^{-1}$, SE < 5%).

charides 73-75%, chlorophyll *a* 74-79%, chlorophyll *b* 63-67%, carotenoids 88-89%, soluble proteins 91-93% and their secretion 85-90% as well as DNA about 90% and RNA 81-85% in relation to the control culture (100%).

DISCUSSION

Of the isomers, *o*-, *m*- and *p*-HBA applied in the optimum physiological concentrations 10^{-4} - 10^{-6} M to the unicellular eukaryotic green alga *C. vulgaris*, the greatest biological activity of stimulative properties was noted under the influence of *o*-HBA while a somewhat weaker activity occurred under that of *p*-HBA. Both isomers were found to exert the greatest biological activity in a concentration of 10^{-4} M. On the other hand, *m*-HBA in the concentration range applied, especially at 10^{-6} M, had a slightly inhibitory effect on the growth of the alga and all the biochemical parameters studied in comparison with the control culture.

Under the influence of *o*- and *p*-HBA the stimulation of the growth and biochemical parameters analysed, that is intensity of growth as expressed by the number of cells and their content of dry mass, soluble proteins, nucleic acids (DNA and RNA), chlorophylls *a* and *b*, carotenoids and monosaccharides was the strongest on the 6th day of the *C. vulgaris* culture, slightly weaker on the 9th day and the weakest during the initial stage, that is on the 3rd day of the alga culture. With a lowering of the concentration of the *o*- and *p*-HBA isomers a gradual weakening of their stimulative effect on the growth and biochemism of the *C. vulgaris* occurred.

The isomer *m*-HBA, on the other hand, was found to have the strongest inhibitory effect in the lowest concentration, 10^{-6} M, particularly in the young alga culture, that is on the 3rd day but on the number of cells and their photosynthesising pigments it had a very weak stimulative effect.

In the defence response SA causes activation of the expression of certain genes responsible for the synthesis of proteins, especially the enzymatic, and promotes the generation of free radicals (Dempsey et al. 1999). In recent studies carried out on tomatoes (*Lycopersicon esculentum*) in which high concentrations of SA 0.5-1.0 mM were applied for a period of 3 to 8 hours, induction of expression of the genes encoding the synthesis of immune proteins Hsp70/Hsc70 was observed. Low concentrations of SA,

however, did not induce the synthesis of these proteins. Studies carried out on mammals also showed that SA not only has an anti-inflammatory effect but also induces the synthesis of antistress proteins Hsp70/Hsc70 related to heat shock and the mechanism of maintaining thermotolerance (Cronje and Bornman 1999).

The studies of Leslie and Romani (1986) demonstrated that SA and acetylsalicylic acid in the optimum concentrations of 10^{-6} - 10^{-4} M are extremely active inhibitors of ethylene synthesis by blocking the conversion of 1-aminocyclopropane-1 carboxylic acid (ACC).

A low pH (acidification) together with SA induced growth and elongation of plant cells and were found to be the key regulating the activity of the enzymes of the pentosophosphate pathway, mainly 6-phosphoglucose dehydrogenase and guaiacolic peroxidase. This occurs particularly during germination and the further elongationary growth of plants as was noted in experiments made on the pea (*Pisum sativum*). The fall in pH is caused by stimulation of phenolic acid synthesis and this is probably the main cause of growth and elongation induction in plants, perhaps by regulation of the pentosophosphate pathway. At the same time an increase in the proline content in a biochemical response to acidification (a fall in pH) and the application of SA particularly in high concentrations was noted (Cvikrova et al. 1996; McCue et al. 2000).

The empirical data we obtained in these studies of the green alga, *C. vulgaris*, differ somewhat from those in the literature concerning vascular plants (Pilet and Gaspar 1965; Pilet 1966; Pancheva et al. 1996, 1998). The latter show that HBA isomers in vascular plants generally exert a slightly inhibitory effect on growth, lowering the activity of some metabolic processes, e.g. photosynthesis. These differences may be due to the taxonomic dissimilarity of the plants, their developmental phase and to different culture conditions and environmental factors.

The biological and metabolic activity of phenolic acids depends mainly on the number and spatial distribution of the hydroxyl groups in relation to the carboxyl group in the aromatic ring. From the data given in the literature it is known that in vascular plants some hydroxyphenylpropene acids, e.g. ferulic, *p*-coumaric and caffeic, form amide compounds with the polyamines in vascular plants: putrescine, spermine and spermidine, which enhances their activity stimulating the induction of genes responsible for growth and some physiological- metabolic processes. It is highly probable that phenolic acids, e.g. the isomers studied *o*-, *m*- and *p*-HBA, can form compounds with polyamines and as phenolamides have a regulatory effect on the synthesis of DNA, RNA and proteins and other metabolites as well as on the activity of enzymes and the structure of cellular membranes (Naguib 1962a, b; Larson 1989).

Some compounds belonging to the phenolic acids together with phytohormones and polyamines exert a significant regulatory effect either inhibitory or stimulative on the growth, development and physiological-metabolic processes of plants. The effectiveness of the action of HBA isomers on the growth and biochemical parameters analysed in the cells of the alga *C. vulgaris* varies depending on the position of the hydroxyl groups in relation to the carboxyl located in the ring portion of the molecule. In the case of the alga *C. vulgaris* and vascular plants, the most biologi-

cally active, usually stimulative, is *o*-HBA (salicylic acid) commonly occurring in the plants. The isomer *p*-HBA had a considerably weaker stimulative activity whereas the isomer *m*-HBA was weakly inhibitory in its action. Vazquez and Mato (1991) demonstrated that *o*-HBA has the greatest activity on the formation of roots on *Phaseolus vulgaris* hypocotyls. *p*-HBA showed a weaker stimulation of the number of roots; the lowest activity was shown by *m*-HBA. HBA participate in the inhibition of the IAA-oxidase activity. The greatest inhibitory effect was shown by *o*-HBA, the lowest by *m*-HBA after 4 h of treatment. On the other hand, the stimulation of IAA-oxidase activity was shown by *p*-HBA in compared to the control. Analogical activity was noted in hydroxyl derivatives of auxins and their chemical analogues, e.g. the isomers *o*-, *m*- and *p*- of hydroxyphenylacetic acid. The *o*-, *p*-hydroxyl isomers of the above mentioned compounds usually have a stimulative effect whereas, as a rule, the isomer *meta* has an inhibitory effect.

LITERATURE CITED

- ABERG B. 1981. Plant growth regulators. XLI. Monosubstituted benzoic acids. Swed. J. Agr. Res., 11: 913-1105.
- BASYOUNI S.L., CHEN D., IBRAHIM R.K., NEISH A.G., TOWERS G.H.N. 1964. The biosynthesis of hydroxybenzoic acids in higher plants. Phytochemistry, 3: 485-492.
- CHEN Z., MALAMY J., HENNIG J., CONRATH U., SANCHEZ-CASAS P., RICIGLIANO J., KLESSIG D.F. 1995. Induction, modification and transduction of the salicylic acid signal in plant defence responses. Proc. Natl. Acad. Sci. USA, 92: 4134-4137.
- CHEN Z., SILVA H., KLESSIG D.F. 1993. Active oxygen species in the induction of plant systemic acquired resistance by salicylic acid. Science, 262: 1883-1886.
- CRONJE M.J., BORNMAN L. 1999. Salicylic acid influences Hsp70/Hsc70 expression in *Lycopersicon esculentum*: dose and time dependent induction or potentiation. Bioch. Biophys. Res. Commun., 265: 422-427.
- CVIKROVA M., HRUBCOWA M., EDER J., BINAROVA P. 1996. Changes the levels of endogenous phenolics, aromatic monoamines, phenylalanine ammonia-lyase, peroxidase and auxine oxidase activities during initiation of alfalfa embryogenic and nonembryogenic calli. Plant Physiol. Biochem., 34: 853-861.
- CZERPAK R., BAJGUZ A. 1998. Physiological and metabolic activity of salicylic acid in plants. Kosmos, 47: 83-93.
- DELANEY T.P., UKNESS S., VERNOOIJ B., FRIEDRICH L., WEYMAN K., NEGROTTO D., GAFFNEY T., KESSMAN H., WARD E., RYALS J. 1994. A central role of salicylic acid in plant disease resistance. Science, 266: 1247-1250.
- DEMPSEY M.A., SHAH J., KLESSIG D.F. 1999. Salicylic acid and disease resistance in plants. Crit. Rev. Plant Sci., 18: 547-575.
- DOARES S.H., NARVAEZ-VASQUEZ J., CONCONI A., RYAN C.A. 1995. Salicylic acid inhibits synthesis of proteinase inhibitors in tomato leaves by systemin and jasmonic acid. Plant Physiol., 108: 1741-1746.
- DURNER J., KLESSIG D.F. 1996. Salicylic acid is a modulator of tobacco and mammalian catalases. J. Biol. Chem., 271: 28492-28501.
- DURNER J., SHAH J., KLESSIG D.F. 1997. Salicylic acid and disease resistance in plants. Trends. Plant Sci., 2: 266-274.
- FERRARESE L., MORETTO P., TRAINOTTI L., RASCIO N., CASADORO G. 1996. Cellulase involvement in the abscission of peach and pepper leaves is affected by salicylic acid. J. Exp. Bot., 47: 251-257.

- KLESSIG D.F., MALAMY J. 1994. The salicylic acid signal in plants. *Plant Mol. Biol.*, 26: 1439-1458.
- KLIEBENSTEIN D.J., DIETRICH R.A., MARTIN A.C., LAST R.L., DANGL J.L. 1999. LSD1 regulates salicylic acid induction of copper zinc superoxide dismutase in *Arabidopsis thaliana*. *Mol. Plant Microb. Interact.*, 12: 1022-1026.
- LARSON L.J. 1989. Effect of phenolic acids on growth of *Chlorella pyrenoidosa*. *Hydrobiologia*, 183: 217-222.
- LEE Y.K., LOW C.S. 1993. Productivity of outdoor algal cultures in unstable weather conditions. *Biotech. Bioeng.*, 41: 1003-1006.
- LESLIE C.A., ROMANI R.J. 1986. Salicylic acid: a new inhibitor of ethylene biosynthesis. *Plant Cell. Rep.*, 5: 144-146.
- LOWRY O.H., ROSEBROUGH N.J., FARR A.L., RANDALL R.J. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, 193: 265-275.
- MACHEIX J.J. 1996. Les composés phénoliques des végétaux. *Acta. Bot. Gal.*, 143: 473-479.
- MALAMY J., KLESSIG D.F. 1992. Salicylic acid and plant disease resistance. *Plant J.*, 2: 643-654.
- MCCUE P., ZHENG Z., PINKHAM J.L., SHETTY K.A. 2000. Model for enhanced pea seedling vigour following low pH and salicylic acid treatments. *Process. Biochem.*, 35: 603-613.
- NAGUIB M.I. 1962a. Effect of benzoic acid and its derivatives on plant metabolism. 1. Effect of benzoic acid and its hydroxyderivatives on respiration of starved and sucrose – fed etiolated barley leaves. *Bull. Fac. Sci. Cairo. Univ.*, 38: 61-72.
- NAGUIB M.I. 1962b. Effect of benzoic acid and its derivatives on plant metabolism. 2. Effect of benzoic acid and its derivatives on sucrose inversion, sugar uptake and excretion by starved and sucrose – fed etiolated barley leaves. *Bull. Fac. Sci. Cairo. Univ.*, 38: 73-86.
- NITSCH J.P., NITSCH C. 1962. Composés phénoliques et croissance végétale. *Ann. Physiol. Veg.*, 4: 211-225.
- PANCHEVA T.V., POPOVA L.P., UZUNOVA A.N. 1998. Effects of salicylic acid of ribulose-1,5-bisphosphate carboxylase/oxygenase in barley leaves. *J. Plant Physiol.*, 152: 381-386.
- PANCHEVA T.V., POPOVA L.P., UZUNOVA A.N. 1996. Effects of salicylic acid on growth and photosynthesis in barley plants. *J. Plant Physiol.*, 149: 57-63.
- Pierpoint W.S. 1994. Salicylic acid and its derivatives in plants: medicines, metabolites and messenger molecules. *Adv. Bot. Res.*, 20: 163-233.
- PILET P.E. 1966. Effect of *p*-hydroxybenzoic acid on growth, auxin content and auxin catabolism. *Phytochemistry*, 5: 77-82.
- PILET P.E., GASPARD T. 1965. Action des acides *o*-, *m*- et *p*-hydroxybenzoïques sur le catabolisme auxinique et la croissance. *Annu. Physiol. Veg.*, 7: 147-155.
- PIRSON A., LORENZEN H. 1966. Synchronized dividing algae. *Ann. Rev. Plant Physiol.*, 17: 439-458.
- RAO M.V., PALIYATH C., ORMROD D.P., MURR D.P., WATKINS C.B. 1997. Influence of salicylic acid on H₂O₂ production oxidative stress and H₂O₂ metabolizing enzymes. *Plant Physiol.*, 115: 137-149.
- RASKIN I. 1992 a. Role of salicylic acid in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 43: 439-463.
- RASKIN I. 1992 a. Salicylate, a new plant hormone. *Plant Physiol.*, 99: 799-803.
- RASKIN I. 1995. Salicylic acid. in: Davies PJ (ed.) *Plant Hormones Physiology, Biochemistry and Molecular Biology*. Kluwer Acad. Publ. pp. 188-205.
- REYMOND P., FARMER E.E. 1998. Jasmonate and salicylate as global signals for defense gene expression. *Curr. Opin. Plant Biol.*, 1: 404-411.
- RHOADS D.M., MCINTOSH L. 1962. Salicylic acid regulation of respiration in higher plants: alternative oxidase expression. *Plant Cell.*, 4: 1131-1139.
- ROMANI R.J., HESS B.M., LESLIE C.A. 1962. Salicylic acid inhibition of ethylene production by apple discs and other plant tissue. *J. Plant Growth. Regul.*, 8: 63-70.
- ROUSTAN J.P., LATCHE A., FALLOT J. 1990. Inhibition of ethylene production and stimulation of carrot somatic embryogenesis by salicylic acid. *Biol. Plant.*, 32: 273-276.
- RUFFER M., STEIPE B., ZENK M.H. 1995. Evidence against specific binding of salicylic acid to plant catalase. *FEBS Lett.*, 377: 175-180.
- SANCHEZ-CASAS P., KLESSIG D.F. 1994. Salicylic acid binding activity correlates with inhibition of catalase activity in various plants. *Plant Physiol.*, 106: 1675-1679.
- SAYEGH A., GREPPIN H. 1973. *Chlorella rubescens* Ch. Essai de synchronisation et mise en évidence de rythmes endogènes. *Arch. Sci. Genève*, 8: 6-18.
- SMITH-BECKER J., MAROIS E., HUGUET E.J., MIDLAND S.L., SIMS J.J., KEEN N.T. 1998. Accumulation of salicylic acid and 4-hydroxybenzoic acid in phloem fluids of cucumber during systemic acquired resistance is preceded by a transient increase in phenylalanine ammonia-lyase activity in petioles and stems. *Plant Physiol.*, 116: 231-238.
- SOMOGYI M. 1954. Notes on sugar determination. *J. Biol. Chem.*, 195: 19-23.
- TOMASZEWSKI M. 1960. The occurrence of *p*-hydroxybenzoic acid and some other simple phenols in vascular plants. *Bull. Acad. Pol. Sci.*, 2: 61-63.
- TOMASZEWSKI M. 1964. The mechanism of synergistic effects between auxin and some natural phenolic substances. Régulateurs naturels de la croissance végétale. *Comp. Natur. Rend. Sci., Paris*, pp. 335-351.
- VAZQUEZ A., MATO M.C. 1991. Effects of hydroxy-benzaldehydes on rooting and indole-3-acetic oxidase activity in bean cuttings. *Physiol. Plant.*, 83: 597-600.
- WELLBURN A.R. 1994. The spectral determination of chlorophylls *a* and *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *J. Plant Physiol.*, 144: 307-313.
- WOBBE K.K., KLESSING D.F. 1996. Salicylic acid – an important signal in plants. in: Dennis ES (ed.) *Plant Gene Research*, Springer-Verlag, pp. 167-196.
- XIE Z., CHEN Z. 1999. Salicylic acid induces rapid inhibition of mitochondrial electron transport and oxidative phosphorylation in tobacco cells. *Plant Physiol.*, 120: 217-225.
- XIE Z., FAN B., CHEN Z. 1998. Induction of PR-1 proteins and potentiation of pathogen signals by salicylic acid exhibit the same dose response and structural specificity in plant cell cultures. *Mol. Plant Microb. Interact.*, 11: 568-571.
- ZHANG S., KLESSIG D.F. 1997. Salicylic acid activates a 48 kDa MAP kinase in tobacco. *Plant Cell.*, 9: 809-824.

WPLYW IZOMERÓW KWASU HYDROKSYBENZOESOWEGO
NA WZROST I METABOLIZM *CHLORELLA VULGARIS* BEIJERINCK (CHLOROPHYCEAE)

STRESZCZENIE

Badano wpływ izomerów *o*-, *m*- i *p*-hydroksybenzoesowego kwasu (HBA) w optymalnym zakresie stężeń 10^{-6} - 10^{-4} M na aktywność biologiczną jednokomórkowej zielenicy *Chlorella vulgaris* (Chlorophyceae). Kwas *o*-hydroksybenzoesowy zwany salicylowym w stężeniu 10^{-4} M najsilniej stymulował wzrost i analizowane parametry biochemiczne, takie jak: zawartość chlorofilu *a* i *b*, karotenoidów, DNA, RNA, monosacharydów, białek rozpuszczalnych i ich sekrecję pozakomórkową. Znacznie słabiej stymulująco na wzrost i zawartość składników chemicznych w *Chlorella vulgaris* działał kwas *p*-hydroksybenzoesowy. Natomiast kwas *m*-hydroksybenzoesowy powodował efekt inhibicyjny na wzrost *Chlorella vulgaris* i wszystkie analizowane parametry biochemiczne w porównaniu z kontrolną hodowlą glonów, pozbawioną egzogenego izomeru HBA.

SŁOWA KLUCZOWE: *Chlorella vulgaris*, *o*-, *m*- i *p*-hydroksybenzoesowy kwas, wzrost, chlorofile, karotenoidy, DNA, RNA, białka, monosacharydy.