# BIOCHEMICAL ACTIVITY OF AUXINS IN DEPENDENCE OF THEIR STRUCTURES IN *WOLFFIA ARRHIZA* (L.) WIMM.

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ABSTRACT

*Wolffia arrhiza* (L.) Wimm. (Lemnaceae) as a mixotrophic plant reacts considerably weaker to used auxins with different chemical structures than typical photosynthetic vascular plants and algae especially from Chlorophyta. Among used auxin compounds, the highest stimulative activity on *W. arrhiza* growth and biochemical parameters which were analysed in biomass, can be attributed to phenylacetic acid (PAA), a somewhat smaller to indole-3-acetic acid (IAA) and the smallest to 2-naphthaleneacetic acid (NAA) used in optimal concentration of $10^{-6}$ M, in comparison with the control culture, devoid of exogenous auxins. The investigated auxins, especially PAA and IAA, were found to have the most powerful stimulative activity (prevailingly between the 10th and the 15th day of cultivation) on the content of reducing sugars between 127 and 169%, chlorophyll *a* and *b* from 117 to 125%, total carotenoids from 115 to 132% and net photosynthetic rate from 127 to 144% in comparison with the control culture, which was treated as 100% for reference. However, the content of water-soluble proteins as well as nucleic acids (DNA and RNA) in the biomass of *W. arrhiza* was less effectively stimulated, hardly from 110 to 116% when compared to the control culture (100%).

KEY WORDS: *Wolffia arrhiza*, auxins, indole-3-acetic acid (IAA), phenylacetic acid (PAA), 2-naphthaleneacetic acid (NAA), proteins, reducing sugars, chlorophylls *a* and *b*, total carotenoids, net photosynthesis rate, nucleic acids (DNA and RNA).

## INTRODUCTION

The rootless wolfia *Wolffia arrhiza* (L.) Wimm. from Lemnaceae is the smallest vascular plant, which – depending on biological environment conditions can be a photoautotroph or a mixotroph (at the same time photo- and heterotroph) or absolutely a heterotroph. Due to adaptation to specific environment conditions, the organism of *W. arrhiza* underwent a high simplification process. The leaf-like body of Lemnaceae species, called a frond, is a complex of tissues with only few differentiation. Therefore there are no roots, leaves or stem and the reduced *W. arrhiza* organism resembles to algae. The *W. arrhiza* frond is covered with epidermis with numerous stomata apparatuses, underneath there is assimilative parenchyma with numerous air spaces filled with carbon dioxide and oxygen, allowing these plants to freely move across water layers or fall down to the bottom of the reservoir (Bhanthumnavin and McGarry 1971; Landolt 1986; Frick and Morley 1995; Mical et al. 1997).

Plants, which belong to the Lemnaceae family, including *W. arrhiza*, possess characteristic flavone pigments, which are one of fundamental chemotaxonomic features. (Frick and Morley 1995; McClure and Alston 1966).

In conditions of our environment, *W. arrhiza* reproduces only vegetatively through gemmation, producing descendant buds and in them pradescendant ones. It is a plant which is very resistant to impact of various stress and toxic conditions such as: lack or excess of light, excess of ammonium ions as well as ions of heavy metals, cyanides and other xenobiotics. In Polish waters it is becoming more and more popular, especially in small and shallow eutrophic reservoirs, rich in organic substances (Frick 1994; Mical et al. 1997).

It is known that a reasonable number of Lemnaceae species, including in that *W. arrhiza*, in environment rich in organic substances, change their way of feeding from photoautotrophic into photoheterotrophic. These plants, thanks to such properties as: possibility of mixotrophic feeding, resistance to numerous toxic substances, fast multiplication in a vegetative way and plaustonic way of life, are used more and more commonly in biotechnology of sewage treatment especially of commune and agricultural origin (Mical et al. 1999; Mical and Krotke 1999).

Vascular plants are characterised by production of various phytohormones with auxins at the head, among which indole-3-acetic acid (IAA) is dominant. Auxins also occur very commonly in many species of bacteria, fungi, micro-

and macroalgae as well as vascular plants. The auxin bio-synthesis in plants from Lemnaceae family e.g. *Spirodela* and *Lemna* has been shown in many studies (Cohen and Bandurski 1982; Slovin and Cohen 1988; Baldi et al. 1991; Gross and Parthier 1994; Davies 1995; Tam et al. 1998; Rapparini et al. 1999).

According to literature data (Millborrow and Purse 1975; Fries and Iwasaki 1976; Fries 1977; Fries and Aberg 1978; Czerpak 1979, 1990; Leuba and Letourneau 1990; Branca et al. 1991; Czerpak et al. 1994; Davies 1995) auxin pro-perties can be found in a number of chemical synthetic analogues, called heteroauxins, such as: phenylacetic acid (PAA), phenoxyacetic acid, 2.4-dichlorophenoxyacetic acid (2.4-D), $\alpha$- and $\beta$-naphthaleneacetic acid (NAA), na-phtoxyacetic acid, *cis*-cinnamic acid and many more. Part of them, just like phenylacetic acid and *p*-hydroksypheny-lacetic acid, occurs besides typical auxins in some species of bacteria, algae and fungi. PAA was also found in vessel plants e.g. pea seedling organs (Shneider et al. 1985). He-teroauxins exhibit stimulative properties which are usually a bit weaker in comparison to commonly naturally present IAA. In turns, NAA and especially its $\beta$-isomer is an au-xin-like synthetic compounds, quite commonly used as growth stimulators in garden and decorative plants (Cohen and Bandurski 1982; Peters et al. 1992; Davies 1995).

Naturally found auxins, especially IAA and PAA as well as their chemical analogues like NAA possess regulative activity, mainly stimulative on biochemical processes with anabolic character, taking place in a unicellular and multi-cellular plant organism. Auxin compounds mainly have in-ducing effects on the following processes: cell elongation and enlargement, cell division, replication, transcription, translation, photosynthesis, and active transport of metabo-lites through membranes and functioning of ion pumps and transduction of cell signals by means of chemical transmit-ters. They also activate numerous enzymes mainly connec-ted with synthesis of nucleic acids and proteins as well as photosynthesis, compounds regulating metabolism, chloro-phylls and carotenoids synthesis and other metabolites im-portant for plant life (Gamburg 1978; Cohen and Bandur-ski 1982; Czerpak 1990; André and Scherer 1991; Venris and Napier 1991; Peters et al. 1992; Guilfoyle et al. 1993; Edgerton et al. 1994; Hobbic et al. 1994; Jones 1994; Czer-pak and Bajguz 1997).

Generally, in photosynthetic plants, typical auxins and auxin-like compounds reduce catabolic processes except for developing plant embryos, which are in heterotrophic phase. However, anabolic processes, especially connected with growth and development of young plants, are intensi-vely activated under the influence of auxin compounds. It is known that auxins possess stimulative effect on the in-crease of intensity of cellular respiration and biosynthesis of ATP, which is necessary to occur many metabolic pro-cesses during plant growth and development (Cohen and Bandurski 1982; Czerpak 1990; Edelman and Kutschera 1993; Davies 1995; Abel and Theologis 1996).

Among others, auxins have a stimulative effect in photo-synthesis process mainly on reactions of bonding $CO_2$ to 1.5-biphosphoribulose; synthesis of reducing sugars espe-cially monosaccharides, photosynthetic phosphorylation and connected with biosynthesis of assimilative pigments – mainly chlorophylls (Adhikary and Pattnaik 1979; Czerpak 1990; Davies 1995; Czerpak et al. 1999).

In connection with the facts mentioned above, compara-tive studies have been performed in order to examine the influence of chemically various auxin compounds like: IAA, PAA and NAA, which vary significantly in terms of chemical structure of aromatic ring, on growth and con-tents of some metabolites in *W. arrhiza*, growing in tap water rich in mineral elements and poor in organic substan-ces. During investigation analysed were the changes in the content of such biochemical parameters as: nucleic acids (DNA and RNA), proteins soluble in water, reducing su-gars, chlorophylls *a* and *b*, total contents of carotenoids and net intensity of photosynthesis in the fresh weight of *W. arrhiza*.

## MATERIALS AND METHODS

### Plant material and growth conditions

The cultivation of *W. arrhiza* was being performed for the period of 20 days under stable conditions of phytotro-ne, temperature at $22\pm1°C$ with 12 hours' long illumination by fluorescent lamps with intensity of photosynthetic acti-ve radiation (PAR) of 50 $\mu mol·m^{-2}·s^{-1}$. Plants were cultiva-ted in glass crystallisers with the capacity of 2 litters and diameter of 20 centimetres, including 1 litre of medium, covered with a polyethylene foil, completely penetrable for light. The culture of *W. arrhiza* was performed in urban water of medium richness in mineral elements and poor in organic substances. The comparative examinations regar-ded the influence of most optimal concentration of $10^{-6}$ M auxins: IAA (indole-3-acetic acid), PAA (phenylacetic acid) and NAA (2-naphthaleneacetic acid), which are diffe-ring significantly in chemical character of aromatic radical, on the intensity of biosynthesis of proteins, reducing su-gars, nucleic acids, photosynthetic pigments and net photo-synthesis rate in *W. arrhiza*. The total content of these bio-logical parameters was determined in mg or mg per g of *W. arrhiza* fresh weight (Hallegraef 1977).

### Determination of water-soluble proteins

The measurement of protein content was done by homo-genisation of *W. arrhiza* biomass and extracting of the frac-tion of water-soluble proteins overnight in 0.1 N NaOH at 4°C. The concentration of protein was determined spectro-photometrically by the Lowry method using Folin phenol reagent (Lowry et al. 1951; Hewitt 1958) with a protein kit calibrated with bovine serum albumin as the standard.

### Reducing sugars determination

A similar preparation procedure was employed for spec-trophotometric determination of reducing sugars present in fresh weight (fr. wt) of *W. arrhiza*, according to the Somo-gyi-Nelson's method (Hodge and Hofreiter 1962).

### Determination of nucleic acids

The total content of nucleic acids (DNA and RNA) in plant biomass was determined spectrophotometrically, using the orcine reagent as described by Ostrowski and Fi-lipowicz (1980) and Rogers and Bendich (1985).

### Determination of photosynthetic pigments

The determination of chlorophyll *a* and *b* and total caro-tenoid contents followed homogenisation of *W. arrhiza*

fresh weight in methanol. The absorbance of the extract was measured with a spectrophotometer at 470.0, 652.4 and 665.2 nm (Goodwin 1980). The amounts of chlorophyll *a* and *b* and total carotenoids present in the extract were calculated according to the equations of Wellburn (1994).

An UV/Vis spectrophotometer (SHIMADZU type 1201, Japan) was used for all measurements.

### Determination of photosynthetic oxygen exchange

Net photosynthesis intensity was determined by measuring amounts of oxygen released by the biomass of plants, using a Clark type oxygen electrode Hansatech Ltd. UK (Lloyd et al. 1977; Walker and Walker 1990). A ml of wolfia's water suspension was incubated in a vessel at 25°C and 1.00 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR (photosynthetically active radiation). The evolution of oxygen in the medium was calculated to represent the activity of photosynthesis of *W. arrhiza*.

### Replication and statistical analysis

Each treatment consisted of 5 replicates and each experiment was carried out at least twice at different times. A 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.

TABLE 1. Chemical characteristics of tap water in Białystok.

| Analysed parameters | Tap water |
|---|---|
| pH | 7.1-7.3 |
| Hardness general (mg/CaCO$_3$/dm$^3$) | 218.5-241.4 |
| Basicity (mval/dm$^3$) | 3.25-3.86 |
| Nitrogen ammonium NH$_4$-N (mg N/dm$^3$) | 0.09-0.12 |
| Nitrogen nitrater (mg N/dm$^3$) | 1.88-2.03 |
| Total Kiejdahl nitrogen (mg N/dm$^3$) | 1.97-2.15 |
| Total phosphorus (mg P/dm$^3$) | 0.02-0.04 |
| Soluble phosphorus (mg PO$_4$$^{-3}$/dm$^3$) | 0.07-0.09 |
| Total iron (mg Fe/dm$^3$) | 0.11-0.13 |
| Chlorides (mg Cl/dm$^3$) | 12.2-14.3 |
| Organic matter (mg/dm$^3$) | 10.8-21.4 |
| Oxidity (mg O$_2$/dm$^3$) | 4.39-4.51 |
| BOD$_5$ (mg O$_2$/dm$^3$) | 7.02-10.36 |
| COD (mg O$_2$/dm$^3$) | 9.6-22.8 |

## RESULTS

Chemical characteristics of tap water from urban network in Białystok, in which *W. arrhiza* grew, with addition of the optimal concentration of $10^{-6}$ M, for structurally varied auxins is presented in Table 1. Whereas Table 2 shows results regarding the influence of used auxins: IAA, PAA and NAA on the contents of analysed biochemical

TABLE 2. The effect of auxins (IAA, PAA, NAA)* on the contents of biochemical parameters in *Wolffia arrhiza*.

| Biochemical parameters | Auxins** and Control | Time of culture (in days) | | | |
|---|---|---|---|---|---|
| | | 5 | 10 | 15 | 20 |
| Soluble proteins (mg·g$^{-1}$ fr. wt) | IAA | 19.95 | 23.05 | 25.41 | 26.41 |
| | PAA | 20.67 | 23.28 | 25.70 | 26.86 |
| | NAA | 19.65 | 22.87 | 25.12 | 26.24 |
| | Control | 18.66 | 22.55 | 24.50 | 25.23 |
| Nucleic acid (DNA+RNA) (mg·g$^{-1}$ fr. wt) | IAA | 3.27 | 4.47 | 5.54 | 5.18 |
| | PAA | 3.36 | 4.62 | 5.71 | 5.33 |
| | NAA | 3.24 | 4.35 | 5.22 | 5.07 |
| | Control | 3.15 | 4.11 | 4.98 | 5.01 |
| Chlorophyll *a* (µg·g$^{-1}$ fr. wt) | IAA | 141.28 | 146.21 | 158.94 | 167.51 |
| | PAA | 150.17 | 159.48 | 165.78 | 172.60 |
| | NAA | 132.37 | 145.42 | 157.55 | 165.14 |
| | Control | 128.23 | 140.80 | 148.12 | 152.09 |
| Chlorophyll *b* (µg·g$^{-1}$ fr. wt) | IAA | 60.76 | 62.52 | 73.18 | 63.08 |
| | PAA | 62.05 | 65.57 | 76.41 | 67.05 |
| | NAA | 55.94 | 60.85 | 69.94 | 62.16 |
| | Control | 54.01 | 58.74 | 61.44 | 59.57 |
| Total carotenoids (µg·g$^{-1}$ fr. wt) | IAA | 23.56 | 28.01 | 32.02 | 34.75 |
| | PAA | 28.95 | 31.50 | 33.59 | 35.46 |
| | NAA | 22.19 | 26.31 | 30.98 | 32.88 |
| | Control | 21.89 | 25.09 | 28.45 | 30.31 |
| Reducing sugars (µg·g$^{-1}$ fr. wt) | IAA | 230.31 | 315.66 | 479.44 | 383.46 |
| | PAA | 274.52 | 348.75 | 506.41 | 442.17 |
| | NAA | 225.40 | 294.55 | 345.68 | 328.81 |
| | Control | 188.43 | 249.53 | 298.15 | 302.49 |
| Net photosynthesis rate (nmol O$_2$·h$^{-1}$·g$^{-1}$ fr. wt) | IAA | 2658 | 3832 | 4461 | 3928 |
| | PAA | 2955 | 4266 | 5038 | 4551 |
| | NAA | 2448 | 3404 | 4171 | 3496 |
| | Control | 2245 | 3013 | 3948 | 3156 |

SE less than 5%; * optimal concentration $10^{-6}$ M; ** IAA – indole-3-acetic acid, PAA – phenylacetic acid, NAA – 2-naphthaleneacetic acid

parameters, expressed in µg or mg per g of *W. arrhiza* fresh weight. Figures 1 to 7 show changes expressed in percentage content of individual biochemical parameters under the influence of auxins in the period of 20 days' long cultivation of *W. arrhiza*, in comparison with the control culture, without auxin addition, taken to be 100%.
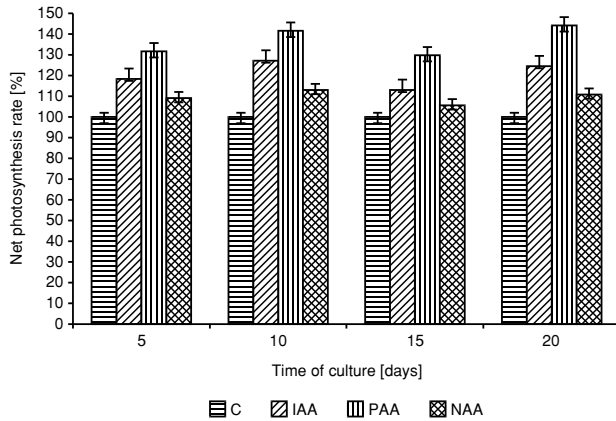


Fig. 1. The stimulation of net photosynthesis rate under the optimal concentration of $10^{-6}$ M different chemical structure auxins (IAA, PAA, NAA) between the 5th-20th day of *Wolffia arrhiza* culture (C – control culture, 100%).
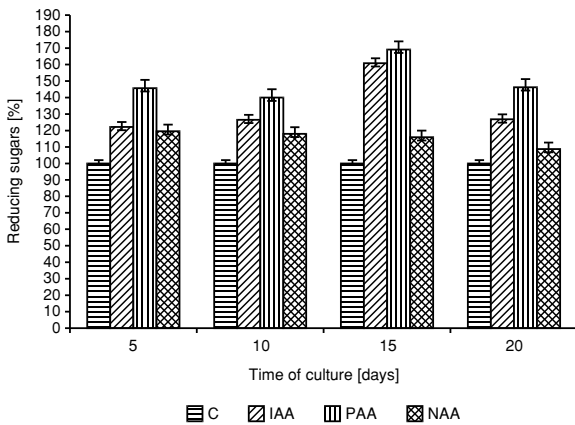


Fig. 2. The stimulation of reducing sugars content under the optimal concentration of $10^{-6}$ M different chemical structure auxins (IAA, PAA, NAA) between the 5th-20th day of *Wolffia arrhiza* culture (C – control culture, 100%).
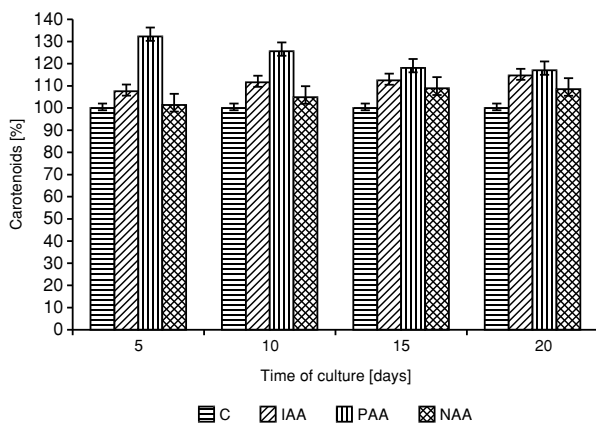


Fig. 3. The stimulation of total carotenoids content under the optimal concentration of $10^{-6}$ M different chemical structure auxins (IAA, PAA, NAA) between the 5th-20th day of *Wolffia arrhiza* culture (C – control culture, 100%).
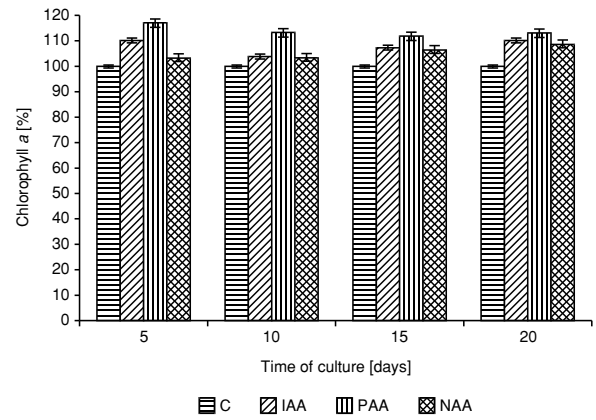


Fig. 4. The stimulation of chlorophyll *a* content under the optimal concentration of $10^{-6}$ M different chemical structure auxins (IAA, PAA, NAA) between the 5th-20th day of *Wolffia arrhiza* culture (C – control culture, 100%).
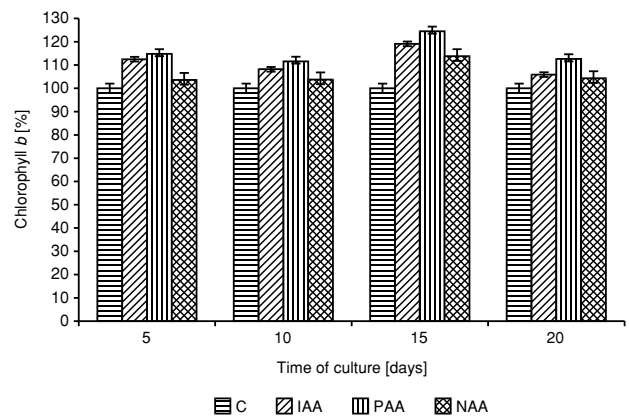


Fig. 5. The stimulation of chlorophyll *b* content under the optimal concentration of $10^{-6}$ M different chemical structure auxins (IAA, PAA, NAA) between the the 5th-20th day of *Wolffia arrhiza* culture (C – control culture, 100%).
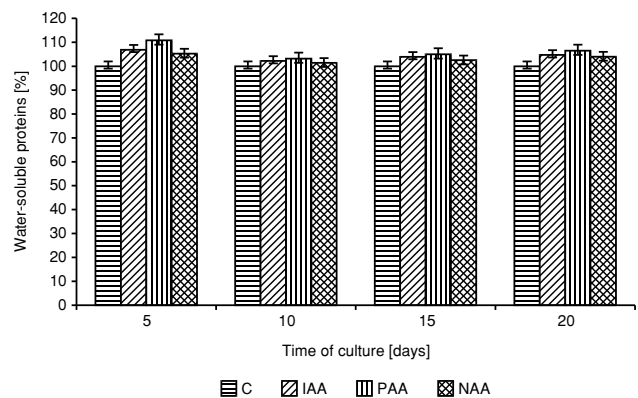


Fig. 6. The stimulation of water-soluble proteins content under the optimal concentration of $10^{-6}$ M different chemical structure auxins (IAA, PAA, NAA) between the 5th-20th day of *Wolffia arrhiza* culture (C – control culture, 100%).

Among used auxin compounds, the highest effectiveness on the content of all analysed biochemical parameters in *W. arrhiza* biomass was exhibited by phenylacetic acid (PAA), somewhat weaker by indole-3-actic acid (IAA) and the weakest by 2-naphthaleneacetic acid (NAA). For all examined auxins: IAA, NAA and PAA applied in the opti-
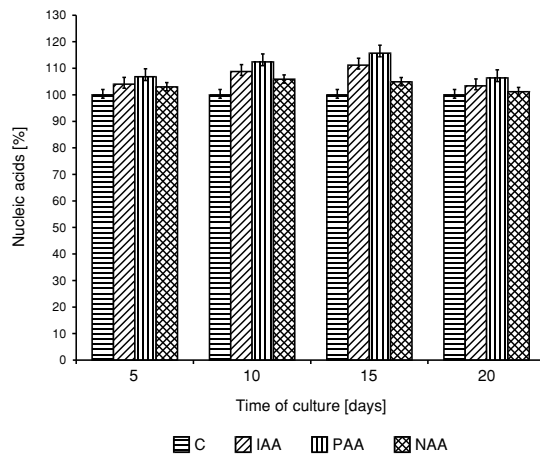
Fig. 7. The stimulation of nucleic acids (DNA and RNA) content under the optimal concentration of $10^{-6}$ M different chemical structure auxins (IAA, PAA, NAA) between the 5th-20th day of *Wolffia arrhiza* culture (C – control culture, 100%).

mal concentration ranges of $10^{-3}$-$10^{-7}$ M to the *W. arrhiza* culture, the greatest biological activity of stimulative properties was noted in the concentration of $10^{-6}$ M (data not shown). The above mentioned auxins exerted the highest stimulative activity in the concentration of $10^{-6}$ M, particularly between the 5th and the 15th day of cultivation, when *W. arrhiza* had reached the maximum level of its development and metabolism. Under their influence – especially PAA and IAA, the most visibly stimulated was the net photosynthesis rate and – connected with it – the content of reducing sugars within 127 to 169% in comparison with the control culture devoid of exogenous auxins, treated as 100%. The maximum increase of net photosynthesis rate to the level of 144.2% under the influence of PAA, to 127.2% under IAA and 124.5% under NAA, was observed (Fig. 1). The reducing sugar content in *W. arrhiza* fresh weight was also the most stimulated under the influence of PAA to 169.1%, slightly weaker to 160.8% under IAA and the weakest under NAA only to 119.6% (Fig. 2). Stimulative effect of examined auxins was a little weaker for the accumulation of analysed photosynthetic pigments such as: chlorophylls *a* and *b* as well as total carotenoids from 115 to 132% in comparison with the control culture (100%). The content of total carotenoids, under the influence of PAA, increased to the level of 132.3%, under IAA to 114.7% and under NAA hardly to 108.9% in biomass of *W. arrhiza* (Fig. 3). The addition of $10^{-6}$ M PAA promoted an increase of the accumulation of chlorophylls *a* to 117.1% and chlorophylls *b* to 124.5%, under the influence of $10^{-6}$ M IAA the chlorophylls *a* the biosynthesis was stimulated to the level of 110.1% and *b* to 119.1%, under $10^{-6}$ M NAA, chlorophyll *a* content reached only 108.6% and *b* – 113.8% (Figs 4-5). The examined auxins showed the weakest stimulative effect on accumulation of water-soluble proteins and nucleic acids (DNA and RNA) in the fresh weight of *W. arrhiza* maximally from 110 to 116% with respect the control culture (100%). It was noted that the content of water-soluble proteins fraction was stimulated to the maximum level of 110.8% under the influence of PAA, to 106.9% under the influence of IAA and only to 105.3% under NAA (Fig. 6). The increase of DNA and RNA contents in biomass to the level of 115.7% when applied $10^{-6}$ M PAA, to 111.2% when applied of $10^{-6}$ M IAA and only

to 105.9% when treated with the $10^{-6}$ M NAA in biomass of *W. arrhiza*, in relation to the control culture (100%), was observed (Fig. 7).

DISCUSSION

From performed examinations it results that rootless wolfia (*Wolffia arrhiza*), which belongs to the family Lemnacae and is characterised by mixotrophic abilities, reacts quite atypical and much weaker to the three applied auxins: IAA, PAA and NAA, which vary in chemical structure, in comparison with typical photosynthetic vascular plants and algae, especially from Chlorophyta. From among exogenously supplied auxins, the highest stimulative effect on the growth of *W. arrhiza* and contents of the analysed biochemical parameters was exhibited by PAA – the chemical analogue of natural auxin with phenol residue, sporadically found in plants, a little weaker by IAA – commonly occurring auxin, and the weakest one by NAA – a stable synthetic auxin. Whereas, in the auxin-treated typical vascular plants, generally the highest stimulative effect on physiologic and metabolic processes was exhibited by IAA or by NAA and much weaker by IPA, ILA, PAA and other related auxin compounds (Gamburg 1978; Cohen and Bandurski 1982; Branca et al. 1991; Davies 1995).

Under the influence of examined auxins: PAA, IAA and NAA – chemical analogue of naturally occurring auxins, used in optimal concentration of $10^{-6}$ M in *W. arrhiza* culture, the highest stimulative effect was visible upon the accumulation of reducing sugars, the intensity of biosynthesis of chlorophylls *a* and *b*, the total contents of carotenoids and the process of photosynthesis from 115 to 170%, in comparison to the control culture (100%). Whereas, the content of water-soluble proteins fraction and total nucleic acids (DNA and RNA) under the influence of auxins mentioned above was relatively stimulated to a small degree, merely in a few to several percent.

The large heterotrophic possibilities of *W. arrhiza* are probably the main reason for significant differences in activities of the used, structurally varied auxins, in comparison to vascular plants, as well as algae from Chlorophyta, for example genus *Chlorella* and *Scenedesmus*, which are only photoautotrophs (Czerpak 1979, 1990; Czerpak et al. 1994, 1997, 1999). In typical photosynthetic plants as well as *Lemna gibba* grown under different light/dark conditions, it was found that light influences the rate of entry of exogenous IAA, the endogenous level of auxins and processes of conjugation and oxidative breakdown of IAA (Tam et al. 1998). In *W. arrhiza*, a mixotrophic plant, which in our experiment is characterised by a lower sensitivity upon IAA in relation to the stronger biochemical response to the same concentration of PAA, the uptake mechanism of applied auxins and their turnover rate can be influenced by other intercellular factors e.g. the level of indolic precursors, vegetative and reproductive phases or environmental conditions.

From the literature (Gamburg 1978; Branca et al. 1991; Davies 1995) it is well known that NAA possesses a more stimulative effect than IAA on some species of vascular plants, especially garden ones. Likewise acids: phenylacetic and *p*-hydroksyphenylacetic, which are derivative metabolites of phenylamine, display an auxin-like activity on

processes of growth and development of plants, but a little weaker in comparison with typical auxins (Millborrow and Purse 1975; Leuba and Letourneau 1990). For example, the experiments of Fries (Fries and Iwasaki 1976; Fries 1977; Fries and Aberg 1978) carried out on algae: *Fucus*, *Porphyra* and *Enteromorpha* from Phaeophyta, Chlorophyta and Rhodophyta exhibited that PAA and *p*-OH-PAA were found to have a marked stimulative effect which resembles typical, natural auxins. It turned out that NAA, the chemical analogue of IAA and PAA, derivative of IAA, sporadically found in plants – acted often much longer, because they are more chemically durable in comparison with IAA and more resistant to activity of degrading them oxidases. Furthermore, it was found that free forms of synthetic auxins are more resistant to the synthesis of inactive conjugates with amino acids or sugars. In this way the concentration of the active form of auxins decreases, what is important for keeping intercellular phytohormonal homeostasis. The correlation between the effect of exogenous auxins and the concentration of endogenous ones, allows to stabilise the steady state level of these compounds in plant cell.

Moreover, earlier examinations performed on unicellular green algae from Chlorophyta: *Chlorella pyrenoidosa* and *Scenedesmus acuminatus* (Czerpak 1979, 1990; Czerpak et al. 1994, 1997, 1999), confirmed the high stimulative activity of NAA and PAA in relation to IAA upon their growth and metabolic processes connected with them. In turn, examining the content of individual types of carotenoids in *Chlorella pyrenoidosa* cells, it was stated that all auxin compounds – regardless differences in their chemical structure – intensify the oxidisation of carotenoids into xanthophylls and especially well oxidised ones, and this way they weaken their metabolic activity. However, IAA possesses a much stronger stimulative activity in comparison with NAA, when it comes to the general content of carotenoids – including in that carotenes and xanthophylls poor in oxygen. Moreover, the synthesis of xanthophylls rich in oxygen is better influenced by the stimulative activity of NAA than of IAA.

In earlier examinations performed on *Chlorella pyrenoidosa* (Czerpak 1990; Czerpak et al. 1994, 1997, 1999) it was proved, that PAA possesses a little lower biological activity, just like in vascular plants in comparison with IAA, on the content of dry mass, chlorophylls ($a + b$) and total carotenoids, but a little stronger on the general content of proteins soluble in water and aldohexoses. However, $\beta$-NAA in *Chlorella pyrenoidosa* cells stimulated slightly stronger in comparison with IAA the following: the content of fresh and dry mass alga, the total accumulation of mineral elements and aldohexoses, but when it comes to the content of proteins, chlorophylls and carotenoids it exhibited a smaller activity when compared with IAA.

Generally, in the rootless wolfia (*W. arrhiza*), which is an atypical mixotrophic plant, the highest stimulative activity was attributed to PAA, which exerted the most influence upon plant growth and the content of biochemical parameters: nucleic acids, water-soluble proteins fraction, the biosynthesis of chlorophylls *a* and *b* as well as total carotenoids, reducing sugars and also net photosynthesis intensity in biomass. However, exogenously supplied IAA have resulted in a less stimulative influence on the growth of *W. arrhiza* and the contents of metabolites, which were analysed in its fresh weight, while the synthetic auxin NAA –

was found to have the weakest activity in comparison with typical photosynthetic vessel plants and algae from Chlorophyta. Overall, our observations suggest the interesting possibility that the mixotrophic wolfia (*W. arrhiza*) demonstrated in some cases an atypical, biochemical reaction and lower sensitivity on the applied auxin IAA – the natural endogenous phytohormone playing a fundamental role in plant growth and development – in relation to metabolic response to PAA, when compared with typical photoautotroph organisms.

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