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Changes in the Growth, Chemical Composition, and Antioxidant Activity in the Aquatic Plant *Wolffia arrhiza* (L.) Wimm. (Lemnaceae) Exposed to Jasmonic Acid

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Abstract The present study was undertaken to test the influence of exogenously applied jasmonic acid (JA) at concentrations of 0.01–100 μM upon the growth and metabolism of the aquatic plant *Wolffia arrhiza* (Lemnaceae). JA acted in a concentration-dependent manner. JA at 0.1 μM stimulated plant growth and accumulation of cellular components (proteins, monosaccharides, chlorophylls, phaeophytins, and carotenoids). Treatment with JA at 0.1 μM enhanced *W. arrhiza* viability by the induction of biomass production and increased the level of photosynthetic pigments, monosaccharides, and soluble proteins. Moreover, JA at 0.1 μM activated the enzymatic (catalase, ascorbate peroxidase, NADH peroxidase) and nonenzymatic antioxidant (ascorbate, glutathione) system in *W. arrhiza* and, therefore, suppressed lipid peroxidation. In contrast, decreases in fresh weight, major photosynthetic pigments, monosaccharides, and soluble protein content were observed in *W. arrhiza* exposed to 100 μM JA. JA applied at 100 μM also stimulated the formation of lipid peroxides which are responsible for membrane damage. In the presence of 100 μM JA, antioxidant enzyme (catalase, ascorbate peroxidase, NADH peroxidase) activity and ascorbate as well as glutathione content were inhibited. The data support the hypothesis that JA plays an important role in *W. arrhiza* growth and metabolism, regulating oxidative status by direct influence on the enzymatic as well as nonenzymatic antioxidant machinery.

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Introduction

The Lemnaceae (duckweeds) comprise a small, aquatic monocotyledon family of five genera and 37 species. Duckweeds are particularly interesting evolutionarily because they are the world's smallest angiosperms (Landolt 1986). Individuals of *Wolffia* (the smallest genera), seldom exceed 1 mm in size and bear little similarity to other flowering plants (Godziemba-Czyż 1970). Duckweeds such as *Wolffia arrhiza* are not simply miniature versions of larger angiosperms; rather, the size reduction in the plant body is associated with a highly modified structural organization that results from the alteration, simplification, or loss of many morphological and anatomical features (Les and others 1997). Therefore, *W. arrhiza* has neither leaves nor a stem and even lacks roots. The whole plant, called a frond, may set flowers and seeds, although rapid multiplication is achieved by budding. *W. arrhiza* plants float individually or as two in a cluster at the surface of the water (Landolt 1986). This plant is also characterized by mixotrophic feeding, a high rate of nutrient absorption from polluted water, quick growth, fast multiplication, and resistance to numerous toxins (Fujita and others 1999; Mical and Krotke 1999). Moreover, *W. arrhiza* is the source of food for aquatic organisms because of the presence of valuable proteins, starch, phytohormones, vitamins, and microelements (Bajguz and Asami 2005). For that reason *W. arrhiza* fronds can be a good model from which to learn the details of phytohormone activity.

(-)-Jasmonic acid (JA) is a widespread phytohormone in the plant kingdom. Endogenous JA was determined in

Lemna minor from the Lemnaceae family (Kristl and others 2005; Krajnčič and others 2006). There is also increasing evidence that JA plays a key role in the growth and metabolism in this group of aquatic plants. For example, exogenously applied JA at low concentrations was found to induce flowering in *Spirodela polyrrhiza* (Krajnčič and Nemec 1995) and *Lemna minor* (Krajnčič and others 2006). Moreover, previous studies indicated that JA stimulated germination of photoblastic light-grown and dark-grown turions of *Spirodela polyrrhiza* (Appenroth and others 1991). Nonetheless, the occurrence of this phytohormone in *W. arrhiza* fronds and its biochemical activity have not yet been detected.

JA is regarded as an important signaling molecule responsible for plant developmental processes such as senescence, cell cycle progression, tuber formation, root growth, fruit ripening, germination, fertility, and ethylene biosynthesis (Miyamoto and others 1997; Świątek and others 2004). In addition, previous studies demonstrated that there is a specific cross-talk between JA and the level of reactive oxygen species (ROS) such as H_2O_2 that plays a key role as a second messenger, able to induce gene expression and protein synthesis (Orozco-Cárdenas and others 2001; Maksymiec and Krupa 2002). The control of oxidative stress is complex and dissection of the mechanism generating and relieving ROS is difficult. To cope with ROS or alleviate their damaging effects, plants have evolved enzymatic and nonenzymatic antioxidant mechanisms. Antioxidants such as ascorbate and glutathione are the main free radical scavengers that can very efficiently remove ROS (Xiang and Oliver 1998). The enzymatic defense system also involves several enzymes, for example, catalase, ascorbate peroxidase, and NADH peroxidase, which are able to scavenge and convert H_2O_2 to less toxic molecules (Nakano and Asada 1981; Aeby 1984; Ishida and others 1987). Moreover, several reports indicate that phytohormones may regulate the synthesis and activity of antioxidant machinery protecting the plant from the oxidative stress, and some of these enzymes are also implicated in the process of plant growth and development (Pasternak and others 2002; Kwak and others 2006).

Therefore, the objective of the present study was to determine the effect of JA at concentrations ranging from 0.01 to 100 μM on the growth (expressed as fresh weight) and the level of cellular components (photosynthetic pigments, monosaccharides, soluble proteins) in *W. arrhiza*. We also tested the hypothesis that JA-induced changes in growth and metabolism may be connected with its influence on the oxidative response, that is, lipid peroxidation, ascorbate and glutathione content, and the activity of catalase, ascorbate peroxidase, and NADH peroxidase. Results may be important for elucidation of the hormone's role in the physiology of *W. arrhiza,* a plant representing the simplest level of organization and highly specialized for life in an aquatic environment (Landolt 1986).

Materials and Methods

Plant Material and Growth Conditions

Wolffia arrhiza (L.) Hork. ex. Wimm. (Lemnaceae) originated from the culture at the Institute of Biology, University of Bialystok. An axenic culture of *W. arrhiza* was grown in 200-ml sterile vessels (Phytatray™, Sigma, St. Louis, MO, USA) containing 100 ml of 1/50 dilution of Hutner's medium (pH 7.0) in the following nutritive solution: 1.3 mM EDTA, 2 mM $MgSO_4 \cdot 7H_2O$, 2.9 mM KH_2PO_4, 1.5 mM $Ca(NO_3)_2 \cdot 4H_2O$, 3.6 mM KOH, 1.7 mM NH_4VO_3, 0.2 mM $ZnSO_4 \cdot 7H_2O$, 0.1 mM $Na_2MoO_4 \cdot 2H_2O$, 0.1 mM $FeSO_4 \cdot 7H_2O$, 0.1 mM $MnCl_2 \cdot 4H_2O$, 0.2 mM H_3BO_3, 20 μM $CuSO_4 \cdot 5H_2O$, 0.7 μM $Co(NO_3)_2 \cdot 6H_2O$ (Hutner 1953). All reagents were purchased from Sigma. Vessels were covered with plastic plates to reduce evaporation of the solutions. *W. arrhiza* was subcultured in 1,000-ml Erlenmeyer flasks containing 500 ml nutrient medium under controlled conditions at 25 ± 0.5°C, with a day/night cycle of 16/8 h (photon flux of 50 μmol m^{-2} s^{-1}). Light was supplied by white fluorescence tubes TLD 18/86 (Philips Aquarelle lamps, Netherlands). Plants were precultivated for 28 days in 1/50 Hutner's medium and the medium was replaced every week. Precultivation was necessary; without this step stress-induced resting form (turion) formation was observed, often giving irregular results. The 0.5 (±0.005) g of *W. arrhiza* fresh weight was treated with JA obtained from Sigma. JA dissolved in 50% ethanol was applied at concentrations of 0.01, 0.1, 1, 10, and 100 μM on the first day of culture. An equal amount of ethanol was added to the control. The final ethanol concentration in the culture media did not exceed 0.025% v/v and this concentration did not affect plant growth. Cultures were conducted in four replications and samples for determination of the biochemical parameters were collected on the 7th and 14th days.

Determination of Plant Growth, Proteins, Monosaccharides, and Photosynthetic Pigments

For fresh weight determination, the fronds were filtered and washed three times with distilled water, kept on filter paper for a few minutes to remove excess liquid, and weighed. The growth rate (k) was estimated using the equation $P_t = P_0 \exp(kt)$, where P_t is the population at the end of the period, P_0 is the population at the beginning of the period, and t is time. Fresh weight was used for this calculation.

For determination of chlorophyll *a* and *b*, total carotenoids, and phaeophytin *a* and *b*, the cultures were first collected by filtration and then the pellets (0.1 g) were homogenized in methanol. The absorbance of the extract was measured at 652.4 and 665.2 nm for chlorophylls, 654.2 and 647.6 nm for phaeophytins, and 470.0 nm for carotenoids. The amounts of photosynthetic pigments present in the methanol extract were calculated using the equations of Wellburn (1994). For sugar determination, the cultures were first collected by filtration and then the pellets (0.1 g) were assessed using the Somogyi (1954) method. Measurement of protein content was done by homogenization of biomass. The homogenate was centrifuged for 10 min at 12,000 *g* and an aliquot of the extract was used to determine protein content following the method of Bradford (1976) using bovine serum albumin as the standard.

Determination of Antioxidants

Extraction and determination of total ascorbate from *W. arrhiza* biomass was carried out following the method of Kampfenkel and others (1995). Plant material (1 g) was harvested by filtration and quickly homogenized in liquid N_2 and thereafter extracted with 5% (w/v) trichloroacetic acid. The homogenate was centrifuged for 5 min at 15,600 *g* (4°C). Then the supernatant was transferred to a new reaction vessel and immediately assayed for the ascorbate content in a reaction mixture containing supernatant, 10 mM dithiothreitol, 0.2 M phosphate buffer (pH 7.4), 0.5% *N*-ethylmaleimide, 10% trichloroacetic acid, 42% H_3PO_4, 4% 2,2′-dipyridyl, and 3% $FeCl_3$. Determination of glutathione was essentially as described by De Kok and others (1986). Briefly, glutathione was extracted from filtered fresh weight in 2 volumes of extracting buffer (2% sulfosalicylic acid, 1 mM Na_2EDTA, and 0.15% ascorbate) and homogenized. The homogenate was centrifuged at 12,000 *g* for 5 min. An aliquot of supernatant was then used for measurement of glutathione content using a glutathione assay kit (Sigma).

Determination of the Antioxidant Enzymes Activities

Enzymatic extracts were obtained from 1 g of fresh weight of *W. arrhiza*. The biomass was filtered and then homogenized in liquid N_2. Next, the samples were homogenized with 0.05 M phosphate buffer (pH 7.0) containing 0.1 mM EDTA and 1% polyvinylpyrrolidone (average molecular weight = 40,000) at 4°C. The plant biomass:extraction buffer (w/v) proportion was 1:5. The homogenate was centrifuged for 10 min at 15,000 *g* (4°C) and the supernatant was dialyzed overnight in phosphate buffer. Estimation of the activity of the selected enzymes was performed as outlined below.

Catalase (EC 1.11.1.6) activity was determined following Aeby (1984). The rate of H_2O_2 decomposition at 240 nm was measured spectrophotometrically and calculated using a molar extension coefficient $\varepsilon = 45.2\ mM^{-1}\ cm^{-1}$. The reaction mixture consisted of 0.05 M phosphate buffer pH 7.0 (2 ml), 0.1 mM H_2O_2 (1 ml), and supernatant (100 µl). One unit of catalase activity (U) was assumed to be the amount of enzyme that decomposed 1 µmol H_2O_2/mg soluble protein per minute at 30°C.

Total ascorbate peroxidase (EC 1.11.1.11) was determined according to the method described by Nakano and Asada (1981). The reaction mixture consisted of 0.05 M phosphate buffer (pH 7.0) (1.8 ml), 5 mM sodium ascorbate (20 µl), 0.1 mM H_2O_2 (100 µl), and supernatant (100 µl). Total ascorbate peroxidase activity was determined as the decrease in absorbance of ascorbate at 290 nm and calculated using a molar extension coefficient $\varepsilon = 2.8\ mM^{-1}\ cm^{-1}$. The enzyme activity (U) was calculated as the amount of the enzyme that oxidizes 1 µmol ascorbate consumed/mg soluble protein per minute at 30°C.

NADH peroxidase (EC 1.11.1.1) activity was determined according to Ishida and others (1987). The reaction mixture consisted of 50 mM pH 6.0 sodium acetate buffer (1.8 ml) and 0.2 mM NADH (100 µl). The reaction was initiated by adding the enzymatic extract (100 µl) and lasted for up to 5 min. The peroxidase activators of 25 µM *p*-cumaric acid (12.5 ml) and 5 nM $MnCl_2$ (12.5 ml) were used. The activity was calculated with a molar extinction coefficient $\varepsilon = 6.300\ mM^{-1}\ cm^{-1}$ for NADH. One unit of NADH peroxidase activity (U) was assumed to be the amount of the enzyme that oxidizes 1 µmol NADH/mg soluble protein per minute at 30°C.

Determination of Lipid Peroxidation

Lipid peroxidation was determined as malondialdehyde (MDA) content by the thiobarbituric acid reaction as described by Heath and Packer (1968). Filtered biomass (1 g) was homogenized in 0.1% trichloroacetic acid. The homogenate was centrifuged at 10,000 *g* for 5 min. The reaction mixture containing supernatant, 20% trichloroacetic acid, and 0.5% thiobarbituric acid was heated at 95°C for 30 min and then quickly cooled on ice. After centrifugation at 10,000 *g* for 10 min, the absorbance at 532 nm was read and the value for the nonspecific absorption at 600 nm was subtracted.

Replication and Statistical Analysis

Each treatment consisted of four replicates and each experiment was carried out at least twice at different times. The data were analyzed by one-way analyses of variance (ANOVA) and the means were separated using Duncan's

multiple-range test (Statistica 6, StatSoft, Tulsa, OK, USA). The level of significance in all comparisons was $P < 0.05$.

Results

Growth of *W. arrhiza*

JA influenced *W. arrhiza* growth in a dose-dependent manner (Fig. 1). Growth stimulation was observed after application of 0.1 μM JA leading to a significant ($P < 0.05$) 25 and 16% increase in the fresh weight on the 7th and the 14th day, respectively. The maximum growth rate was also obtained in 0.1 μM JA treated fronds; however, this effect was not statistically significant (Fig. 2a). In contrast, JA at the highest concentration range of 10–100 μM considerably suppressed biomass production. For example, significant ($P < 0.05$) decreases in fresh weight by 43 and 61% on the 7th and the 14th day of culture, respectively, were obtained in the cultures treated with 100 μM JA. Moreover, a negative growth rate indicates that the culture declines under the influence of 100 μM JA (Fig. 2a).

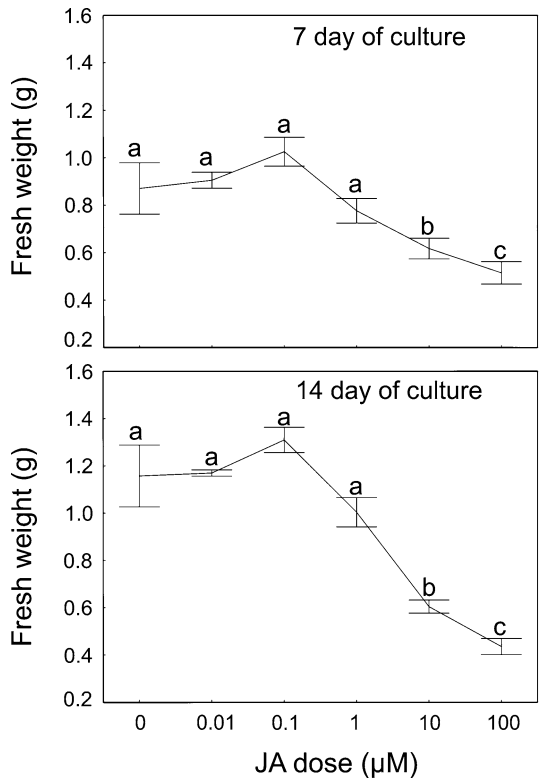

Fig. 1 The relationships between the dose of jasmonic acid and *Wolffia arrhiza* response expressed as fresh weight on the 7th and the 14th day of culture. Fresh weight on the first day of culture was 0.50 ± 0.05 g. Data are the means ± standard deviation of four independent experiments. Treatments with at least one letter the same are not significantly different according to Duncan's test

Protein Content

Stimulation of protein accumulation in response to 0.01–1 μM JA during 14 days of culture was observed (Fig. 2b). A significant ($P < 0.05$) increase of 30% in average protein level was found in *W. arrhiza* fronds after application of 0.1 μM JA. By contrast, treatment with 10–100 μM JA resulted in inhibition of water-soluble protein accumulation in *W. arrhiza* culture. JA at an extremely inhibitory dose for plant growth (100 μM) reduced the protein content by more than 26 and 40% on the 7th and the 14th day of the experiment, respectively.

Photosynthetic Pigment Content

The photosynthetic apparatus is often highly sensitive to exogenous JA; therefore, the effect of this phytohormone on the photosynthetic pigment content was examined. Under the influence of 0.01–0.1 μM JA an increase in photosynthetic pigment content over the control was found (Fig. 2c–f). JA at 0.1 μM induced a statistically significant ($P < 0.05$) 10–20% average increase in chlorophyll and phaeophytin accumulation. However, JA in the 1–100-μM concentration range inhibited photosynthetic pigment content in *W. arrhiza* during the whole 14-day culture. The most dramatic decrease in chlorophyll *a* by 30–34%, chlorophyll *b* by 27–33%, phaeophytin *a* by 30–38%, and phaeophytin *b* by 26–35% was found in fronds in response to 100 μM JA on the 7th and the 14th day of culture. In addition, the chlorophyll *a*/*b* ratio decreased with the increase in exogenous JA concentrations at all exposure periods in comparison with the control (Table 1). JA at the 0.01–10-μM concentration range stimulated carotenoid content (Fig. 2g). For example, *W. arrhiza* treated with 0.1 μM JA contained about twice as much carotenoids on the 14th day as the control plants. By contrast, application of 100 μM JA did not alter carotenoid accumulation in culture.

Monosaccharides Content

JA at the 0.01–10-μM concentration range enhanced monosaccharide content in the fronds (Fig. 2h). For example, exposure of *W. arrhiza* to 0.1 μM JA caused more than a 70 and 60% increase in the monosaccharide level on the 7th and the 14th day of cultivation, respectively. On the other hand, JA at the highest concentration (100 μM) had an inhibitory effect on the accumulation of monosaccharides in *W. arrhiza* culture because a statistically significant ($P < 0.05$) decrease of 45% in their content was found on the 14th day.

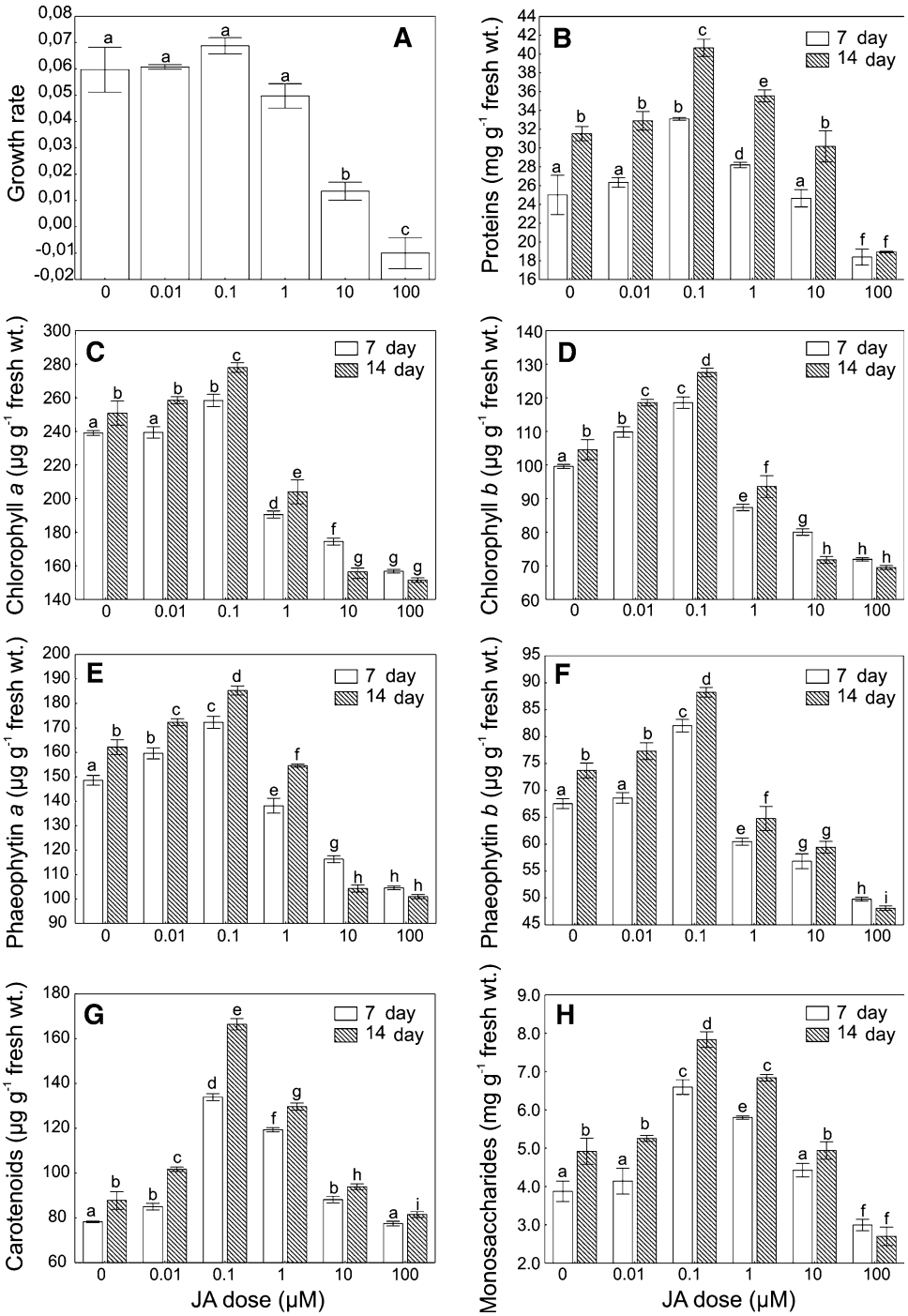

Fig. 2 The effect of jasmonic acid on the growth rate, protein, chlorophyll *a* and *b*, phaeophytin *a* and *b*, carotenoids, and monosaccharide content in *Wolffia arrhiza*. Biochemical parameter levels on the first day of culture: proteins, 24.77 ± 0.93 mg g^{-1} fresh wt; chlorophyll *a*, 205.04 ± 4.36; chlorophyll *b*, 88.43 ± 2.25; phaeophytin *a*, 140.61 ± 3.09; phaeophytin *b*, 60.55 ± 2.69; carotenoids, 65.41 ± 2.35 µg g^{-1} fresh wt; and monosaccharides 3.11 ± 0.35 mg g^{-1} fresh wt. Data are the means ± standard deviation of four independent experiments. Treatments with at least one letter the same are not significantly different according to Duncan's test

Table 1 Chlorophyll a/b ratio in *W. arrhiza* fronds exposed to 0.01–100 µM JA on the 7th and the 14th day of culture

Day of culture	JA concentration (µM)				
	0	0.01	0.1	1	100
7	2.42 ± (0.09)[a]	2.20 ± (0.05)[b]	2.19 ± (0.06)[b]	2.19 ± (0.05)[b]	2.18 ± (0.04)[c]
14	2.41 ± (0.05)[a]	2.19 ± (0.07)[b]	2.19 ± (0.08)[b]	2.18 ± (0.09)[c]	2.17 ± (0.06)[c]

Data are the means of four independent experiments ± SD. Treatments with at least one letter the same are not significantly different according to Duncan's test

Levels of Ascorbate and Glutathione

To determine whether JA activates the nonenzymatic antioxidant system, we conducted an experiment in which cultures were treated with 0.01–100 μM JA and plants were sampled at various times to measure glutathione and ascorbate levels. The results demonstrate that fronds in the presence of 0.1 μM JA produced 24 and 33% more glutathione than controls on the 7th and the 14th day of cultivation, respectively (Fig. 3b). In addition, the exposure of *W. arrhiza* to 0.1 μM JA caused a weaker but statistically significant ($P < 0.05$) increase (7–24%) in the total ascorbate level during 14 days of culture (Fig. 3a). Furthermore, JA in the 10–100-μM concentration range showed an inhibitory effect on the content of antioxidants. The statistically significant ($P < 0.05$) decrease in the level of ascorbate by 63% and glutathione by 50% was observed on the 14th day of the experiment under the influence of JA, especially at 100 μM.

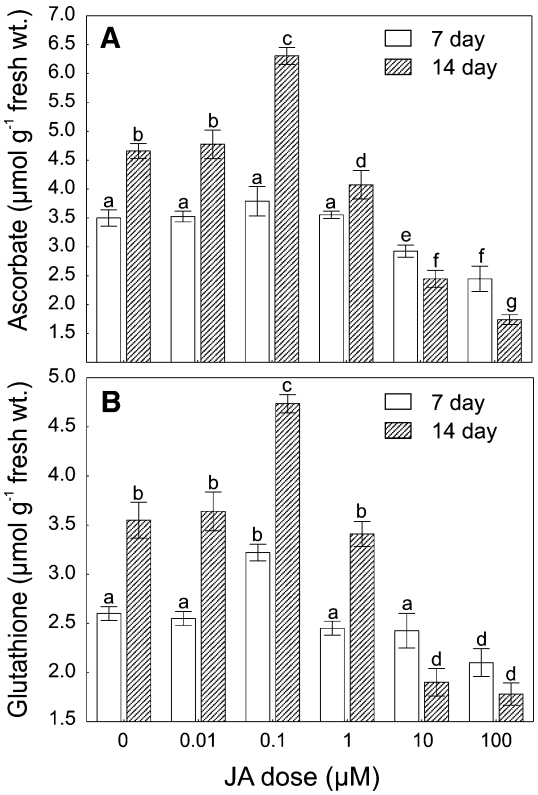

Fig. 3 The effect of JA on the antioxidant content in *Wolffia arrhiza*. The antioxidant level on the first day of culture: glutathione, 0.75 ± 0.09, and ascorbate, 3.10 ± 0.45 μmol g^{-1} fresh wt. Data are the means ± standard deviation of four independent experiments. Treatments with at least one letter the same are not significantly different according to Duncan's test

Activities of Antioxidant Enzymes

Similarly, JA affected the changes in the activity of antioxidant enzymes involved in scavenging of reactive oxygen species (Fig. 4a–c). The application of 0.1 μM JA induced the activity of catalase, a key enzyme responsible for H_2O_2 degradation, on the 14th day of cultivation almost twice as great as in the control culture. *W. arrhiza* treated with 0.1 μM JA also had greater peroxidase activity. JA stimulated ascorbate peroxidase activity by 10 and 16% on the 7th and the 14th day of culture, respectively, compared

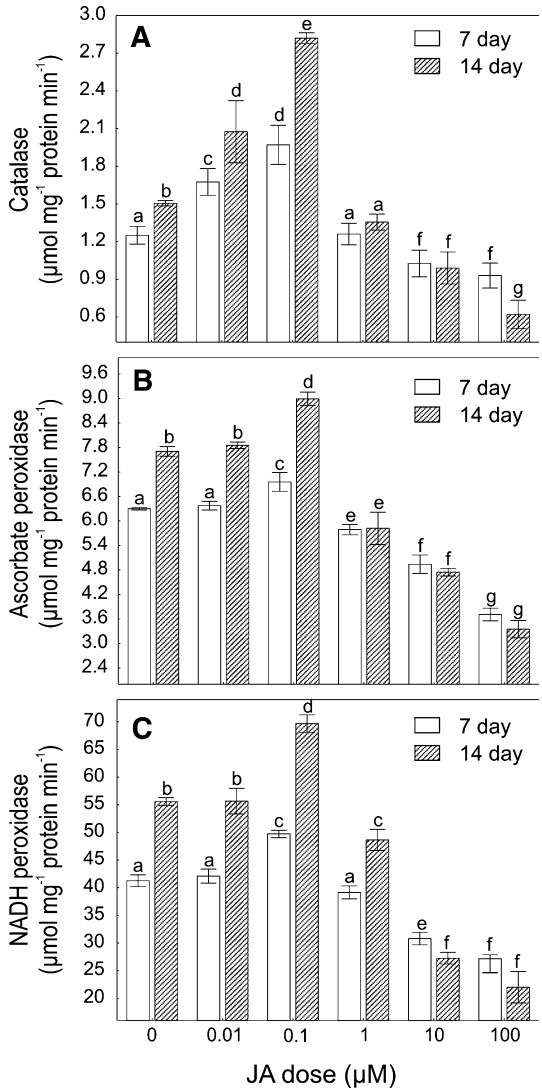

Fig. 4 The effect of JA on the antioxidant enzyme activity in *Wolffia arrhiza*. The antioxidant enzyme activity on the first day of culture: catalase, 0.58 ± 0.03; ascorbate peroxidase, 4.11 ± 0.21; and NADH peroxidase, 32.93 ± 1.91 μmol mg^{-1} protein min^{-1}. Data are the means ± standard deviation of four independent experiments. Treatments with at least one letter the same are not significantly different according to Duncan's test

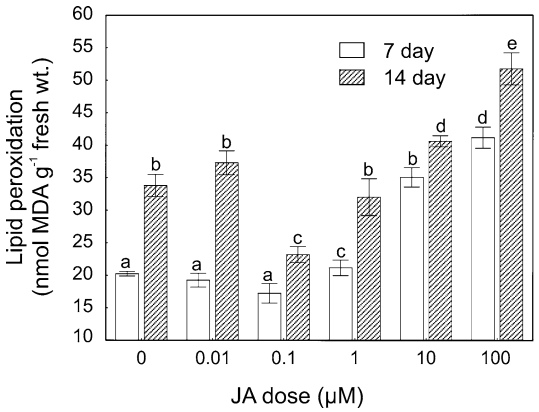

Fig. 5 The effect of JA on lipid peroxidation in *Wolffia arrhiza*. The MDA level on the first day of culture was 15.09 ± 0.9 nmol g^{-1} fresh wt. Data are the means ± standard deviation of four independent experiments. Treatments with at least one letter the same are not significantly different according to Duncan's test

to the control culture. Analogously, the activity of NADH peroxidase was significantly ($P < 0.05$) higher by 21–25% under the influence of 0.1 μM JA in plants grown for 14 days. On the other hand, the enzymatic activities, like those of catalase, ascorbate peroxidase, and NADH peroxidase, in *W. arrhiza* culture were all significantly ($P < 0.05$) inhibited twofold at JA concentrations of 100 μM on the 14th day of cultivation.

Lipid Peroxidation

The increase in the level of metabolite scavenging of reactive oxygen species and antioxidant enzyme activity may be linked to the decrease in lipid peroxidation. Therefore, the results of our experiments have shown that the content of lipid peroxides, measured as the concentration of MDA (malondialdehyde), the cytotoxic product of lipid peroxidation, was reduced by 15% on the 7th day and decreased significantly by 32% on the 14th day of *W. arrhiza* cultivation under the influence of 0.1 μM JA (Fig. 5). However, JA at the extremely inhibitory dose of 100 μM significantly ($P < 0.05$) stimulated the ratio of the formation of lipid peroxides involved in oxidative cellular destruction by 103 and 53% on the 7th and the 14th day of culture, respectively.

Discussion

The activity of exogenously applied JA displays a biphasic character in *W. arrhiza* fronds. Stimulation of the growth rate was obtained in plants exposed to 0.1 μM JA. This is not surprising because previous experiments performed on *Chlorella vulgaris* (Chlorophyceae) showed enhanced cell division, biomass production, and algal viability in response to exogenous 0.01–10 μM JA. In particular, JA at 0.1 μM stimulated cell number and metabolite accumulation in the algal culture (Czerpak and others 2006). In addition, experiments performed on Lemnaceae plants showed that exogenous JA at lower concentrations (0.475–47.5 nM) induced flowering in *Lemna minor* under long-day, in the photoperiodically neutral plant *Spirodela polyrrhiza*, and in the long–short-day plant *W. arrhiza*. In response to exogenous JA, the promotion of photoperiodic floral induction, apical floral induction (evocation), and floral differentiation were observed in these species (Landolt and Kandeler 1987; Krajnčič and Nemec 1995; Krajnčič and others 2006). Moreover, the germination of photoblastic light-grown as well as dark-grown turions of *Spirodela polyrrhiza* and the formation of two daughter fronds was stimulated by JA and its methyl ester (MeJA) (Appenroth and others 1991).

Growth of *W. arrhiza* expressed as fresh weight depends on the synthesis of nucleic acids and proteins. An increase in soluble protein level is observed in response to 0.1 μM JA. Our results are in agreement with observations that indicate that one striking effect of jasmonates is the induction of synthesis of jasmonate-induced proteins (JIPs) which were identified, for example, as proteinase inhibitors, pathogenesis-related and antifungal proteins (Reinbothe and others 1993; Wasternack 2007). Soluble-protein content in plants, an important indicator of reversible and irreversible changes in metabolism, is known to respond to a wide variety of phytohormones. For this reason, our observation supports the suggestion that protein accumulation is the specific *W. arrhiza* response to exogenous 0.1 μM JA, which acts as a signaling molecule at the molecular level.

The application of JA at 0.1 μM promotes the accumulation of photosynthetic pigments in *W. arrhiza* fronds. Because of the photosynthetic pigments' protection function, the increase in their content has a positive effect on the ability of *W. arrhiza* plants to adapt to conditions in the aquatic environment. Stimulation of anthocyanins and *β*-carotene biosynthesis was confirmed by Saniewski and others (1998) and Curtin and others (2003). Monosaccharides are building substances for a plant as well as a key source of energy necessary for stimulating all biochemical processes. *W. arrhiza* cultures treated with 0.1 μM JA are characterized by a significant increase in the monosaccharide level. Bogatek and others (2002) indicated that the sharp increase in monosaccharide content in response to JA may result from the formation of these sugars during the hydrolysis of polysaccharides. In summary, our data suggest that JA applied at low concentrations is a signaling molecule that stimulates the growth and accumulation of proteins, monosaccharides, and photosynthetic pigments in *W. arrhiza*.

Several reports indicate there is cross-talk between the jasmonate, oxidative response, and metabolic processes. For example, methyl jasmonate (MeJA) may regulate the level of reactive oxygen species (ROS) in *Arabidopsis thaliana* by directly influencing the antioxidant enzymes' activities (Maksymiec and Krupa 2002). Given that the highly reactive oxygen species may cause lipid peroxide formation, the content of MDA (malondialdehyde), a cytotoxic product of lipid peroxidation, was determined. Our results revealed that JA at 0.1 μM inhibited lipid peroxide generation, which was obvious from lower MDA content in *W. arrhiza* fronds. Probably JA diminishes lipid peroxidation through stimulation of nonenzymatic (ascorbate, glutathione) antioxidants tightly regulating ROS homeostasis.

Ascorbate is known to operate as an antioxidant either in direct chemical interaction with ROS or during the reaction catalyzed by ascorbate peroxidase (Kampfenkel and others 1995). Ascorbate derives its role from its sensitivity to ROS and from the fact that its oxidation affects the redox balance of other metabolites such as glutathione, which, themselves being involved in the perception of cellular redox imbalance, contributed to signal amplification (Apel and Hirt 2004). Glutathione is an important water-phase antioxidant with proposed roles in the storage and transport of reduced sulfur, in the synthesis of proteins and nucleic acids, and as a modulator of enzyme activity (De Kok and others 1986). The stimulation of glutathione levels in JA-treated plants is supported by the work of Xiang and Oliver (1998), which showed that JA induced the expression of genes for glutathione synthesis and recycling in *Arabidopsis thaliana*, providing protection against oxidative stress. Moreover, JA at 0.1 μM enhanced the capacity for glutathione synthesis when there is no demand for this substance in our experimental system. It is expected that glutathione accumulation should be faster and more responsive when oxidative stress is imposed upon the organism. Results indicating that JA stimulates nonenzymatic antioxidant systems in *W. arrhiza* are not surprising because it was previously shown that JA can either induce or act antagonistically in the oxidative-dependent cell death cycle, depending on whether the initial ROS signal is a singlet oxygen or superoxide (Danon and others 2005; Van Breusegen and Dat 2006).

Apart from the positive effect of 0.1 μM JA on nonenzymatic antioxidants, this phytohormone stimulated the activity of enzymatic antioxidants leading to a decrease in lipid peroxide generation in *W. arrhiza*. The majority of ROS-scavenging pathways of plants include ascorbate peroxidase, catalase, and NADH peroxidase which can prevent H_2O_2 accumulation. Enhanced activity of these enzymes is generally associated with an acclimation to elevated amounts of active oxygen species and plant adaptation to environmental changes. However, an association between the activity of catalase and peroxidases involved in ROS metabolism and physiological as well as morphological responses in plants is often observed. Carpin and others (2001) suggested that the epidermis of zucchini (*Cucurbita pepo*) has strong peroxidase activity which stimulates polymerization of phenolics. Peroxidases are also known to participate in lignin and suberin formation in the cell walls and in the establishment of covalent bonds between hydroxycinnamate ester moieties or flavonoids associated with pectins or hemicelluloses as well as in the catabolism of auxins (Gazaryan and others 1996). Phytohormones may also regulate morphological and physiological processes that influence antioxidant enzymes' activities and ROS, especially H_2O_2, levels (Kwak and others 2006). For example, auxin-induced increases in the activity of these enzymes is closely associated with activation of embryo- and organogenesis in leaf protoplasts (Pasternak and others 2002). Therefore, the activation of the antioxidant enzymatic system in *W. arrhiza* by exogenous 0.1 μM JA can be involved in metabolic processes leading to stimulation of plant growth and cellular component accumulation.

The second type of *W. arrhiza* response to JA is the suppression of plant growth, especially at 100 μM JA. JA at the highest dose has been shown to reduce biomass and primary metabolite accumulation in comparison to the observed weight at the start of the experiments. However, microscopic observations indicate that there are no significant differences in morphology between 100 μM JA-treated *W. arrhiza* cultures and controls (data not shown). Our finding also corresponds well with literature data regarding jasmonate activity in plants from the Lemnaceae family. For example, JA at high concentrations (475 nM) may induce a yellowing of *Lemna minor* fronds, a decrease in frond size and root length, inhibition and blocking of flowering, and formation of densely packed colonies composed of 10–15 fronds (Krajnčič and others 2006). The inhibitory activity of 100 μM JA was also confirmed by Świątek and others (2004), who indicated that 100 μM JA reduced synchronized BY-2 tobacco callus cell growth, blocked mitosis, inhibited DNA replication, and kept the cells in the G1 stage when JA was applied just before the G1/S transition. The growth inhibition as a result of breakdown of cell integrity and cell membranes as well as the degradation of cell wall polysaccharides induced by jasmonates has also been reported in several plant species (Miyamoto and others 1997).

The loss of more than 30% in chlorophyll and phaeophytin content in *W. arrhiza* supports the hypothesis that JA plays a key role as a signaling molecule triggering plant senescence. Most studies have dealt with a marked loss of chlorophyll resulting in yellowing of leaf tissue because carotenoids are less affected by jasmonate treatments

(Saniewski and others 1998). The literature data confirm our results that indicate that 100 μM JA did not significantly effect the carotenoid level in *W. arrhiza* culture. JA-induced rapid reduction in the chlorophyll level is the typical response observed in many species of plants (Czerpak and others 2006). Therefore, it is most likely that the reduction in the sugar content in *W. arrhiza* is closely related to the inhibitory effect of 100 μM JA on photosynthetic pigment levels leading to inhibition of monosaccharide production. Alterations in the photosynthetic apparatus of *W. arrhiza* treated with JA were related to modifications of the photosynthetic pigment mass ratio. It seems that 100 μM JA has a higher toxic effect on chlorophyll *a* than *b*. The relatively higher increase in chlorophyll *b* suggests also that JA, especially at 100 μM, is probably involved in the stimulation of photosystem I function with simultaneous loss in photosystem II activity. However, further investigations are needed to resolve this question.

The cultivation of *W. arrhiza* fronds with 100 μM JA led to a significant decrease in the soluble-protein level. The results confirm the suggestion that 100 μM JA may induce protein hydrolysis by specific proteases. This activity was shown in cases of reserve proteins in germinating apple embryos (Ranjan and Lewak 1995). The decline in protein content may also be explained by the observation that 100 μM JA enhances lipid peroxide formation leading to oxidative destruction of proteins and others cellular components.

Some data indicate that lipid peroxidation, expressed as MDA level, can be activated by the action of lipoxygenase, which is stimulated by jasmonates (Vèronèsi and others 1999; He and others 2002). MDA is the product of the decomposition of polysaturated fatty acids of biomembranes and its increase shows that plants are under high-level oxidative stress (Apel and Hirt 2004). Probably, an oxidative burst leading to lipid peroxide formation is closely connected with decreased activities of antioxidant enzymes and the levels of antioxidants in *W. arrhiza* fronds exposed to 100 μM JA. Similarly, inhibition of the activity of antioxidant enzymes and a significant decrease in ascorbate level in response to the highest concentration of methyl jasmonate leading to a hydrogen peroxide burst were also observed in *Arabidopsis thaliana* leaves (Maksymiec and Krupa 2002). Although the rapid increase in the accumulation of ROS after exposure of plants to jasmonates has been confirmed (Orozco-Cárdenas and others 2001), the mechanism of this increase is not resolved. The decrease in peroxidases and catalase activity and the decline in ascorbate and glutathione levels in *W. arrhiza* treated with 100 μM JA appear to be symptoms of stress damage. The decrease in antioxidant enzyme activities may also be due to the harmful effect of oxygen species overproduction or inhibition of the synthesis of enzymes. Therefore, we can conclude that JA at the highest dose generates oxidative stress via interference with the plant antioxidant defense system finally leading to growth inhibition and metabolite degradation in *W. arrhiza* culture.

The data suggest that JA plays a key role in the growth and metabolism of the aquatic plant *W. arrhiza* from the Lemnaceae family. Results suggest that there is link between the perception of JA, oxidative status of the cell, and plant growth as well as metabolite content in *W. arrhiza*. However, further studies on the molecular level are necessary to validate this hypothesis.

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