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Optimization of Indole-3-Acetic Acid Concentration in a Nutrient Solution for Increasing Bioactive Compound Accumulation and Production of *Agastache rugosa* in a Plant Factory

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Abstract: This study aimed to determine the optimal indole-3-acetic acid (IAA) concentration in a nutrient solution to increase the bioactive compounds while enhancing the plant growth of *A. rugosa* grown hydroponically. Twenty-eight-day-old plants were transplanted in a plant factory for 32 days. The plants were subjected to various IAA concentrations (10^{-11}, 10^{-9}, 10^{-7}, and 10^{-5} M) from 8 days after transplanting, and the control treatment (without IAA). Shoot and root fresh weights were effectively improved under 10^{-7} and 10^{-9} IAA treatments. Leaf gas exchange parameters were increased under 10^{-7} and 10^{-9} IAA treatments. Four of the IAA treatments, except 10^{-11} IAA treatment, significantly increased the rosmarinic acid (RA) concentration, as well as the tilianin concentration was significantly increased at all IAA treatments, compared with that of the control. Especially, the tilianin concentration of the 10^{-11} IAA treatment was significantly (1.8 times) higher than that of the control. The IAA treatments at 10^{-5} and 10^{-7} significantly raised the acacetin concentrations (1.6- and 1.7-times, respectively) compared to those of the control. These results suggested that 10^{-7} concentration of IAA in a nutrient solution was effective for enhancing plant growth and increasing bioactive compounds in *A. rugosa*, which offers an effective strategy for increasing phytochemical production in a plant factory.

Keywords: acacetin; growth parameter; phytochemical; rosmarinic acid; tilianin

1. Introduction

The requirement for medicinal plants on a global scale such as *Agastache rugosa* is increasing [1,2]. *A. rugosa* (Lamiaceae family) is a popular herbaceous plant, and its leaves have been largely used as food flavor in daily cuisines. All parts of this plant are used as traditional pharmaceuticals to treat different disorders in various civilizations' medical systems [3]. *A. rugosa* is rich in phytochemicals such as chlorogenic, ferulic, caffeic, and rosmarinic acid (RA), as well as flavone glycosides, sesquiterpenes, tilianin, diterpenes, acacetin, and triterpene [4–6]. Furthermore, the previous reports indicated that rosmarinic acid, tilianin, and acacetin are the main active compounds of *A. rugosa* [5,7]. They are famous for their pharmacological activities such as anti-allergic, antioxidant, anti-microbial, anti-tumor, anti-depression, anti-cancer, anti-viral, antioxidant activities, and anti-asthmatic [3,8–12]. The quality of these herb plants enables assessment of their concentrations of secondary metabolic compounds [13].

Food production with high quality and productivity can be achieved by growing in a plant factory, which is one way to satisfy very specific requirements for plant growth and secondary metabolite accumulation [14]. Various external factors such as the light, temperature, electrical conductivity, root temperature, and hormones affect the accumulation of bioactive compounds in *A. rugosa* [15–18]. Furthermore, plant hormones such as indole-3-butyric acid (IBA), 1-naphtha-leneacetic acid (NAA), indole-3-acetic acid (IAA), and phenylacetic acid (PAA) play an essential role not only in the growth but also in the accumulation of antioxidant enzymes and bioactive compounds in plants [19,20]. Plant hormones can change the activity of antioxidant enzymes and the antioxidant synthesis, and some of these enzymes are related to phytohormone synthesis [21]. For example, the activity of antioxidant enzymes was increased by auxin to adjust reactive oxygen species levels, which may be related to the activation of embryo/organogenesis [21]. The growth and amounts of bioactive contents in plants rely on the concentrations of hormones [22]. IAA is an important auxin that has a strong influence on the growth and development of *Acutodesmus obliquus* and IAA-enhanced cell proliferation and antioxidant properties in plants [23]. Rutin and protein contents of the white mulberry tree were increased by increasing the IAA concentration into the incubation medium [22]. The ajmalicine concentration in the shoot of *Catharanthus roseus* was increased under high IAA concentration (11.42 μM) [24]. The highest monosaccharide content (73%) in *Chlorella vulgaris* was obtained under 0.1 μM IAA concentration compared with the control [20]. These results showed that it is possible to improve the nutritional quality in terms of phytochemicals in plants by controlling the IAA concentration levels. Thus, the application of IAA could be a beneficial tool for increasing the bioactive compounds in *A. rugosa* for fresh consumption or functional ingredients for nutraceutical food.

To date, several studies have examined the effects of IAA concentration on the accumulation of bioactive compounds in plants; however, most were performed in vitro cultivation [22,24]. No report has shown whether the IAA dissolved in nutrient solution stimulates bioactive compounds in *A. rugosa* grown under a deep flow technique system in a plant factory with artificial light. Therefore, this study aimed to evaluate the effects of IAA concentration levels in Hoagland's nutrient solution on the chlorophyll content, leaf gas exchange parameters, growth, and accumulation of bioactive compounds in *A. rugosa* grown in a closed plant factory and to try to determine the optimal IAA concentration treatment to raise the tilianin, RA, and acacetin concentrations with advantageous effects on plant growth.

2. Materials and Methods

2.1. Plant Materials and Seedling Conditions

A. rugosa (Danong Seed Co., Ltd., Seoul, Korea) seeds were sown according to the method described in Lam et al. (2020) [18]. Germinated seeds were grown for 4 weeks in a closed plant factory with environmental control of air temperature at 22/18 °C (light/dark), a relative humidity of 60–80%, photoperiod period of 16 h per day, and photosynthetic photon flux density (PPFD) of 180 ± 10 μmol·m^{-2}·s^{-1} irradiated by fluorescent lamps (TL5 14W/865 Philips, Amsterdam, Netherlands) for 28 days. The seedlings were watered by the Hoagland solution from 15 days after sowing [18,25]. The pH and electrical conductivity (EC) values of the nutrient solution (NS) were controlled to around 6.0 and 1.1 dS·m^{-1}, respectively.

2.2. Auxin Experiment and Growth Conditions

At 28 days after sowing, eighteen *A. rugosa* young plants at the four leaves stage were transferred into a deep flow technique system (1.0 × 1.29 × 0.11 m; W × L × H) in a plant factory (Figure 1) and subjected to PPFD of 200 ± 10 μmol·m^{-2}·s^{-1} with a photoperiod of 14 h for 32 days, using light-emitting diode lamps (TL5 14W/865 Philips, Amsterdam, Netherlands). The average air temperature and relative humidity of the plant factory were maintained at 22 ± 3 °C and 70 ± 10%, respectively. Hoagland nutrient solution with the EC of 2.0 dS.m^{-1} and pH value around 6.0 was provided for all *A. rugosa*

plants during the experiment period. Eight days after transplanting, plants were treated with four different concentrations of IAA (10^{-11}, 10^{-9}, 10^{-7}, and 10^{-5} M; 0.00000000175, 0.000000175, 0.0000175, 0.00175 g·L^{-1}, respectively) while control plants were treated with similar volume without IAA concentration. Indole-3-acetic acid (IAA) stock solution was prepared according to the manufacturer's instructions (Gold Biotechnology, St. Louis, MO, USA). Briefly, 0.14 g of indole-3-acetic acid powder was dissolved in 1.5 mL of ethanol (EtOH) and diluted to 100 mL with deionized water. The nutrient solution reservoir had a volume of 80 L. Both nutrient reservoir containing IAA and the grow trays were covered by the silver paper to protect IAA from degradation. This experiment was repeated twice with the same experimental unit (a hydroponic culture system) at the same location under the same environmental conditions.

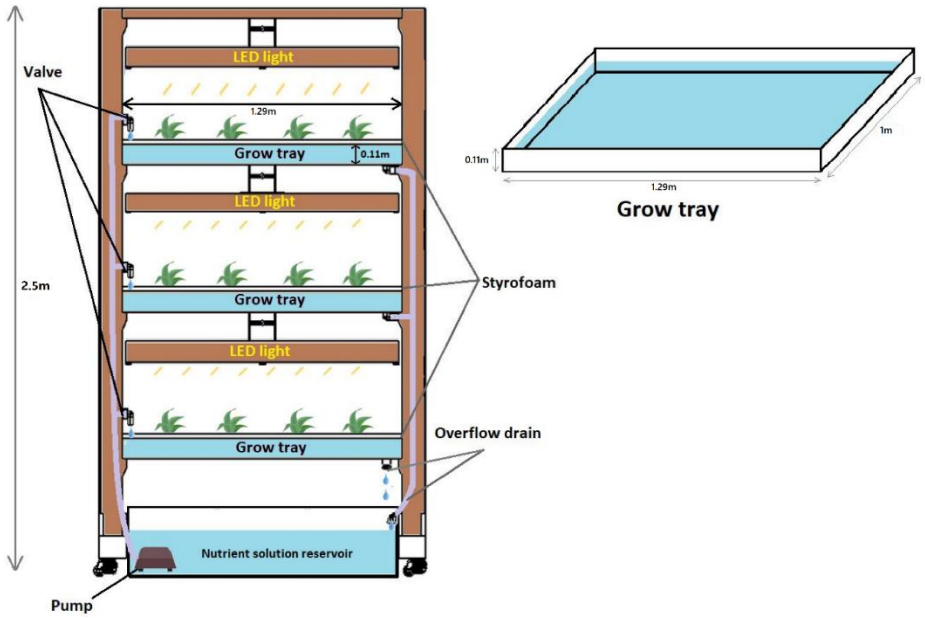

Figure 1. A deep flow technique (DFT) system in a plant factory. The plants were cultivated on a styrofoam board and placed on the surface of the nutrient solution with dissolved indole-3-acetic acidand the control treatment (without dissolved IAA).

2.3. Measurement of Growth Parameters

All plants for the IAA experiment were harvested at 32 days after transplanting and 8 plants were used for measuring all plant growth parameters per each replication. The number of leaves, which are longer than 1 cm and broader than 1 cm were counted. Leaf area was measured using a Li-3100 leaf area meter (LiCor, Lincoln, NE, USA). A tape measure was used to record leaf width and length, and stem and root lengths. The fresh weights of shoots and roots were separately determined using a micro weighing scale (CAS MW-II, CAS Co., Ltd., East Rutherford, NJ, USA). The fresh shoots and roots were dried at 70 °C in a drying oven (HB-502M, Hanback Sci, Suwon, Korea) for seven days to determine shoot and root dry weights.

2.4. Chlorophyll Fluorescence (Fv/Fm) and Relative Chlorophyll Values

The ratio of variable to maximum fluorescence (F_v/F_m) and relative chlorophyll values were measured by a portable fluorometer (Fluorpen Pen FP 100, Photon System Instruments Ltd., Drasov, Czech Republic) and a portable chlorophyll meter (502, Minolta Camera Co., Ltd., Tokyo, Japan), respectively. Chlorophyll fluorescence (Fv/Fm) and the relative chlorophyll values were measured using 3 and 8 plants (n = 3 and n = 8) in each replication at 32 days after transplanting, respectively.

2.5. Measurement of Leaf Gas Exchange Parameters

A portable photosynthesis system LI-6400 (IRGA, Licor. Inc. Nebraska, NE, USA) was used to determine the transpiration rate (T_r), net photosynthetic rate (P_n), intercellular CO_2 concentration (C_i), and stomatal conductance (g_s) at 31 days after transplanting on the third leaf from the plant apex. The airflow rate, block temperature, and CO_2 concentration were set at 500 $cm^3 \cdot s^{-1}$, 25 °C, and 400 $\mu mol \cdot mol^{-1}$, respectively. These parameters were measured on five plants per each replication and for each IAA treatment. Plants used for measuring chlorophyll fluorescence (Fv/Fm), relative chlorophyll values, and leaf gas exchange parameters belonged to the list of eight plants that were used to measure plant growth parameters. All samples were selected by the randomization method.

2.6. Analysis of Acacetin, Tilianin, and Rosmarinic Acid (RA) Concentrations and Contents

The acacetin, tilianin, and RA of *A. rugosa* were analyzed according to the literatures [5,18] with modifications. Roots, stems, leaves, and flowers of three plant samples per replication from each IAA treatment group were placed in liquid nitrogen immediately after harvest and stored at −70 °C in a deep freezer and then put into a dry freezer. Materials for analysis were obtained from three randomly selected plants (n = 3). After the dried *A. rugosa* samples of root, stem, leaf, and flower were ground to a fine powder and filtered by a sieve, a 100 mg sample was accurately weighed and placed in a 2.0 mL tube, to which 80% (*v*/*v*) methanol (1.5 mL) was added and blended by a vortex, with sonication being conducted for 60 min. The mixed solution was centrifuged for 10 min at 13,000× *g* by a microcentrifuge (R17 Plus, Hanil Scientific Co., Ltd., Gimpo, Korea). Then the supernatant was filtered by a 0.45 μm filter for high-performance liquid chromatography (HPLC) analysis. The HPLC conditions for *A. rugosa* were C18 column (250 mm × 4.6 mm, 5 μm; RS tech, Daejeon, Korea); flow rate, one mL/min; temperature, 30 °C; run time, 60 min; detector wavelength, 275 nm; mobile phase, 100% methanol and 0.20% (*v*/*v*) acetic acid; and injection volume, 20 μL. The tilianin, RA, and acacetin analytical standards were purchased from (Sigma-Aldrich, Co., Ltd., Seoul, Korea). HPLC was performed by reversed-phase HPLC-ultraviolet analysis (1260 Infinity, Agilent Technologies, Santa Clara, CA, USA) to determine the acacetin, RA, and tilianin concentrations, which were expressed as milligrams of acacetin, tilianin, and RA concentrations equivalent per gram of each plant organ dry weight ($mg \cdot g^{-1}$ DW) [17]. Whole plant concentration ($mg \cdot g^{-1}$ plant DW) means the total of tilianin, acacetin, and RA concentrations of all plant organs. The RA, acacetin, and tilianin contents in stems, roots, leaves, and flowers (mg/plant organs DW) mean the RA, acacetin, and tilianin concentrations in the plant organs ($mg \cdot g^{-1}$ DW) multiplied by plant organs DW (g). The RA, acacetin, and tilianin contents in the whole plant (mg/plant DW) mean the RA, acacetin, and tilianin concentrations ($mg \cdot g^{-1}$ plant DW) multiplied by whole plant DW (g).

2.7. Statistical Analysis

The IAA experiment was repeated twice by a completely randomized design. Statistical analysis was performed by SPSS 20.0 software program (SPSS 20, SPSS Inc., Chicago, IL, USA). An analysis of variance (ANOVA) was conducted. The significant differences among the means of IAA treatments were determined by Tukey's multiple range test at 5%. SigmaPlot 10 (Systat Software Inc., San Jose, CA, USA) was used to build the graphs.

3. Results

3.1. Plant Growth Parameters

At 32 days after transplanting, leaf length and width, the number of leaves, shoot and root fresh weights, and root dry weight differed significantly according to the IAA concentrations and from that of the control (Table 1). Of the four IAA treatments, 10^{-11} M induced the maximum leaf length and width, which was significantly higher (1.05- and 1.13-times, respectively) than that of the control. In contrast, 10^{-11} M IAA treatment showed the fewest leaves compared to the other IAA treatments

and the control. The leaf area, stem and root lengths, and shoot dry weight did not differ significantly with IAA treatments and the control. The 10^{-5} M IAA treatment tended to inhibit root fresh weight, shoot and root dry weights, although no significant difference was observed compared with the control. Plants under 10^{-11} M IAA treatments did not exhibit improved shoot and root fresh weights compared with the control, whereas 10^{-7} and 10^{-9} M IAA treatments improved shoot and root fresh weights compared with the control. There was no significant difference in some growth parameters between IAA treatments and the control.

3.2. Relative Chlorophyll Value, Chlorophyll Fluorescence (Fv/Fm), and Leaf Gas Exchange Parameters

There was no significant difference in relative chlorophyll and Fv/Fm ratio values between IAA treatments and the control (Figure 2A,B). The net photosynthesis rate of *A. rugosa* treated with 10^{-7} and 10^{-9} M IAA concentration was significantly higher than that of the control and other IAA treatments, and the 10^{-5} M IAA concentration treatment showed the lowest net photosynthesis rate among all treatments (Figure 3A). This trend was observed in the stomatal conductance (g_s) (Figure 2B), intercellular CO_2 concentration (C_i) (Figure 3C), and transpiration rate (T_r) (Figure 3D), which were significantly increased under 10^{-7} and 10^{-9} M IAA treatments compared with the other IAA treatments and the control.

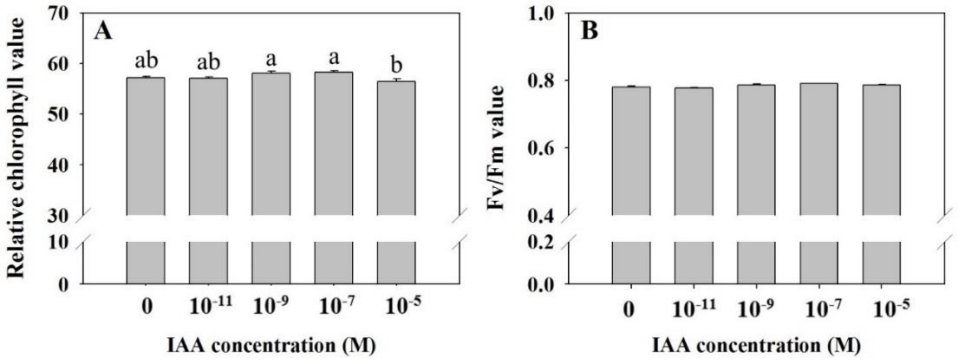

Figure 2. Relative chlorophyll value (**A**) and Fv/Fm value ratio (**B**) of *A. rugosa* subjected to the control (without IAA) and four IAA treatments. Different letters (a–b) indicate significant differences among treatments at $p \leq 0.05$ ($n = 8$ and $n = 3$).

Table 1. Plant growth parameters of *Agastache rugosa* subjected to the control (without IAA) and four IAA treatments.

IAA Concentration [w] (M)	Leaf Length (cm)	Leaf Width (cm)	Number of Leaves	Leaf Area (cm^2)	Stem Length (cm)	Root Length (cm)	Fresh Weight (g/plant)		Dry Weight (g/plant)	
							Shoot	Root	Shoot	Root
Control	9.09bc	7.54c	78.50ab	860.25	41.86	54.85	25.56bc	15.69ab	3.12	0.80ab
10^{-11}	9.51a	8.52a	76.50b	845.56	41.19	50.74	25.74abc	14.98ab	3.01	0.78ab
10^{-9}	9.45ab	8.10b	84.87a	893.53	40.46	55.34	28.65a	16.69a	3.14	0.81a
10^{-7}	9.06bc	8.06b	85.50a	925.99	41.61	57.49	28.38ab	16.76a	3.15	0.81a
10^{-5}	8.74c	7.91b	78.25ab	858.28	40.97	50.91	25.09c	14.70b	2.95	0.75b
Significance [z]	***	***	**	NS	NS	NS	**	**	NS	**
L [y]	*	***	NS	NS	NS	NS	NS	NS	NS	NS
Q [x]	***	***	NS	NS	NS	NS	NS	NS	NS	NS

[w] IAA concentration treatments. Data are the mean of eight separate plants ($n = 8$). Different letters (a–c) indicate significant differences among treatments at the level of 5% according to Tukey's test. NS: Not significant ($p > 0.05$), [z] significant at * $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$. [y] L: linear, [x] Q: quadratic in regression analysis.

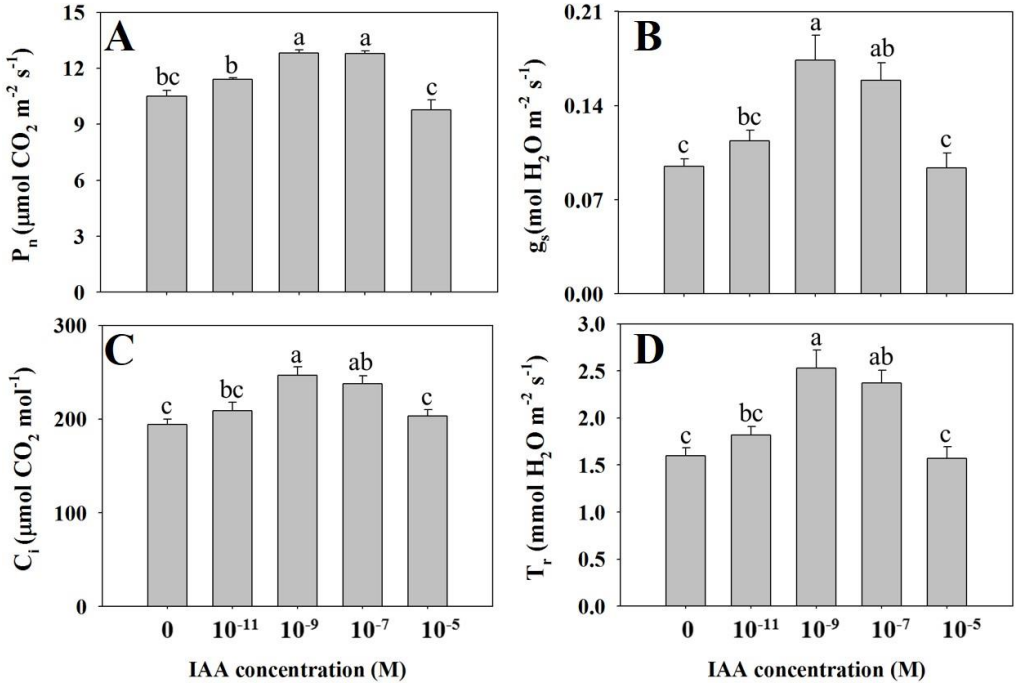

Figure 3. Net photosynthetic rate (P_n) (**A**), stomatal conductance (g_s) (**B**), intercellular CO_2 concentration (C_i) (**C**), and transpiration rate (T_r) (**D**) of *A. rugosa* subjected to the control (without IAA) and four IAA treatments. Different letters (a–c) indicate significant differences among treatments at $p \leq 0.05$ ($n = 5$).

3.3. Acacetin, Rosmarinic Acid (RA), and Tilianin Concentrations and Contents

The tilianin, RA, and acacetin concentrations were separately analyzed in the leaves, roots, flowers, and stems of the *A. rugosa* plants treated with different IAA concentrations and they showed significant differences (Table 2). Among the IAA treatments, RA concentrations in leaves at 10^{-9} M and in stem at 10^{-7} M were the highest amount compared with other treatments, which were significantly higher (1.7 and 1.4 times, respectively) than that of the control. However, the RA concentration in flowers was lower under 10^{-7} and 10^{-9} M IAA concentrations compared with the control and other IAA treatments. Furthermore, the 10^{-5} M IAA treatment showed the highest RA concentration in roots, which was 1.6 times higher than the control (Table 2). Table 3 shows that the RA content in leaves and stems exposed to 10^{-9} and 10^{-7} M IAA was significantly increased by 1.7- and 1.4-times, respectively, compared to that of the control. There was no significant difference in RA contents in the flowers at $p > 0.05$ ($p = 0.077$) between four IAA treatments and the control; however, RA contents in roots was the highest amount at the treatment of 10^{-5} M IAA compared to the control and other IAA treatments (Table 3).

Tilianin concentration was significantly increased up to 1.6-time (in leaves) and 1.8-time (in stems) at 10^{-11} M IAA, respectively, compared with the control. Most notably, all IAA treatments had a significantly higher tilianin concentration in leaves and stems than that of the control. Tilianin concentration in flowers exhibited a similar trend: Plants exposed to IAA treatments exhibited significantly increased tilianin concentrations compared to those of the control. The roots under 10^{-5} M IAA treatment reached their highest levels of tilianin concentration, which was significantly (2.2 times) higher than that of the control (Table 2). Table 3 shows that tilianin content in leaves, stems, and roots exhibited a tendency similar to the results reported for tilianin concentration. Tilianin content in leaves and stems was the highest at 10^{-11} M IAA, and in roots at 10^{-5} M IAA compared to the control and other treatments. Especially, the flower's tilianin contents in all IAA treatments were higher than the control.

Table 2. Rosmarinic acid (RA), tilianin, and acacetin concentrations (mg·g^{-1} DW) in flowers, leaves, roots, and stems of *A. rugosa* subjected to the control (without IAA) and four IAA treatments.

IAA Concentration [w] (M)	RA Concentration in Plant Organs (mg·g^{-1} DW)				Tilianin Concentration in Plant Organs (mg·g^{-1} DW)				Acacetin Concentration in Plant Organs (mg·g^{-1} DW)			
	Leaves	Flowers	Stems	Roots	Leaves	Flowers	Stems	Roots	Leaves	Flowers	Stems	Roots
Control	3.718d	6.163a	6.379bc	19.153b	1.480c	2.919b	0.931d	0.033c	ND	0.064b	ND	ND
10^{-11}	4.604c	5.304ab	5.495d	10.348c	2.311a	5.781a	1.715a	0.034c	ND	0.059b	ND	ND
10^{-9}	6.392a	5.076b	6.809b	24.307ab	1.908b	5.011a	1.150c	0.038c	ND	0.033c	ND	ND
10^{-7}	5.919b	4.905b	9.075a	23.218b	1.706b	4.817a	1.209bc	0.055b	ND	0.110a	ND	ND
10^{-5}	6.041b	5.606ab	6.068cd	29.925a	1.744b	4.399a	1.345b	0.074a	ND	0.104a	ND	ND
Significance [z]	***	**	***	**	***	**	***	***	ND	***	ND	ND
L [y]	NS	**	NS	NS	***	***	***	NS	ND	NS	ND	ND
Q [x]	***	**	NS	***	***	***	**	***	ND	*	ND	ND

[w] IAA concentration treatments. Data are the mean of three separate plants ($n = 3$). Different letters (a-d) indicate significant differences among treatments at the level of 5% according to Tukey's test. NS: Not significant ($p > 0.05$), [z] significant at * $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$. [y] L: linear, [x] Q: quadratic in regression analysis. ND: not detected; DW: dry weight.

Table 3. Rosmarinic acid (RA), tilianin, and acacetin contents (mg/plant organs DW) in stems, leaves, roots, and flowers of *A. rugosa* subjected to the control (without IAA) and four IAA treatments.

IAA Concentration [w] (M)	RA Content in Plant Organs (mg/plant organs DW)				Tilianin Content in Plant Organs (mg/plant organs DW)				Acacetin Content in Plant Organs (mg/plant organs DW)			
	Leaves	Flowers	Stems	Roots	Leaves	Flowers	Stems	Roots	Leaves	Flowers	Stems	Roots
Control	7.325d	1.309a	5.763c	15.429b	2.915c	0.601b	0.841c	0.027c	ND	0.014b	ND	ND
10^{-11}	8.670c	1.061b	4.962d	8.139c	4.353a	1.156a	1.549a	0.026c	ND	0.012b	ND	ND
10^{-9}	12.231a	1.083ab	6.582b	20.101ab	3.651b	1.068a	1.112b	0.031c	ND	0.007c	ND	ND
10^{-7}	11.719b	1.030b	8.228a	19.119ab	3.378b	1.011a	1.096b	0.045b	ND	0.023a	ND	ND
10^{-5}	11.559b	1.156ab	5.099d	22.745a	3.337bc	0.909ab	1.131b	0.056a	ND	0.021a	ND	ND
Significance [z]	***	NS	***	***	***	**	***	***	ND	***	ND	ND
L [y]	NS	**	NS	NS	***	***	***	NS	ND	NS	ND	ND
Q [x]	***	**	NS	***	***	***	***	***	ND	*	ND	ND

[w] IAA concentration treatments. Data are the mean of three separate plants ($n = 3$). Different letters (a–d) indicate significant differences among treatments at the level of 5% according to Tukey's test. NS: Not significant ($p > 0.05$), [z] significant at * $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$. [y] L: linear, [x] Q: quadratic in regression analysis. ND: not detected; DW: dry weight.

Acacetin concentration in flowers was significantly higher in 10^{-5} and 10^{-7} M IAA treatments compared with the control and other IAA treatments (Table 2). The acacetin concentration in stems was detected a minimal amount in 10^{-5}, 10^{-7}, and 10^{-9} M IAA treatments (data not shown). The acacetin concentration was undetectable in roots and leaves. The acacetin content in flowers showed a similar trend to the results reported for acacetin concentration, which was significantly higher under 10^{-5} and 10^{-7} M IAA treatments compared with the control and other IAA treatments (Table 3). In addition, the RA concentration was the highest in the roots and the tilianin concentration was the highest in the flowers. The RA concentration was greater than the acacetin and tilianin concentrations in *A. rugosa* (Table 2 and Figure 4).

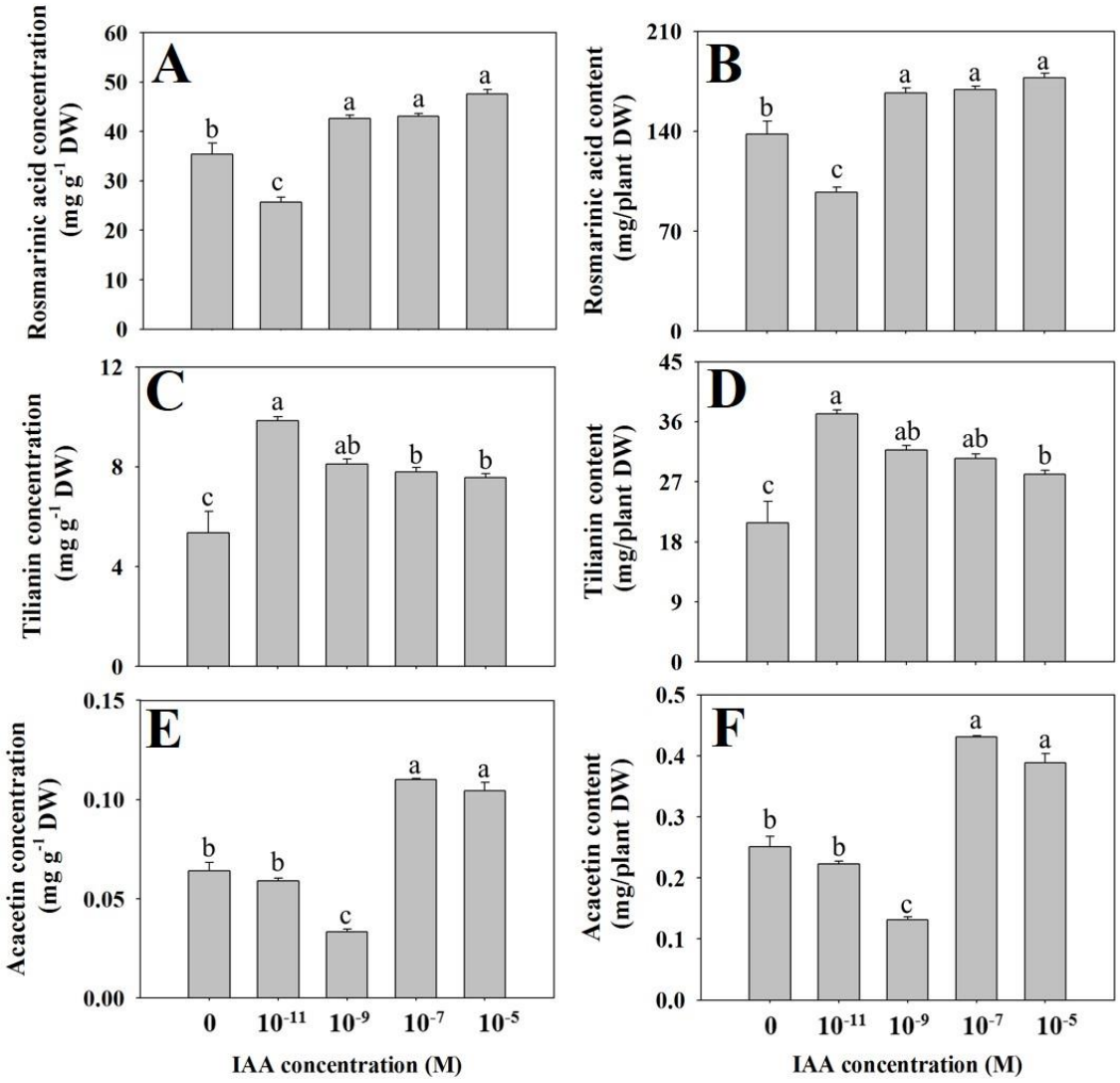

Figure 4. Rosmarinic acid (**A**,**B**), tilianin (**C**,**D**), and acacetin (**E**,**F**) concentrations and contents in the whole plant of *A. rugosa* subjected to the control (without IAA) and four IAA treatments. Different letters (a–c) indicate significant differences among treatments at $p \leq 0.05$ ($n = 3$).

Three of the four IAA treatments, except 10^{-11} M, significantly increased the RA concentration per plant compared with that of the control. The RA concentration per plant was the significantly lowest under 10^{-11} M IAA treatment (Figure 4A). The RA content per plant also showed a similar trend to RA concentration (Figure 4B). All four IAA treatments significantly increased the tilianin concentration of *A. rugosa* compared with that of the control. Especially, 10^{-11} M maximized the tilianin concentration per plant, which was significantly (1.8 times) higher than that in the control (Figure 4C). The tilianin

content per plant in response to IAA concentrations followed the same trend as that for the tilianin concentration (Figure 4D). The 10^{-5} and 10^{-7} M IAA treatments induced significantly higher acacetin concentration (1.6- and 1.7-time, respectively) than that of the control (Figure 4E). A similar trend was observed for acacetin content (Figure 4F).

4. Discussion

4.1. Plant Growth Parameters

To understand the suitability of IAA concentration for *A. rugosa* growth, we investigated the growth parameters of plants grown under IAA dissolved in Hoagland nutrient solution compared to the control (without IAA). There was no significant difference in some growth parameters between IAA treatments and the control. However, shoot fresh weight under 10^{-9} M IAA treatment was significantly higher than that of the control. Treatments at 10^{-7} and 10^{-9} M IAA tended to increase the leaf number and leaf area compared with other IAA treatments and the control. IAA's primary efficiency was to stimulate the development of stems and roots, lateral root production, and root elongation by extending the new cells in the meristem [26]. The growth-stimulating phytohormone auxin is known to cause root growth by increasing cell division and cell extension [27]. The lateral root density per plant of *Trichoderma virens* was doubled under IAA treatment compared with that of the control (without IAA) [28].

In contrast, the IAA concentration at 10 μM reduced shoot and root growth of chickpea after 10 days of treatment [29]. The influence of IAA has been determined based on the concentration, with low and high IAA concentrations increasing and restricting plant growth, respectively [20,29]. For example, root and shoot growth of sunflower plants grown under a hydroponic system was effectively enhanced under 10^{-10} M IAA concentration [30]. The number of cells in *Chlorella vulgaris* was the highest under an IAA concentration of 0.1 μM [20]. A higher concentration of IAA at 10.0 μM restricted the seedling's growth of chickpea when compared with lower IAA concentration at 0.5 and 1.0 μM IAA [29]. Shoot and root growth of sunflower were effectively increased in 10^{-10} M IAA concentration [30]. The IAA concentration in nutrient solution at 10^{-6} M reduced K and Mg contents and increased Ca in leaves, but increased N, P, K Ca, Fe, Cu, Zn, and Mn contents in the roots of pepper plants, probably due to the increase of plasmalemma H^+ pump activity in the cells [31]. Plant height, the number of leaves, leaf dry weight, root and stem dry weights, and leaf water content were increased under 10^{-6} M IAA concentration compared with those of the control (without IAA). The same patterns were observed for l-tryptophan and indole treatments [31]. This is in agreement with our results that the number of leaves, leaf area, shoot fresh and dry weights of *A. rugosa* were increased at medium IAA concentration (10^{-7} and 10^{-9} M), and leaf length and width were increased at low IAA concentration (10^{-11} M) compared to the control and other IAA treatments; however, high IAA concentration (10^{-5} M) tended to reduce plant growth, probably ion imbalance occurred at high IAA concentration. Use of 10^{-10} M IAA decreased the toxic influences of Pb and Zn on the root and shoot growth [30]. Therefore, the optimum IAA concentration for growth depends on the kind of plants and cultivation methods.

4.2. Chlorophyll Fluorescence (F_v/F_m), Relative Chlorophyll Value, and Leaf Gas Exchange Parameters

This study has demonstrated that the relative chlorophyll values at 10^{-7} and 10^{-9} M IAA treatments were higher than those at the other IAA treatments and the control, but not significantly. There was no significant difference in Fv/Fm ratio between IAA treatments and the control. However, plants cultivated *in-vitro* method exhibited stimulated levels of chlorophyll synthesis at high IAA concentrations. For example, the chlorophyll synthesis in *Scenedesmus quadricauda* and *Chlorella pyrenoidosa* was strongly enhanced at high IAA concentrations (40 and 60 $mg{\cdot}L^{-1}$, respectively) [32]. In contrast, IAA concentration at 0.1 μM exerted a positive effect on chlorophyll in *chlorella vulgaris* after 48 h of cultivation [20], whereas 1 μM IAA treatment did not influence chlorophyll synthesis in *Arabidopsis* [33].

All leaf gas exchange parameters were higher at 10^{-7} and 10^{-9} M IAA treatments compared with the control and other IAA treatments. The optimal IAA positively affected plant growth by extending leaves and raising the photosynthetic rate of plants, and it also induced the translocation of carbohydrates in the synthesis process. [34]. Similarly, auxin concentration at 100 mg·L^{-1} stimulated and thus increased the photosynthetic rate and stomatal conductance in mustard [34]. Auxins (IAA, IBA, and NAA) had a positive influence on photosynthesis [32], probably because auxin enhances nutrient uptake such as N, P, and K [31]. The IAA solution augments the rate of CO_2 assimilation and photosynthesis rate in leaves [35]. In this study, the optimal IAA concentrations at 10^{-7} and 10^{-9} M increased leaf gas exchange parameters.

4.3. Rosmarinic Acid (RA), Tilianin, and Acacetin Concentrations and Contents

The RA concentration per plant was significantly higher under almost all IAA treatments except 10^{-11} M IAA treatment compared with that of the control (Figure 4A). In addition, all IAA treatments significantly increased the concentration of tilianin in *A. rugosa* compared to the control, and 10^{-11} M IAA treatment showed the highest value of tilianin compared to the control and other IAA treatments (Figure 4C). The 10^{-5} and 10^{-7} M IAA treatments showed significantly higher acacetin concentration than the control and other IAA treatments. A similar trend was observed for RA, tilianin, and acacetin contents in *A. rugosa* (Figure 4B,D,F). Because auxins (IAA and IBA) increased the activities of CAT, SOD, and APX [20], the increase in SOD activity can be used to adjust H_2O_2 production [36]. Auxin enhanced antioxidant activity to control reactive oxygen species levels, which may correlate to the activity of embryo/organogenesis [21]. IAA enhances the antioxidant enzyme activities such as SOD, CAT, and peroxidases in *Helianthus annuus* L [37]. Glutathione and ascorbate in *Chlorella vulgaris* were increased under the IAA treatment compared with the control (without IAA) [20].

The contents of total phenol and flavonoid in the white mulberry tree were increased under the different concentrations of auxins such as IBA, IAA, and NAA [38]. The phenolic compound and flavonoid in gherkin pickles (*Cucumis anguria* L.) were improved by using IBA [39]. Protein and rutin contents in the white mulberry tree were increased by raising the IAA concentration [22]. Auxin increased the production of secondary metabolites and antioxidant activities in *Aloe arborescens* Mill [10]. In addition, IAA stress may lead to ion imbalance [31], which increased antioxidant enzymes and bioactive compounds in plants [25,40]. In this study, IAA treatments from 10^{-5} to 10^{-9} M significantly increased RA, 10^{-9} to 10^{-11} M significantly increased tilianin, and *A. rugosa*'s acacetin concentration from 10^{-5} to 10^{-7} M significantly increased compared to the control and other IAA treatments. Furthermore, the RA concentration was greater than tilianin and acacetin concentrations in the overall plant. Similar results were reported by Tuan et al. (2012) [5], who reported that RA concentration, which was higher than the acacetin and tilianin concentrations in *A. rugosa*. The same results were found by Lee et al. (2008) [4], who reported that RA was a main phenolic compound and concentrated in hairy roots of *A. rugosa.* In our study, acacetin and tilianin were mostly concentrated in flowers of *A. rugosa* while acacetin was undetectable in leaves, roots, and stems. Similar results were found by Tuan et al. (2012) [5] who reported that acacetin and tilianin were mainly extracted from the flowers of *A. rugosa* while acacetin was detected with very low amount in leaves (0.06 μg·g^{-1}) and undetectable in stems and roots.

These results showed that auxin dissolved in the nutrient solution could increase bioactive compounds in plants, but they mainly depend on plant species and auxin concentration levels. No report is presently available on the influence of IAA concentrations on the accumulation of bioactive compounds in *A. rugosa*. Our present results indicate that RA, tilianin, and acacetin concentrations were increased at 10^{-9}; 10^{-7}; 10^{-5}, 10^{-11};10^{-9}; 10^{-7}; 10^{-5}, and 10^{-7}; 10^{-5} IAA treatments, respectively, compared with those of the control. However, the IAA concentration at 10^{-7} M significantly increased RA, acacetin, and tilianin contents (Figure 4 B,D,F), net photosynthesis rate, and shoot fresh weight of *A. rugosa* compared with those of the control and other IAA treatments (Table 1 and Figure 3). IAA treatment at 10^{-11} M reduced RA and acacetin content, and net photosynthesis rate, at 10^{-9} M reduced

acacetin content, and at 10^{-5} M decreased tilianin content and shoot fresh weight compared with the other IAA treatments.

5. Conclusions

IAA dissolved in the nutrient solution improved several growth parameters and increased the production of bioactive compounds in *A. rugosa* while these effects were most pronounced at 10^{-7} M IAA treatment. The results showed that IAA treatment at optimized concentration is most beneficial for enhancing plant growth and bioactive compound production in *A. rugosa*. Therefore, these results could be applied to optimize plant growth and bioactive compounds of *A. rugosa* in a plant factory. The IAA dissolved in the nutrient solution for herb plants in a plant factory can be used to produce ingredients for extractable nutraceutical in the drug industry and thereby enhance the healthy properties.

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References

1. Yamani, H.; Mantri, N.; Morrison, P.D.; Pang, E. Analysis of the volatile organic compounds from leaves, flower spikes, and nectar of Australian grown *Agastache Rugosa*. *BMC Complement. Altern. Med.* **2014**, *14*, 1–6. [CrossRef]
2. Oliver, S.J. The role of traditional medicine practice in primary health care within Aboriginal Australia: A review of the literature. *J. Ethnobiol. Ethnomed.* **2013**, *9*, 46. [CrossRef]
3. Zielinska, S.; Matkowski, A. Phytochemistry and bioactivity of aromatic and medicinal plants from the genus *Agastache* (Lamiaceae). *Phytochem. Rev.* **2014**, *13*, 391–416. [CrossRef]
4. Lee, S.Y.; Xu, H.; Kim, Y.K.; Park, S.U. Rosmarinic acid production in hairy root cultures of *Agastache rugosa* Kuntze. *World J. Microbiol. Biotechnol.* **2008**, *24*, 969–972. [CrossRef]
5. Tuan, P.A.; Park, W.T.; Xu, H.; Park, N.I.; Park, S.U. Accumulation of tilianin and rosmarinic acid and expression of phenylpropanoid biosynthetic genes in *Agastache Rugosa*. *J. Agric. Food Chem.* **2012**, *60*, 5945–5951. [CrossRef] [PubMed]
6. Carmona-Castro, G.; Estrada-Soto, S.; Arellano-Garcia, J.; Arias-Duran, L.; Valencia-Diaz, S.; Perea-Arango, I. High accumulation of tilianin in in-vitro cultures of *Agastache mexicana* and its potential vasorelaxant action. *Mol. Biol. Rep.* **2019**, *46*, 1107–1115. [CrossRef] [PubMed]
7. Wei, J.F.; Cao, P.R.; Wang, J.M.; Kang, W.Y. Analysis of tilianin and acacetin in *Agastache rugosa* by high-performance liquid chromatography with ionic liquids-ultrasound based extraction. *Chem. Cent. J.* **2016**, *10*, 1–9. [CrossRef] [PubMed]
8. Guo, K.J.; Xu, S.F.; Yin, P.; Wang, W.; Song, X.Z.; Liu, F.H.; Xu, J.Q.; Zoccarato, I. Active components of common traditional Chinese medicine decoctions have antioxidant functions. *J. Anim. Sci.* **2011**, *89*, 3107–3115. [CrossRef]
9. Nadeem, M.; Imran, M.; Gondal, T.A.; Imran, A.; Shahbaz, M.; Amir, R.M.; Sajid, M.W.; Qaisrani, T.B.; Atif, M.; Hussain, G.; et al. Therapeutic potential of rosmarinic acid: A comprehensive review. *Appl Sci.* **2019**, *9*, 3139. [CrossRef]
10. Amoo, S.O.; Aremu, A.O.; Van Staden, J. In vitro plant regeneration, secondary metabolite production and antioxidant activity of micropropagated *Aloe arborescens* Mill. *Plant Cell Tissue Organ Cult.* **2012**, *111*, 345–358. [CrossRef]
11. Akanda, M.R.; Uddin, M.N.; Kim, I.S.; Ahn, D.; Tae, H.J.; Park, B.Y. The biological and pharmacological roles of polyphenol flavonoid tilianin. *Eur. J. Pharm.* **2019**, *842*, 291–297. [CrossRef] [PubMed]

12. Semwal, R.B.; Semwal, D.K.; Combrinck, S.; Trill, J.; Gibbons, S.; Viljoen, A. Acacetin-A simple flavone exhibiting diverse pharmacological activities. *Phytochem. Lett.* **2019**, *32*, 56–65. [CrossRef]
13. Wink, M. Modes of action of herbal medicines and plant secondary metabolites. *Medicines* **2015**, *2*, 251–286. [CrossRef] [PubMed]
14. Kozai, T.; Niu, G.; Takagaki, M. Plant factory: An indoor vertical farming system for efficient quality food production. In *Plant. Factory as a Resource-Efficient Closed Plant Production System*, 2nd ed.; Kozai, T., Niu, G., Takagaki, M., Eds.; Academic Press: Cambridge, MA, USA, 2015; pp. 69–90.
15. Kim, Y.B.; Kim, J.K.; Uddin, M.R.; Xu, H.; Park, W.T.; Tuan, P.A.; Li, X.; Chung, E.; Lee, J.H.; Park, S.U. Metabolomics analysis and biosynthesis of rosmarinic acid in *Agastache rugosa* Kuntze treated with methyl jasmonate. *PLoS ONE.* **2013**, *8*, e64199. [CrossRef]
16. Kim, S.J.; Bok, K.J.; Lam, V.P.; Park, J.S. Response of nutrient solution and photosynthetic photon flux density for growth and accumulation of antioxidant in *Agastache rugosa* under hydroponic culture systems. *Prot. Hortic. Plant. Fact.* **2017**, *26*, 249–257. [CrossRef]
17. Lam, V.P.; Kim, S.J.; Bok, G.J.; Lee, J.W.; Park, J.S. The effects of root temperature on growth, physiology, and accumulation of bioactive compounds of *Agastache Rugosa*. *Agriculture* **2020**, *10*, 162. [CrossRef]
18. Lam, V.P.; Kim, S.J.; Park, J.S. Optimizing the electrical conductivity of a nutrient solution for plant growth and bioactive compounds of *Agastache rugosa* in a plant factory. *Agronomy* **2020**, *10*, 76. [CrossRef]
19. Bais, H.P.; Sudha, G.; George, J.; Ravishankar, G.A. Influence of exogenous hormones on growth and secondary metabolite production in hairy root cultures of *Cichorium intybus* L. *cv. Lucknow Local. Vitr. Cell. Dev. Biol. Plant.* **2001**, *37*, 293–299. [CrossRef]
20. Piotrowska-Niczyporuk, A.; Bajguz, A. The effect of natural and synthetic auxins on the growth, metabolite content and antioxidant response of green alga *Chlorella vulgaris* (Trebouxiophyceae). *J. Plant. Growth Regul.* **2014**, *73*, 57–66. [CrossRef]
21. Synkova, H.; Semoradova, S.; Schnablova, R.; Witters, E.; Husak, M.; Valcke, R. Cytokinin-induced activity of antioxidant enzymes in transgenic Pssu-ipt tobacco during plant ontogeny. *Biol. Plant.* **2006**, *50*, 31–41. [CrossRef]
22. Lee, Y.; Lee, D.E.; Lee, H.S.; Kim, S.K.; Lee, W.; Kim, S.H.; Kim, M.W. Influence of auxins, cytokinins, and nitrogen on production of rutin from callus and adventitious roots of the white mulberry tree (*Morus alba* L.). *Plant. Cell Tissue Organ. Cult.* **2011**, *105*, 9–19. [CrossRef]
23. Piotrowska-Niczyporuk, A.; Bajguz, A.; Kotowska, U.; Bralska, M.; Talarek-Karwel, M. Growth, metabolite profile, oxidative status, and phytohormone levels in the green alga *Acutodesmus obliquus* exposed to exogenous auxins and cytokinins. *J. Plant. Growth Regul.* **2018**, *37*, 1159–1174. [CrossRef]
24. Satdive, R.K.; Fulzele, D.P.; Eapen, S. Studies on production of ajmalicine in shake flasks by multiple shoot cultures of *Cathar. Roseus. Biotechnol. Prog.* **2003**, *19*, 1071–1075. [CrossRef] [PubMed]
25. Ding, X.; Jiang, Y.; Zhao, H.; Guo, D.; He, L.; Liu, F.; Zhou, Q.; Nandwani, D.; Hui, D.; Yu, J. Electrical conductivity of nutrient solution influenced photosynthesis, quality, and antioxidant enzyme activity of pakchoi (*Brassica campestris* L. ssp. *Chinensis*) in a hydroponic system. *PLoS ONE* **2018**, *13*, e0202090. [CrossRef] [PubMed]
26. Zhao, Y.D. Auxin biosynthesis and its role in plant development. *Annu. Rev. Plant. Biol.* **2010**, *61*, 49–64. [CrossRef]
27. Haber, A.H. Effects of indoleacetic acid on growth without mitosis & on mitotic activity in absence of growth by expansion. *Plant. Physiol.* **1962**, *37*, 18–26.
28. Contreras-Cornejo, H.A.; Macías-Rodríguez, L.; Cortés-Penagos, C.; López-Bucio, J. *Trichoderma virens*, a plant beneficial fungus, enhances biomass production and promotes lateral root growth through an auxin-dependent mechanism in Arabidopsis. *Plant. Physiol.* **2009**, *149*, 1579–1592. [CrossRef]
29. Malik, D.K.; Sindhu, S.S. Production of indole acetic acid by *Pseudomonas* sp.: Effect of coinoculation with *Mesorhizobium* sp. Cicer on nodulation and plant growth of chickpea (*Cicer arietinum*). *Physiol. Mol. Biol. Plants.* **2011**, *17*, 25–32.
30. Fassler, E.; Evangelou, M.W.; Robinson, B.H.; Schulin, R. Effects of indole-3-acetic acid (IAA) on sunflower growth and heavy metal uptake in combination with ethylene diamine disuccinic acid (EDDS). *Chemosphere* **2010**, *80*, 901–907. [CrossRef]

31. San-Francisco, S.; Houdusse, F.; Zamarreno, A.; Garnica, M.; Casanova, E.; Garcia-Mina, J. Effects of IAA and IAA precursors on the development, mineral nutrition, IAA content and free polyamine content of pepper plants cultivated in hydroponic conditions. *Sci. Hortic.* **2005**, *106*, 38–52. [CrossRef]
32. Liu, J.; Qiu, W.; Song, Y. Stimulatory effect of auxins on the growth and lipid productivity of *Chlorella pyrenoidosa* and *Scenedesmus Quadricauda*. *Algal Res.* **2016**, *18*, 273–280. [CrossRef]
33. Kobayashi, K.; Baba, S.; Obayashi, T.; Sato, M.; Toyooka, K.; Keränen, M.; Aro, E.M.; Fukaki, H.; Ohta, H.; Sugimoto, K.; et al. Regulation of root greening by light and auxin/cytokinin signaling in *Arabidopsis*. *Plant Cell* **2012**, *24*, 1081–1095. [CrossRef] [PubMed]
34. Khan, N.A.; Khan, M.; Ansari, H.R. Auxin and defoliation effects on photosynthesis and ethylene evolution in mustard. *Sci. Hortic.* **2002**, *96*, 43–51. [CrossRef]
35. Bidwell, R.G.S.; Turner, W.B. Effect of growth regulators on CO_2 assimilation in leaves, and its correlation with the bud break response in photosynthesis. *Plant. Physiol.* **1966**, *41*, 267–270. [CrossRef]
36. Wang, Y.; Branicky, R.; Noë, A.; Hekimi, S. Superoxide dismutases: Dual roles in controlling ROS damage and regulating ROS signaling. *J. Cell Biol.* **2018**, *217*, 1915–1928. [CrossRef]
37. Kirecci, O.A. The effects of salt stress, SNP, ABA, IAA and GA applications on antioxidant enzyme activities in *Helianthus Annuus* L. *Fresenius Environ. Bull.* **2018**, *27*, 3783–3788.
38. Lee, E.J.; Kim, M.K.; Paek, K.Y. Auxin and cytokinin affect biomass and bioactive compound production from adventitious roots of *Eleutherococcus Koreanum*. *Korean J. Hortic. Sci. Technol.* **2010**, *28*, 678–684.
39. Thiruvengadam, M.; Chung, I.M. Phenolic compound production and biological activities from in vitro regenerated plants of gherkin (*Cucumis anguria* L.). *Electron. J. Biotechnol.* **2015**, *18*, 295–301. [CrossRef]
40. Galieni, A.; Di Mattia, C.; De Gregorio, M.; Speca, S.; Mastrocola, D.; Pisante, M.; Stagnari, F. Effects of nutrient deficiency and abiotic environmental stresses on yield, phenolic compounds and antiradical activity in lettuce (*Lactuca sativa* L.). *Sci. Hortic.* **2015**, *187*, 93–101. [CrossRef]