forests

MDPI

Article

Effect of Multi-Walled Carbon Nanotubes on the Growth and Expression of Stress Resistance Genes in Birch

Konstantin V. Zhuzhukin [1,*], Peter M. Evlakov [2], Tatiana A. Grodetskaya [2], Alexander A. Gusev [2,3,4], Olga V. Zakharova [3,5], Aleksey V. Shuklinov [3] and Elena V. Tomina [1,6]

[1] Department of Chemistry, Voronezh State University of Forestry and Technologies Named after G.F. Morozov, 8, Timiryazeva, 394087 Voronezh, Russia
[2] Laboratory of PCR Analysis, Research Institute of Innovative Technologies of the Forestry Complex, Voronezh State University of Forestry and Technologies Named after G. F. Morozov, 394087 Voronezh, Russia
[3] Institute for Environmental Science and Biotechnology, Derzhavin Tambov State University, 392020 Tambov, Russia
[4] Department of Functional Nanosystems and High-Temperature Materials, National Research Technological University "MISiS", 119049 Moscow, Russia
[5] Engineering Center, Plekhanov Russian University of Economics, 117997 Moscow, Russia
[6] Voronezh State University, 394018 Voronezh, Russia
* Correspondence: kinkon18@yandex.ru

Abstract: Recent studies have shown that nanomaterials, including carbon nanotubes, are associated with a wide range of effects on living organisms, from stimulation to toxic effects. Plants are an important object of such research, which is associated with the potential use of carbon nanomaterials in agriculture and environmental protection. At the same time, the specific mechanisms of formation of plant resistance to the effects of carbon nanotubes remain not fully understood, especially in woody plants. Therefore, we studied the effect of aqueous colloids of multi-walled carbon nanotubes (MWCNTs) with an outer diameter of 10–30 nm and a length of about 2 µm at a concentration of 1, 10, 50, and 100 mg/L on morphometric parameters and the level of expression of stress resistance genes in *Betula pubescens* Ehrh. and *B. pendula* Roth. plants in greenhouse conditions. The results showed an increase in the length and diameter of the shoot in the studied plants. The dry biomass of the leaf increased by 30%, the stem by 42%, and the root by 49% when using MWCNTs at a concentration of 10 mg/L. The expression of the stress resistance genes *DREB2* and *PR-10* significantly increased under the influence of 1 mg/L MWCNTs on plants of both species. At the same time, the use of 100 mg/L nanoparticles led to a decrease in the studied parameters in *Betula pendula*, which may be associated with the negative effect of MWCNTs in high concentrations. The revealed positive effects of low concentrations of MWCNTs on morphometric parameters and stimulation of stress resistance genes by nanotubes open up prospects for their use in woody plant biotechnology.

Keywords: carbon nanomaterials; changes in growth rates; gene expression; birch plants; growth stimulation

check for updates

Citation: Zhuzhukin, K.V.; Evlakov, P.M.; Grodetskaya, T.A.; Gusev, A.A.; Zakharova, O.V.; Shuklinov, A.V.; Tomina, E.V. Effect of Multi-Walled Carbon Nanotubes on the Growth and Expression of Stress Resistance Genes in Birch. *Forests* **2023**, *14*, 163. https://doi.org/10.3390/f14010163

Academic Editor: Rita Lourenço Costa

Received: 21 October 2022
Revised: 6 January 2023
Accepted: 13 January 2023
Published: 16 January 2023

Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

The potential for using nanomaterials in crop production is very wide. Nanofertilizers improve plant growth and yield, pesticides control pests and diseases, and nanosensors contribute to plant health and regulate soil quality [1–3]. A significant part of such research is focused on carbon materials, in particular, carbon nanotubes, which have outstanding physical and mechanical properties and ease of functionalization [4–7]. The results assessing the impact of carbon nanomaterials in growth and development are contradictory [8–10]; however, the authors of a number of works emphasize the positive influence on plant objects [11,12]. Carbon nanotubes (CNTs) are sp^2-hybridized carbon atoms arranged in a cylindrical nanostructure. Since Iijima's seminal work in 1991, which detailed their structure and properties [13], CNTs have become widespread due to their outstanding electrical,

mechanical and thermal properties [2]. One of the main areas of potential application of CNTs is agriculture. Single-walled carbon nanotubes (SWCNTs) accelerated growth of maize seed roots by the change in the related gene expression [14]. The similar effect was also observed for rice [15], carrot, cabbage, onion, lettuce, and tomato seedlings [16]. SWCNTs improved germination of sage and pepper at low concentrations of 30 mg/L and 10 mg/L, respectively [17]. In [17,18], similar SWCNT concentrations were used to improve the germination of tomato and onion seedlings. The same result was obtained by Flores et al. [19] when using functionalized SWCNTs on *Rubus adenotrichos*, which positively affected the elongation of roots and shoots. An increase in the biomass of tobacco plants was confirmed upon treatment with SWCNTs [20,21]. It was shown that SWCNTs enhanced plants tolerance in salt and drought stress conditions [22,23].

In the last decade, the impact of multi-walled carbon nanotubes (MWCNTs) on plants has attracted interest because they are easier to produce and cheaper than single-walled carbon nanotubes. MWCNTs penetrate into the structure of cell walls, facilitate the absorption of water and increase growth in broccoli [24], chickpea (when using hydrophilic MWCNTs) [25,26], barley, soybeans, and corn [27]. A positive effect of functionalized MWCNTs on the growth of tobacco cells was also shown [20]. MWCNTs penetrate into plant seeds due to the formation of new pores, which contributes to the absorption of water and an increase in the germination rate [28]. In addition, it has also been observed that CNTs have the ability to penetrate the seed coat of plants [29,30]. In [31], it was found that MWCNTs affect root elongation and increase in biomass in rapeseed and wheat, but no effect on photosynthetic activity was found.

A number of works report on the toxicity of CNTs for biological objects. Wang et al. [32] observed that carboxylated MWCNTs cause biochemical and subcellular damage in the leaves of bean seedlings (*Vicia faba* L.). The use of SWCNTs resulted in a delay in flowering and a decrease in rice yield [33]. Researchers reported a decrease in squash biomass [34], root growth inhibition in lettuce [35], and DNA damage in *Allium cepa* [36]. The treatment of maize and soybean with 10–50 mg/L MWCNTs caused cellular, charge, and size selectivity and inhibited growth in soybean [37].

The interaction of CNTs with plants is a complex phenomenon that causes a number of physiological and morphological changes [18], which depend on the size, concentration, and type of CNTs, as well as the plant species and stage of their development. As a result of this interaction, a change in the expression of various genes can be observed. For example, in [14] the authors reported a significant increase in the expression of genes responsible for the development of seminal root in maize plants exposed to SWCNTs. Khodakovskaya et al. showed that MWCNTs affect gene expression in tobacco cells and tomato tissues [20,38]. Fullerenes upregulated protective genes involved in both abiotic and biotic stress in *Arabidopsis.* In addition, upregulation was also noted in rice [39,40]. The study [41] reported on the influence of MWCNTs on plant physiology, gene expression, and the composition of the soil bacterial community in the rice–soil–bacterial ecosystem.

Despite the availability of a significant amount of data, the exact mechanism of the impact of carbon nanomaterials (CNMs) on biological objects, especially woody plants, is not fully understood. Forest species play a key role in almost all terrestrial ecosystems, and the influence of nanomaterials on them should also be analyzed. Therefore, in this study we used as objects the deciduous tree species of silver birch (*Betula pendula* Roth.) and downy birch (*Betula pubescens* Ehrh.). Widespread in Europe, their planting area is estimated at 80 million hectares, with a timber reserve of about 6 billion m^3.

The aim of this work was to study the effect of MWCNTs on growth rates and the level of expression of stress resistance genes in two birch species under greenhouse conditions.

2. Materials and Methods

2.1. Analysis of MWCNTs

We used MWCNTs purchased from MST-Nano (Riga, Latvia). According to the manufacturer's data, the MWCNT sample had a specific surface area of $\geq$270 m^2 g^{-1} and a distance between layers of 0.34 nm.

The Raman scattering spectrum (RSS) was obtained using the scanning probe complex for micro-Raman spectroscopy INTEGRA Spektra (NT-MDT, Moscow, Russia) at an exciting laser wavelength of 532 nm.

Fourier transform IR spectroscopic analysis was carried out on a VERTEX 70 spectrometer (Bruker, Germany) by the method of frustrated total internal reflection using a diamond prism in the frequency range from 400–4000 cm^{-1} with a resolution of 2 cm^{-1} in transmission mode.

The dimensional characteristics of MWCNTs were analyzed on a transmission-(TEM) (Carl Zeiss Libra 120, Jena, Germany) and scanning (JSM-6380LV) electron microscope (SEM). A conductive layer of gold 40 nm thick was deposited on the samples to prevent electrization. The deposition of a conductive layer of gold was carried out in order to exclude the accumulation of charges on MWCNTs and to obtain a sufficiently contrasting pattern on the sample surface.

The elemental composition of the sample was analyzed by energy dispersive X-ray spectroscopy on an attachment to an X-flash scanning electron microscope (Bruker, Germany).

2.2. Obtaining and Studying Colloidal Solutions of MWCNTs

Aqueous solutions of MWCNTs were obtained using double purified distilled water with the Milly-Q system and sodium dodecyl sulfate (SDS) (Sigma Aldrich, St. Louis, MO, USA) as a stabilizer [42]. A portion of SDS (0.5 g) was weighed on a ViBRA HT 224CE analytical balance (Japan), added to 95.5 mL of distilled water, stirred with a glass rod, and then brought to complete dissolution with a magnetic stirrer (OLDIS, Moscow, Russia) for 30 min at a stirring speed of 2000 rpm. During the preparation of colloidal solutions, a suspension with a concentration of 1000 mg/L was obtained, which was subsequently diluted to the final concentrations (1, 10, 50, 100 mg/L) and subjected to intensive dispersion on an ultrasonic homogenizer Sonicator Q500 (QSonica, Newtown, CT, USA) for 30 min with a power of 100 W and a frequency of 22 kHz. The exact values of the concentrations of the obtained control solutions were determined on a PE-5400VI spectrophotometer (Russia) at a wavelength of 660 nm [43].

The concentrations of MWCNTs for the study were chosen based on the data from previously published works [9,38,41,44–46].

The stability of colloidal solutions was evaluated by measuring the zeta potential (ζ) of particles of the dispersed phase directly in solution using a Zetasizer Nano ZSP instrument (Malvern Instruments Ltd., Malvern, UK). The analysis of solutions was carried out after their settling for 20 days at a temperature of 25 °C, and the sample volume was 50 µL. A helium-neon laser with a wavelength of 633 nm and a power of 10 mW was used for the research. Studies were conducted for all the prepared concentrations.

2.3. Objects of Study and Methodology for Conducting the Experiment

For the experiment, plants of downy birch (*Betula pubescens* Ehrh.) and silver birch (*Betula pendula* Roth.) obtained by in vitro clonal micropropagation were used. Microplants were transplanted into 500 cm^3 plastic containers filled with a nutrient substrate based on neutralized high-moor peat "Pelgorskaya-M" (Russia) and perlite in a ratio of 3:1. The experimental substrate was evenly moistened with 50 mL of MWCNT colloidal solutions at a concentration of 1, 10, 50, and 100 mg/L and thoroughly mixed. The control substrate was moistened with distilled water in the same volume. Each experimental group contained 25 plants in 3 repetitions. Growing conditions were maintained at 25 °C day and 15 °C night and a relative humidity of at least 85%. Plant care consisted of regular watering and loosening the soil. Growth parameters were measured after 30 days of germination in the greenhouse.

To obtain absolutely dry weight, leaves, stems, and roots were dried in an RS422 oven (Binder, Germany) at a temperature of 102 °C.

To determine the changes in growth parameters of MWCNT-treated and untreated plants, we measured the height, the number of leaves per plant, the total area of the assimilation surface, and the wet and dry biomass of the leaves, stem, and root. To measure the leaf area and the assimilation surface of the plant, a CI-202 portable laser leaf area meter (CID Bio-Science, Camas, WA, USA) was used.

2.4. Conducting Gene Expression Analysis for the Effects of MWCNTs

RNA extraction was performed by a modified CTAB method [47]. The isolation products were visualized in a 1% agarose gel stained with ethidium bromide intercalating dye (PanReac Applichem, Darmstadt, Germany); RNA quality and homogeneity were assessed using an Infinity VX2 1126MX X-Press gel documentation system (Vilber Lourmat, Collegien, France).

The RNA concentration was determined using a Qubit 2.0 fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions.

cDNA synthesis was performed using a standard kit with MMLV-RH (Dia-M, Moscow, Russia) and 0.5–1 µg of total RNA.

Primers for resistance genes, regarded as markers [48–52], were selected based on sequences from the NCBI database using the Primer3 program (Table 1).

Table 1. Primer sequences for stress resistance genes.

№	Gene	Sequence (5′→3′)
1	*Pal*	F: CTGTGGCTGCAACGGTTT
		R: TCAATTTGAGGTCCGAGCCA
2	*PR-10*	F: GGCCCGGAACCATTAAGAAG
		R: CCACCCTCGATCAAGCTGTA
3	*PR-1*	F: CCTCAAAGCCCACAATGACG
		R: TCTCGTCCACCCATAGCTTC
4	*lea8*	F: AATGACTTTGACATGGGCGT
		R: TATCCCAAACTGCAGAGCCA
5	*GAPDH*	F: CAGCCGAAGATGTCAATGCA
		R: GGCCACTTGTTTGCTACCAA
6	*DREB2*	F: AGGCAGAGAACATGGGGAAA
		R: GAAAGTTGAGGCGAGCGTAA

After optimization of the primer annealing conditions, the PCR protocol included preliminary annealing at 95 °C for 3 min, then 45 cycles from the stage 30 s at 95 °C, 30 s at 60 °C, 30 s at 72 °C, and the final elongation at 72 °C for 2 min. The reaction was carried out using a standard set of reagents containing SYBR Green I dye (Evrogen, Moscow, Russia) in real time.

Resistance gene expression was analyzed by the 2-ΔΔCt method using LightCycler480 II v 1.5.1 software (Roche, Basel, Switzerland) [53]. The *GAPDH* gene was used as a reference.

2.5. Statistical Analysis

To test the statistical significance of the change in growth parameters (growth height, number of leaves per plant, total assimilation surface area, wet biomass of leaves, stem, root, dry biomass of leaves, stem, root) between control and exposed-to-MWCNTs samples, VASSARSTAT (http://vassarstats.net/anova1u.html, accessed on 24 May 2021) was used to perform a one-way variance analysis. All data are expressed as means ±(SD) SE (standard

deviation). The mean and standard deviation data obtained from the measurements of various treatments in replicates were statistically analyzed using ANOVA (analysis of variance) and Tukey's multiple comparison tests to determine the significant difference among different treatments. A probability value (p) of 0.05 and lower was considered significant.

3. Results

3.1. Characterization of MWCNTs

Raman spectroscopy (Figure 1) recorded two characteristic maxima, the G peak (~1582 cm^{-1}), corresponding to the tangential graphite-like mode, and the D peak (~1334 cm^{-1}), called the "defective Raman zone", which is activated as a result of the first-order sp^2 carbon scattering process the presence of heteroatoms, vacancies, grain boundaries, or other defects in the substitution plane, as well as finite size effects [54–56]. The observed band in the region of 2729 cm^{-1} can be attributed to the second-order Raman scattering modes (G') [57].

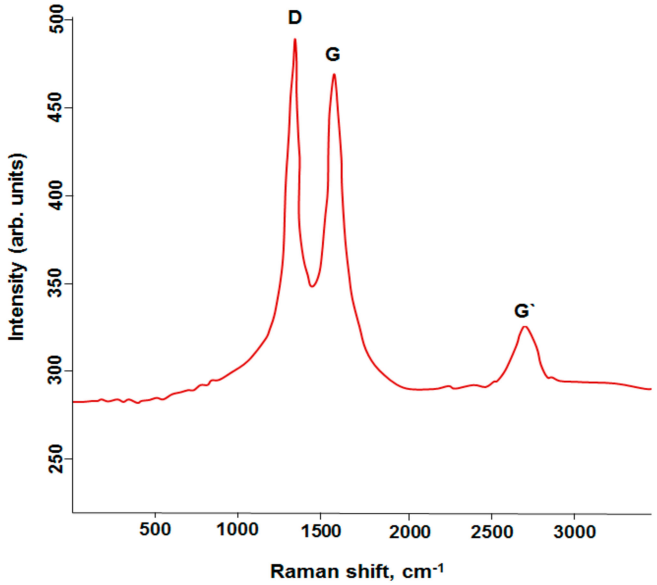

Figure 1. Raman spectrum of a MWCNT sample.

In order to determine the functional groups presented in MWCNTs, an IR spectroscopic analysis was carried out. Figure 2 shows the FTIR spectrum of MWCNTs in the frequency range 400–4000 cm^{-1} used for the study.

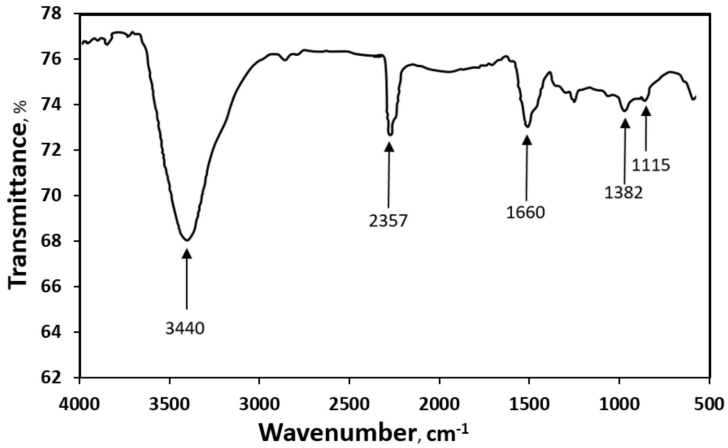

Figure 2. FTIR spectrum of MWCNTs.

The obtained FTIR spectrum has a peak corresponding to the stretching region of the C=C double bond in the region of 1630 cm^{-1}, which forms the skeleton of MWCNTs [58,59]. The peak at 3440 cm^{-1} appears in a presence of hydroxyl groups (−OH) on MWCNTs due to the moisture in the sample. The peaks at 2922 and 2852 cm^{-1} indicate the C–H stretching vibration of the methylene groups formed in defective regions, and the peak at 1382 cm^{-1} corresponds to the deviating C–H methyl group [60].

To determine the dimensional characteristics of MWCNTs, morphology studies were carried out using transmission and scanning electron microscopes; the results are shown in Figure 3.

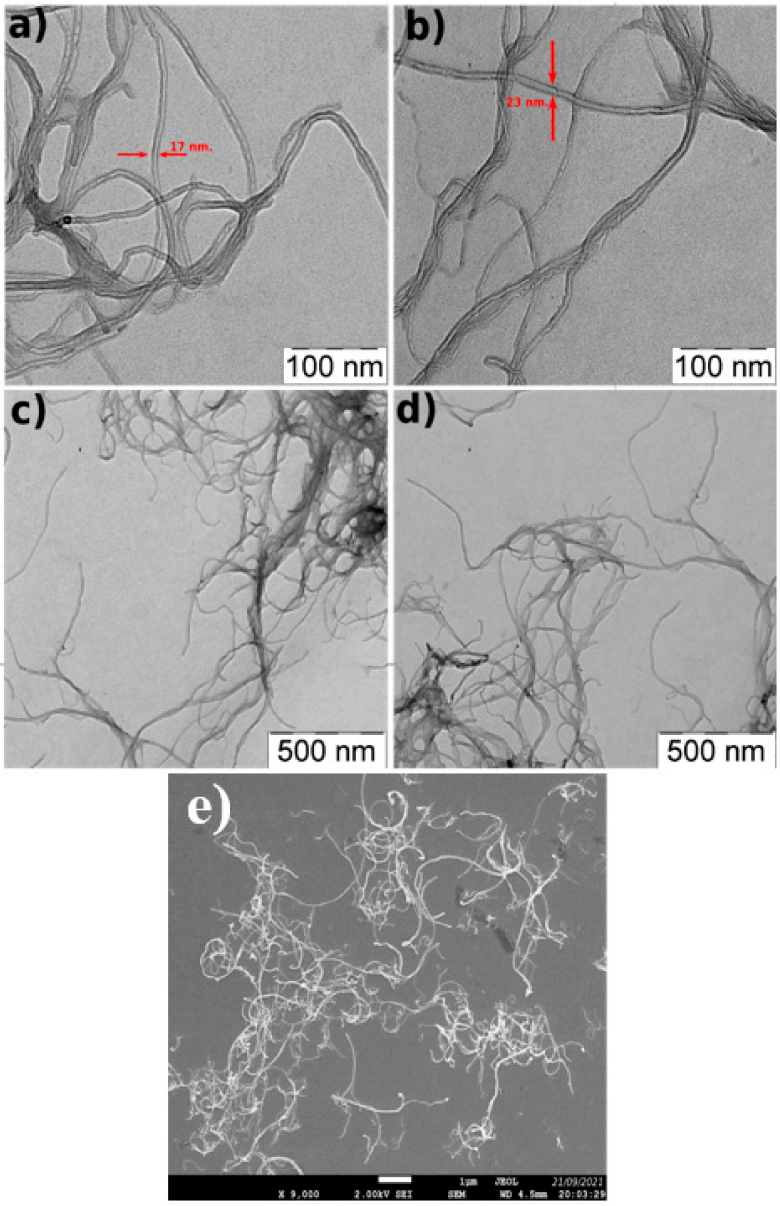

Figure 3. Image in MWCNT in TEM (**a**–**d**) and SEM (**e**).

As a result of TEM analysis, bulk tubular filaments about 2 µm long were observed. Images of MWNTs consisted of threads intertwined with closed ends. In addition, significant voids were observed between the weaves. The average outer diameter of the nanotubes was 10–30 nm, and the inner diameter was 5–15 nm. The results of energy dispersive analysis are presented in Table 2.

Table 2. Results of energy dispersive analysis of MWCNTs.

Element	C	Al	Cl	Co	S
Content, %	97.34	0.25	0.98	1.16	0.27

As can be seen from the table, the carbon content in the samples was more than 97%. The presence of other elements may be related to the peculiarities of the synthesis of MWCNTs.

3.2. Results of Determining the Stability of Colloidal Solutions

Twenty days after preparation, the stability of MWCNT suspensions was evaluated. The appearance of the analyzed colloidal systems is shown in Figure 4a; the results of zeta potential measurements are presented in Figure 4b.

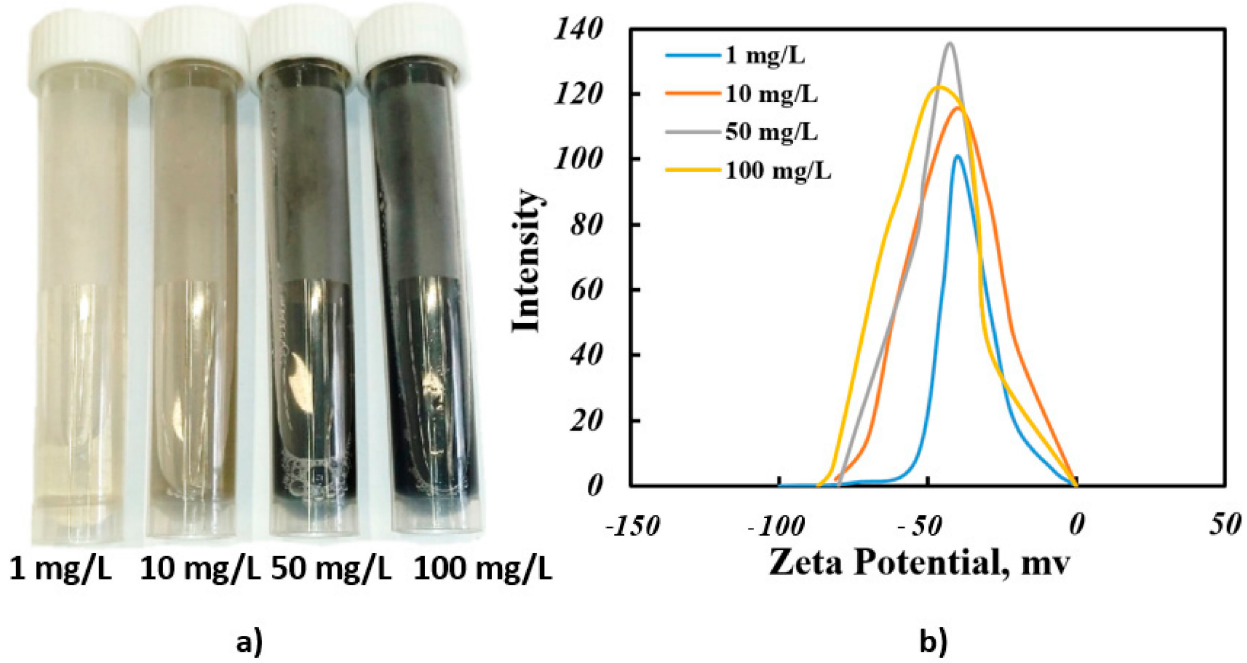

Figure 4. General view of colloidal solutions with different concentrations of MWCNTs (**a**) and the results of determining the zeta potential (**b**), certain concentrations of solutions.

As can be seen from Figure 4a, no visible precipitate was observed in the obtained suspensions. As a result of determining the zeta potential, it was found that for all the studied concentrations, the values were above the threshold (30 mV) (Figure 4b). For colloidal solutions of 1, 10, 50 and 100 mg/L, the zeta potential had the values of −39.7 mV, −41.2 mV, −43.2 mV, and −48.8 mV, respectively, i.e., the stability of colloidal systems enhanced with increasing concentration.

3.3. Results of Determining the Morphometric Parameters of Silver Birch (B. pendula Roth.) and Downy Birch (B. pubescens Ehrh.)

The effect of MWCNTs on the morphometric parameters of birch was studied in birch plants after 30 days of growing in a greenhouse (Figures 5 and 6, $p < 0.05$).

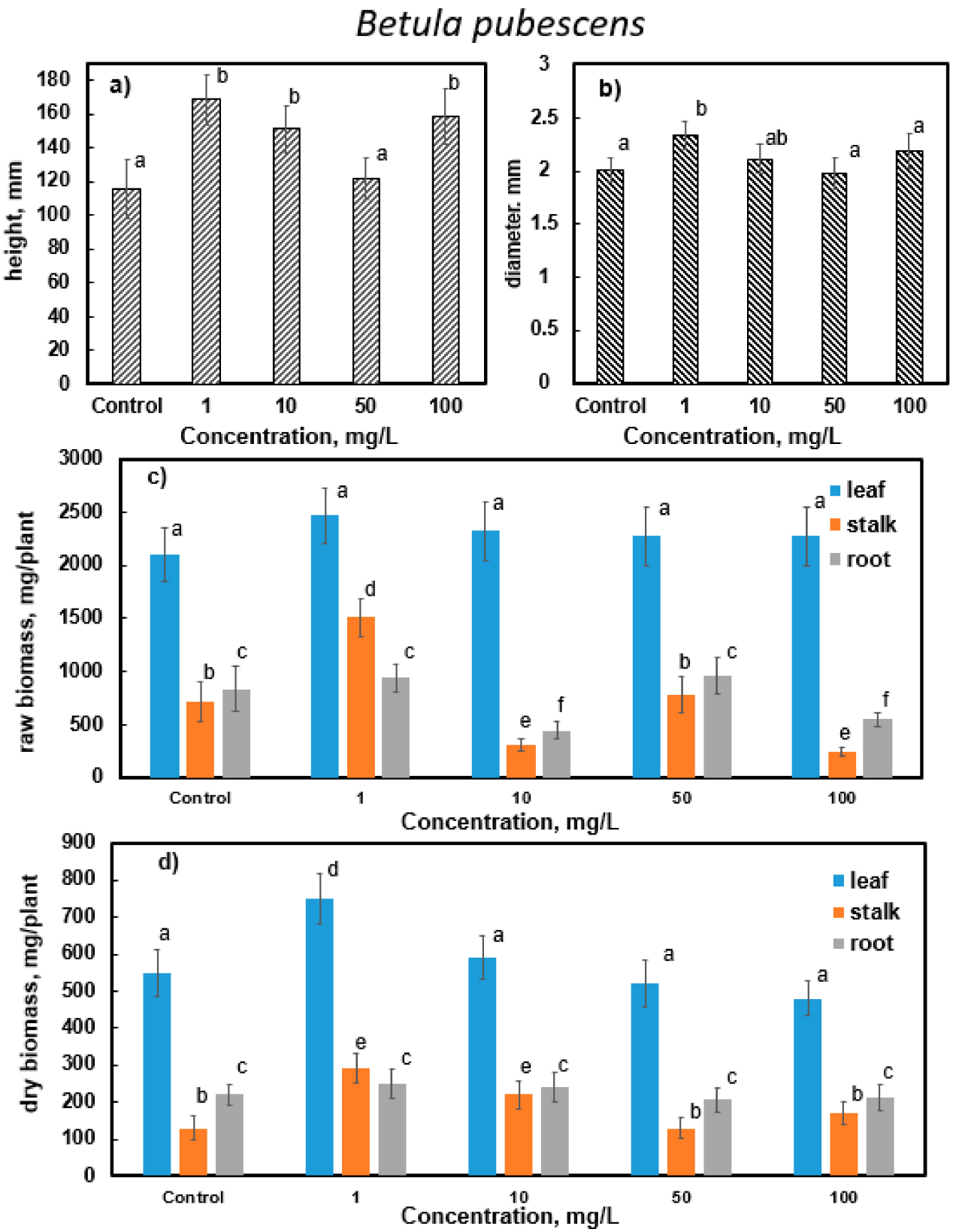

Figure 5. Effect of MWCNTs on height (**a**), diameter (**b**), parameters of wet (**c**) and dry (**d**) biomass of downy birch (*B. pubescens*) plants. Bars represent the ±SE of three replicates. Letters on vertical bars represent significant differences between groups according to Duncan's multiple range test at $p < 0.05$.

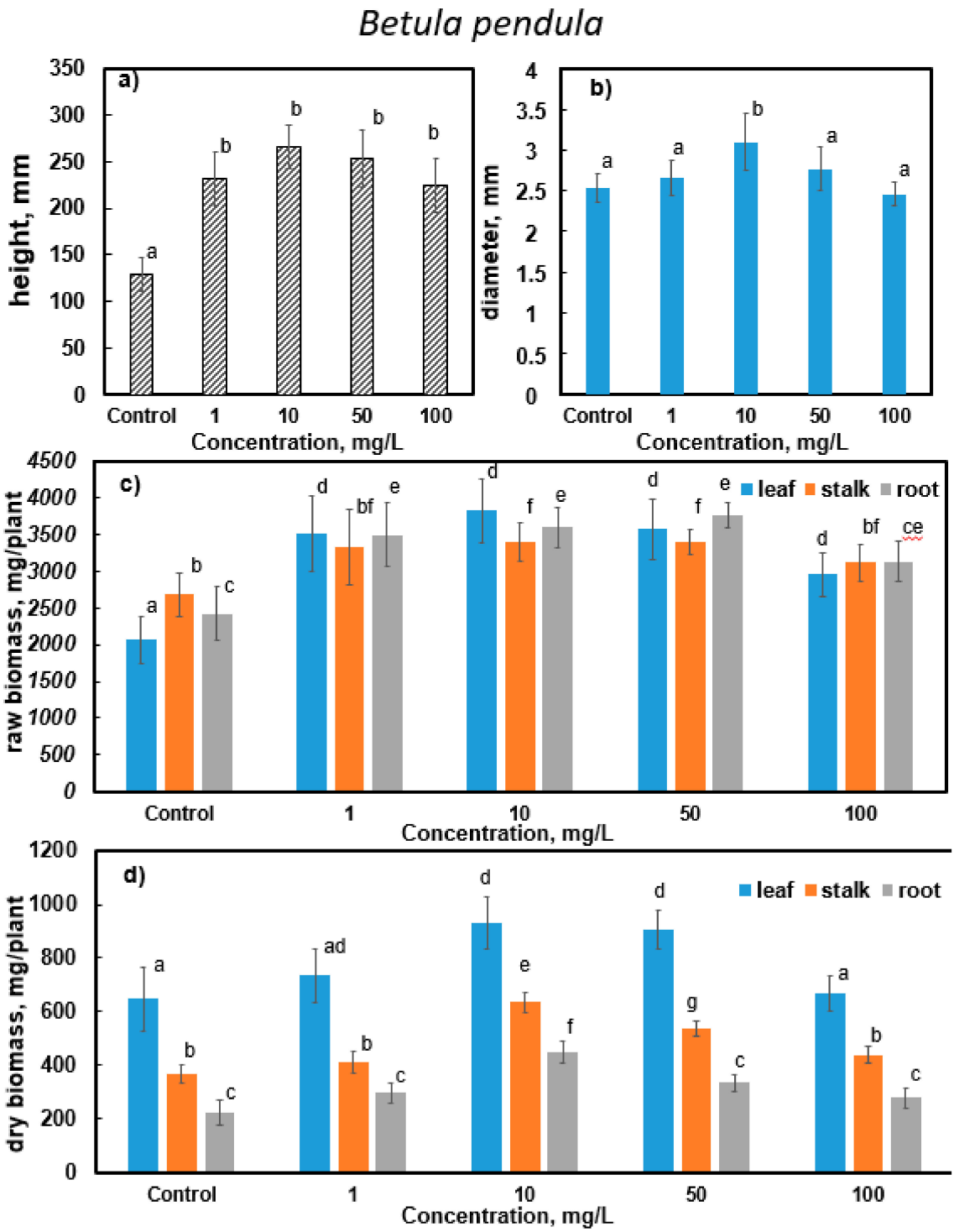

Figure 6. Effect of MWCNTs on height (**a**), diameter (**b**), parameters of wet (**c**) and dry (**d**) biomass of silver birch (*B. pendula*) plants. Bars represent the ±SE of three replicates. Letters on vertical bars represent significant differences between groups according to Duncan's multiple range test at $p < 0.05$.

Treatment with MWCNTs stimulated shoot growth in downy and silver birch (Figures 5a and 6a) in a concentration-dependent manner. The most significant change was shown when using 1 mg/L nanoparticles for downy birch (32%) and 10 mg/L for silver birch. Higher concentration of MWCNTs slightly decreased the shoot length of downy birch, and 50 mg/L did not affect significantly experimental plants. At the same time, treatment of birch samples with 100 mg/L of nanotubes caused the increase in the shoot length relative

to control by 26%. Exposure to all studied concentrations of MWCNT-stimulated shoot growth by 43%–52% in silver birch (Figure 6a).

Shoot diameter increased with the treatment of 1 mg/L MWCNTs in downy birch, while 19% growth in stalk diameter in silver birch was observed at 10 mg/L (Figures 5b and 6b). Other concentrations of nanoparticles did not significantly influence the studied plants.

Nanoparticles treatment increased leaf and stem mass in the studied plants. Significant raw biomass accumulation was shown when using 1 mg/L nanoparticles for downy birch plants (Figure 5c). At the same time, raw biomass of leaves, stem and root of silver birch augmented under the influence of nanoparticles at a concentration of 10 mg/L (Figure 6c).

MWCNTs of 1 mg/L significantly stimulated accumulation of leaves and stems dry weight in downy birch (Figure 5d). The use of higher concentrations of MWCNTs did not cause any changes in the studied parameters. In silver birch, the dry weight of the leaf, stem, and root increased when exposed to nanoparticles of 1–100 mg/L, although the values of the experimental samples were close to the control when using the highest concentration (Figure 6d). The maximum value of these indicators was revealed when birch plants were treated with 10 mg/L of MWCNTs, the dry weight of the leaf increased by 30%, the stem by 42%, the root by 49% relative to the control samples.

The low concentrations of MWCNTs (the highest values at 1 mg/L) stimulated the leaf area and the total leaf surface growth in *B. pubescens*, while higher concentrations did not show significant influence on studied plants. The minimal effect was shown with the use of 100 mg/L (Figure 7).

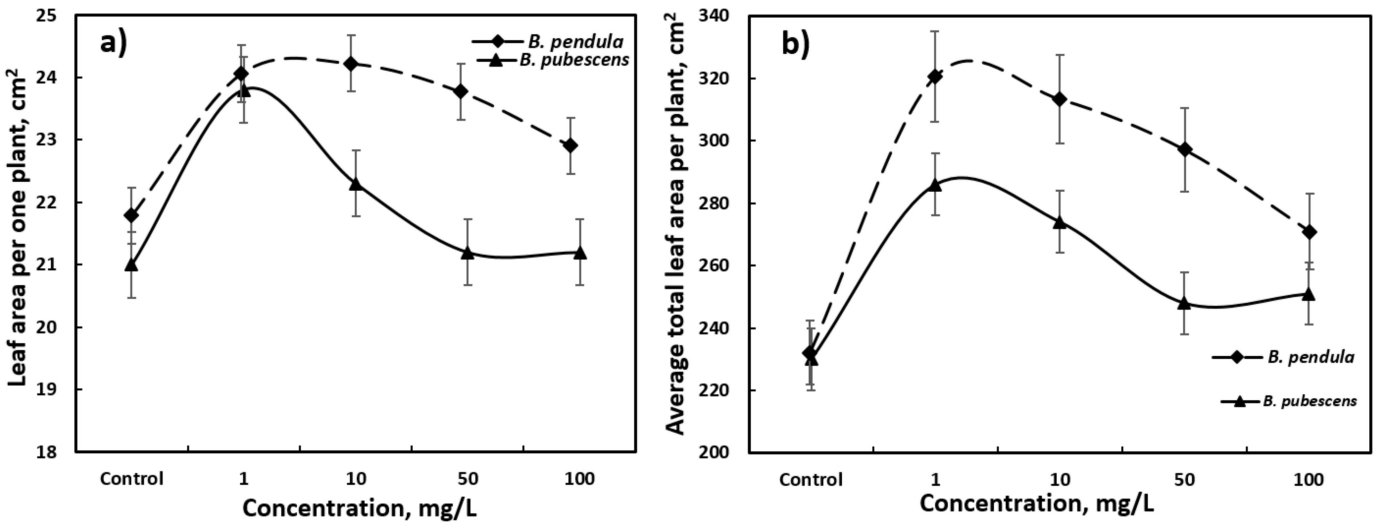

Figure 7. Leaf area (**a**) and total leaf surface (**b**).

Similar results were obtained for silver birch; the leaf area increased by 1.4–1.8 times, with the highest values at 1 and 10 mg/L, and the total leaf area was maximal at a concentration of 1 mg/L nanoparticles.

The use of 1, 10, and 100 mg/L showed a similar effect; an average number of leaves per plant increased from 8 to 10 after MWCNTs treatment of downy birch (Figure 8).

For silver birch, the highest leaf area value was shown at 10 mg/L with an increase of 26% relative to the control. The number of leaves reached a peak at a concentration of 10 mg/L, while 50 and 100 mg/L reduced their number.

The study of the effect of carbon nanotubes at different concentrations on downy and silver birch plants revealed a concentration-dependent change in the expression of stress resistance genes. The impact of 1 mg/L nanoparticles on downy birch plants contributed to an increase in the expression of the *DREB2*, *PR-1,* and *PR-10* genes by 2.1, 8.1, and 3.3 times, respectively (Figure 9a).

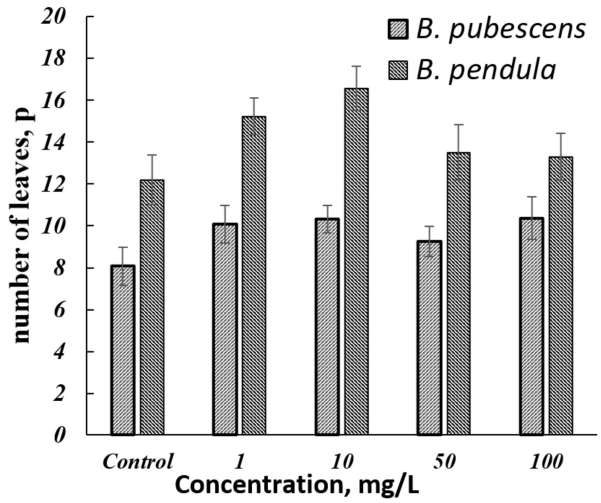

Figure 8. Average number of leaves per plant.

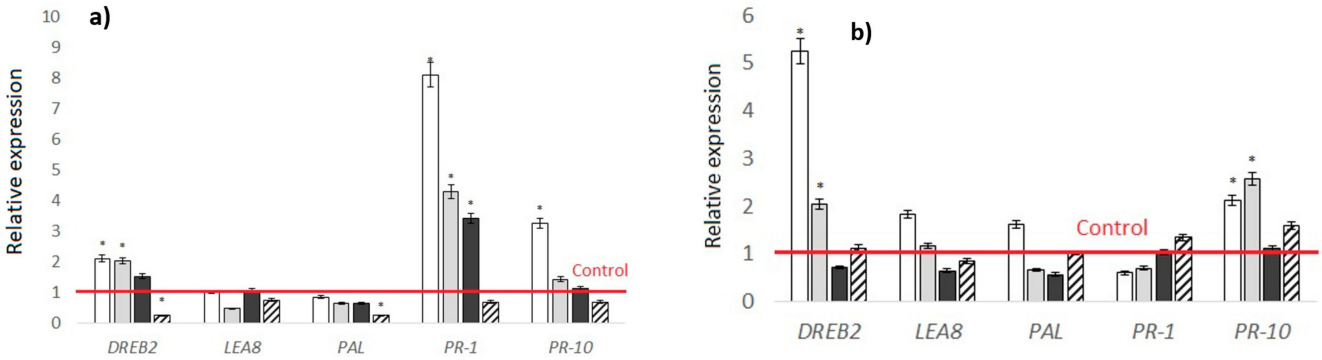

Figure 9. Expression of stress resistance genes in downy birch (**a**) and silver birch (**b**) plants: white columns—nanotubes at a concentration of 1 mg/L, gray columns—10 mg/L, black columns—50 mg/L and shaded columns—100 mg/L. Asterisks in the vertical bars represent significant differences between groups according to Duncan's multiple range test at $p < 0.05$.

With an increase in the concentration of MWCNTs to 10 mg/L, the response of plants was observed at the level of *DREB2* and *PR-1*, the expression of which increased by 2.4 and 4.3 times, respectively. A further elevation of MWCNTs concentration contributed to the upregulation of *PR-1* gene, the expression of which increased by 3.4 times relative to the control.

In silver birch, a significant upregulation of *DREB2*, *LEA8*, *PAL*, and *PR-10* genes was observed under 1 mg/L MWCNTs treatment, while 10 mg/L stimulated only *DREB2* and *PR-10* (Figure 9b). Higher concentrations did not regulate the expression of the analyzed genes. Although 100 mg/L nanotubes treatment did not cause significant changes in gene expression in silver birch relative to the control, *DREB2* and *PAL* were significantly downregulated in downy birch.

The results obtained indicate a stimulating effect of MWCNTs at low concentrations (1 and 10 mg/L) on growth parameters and expression of resistance genes in downy and silver birch plants. The concentration of 100 mg/L downregulated growth rate and *DREB2* and *PAL* genes expression in downy birch, which indicates a greater sensitivity of this genotype to high concentrations of MWCNTs.

4. Discussion

The results obtained in two species of birch once again confirm the role of MWCNTs in growth stimulation, which has been reported extensively in various plant species such as wheat [61], bitter melon [62], sorghum [27,37], and tobacco [20,25]. Improvement in growth

rates can occur due to elongation of the root system [14,63], changes in the cell cycle [62], plasma membranes [24], nutrition, and water absorption [27], differentiation of xylem and phloem conductive tissues [64], photosynthesis efficiency [63,65], and metabolism [22].

Nanotubes have a high activity due to their small size, penetrating capability, and availability for plants. They also act at the genetic level, directly regulating gene expression [30]. In our study, the effect of MWCNTs was concentration-dependent. Thus, low concentrations of 1 and 10 mg/L stimulated growth and expression of stress-resistance genes in downy and silver birch. At the same time, the positive influence on morphological parameters and gene expression decreased with the use of elevated MWCNTs concentrations. The data obtained are consistent with the results of other studies [12,30,66]. The positive or negative effect of exposure to nanotubes during seed germination and at the initial stages of shoot growth depended on the size and concentration of nanoparticles [67]. The use of MWCNTs up to 250 μg mL^{-1} stimulated biomass and secondary metabolites accumulation and upregulated biosynthesis of antioxidants in thyme, while the treatment with higher concentrations significantly decreased morphometric parameters [68]. Although exposure to 100 mg/L nanotubes contributed to an increase in morphometric parameters, such as stem length in birch of both studied species and biomass in silver birch, no stimulating effect of high concentration on gene expression was found. The level of *DREB2* and *PAL* transcripts was reduced in downy birch, and the expression of other genes did not differ significantly from the control.

Low content of MWCNTs can show a stimulating effect, while a significant increase in concentrations leads to inhibition of germination and growth [69], intoxication, and oxidative stress [9,70] in plant species. As a result, nanoparticles cause cytotoxic and genotoxic effects [71], disrupt the integrity of cell membranes, and cause chromosomal aberrations, cell division, and DNA damage [72].

It has been shown that the use of both metallic and non-metallic nanoparticles can help reduce the negative impact of abiotic stressors such as drought [73–75], high [76] and low temperatures, heavy metals [77], and salinity [78], and increase resistance to phytopathogens [77] in plants. A positive effect is observed, among other things, due to the activation of protein factors (for example, the LEA family), which protect cells and cellular structures by directlly binding to the surface of proteins or ordering water around bound macromolecules [79]. The LEA genes contain the DRE/CRT (drought-responsive/C-repeat) cis-element promoter [80], which binds to and regulates their expression, and representatives of the DREB (dehydration responsive element binding) transcription factor family [81], which are activated under abiotic stress and exposure to nanoparticles [82]. Thus, exposure to heat contributed to an increase in the expression of *DREB2* in lilies [83], different genotypes of crested wheatgrass [84], concentration of mannitol in lilies [83], cadmium in sorghum [85], and salinity in a number of plants [83,85,86].

Stress induces the synthesis of ROS by plants, which helps to reduce the infection and spread of phytopathogens and raise systemic and local defense mechanisms, such as the activation of pathogenesis-related (PR) genes [87]. In high concentrations, ROS become detrimental to cellular structures. Synthesis of phenolic compounds, activation of enzymes for the synthesis of phenylpropanoids (phenylalanine ammonium lyase (PAL)) and flavonoids under stress contributes to neutralization of ROS, protecting plants from the effects of heavy metals influence [88], damage, pathogens, mineral deficiency, and temperature stress [89]. Treatment with 4 and 40 mg L^{-1} selenium nanoparticles (nSe) and 25 μM nitric oxide (NO) of chicory seedlings [82] and exposure of momordica seedlings to 1, 4, 10, 30, and 50 mg L^{-1} nSe of momordica seedlings [90] contributed to a significant increase in gene expression and activity of the PAL enzyme, the accumulation of dissolved phenols. The use of selenium nanoparticles contributed to an increase in *PAL* expression in oats [91].

Thus, low concentrations of nanoparticles contribute to the protection of plants from stress and maintain the ratio between the level of ROS and neutralizing factors, stimulating the synthesis of antioxidant enzymes. At the same time, elevated concentrations of

nanoparticles act as a stress factor, provoking excessive synthesis of ROS, causing damage and cell death.

Plant genotypic differences can also affect the ability to receive external and internal stimuli, including exposure to nanoparticles. Although treatment with the lowest doses, 1 and 10 mg/L MWCNTs, had the greatest stimulating effect on the expression of stress resistance genes in both birch species, increasing the concentration to 10 mg/L turned out to be favorable for growth processes and biomass accumulation in silver birch, but not in downy birch. Treatment with 100 mg/L nanotubes did not affect the expression of *Betula pendula* genes, but promoted growth and biomass. At the same time, when the concentration was increased to 100 mg/L, downy birch showed a tendency to decrease in biomass, and the expression of the *DREB2* and *PAL* genes decreased significantly relative to the control, demonstrating a greater sensitivity of this species to the effect of nanotubes.

MWCNTs can act as signaling molecules in plants, initiating the synthesis of secondary metabolites [68]. The scheme of this process is shown in Figure 10.

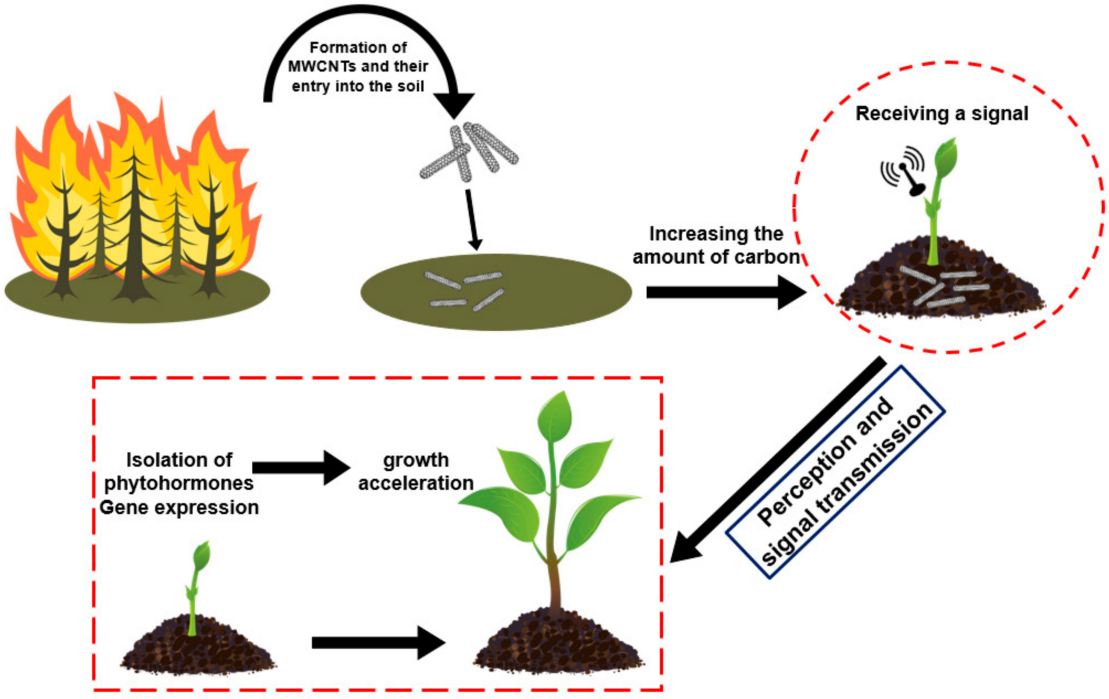

Figure 10. Possible process of the influence of MWCNTs on the synthesis of secondary metabolites.

During forest fires, the combustion of wood produces a certain amount of carbon nanomaterials, including CNTs. It is possible that the presence of an increased content of carbon nanotubes in the soil is perceived by a woody plant as a signal for the possibility of occupying a free area. In addition, forest fires are more likely to occur in warmer areas, so it is possible that southern plant species are more sensitive to carbon nanomaterials.

A significant improvement in growth rates and activation of stress resistance genes over a short period of study indicate a positive effect of MWCNTs in low concentrations on downy and silver birch plants and open up the prospect of their use in the cultivation of woody plants.

5. Conclusions

In this work, we studied the effect of aqueous colloids of multi-walled carbon nanotubes with an outer diameter of 10–30 nm and a length of about 2 μm at a concentration of 1, 10, 50, and 100 mg/L on morphometric parameters and the level of expression of stress resistance genes in *Betula pubescens* Ehrh. and *B. pendula* Roth. plants in greenhouse conditions. MWCNTs were characterized by FTIR analysis, Raman spectroscopy, and trans-

mission and scanning electron microscopy. A high stability of MWCNT colloidal solutions was established by zeta potential measurement (above −39 mV for all concentrations). The study of growth parameters showed a significant increase in the length and diameter of the shoot in the studied plants ($p < 0.05$). The dry biomass of the leaf increased by 30%, the stem by 42%, and the root by 49% when using MWCNTs at a concentration of 10 mg/L ($p < 0.05$). A concentration-dependent effect was noted upon treatment of the studied plants with nanoparticles. The influence of nanoparticles also depended on the genotype, and *Betula pubescens* was more sensitive to the effects of high and low concentrations. The expression of the stress resistance genes *DREB2* and *PR-10* significantly increased under the influence of 1 mg/L MWCNTs on plants of both species. At the same time, the decrease in gene expression upon treatment with 100 mg/L MWCNTs may be associated with their negative effect at high concentrations. The stimulation of growth and stress resistance genes expression by nanotubes open up prospects for their use in biotechnology of woody plants. A possible pre-adaptation signaling mechanism that may be associated with forest fires has also been noted. The results obtained provide a new alternative way of using carbon nanomaterials in woody crop production. Changes in growth rates and activation of stress resistance genes over a short period of study indicate a positive effect of MWCNTs at low concentrations (1 and 10 mg/L) on woody plants, opening up the prospect of their use in the cultivation of woody plants ($p < 0.05$). However, there is still a gap in knowledge, especially at the molecular level, and more research is needed in this regard.

Author Contributions: Conceptualization, K.V.Z., P.M.E., T.A.G. and A.A.G.; methodology, K.V.Z., P.M.E. and T.A.G.; software, K.V.Z. and E.V.T.; validation, E.V.T. and O.V.Z.; formal analysis, A.A.G., O.V.Z. and A.V.S.; investigation, K.V.Z., P.M.E., T.A.G. and A.V.S.; resources, E.V.T., P.M.E. and A.A.G.; data curation, O.V.Z. and A.V.S.; writing—original draft preparation, K.V.Z. and T.A.G.; writing—review and editing, O.V.Z. and A.A.G.; visualization, K.V.Z. and T.A.G.; supervision, A.V.S. and E.V.T.; project administration, P.M.E., A.A.G. and E.V.T.; funding acquisition, P.M.E. and K.V.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Russian Scientific Foundation grant 21-14-00233 (research on tree crops), and by the Ministry of Science and Higher Education of the Russian Federation, the contract 075-15-2021-709, unique identifier of the project RF-2296.61321X0037 (equipment maintenance).

Acknowledgments: Voronezh State University of Forestry and Technologies named after G.F. Morozov (VSUFT) for the opportunity to conduct research.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Mishra, S.; Keswani, C.; Abhilash, P.C.; Fraceto, L.F.; Singh, H.B. Integrated Approach of Agri-nanotechnology: Challenges and Future Trends. *Front. Plant Sci.* **2017**, *8*, 471. [CrossRef]
2. Abideen, Z.; Hanif, M.; Munir, N.; Nielsen, B.L. Impact of Nanomaterials on the Regulation of Gene Expression and Metabolomics of Plants under Salt Stress. *Plants* **2022**, *11*, 691. [CrossRef] [PubMed]
3. Wu, H.; Li, Z. Recent advances in nano-enabled agriculture for improving plant performance. *Crop J.* **2022**, *10*, 1–12. [CrossRef]
4. Khan, F.S.A.; Mubarak, N.M.; Khalid, M.; Walvekar, R.; Abdullah, E.C.; Ahmad, A.; Karri, R.R.; Pakalapati, H. Functionalized multi-walled carbon nanotubes and hydroxyapatite nanorods reinforced with polypropylene for biomedical application. *Sci. Rep.* **2021**, *11*, 843. [CrossRef]
5. Vardharajula, S.; Ali, S.Z.; Tiwari, P.M.; Eroğlu, E.; Vig, K.; Dennis, V.A.; Singh, S.R. Functionalized carbon nanotubes: Biomedical applications. *Int. J. Nanomed.* **2012**, *7*, 5361–5374. [CrossRef]
6. Díez-Pascual, A.M. Chemical Functionalization of Carbon Nanotubes with Polymers: A Brief Overview. *Macromol* **2021**, *1*, 64–83. [CrossRef]
7. Subagio, A.; Prihastanti, E.; Ngadiwiyana, N. Application of Functionalized Multi-Walled Carbon Nanotubes for Growth Enhancement of Mustard Seed Germination. *Indones. J. Chem.* **2019**, *20*, 120. [CrossRef]
8. Sharifi, P.; Bidabadi, S.S.; Zaid, A.; Abdel Latef, A.A.H. Efficacy of multi-walled carbon nanotubes in regulating growth performance, total glutathione and redox state of *Calendula officinalis* L. cultivated on Pb and Cd polluted soil. *Ecotoxicol. Environ. Saf.* **2021**, *213*, 112051. [CrossRef]
9. Rahmani, N.; Radjabian, T.; Soltani, B.M. Impacts of foliar exposure to multi-walled carbon nanotubes on physiological and molecular traits of *Salvia verticillata* L., as a medicinal plant. *Plant Physiol. Biochem.* **2020**, *150*, 27–38. [CrossRef]

10. Zaytseva, O. Analysis of Phytotoxicity and Plant Growth Stimulation by Multi-Walled Carbon Nanotubes. Ph.D. Thesis, University of Hohenheim, Stuttgart, Germany, 2017.
11. Joshi, A.; Sharma, L.; Kaur, S.; Dharamvir, K.; Nayyar, H.; Verma, G. Plant Nanobionic Effect of Multi-walled Carbon Nanotubes on Growth, Anatomy, Yield and Grain Composition of Rice. *BioNanoScience* **2020**, *10*, 430–445. [CrossRef]
12. Ghasempour, M.; Iranbakhsh, A.; Ebadi, M.; Oraghi Ardebili, Z. Multi-walled carbon nanotubes improved growth, anatomy, physiology, secondary metabolism, and callus performance in *Catharanthus roseus*: An in vitro study. *3 Biotech* **2019**, *9*, 404. [CrossRef] [PubMed]
13. Iijima, S. Helical microtubules of graphitic carbon. *Nature* **1991**, *354*, 56–58. [CrossRef]
14. Yan, S.; Zhao, L.; Li, H.; Zhang, Q.; Tan, J.; Huang, M.; He, S.; Li, L. Single-walled carbon nanotubes selectively influence maize root tissue development accompanied by the change in the related gene expression. *J. Hazard. Mater.* **2013**, *246–247*, 110–118. [CrossRef] [PubMed]
15. Zhang, H.; Yue, M.; Zheng, X.; Xie, C.; Zhou, H.; Li, L. Physiological Effects of Single- and Multi-Walled Carbon Nanotubes on Rice Seedlings. *IEEE Trans. Nanobiosci.* **2017**, *16*, 563–570. [CrossRef]
16. Sasidharan, A.; Panchakarla, L.S.; Chandran, P.; Menon, D.; Nair, S.; Rao, C.N.; Koyakutty, M. Differential nano-bio interactions and toxicity effects of pristine versus functionalized graphene. *Nanoscale* **2011**, *3*, 2461–2464. [CrossRef]
17. Pourkhaloee, A.; Haghighi, M.; Saharkhiz, M.J.; Jouzi, H.; Doroodmand, M.M. Carbon Nanotubes Can Promote Seed Germination via Seed Coat Penetration. *Seed Technol.* **2011**, *33*, 155–169.
18. Khodakovskaya, M.V.; Kim, B.-S.; Kim, J.N.; Alimohammadi, M.; Dervishi, E.; Mustafa, T.; Cernigla, C.E. Carbon Nanotubes as Plant Growth Regulators: Effects on Tomato Growth, Reproductive System, and Soil Microbial Community. *Small* **2013**, *9*, 115–123. [CrossRef]
19. Flores, D.; Schmidt-Duran, A.; Alvarado, L. Effect of Using Two Different Types of Carbon Nanotubes for Blackberry (*Rubus adenotrichos*) in Vitro Plant Rooting, Growth and Histology. *Am. J. Plant Sci.* **2014**, *5*, 3510–3518. [CrossRef]
20. Khodakovskaya, M.V.; de Silva, K.; Biris, A.S.; Dervishi, E.; Villagarcia, H. Carbon Nanotubes Induce Growth Enhancement of Tobacco Cells. *ACS Nano* **2012**, *6*, 2128–2135. [CrossRef]
21. Wu, H.; Li, Z. Nano-Enabled Agriculture: How Do Nanoparticles Cross Barriers in Plants? *Plant Commun.* **2022**, *3*, 100346. [CrossRef]
22. Hatami, M.; Hadian, J.; Ghorbanpour, M. Mechanisms underlying toxicity and stimulatory role of single-walled carbon nanotubes in Hyoscyamus niger during drought stress simulated by polyethylene glycol. *J. Hazard. Mater.* **2017**, *324*, 306–320. [CrossRef] [PubMed]
23. Sun, W.; Shahrajabian, M.H.; Huang, Q. Soybean seeds treated with single walled carbon nanotubes (SwCNTs) showed enhanced drought tolerance during germination. *Int. J. Adv. Biol. Biomed. Res.* **2020**, *8*, 9–16. [CrossRef]
24. Martínez-Ballesta, M.C.; Zapata, L.; Chalbi, N.; Carvajal, M. Multiwalled carbon nanotubes enter broccoli cells enhancing growth and water uptake of plants exposed to salinity. *J. Nanobiotechnol.* **2016**, *14*, 42. [CrossRef] [PubMed]
25. Martinez-Ballesta, M.C.; Chelbi, N.; Lopez-Zaplana, A.; Carvajal, M. Discerning the mechanism of the multiwalled carbon nanotubes effect on root cell water and nutrient transport. *Plant Physiol. Biochem.* **2020**, *146*, 23–30. [CrossRef] [PubMed]
26. Tripathi, S.; Sonkar, S.K.; Sarkar, S. Growth stimulation of gram (*Cicer arietinum*) plant by water soluble carbon nanotubes. *Nanoscale* **2011**, *3*, 1176–1181. [CrossRef]
27. Lahiani, M.H.; Dervishi, E.; Chen, J.; Nima, Z.; Gaume, A.; Biris, A.S.; Khodakovskaya, M.V. Impact of Carbon Nanotube Exposure to Seeds of Valuable Crops. *ACS Appl. Mater. Interfaces* **2013**, *5*, 7965–7973. [CrossRef]
28. Yousefi, S.; Kartoolinejad, D.; Naghdi, R. Effects of priming with multi-walled carbon nanotubes on seed physiological characteristics of Hopbush (*Dodonaeaviscosa* L.) under drought stress. *Int. J. Environ. Stud.* **2017**, *74*, 528–539. [CrossRef]
29. Khodakovskaya, M.; Dervishi, E.; Mahmood, M.; Xu, Y.; Li, Z.; Watanabe, F.; Biris, A.S. Carbon Nanotubes Are Able to Penetrate Plant Seed Coat and Dramatically Affect Seed Germination and Plant Growth. *ACS Nano* **2009**, *3*, 3221–3227. [CrossRef]
30. Mathew, S.; Tiwari, D.K.; Tripathi, D. Interaction of carbon nanotubes with plant system: A review. *Carbon Lett.* **2021**, *31*, 167–176. [CrossRef]
31. Larue, C.; Pinault, M.; Czarny, B.; Georgin, D.; Jaillard, D.; Bendiab, N.; Mayne-L'Hermite, M.; Taran, F.; Dive, V.; Carrière, M. Quantitative evaluation of multi-walled carbon nanotube uptake in wheat and rapeseed. *J. Hazard. Mater.* **2012**, *227–228*, 155–163. [CrossRef]
32. Wang, C.; Liu, H.; Chen, J.; Tian, Y.; Shi, J.; Li, D.; Guo, C.; Ma, Q. Carboxylated multi-walled carbon nanotubes aggravated biochemical and subcellular damages in leaves of broad bean (*Vicia faba* L.) seedlings under combined stress of lead and cadmium. *J. Hazard. Mater.* **2014**, *274*, 404–412. [CrossRef] [PubMed]
33. Xin, X.; Zhao, F.; Judy, J.D.; He, Z. Copper Stress Alleviation in Corn (*Zea mays* L.): Comparative Efficiency of Carbon Nanotubes and Carbon Nanoparticles. *NanoImpact* **2022**, *25*, 100381. [CrossRef] [PubMed]
34. Stampoulis, D.; Sinha, S.K.; White, J.C. Assay-Dependent Phytotoxicity of Nanoparticles to Plants. *Environ. Sci. Technol.* **2009**, *43*, 9473–9479. [CrossRef] [PubMed]
35. Lin, D.; Xing, B. Phytotoxicity of nanoparticles: Inhibition of seed germination and root growth. *Environ. Pollut.* **2007**, *150*, 243–250. [CrossRef] [PubMed]
36. Ghosh, M.; Chakraborty, A.; Bandyopadhyay, M.; Mukherjee, A. Multi-walled carbon nanotubes (MWCNT): Induction of DNA damage in plant and mammalian cells. *J. Hazard. Mater.* **2011**, *197*, 327–336. [CrossRef]

37. Zhai, G.; Gutowski, S.M.; Walters, K.S.; Yan, B.; Schnoor, J.L. Charge, Size, and Cellular Selectivity for Multiwall Carbon Nanotubes by Maize and Soybean. *Environ. Sci. Technol.* **2015**, *49*, 7380–7390. [CrossRef]
38. Khodakovskaya, M.V.; de Silva, K.; Nedosekin, D.A.; Dervishi, E.; Biris, A.S.; Shashkov, E.V.; Galanzha, E.I.; Zharov, V.P. Complex genetic, photothermal, and photoacoustic analysis of nanoparticle-plant interactions. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 1028–1033. [CrossRef]
39. Landa, P.; Vankova, R.; Andrlova, J.; Hodek, J.; Marsik, P.; Storchova, H.; White, J.C.; Vanek, T. Nanoparticle-specific changes in Arabidopsis thaliana gene expression after exposure to ZnO, TiO_2, and fullerene soot. *J. Hazard. Mater.* **2012**, *241–242*, 55–62. [CrossRef]
40. Jordan, J.T.; Singh, K.P.; Cañas-Carrell, J.E. Carbon-based nanomaterials elicit changes in physiology, gene expression, and epigenetics in exposed plants: A review. *Curr. Opin. Environ. Sci. Health* **2018**, *6*, 29–35. [CrossRef]
41. Hao, Y.; Ma, C.; Zhang, Z.; Song, Y.; Cao, W.; Guo, J.; Zhou, G.; Rui, Y.; Liu, L.; Xing, B. Carbon nanomaterials alter plant physiology and soil bacterial community composition in a rice-soil-bacterial ecosystem. *Environ. Pollut.* **2018**, *232*, 123–136. [CrossRef]
42. Green, M.J. Analysis and measurement of carbon nanotube dispersions: Nanodispersion versus macrodispersion. *Polym. Int.* **2010**, *59*, 1319–1322. [CrossRef]
43. Irin, F.; Shrestha, B.; Cañas, J.E.; Saed, M.A.; Green, M.J. Detection of carbon nanotubes in biological samples through microwave-induced heating. *Carbon* **2012**, *50*, 4441–4449. [CrossRef]
44. Mukherjee, A.; Majumdar, S.; Servin, A.D.; Pagano, L.; Dhankher, O.P.; White, J.C. Carbon Nanomaterials in Agriculture: A Critical Review. *Front. Plant Sci.* **2016**, *7*, 172. [CrossRef] [PubMed]
45. Liné, C.; Larue, C.; Flahaut, E. Carbon nanotubes: Impacts and behaviour in the terrestrial ecosystem—A review. *Carbon* **2017**, *123*, 767–785. [CrossRef]
46. Husen, A.; Siddiqi, K.S. Carbon and fullerene nanomaterials in plant system. *J. Nanobiotechnol.* **2014**, *12*, 16. [CrossRef]
47. Grodetskaya, T.; Fedorova, O.; Evlakov, P. Optimized method for RNA extraction from leaves of forest tree species. *IOP Conf. Ser. Earth Environ. Sci.* **2021**, *875*, 012008. [CrossRef]
48. Grodetskaia, T.A.; Fedorova, O.A.; Evlakov, P.M.; Baranov, O.Y.; Zakharova, O.V.; Gusev, A.A. Effect of Copper Oxide and Silver Nanoparticles on the Development of Tolerance to *Alternaria alternata* in Poplar in Vitro Clones. *Nanobiotechnol. Rep.* **2021**, *16*, 231–238. [CrossRef]
49. Ritonga, F.; Ngatia, J.; Song, R.; Farooq, U.; Somadona, S.; Lestari, A.T.; Chen, S. Abiotic stresses induced physiological, biochemical, and molecular changes in *Betula platyphylla*: A review. *Silva Fenn.* **2021**, *55*, 10516. [CrossRef]
50. Wen, X.; Wang, J.; Zhang, D.; Wang, Y. A Gene Regulatory Network Controlled by BpERF2 and BpMYB102 in Birch under Drought Conditions. *Int. J. Mol. Sci.* **2019**, *20*, 3071. [CrossRef]
51. Zan, T.; Li, L.; Li, J.; Zhang, L.; Li, X. Genome-wide identification and characterization of late embryogenesis abundant protein-encoding gene family in wheat: Evolution and expression profiles during development and stress. *Gene* **2020**, *736*, 144422. [CrossRef]
52. Moradbeygi, H.; Jamei, R.; Heidari, R.; Darvishzadeh, R. Fe_2O_3 nanoparticles induced biochemical responses and expression of genes involved in rosmarinic acid biosynthesis pathway in Moldavian balm under salinity stress. *Physiol. Plant.* **2020**, *169*, 555–570. [CrossRef] [PubMed]
53. Yao, J.; Luo, Y.; Zhang, Z.; Li, J.; Li, C.; Li, C.; Guo, Z.; Wang, L.; Zhang, W.; Zhao, H.; et al. The Development of Real-Time Digital PCR Technology Using an Improved Data Classification Method. *Biosens. Bioelectron.* **2022**, *199*, 113873. [CrossRef] [PubMed]
54. Jorio, A.; Saito, R. Raman spectroscopy for carbon nanotube applications. *J. Appl. Phys.* **2021**, *129*, 021102. [CrossRef]
55. Goh, B.; Kim, K.J.; Park, C.-L.; Kim, E.S.; Kim, S.H.; Choi, J. In-Plane Thermal Conductivity of Multi-Walled Carbon Nanotube Yarns under Mechanical Loading. *Carbon* **2021**, *184*, 452–462. [CrossRef]
56. Dobrzańska-Danikiewicz, A.; Wolany, W.; Lukowiec, D.; Jurkiewicz, K.; Niedziałkowski, P. Characteristics of multiwalled carbon nanotubes-rhenium nanocomposites with varied rhenium mass fractions. *Nanomater. Nanotechnol.* **2017**, *7*, 184798041770717. [CrossRef]
57. Shuaib, M.; Mohammed, N.; Burhanudin, Z. Optimization of the Production of Aligned CNTs Array as the Gas Sensing Element. *Mater. Sci. Forum* **2013**, *756*, 156–163. [CrossRef]
58. Kumar, A.; Ghosh, P.K.; Yadav, K.L.; Kumar, K. Thermo-mechanical and anti-corrosive properties of MWCNT/epoxy nanocomposite fabricated by innovative dispersion technique. *Compos. Part B Eng.* **2017**, *113*, 291–299. [CrossRef]
59. Chen, J.; Han, J. Effect of Hydroxylated Carbon Nanotubes on the Thermal and Electrical Properties of Derived Epoxy Composite Materials. *Results Phys.* **2020**, *18*, 103246. [CrossRef]
60. Nitesh; Kumar, A.; Saini, S.; Yadav, K.L.; Ghosh, P.K.; Rathi, A. Morphology and tensile performance of MWCNT/TiO_2-epoxy nanocomposite. *Mater. Chem. Phys.* **2022**, *277*, 125336. [CrossRef]
61. Joshi, A.; Kaur, S.; Dharamvir, K.; Nayyar, H.; Verma, G. Multi-walled carbon nanotubes applied through seed-priming influence early germination, root hair, growth and yield of bread wheat (*Triticum aestivum* L.). *J. Sci. Food Agric.* **2018**, *98*, 3148–3160. [CrossRef]
62. Seddighinia, F.S.; Iranbakhsh, A.; Oraghi Ardebili, Z.; Nejad Satari, T.; Soleimanpour, S. Seed Priming with Cold Plasma and Multi-walled Carbon Nanotubes Modified Growth, Tissue Differentiation, Anatomy, and Yield in Bitter Melon (*Momordica charantia*). *J. Plant Growth Regul.* **2020**, *39*, 87–98. [CrossRef]

63. Joshi, A.; Kaur, S.; Singh, P.; Dharamvir, K.; Nayyar, H.; Verma, G. Tracking multi-walled carbon nanotubes inside oat (*Avena sativa* L.) plants and assessing their effect on growth, yield, and mammalian (human) cell viability. *Appl. Nanosci.* **2018**, *8*, 1399–1414. [CrossRef]
64. Agrawal, S.; Kumar, V.; Kumar, S.; Shahi, S.K. Plant development and crop protection using phytonanotechnology: A new window for sustainable agriculture. *Chemosphere* **2022**, *299*, 134465. [CrossRef] [PubMed]
65. Lahiani, M.H.; Nima, Z.A.; Villagarcia, H.; Biris, A.S.; Khodakovskaya, M.V. Assessment of Effects of the Long-Term Exposure of Agricultural Crops to Carbon Nanotubes. *J. Agric. Food Chem.* **2018**, *66*, 6654–6662. [CrossRef] [PubMed]
66. Zhang, X.; Cao, H.; Wang, H.; Zhang, R.; Jia, H.; Huang, J.; Zhao, J.; Yao, J. Effects of graphene on morphology, microstructure and transcriptomic profiling of *Pinus tabuliformis* Carr. roots. *PLoS ONE* **2021**, *16*, e0253812. [CrossRef]
67. Szőllősi, R.; Molnár, Á.; Kondak, S.; Kolbert, Z. Dual Effect of Nanomaterials on Germination and Seedling Growth: Stimulation vs. Phytotoxicity. *Plants* **2020**, *9*, 1745. [CrossRef]
68. Samadi, S.; Saharkhiz, M.J.; Azizi, M.; Samiei, L.; Ghorbanpour, M. Multi-walled carbon nanotubes stimulate growth, redox reactions and biosynthesis of antioxidant metabolites in *Thymus daenensis* Celak. In vitro. *Chemosphere* **2020**, *249*, 126069. [CrossRef]
69. Chen, M.; Zhou, S.; Zhu, Y.; Sun, Y.; Zeng, G.; Yang, C.; Xu, P.; Yan, M.; Liu, Z.; Zhang, W. Toxicity of carbon nanomaterials to plants, animals and microbes: Recent progress from 2015-present. *Chemosphere* **2018**, *206*, 255–264. [CrossRef]
70. Samadi, S.; Asgari Lajayer, B.; Moghiseh, E.; Rodríguez-Couto, S. Effect of carbon nanomaterials on cell toxicity, biomass production, nutritional and active compound accumulation in plants. *Environ. Technol. Innov.* **2021**, *21*, 101323. [CrossRef]
71. Giorgetti, L. Chapter 4—Effects of Nanoparticles in Plants: Phytotoxicity and Genotoxicity Assessment. In *Nanomaterials in Plants, Algae and Microorganisms*; Tripathi, D.K., Ahmad, P., Sharma, S., Chauhan, D.K., Dubey, N.K., Eds.; Academic Press: Cambridge, MA, USA, 2019; pp. 65–87.
72. Pacheco, I.; Buzea, C. Nanoparticle Uptake by Plants: Beneficial or Detrimental? In *Phytotoxicity of Nanoparticles*; Faisal, M., Saquib, Q., Alatar, A.A., Al-Khedhairy, A.A., Eds.; Springer International Publishing: Cham, Switzerland, 2018; pp. 1–61.
73. Raeisi Sadati, S.Y.; Jahanbakhsh Godehkahriz, S.; Ebadi, A.; Sedghi, M. Zinc Oxide Nanoparticles Enhance Drought Tolerance in Wheat via Physio-Biochemical Changes and Stress Genes Expression. *Iran. J. Biotechnol.* **2022**, *20*, 12–24. [CrossRef]
74. Linh, T.; Mai, N.; Hoe, P.; Lien, L.; Ban, N.; Hien, L.; Hoai Chau, N.; Van, N. Metal-Based Nanoparticles Enhance Drought Tolerance in Soybean. *J. Nanomater.* **2020**, *2020*, 4056563. [CrossRef]
75. Attaran Dowom, S.; Karimian, Z.; Mostafaei Dehnavi, M.; Samiei, L. Chitosan nanoparticles improve physiological and biochemical responses of *Salvia abrotanoides* (Kar.) under drought stress. *BMC Plant Biol.* **2022**, *22*, 364. [CrossRef]
76. Saha, G.; Mostofa, M.G.; Rahman, M.M.; Tran, L.-S.P. Silicon-mediated heat tolerance in higher plants: A mechanistic outlook. *Plant Physiol. Biochem.* **2021**, *166*, 341–347. [CrossRef]
77. Ali, F.; Bano, A.; Fazal, A. Recent methods of drought stress tolerance in plants. *Plant Growth Regul.* **2017**, *82*, 363–375. [CrossRef]
78. Moradi, P.; Vafaee, Y.; Mozafari, A.A.; Tahir, N.A.-r. Silicon Nanoparticles and Methyl Jasmonate Improve Physiological Response and Increase Expression of Stress-related Genes in Strawberry cv. Paros Under Salinity Stress. *Silicon* **2022**, *14*, 10559–10569. [CrossRef]
79. Mertens, J.; Aliyu, H.; Cowan, D.A. LEA Proteins and the Evolution of the WHy Domain. *Appl. Environ. Microbiol.* **2018**, *84*, e00539-18. [CrossRef] [PubMed]
80. Yumurtaci, A. Diverse Aspects of ABA Signaling under Drought Stress in Wheat *Glob. J. Bot. Sci.* **2020**, *8*, 30–39. [CrossRef]
81. Erpen, L.; Devi, H.S.; Grosser, J.W.; Dutt, M. Potential use of the DREB/ERF, MYB, NAC and WRKY transcription factors to improve abiotic and biotic stress in transgenic plants. *Plant Cell Tissue Organ Cult.* **2018**, *132*, 1–25. [CrossRef]
82. Abedi, S.; Iranbakhsh, A.; Oraghi Ardebili, Z.; Ebadi, M. Nitric oxide and selenium nanoparticles confer changes in growth, metabolism, antioxidant machinery, gene expression, and flowering in chicory (*Cichorium intybus* L.): Potential benefits and risk assessment. *Environ. Sci. Pollut. Res.* **2021**, *28*, 3136–3148. [CrossRef]
83. Wu, Z.; Liang, J.; Zhang, S.; Zhang, B.; Zhao, Q.; Li, G.; Yang, X.; Wang, C.; He, J.; Yi, M. A Canonical DREB2-Type Transcription Factor in Lily Is Post-translationally Regulated and Mediates Heat Stress Response. *Front. Plant Sci.* **2018**, *9*, 243. [CrossRef]
84. Sheikh-Mohamadi, M.-H.; Etemadi, N.; Arab, M.M.; Aalifar, M.; Arab, M. Physiological and Ascorbate-Glutathione pathway-related genes responses under drought and heat stress in crested wheatgrass. *Sci. Hortic.* **2018**, *242*, 195–206. [CrossRef]
85. Akbudak, M.A.; Filiz, E.; Kontbay, K. DREB2 (dehydration-responsive element-binding protein 2) type transcription factor in sorghum (*Sorghum bicolor*): Genome-wide identification, characterization and expression profiles under cadmium and salt stresses. *3 Biotech* **2018**, *8*, 426. [CrossRef] [PubMed]
86. Arabbeigi, M.; Arzani, A.; Majidi, M.M. Expression Profiles of *P5CS* and *DREB2* Genes under Salt Stress in *Aegilops cylindrica*. *Russ. J. Plant Physiol.* **2019**, *66*, 583–590. [CrossRef]
87. Vargas-Hernandez, M.; Macias-Bobadilla, I.; Guevara-Gonzalez, R.G.; Rico-Garcia, E.; Ocampo-Velazquez, R.V.; Avila-Juarez, L.; Torres-Pacheco, I. Nanoparticles as Potential Antivirals in Agriculture. *Agriculture* **2020**, *10*, 444. [CrossRef]
88. Mitra, M.; Agarwal, P.; Roy, S. Plant Response to Heavy Metal Stress: An Insight into the Molecular Mechanism of Transcriptional Regulation. In *Plant Transcription Factors*; Elsevier: Amsterdam, The Netherlands, 2023; pp. 337–367.
89. Chalker-Scott, L.; Fuchigami, L.H. The role of phenolic compounds in plant stress responses. In *Low Temperature Stress Physiology in Crops*; CRC Press: Boca Raton, FL, USA, 1989; pp. 68–76.

90. Rajaee Behbahani, S.; Iranbakhsh, A. Red elemental selenium nanoparticles mediated substantial variations in growth, tissue differentiation, metabolism, gene transcription, epigenetic cytosine DNA methylation, and callogenesis in bittermelon (*Momordica charantia*); an in vitro experiment. *PLoS ONE* **2020**, *15*, e0235556. [CrossRef] [PubMed]
91. Asgari, F.; Majd, A.; Jonoubi, P.; Najafi, F. Effects of silicon nanoparticles on molecular, chemical, structural and ultrastructural characteristics of oat (*Avena sativa* L.). *Plant Physiol. Biochem.* **2018**, *127*, 152–160. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.