Article

Titanium Trisulfide Nanoribbons Affect the Downy Birch and Poplar × Aspen Hybrid in Plant Tissue Culture via the Emission of Hydrogen Sulfide

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Abstract: Plant tissue culture is an important tool for accelerated vegetative reproduction of woody plants. To increase the efficiency of this method, it is necessary to search for new growth stimulators and protectors of microshoots. Two-dimensional (2D) nanomaterials are highly promising for applications in medicine and biotechnologies. We have studied the effects of TiS₃ nanoribbons with the following mean dimensions: thickness less than 100 nm, length 1–10 µm and width 0.4–1 µm upon poplar × aspen hybrid and downy birch microclones in plant tissue culture. We have found the effectiveness of this particular nanomaterial as a sterilizing and stimulating agent in the initial growth stage and as a rhizogenesis-activating agent in the rooting stage. We analyzed a wide range of TiS₃ concentrations in the nutrient media and identified 1.5 and 3 µg/L as optimal. However, concentration-dependent toxic effects manifesting themselves through microclones viability suppression have been discovered in the groups exposed to 6 and 15 µg/L TiS₃. We have established that plant reaction to TiS₃ treatment is specific for different plant species, i.e., downy birch is generally more tolerant than poplar × aspen hybrid. Here, we propose that the protective and stimulating effects of titanium trisulfide as well as its toxicity in high concentrations can be explained by the release of hydrogen sulfide as a result of aqueous hydrolysis of nanoribbons and its effect on plants. Additional studies are required in order to assess all biological effects produced by TiS₃ nanoribbons at further stages of ontogenetic development and to identify the mechanisms of their action.

Keywords: downy birch; poplar × aspen hybrid; plant tissue culture; 2D transition metal chalcogenides; TiS₃ nanoribbons; sterilizing and stimulating agent; hydrogen sulfide

1. Introduction

The downy birch (*B. pubescens* Ehrh.) is typical in northern regions [1] and provides raw material for paper and paperboard, pulp, plywood, veneer and sawn goods, particle board, fiberboard and firewood [2]. Poplars (genus *Populus*) are the model organisms for forest biotechnology, and genetic modification is more advanced for this genus than other trees [3]. Poplar and its hybrids are useful for energetic plantations [4] and the phytoremediation of soils [5] and as ornamental plants [6]. The selection of the best clonal lines and genotypes for more effective breeding of species such as birch, poplar and its hybrids should be accompanied by more convenient and productive methods of vegetative propagation. Despite many years of research in the field of plant tissue culture, callus induction, organogenesis and somatic embryogenesis of adult birches and poplars, like

other hardwood trees, are still limited by genotypic, physiological and environmental factors [7–10]. Here, the main problem is the low production of viable plantlets from somatic embryos. The culture media and environmental conditions have not yet been optimized for many species/genotypes, and the use of alternative or new chemicals may prove to be more effective than conventional culture media [11]. Therefore, many studies have been devoted to the search for new stimulators of micropropagation of these species [12–16].

Multiple examples prove that nanomaterials can be successfully applied in plant tissue culture [17,18]. Nanoparticles (NPs) are widely used to boost seed germination and enhance plant growth and yield, and they can facilitate genetic modifications, improve bioactive substances production and provide plant protection against diseases [19–21]. There are reports showing that treatment with NPs removes microbial contaminants from explants and promotes such processes as callus induction, organogenesis, somatic embryogenesis, etc. [22]. It has been shown that the treatment of arabidopsis seeds, potato leaves and tomato cotyledons with 100 mg/L silver NPs for 1 and 5 min resulted in 100% decontamination of the samples without affecting their viability [23]. Silver NPs added at 4 mg/L to the growing medium reduced phytopathogenic activity in olive explants and had no adverse effects on the plantlets [24]. Additionally, pathogen-free cultures were obtained when potato and tobacco axillary buds were disinfected and cultivated in MS media with added titanium dioxide [25]. A number of research works have revealed a positive effect of nanoparticles on callus induction and on shoots regeneration and growth. For example, the visual inspection of *Tecomella undulata* explants grown in MS media with 10 mg/L silver NPs, 2.5 mg/L 6-benzylaminopurine (BAP) and 0.1 mg/L indole acetic acid (IAA) revealed an increase in the mean number of fresh shoots per explants, in the percentage of explants producing shoots and in callus formation [26]. Copper NPs at 0.5 mg/L and cobalt NPs at 0.8 mg/L were added to MS nutrient medium and led to an increase in microplant height, the quantity of internodes and the quantity of shoots and to the improved rooting activity of *Mentha longifolia* [27]. In the study performed on *Stevia rebaudiana* internode explants, the highest percentage of shoot formation (89.6%) was obtained at 1 mg/L of ZnO nanoparticles concentration in MS medium [28]. The exploration of the potential effects of multiwalled carbon nanotubes (MWCNTs) on callus induction in *Satureja khuzestanica* leaf explants has revealed a significant improvement in callus growth in Gamborg's B5 medium treated with 25–50 µg/mL of the nanomaterial, while higher concentrations of the material (100–500 µg/mL) reduced callus biomass [29]. Khodakovskaya et al. [30] reported a 64% increase in tobacco explants callus growth upon treatment with 100 µg/mL MWCNTs compared to the control. The authors found a correlation between the activation of cell growth exposed to MWCNTs and the upregulation of genes involved in cell division (CycB), cell wall formation (NtLRX1) and water transport (NtPIP1).

At the same time, there is evidence of the phytotoxicity of nanoparticles of various types [31,32], including titanium-based nanoparticles. TiO₂ nanoparticles inhibited the rate of seed germination of tomatoes [33], peppermint [34], maize and Narbonne peas [35] and disrupted microtubular networks in *Arabidopsis thaliana* root cells [36].

There are a limited number of research works on the effects of transition metal di- and trichalcogenides on plants. For example, one study showed that MoS₂ had a negligible impact on rice seed germination rate, malonaldehyde (MDA) content and antioxidant enzyme activity [37]. However, the length and biomass of rice root and shoot, chlorophyll content index and expression of aquaporin genes were significantly increased. The authors concluded that MoS₂ promoted rice growth through the promotion of nitrogen source assimilation, the enhancement of photosynthesis, enzymatic-related biochemical reactions, metabolic processes and, subsequently, the acceleration of cell division and expansion.

We screen a wide range of nanoparticles as potential growth promoters and protectors of woody plants during micropropagation [38,39]. A previous study has revealed the positive effect of zirconium trisulfide (ZrS₃) on explants of white poplar × aspen hybrid, birch, willow, oak and pine [39]. At the same time, in another recent study on bacte-

ria [40], we discovered an interesting mechanism of the biological effects of transition metal trichalcogenides, associated with the slow emission of hydrogen sulfide from the surface of the nanomaterial. As the first study of this phenomenon in plants, here we evaluated the effects of a promising 2D material, namely, titanium trisulfide (TiS_3) nanoribbons, on white poplar $\times$ aspen (*Populus alba* $\times$ *Populus tremula*) hybrid and downy birch (*Betula pubescens*) regenerants at various microcloning stages to search for new effective and safe stimulants of vegetative propagation for these important forest trees.

2. Materials and Methods

The studied TiS_3 sample was fabricated by a direct reaction of metallic titanium with elemental sulfur: $\text{Ti} + 3\text{S} = \text{TiS}_3$ in evacuated ampoules at 500–600 °C [41,42].

The obtained nanomaterial sample was characterized via scanning electron (SEM) microscopy (Vega3 Tescan, Brno, Czech Republic), atomic force (AFM) microscopy (AIST-NT, Zelenograd, Russia) and Raman-scattering spectroscopy (Thermo DXR, Thermo Scientific, Waltham, MA, USA).

The analysis of the distribution of metals in the tissues of experimental plants was carried out on scanning electron microscope Neon 40 (Carl Zeiss, Jena, Germany) by energy dispersive (EDS) spectrum analysis. Sample preparation: a weighed portion of plant tissue weighing 250 mg was ground in a cooled mortar in 0.5 mL of distilled water. The resulting homogenate was applied to a substrate, dried at room temperature and placed in the SEM to study.

The concentration of hydrogen sulfide in the solution was measured by the method of reverse iodometric titration. One gram of titanium trisulfide nanoribbons was added to 100 mL of bidistilled water, and the concentration of hydrogen sulfide in water was measured daily for 7 days.

To prepare the colloidal solution, 0.3 g nanoparticles portion was weighed using an analytical balance ViBRA HT (Shinko Denshi, Tokyo, Japan, (accuracy ± 0.0001 g)), poured into 100 mL distilled water ($\text{pH} = 7.1 \pm 0.2$), stirred with a glass rod for 20 s and treated in an ultrasound bath, the Ultrasonic Cleaner CD-4800 (Codyson, Guangdong, China), for 2 min (70 W, 44 Hz, volume—1.4 L). The solution was added to the growth medium to obtain 0.75, 1.5, 3, 6 and 15 $\mu\text{g/L}$ nanomaterial concentrations.

For the in vitro culture initiation, plant cuttings with apical and axillary buds were used. The cuttings were washed with a surface-active agent (Tween-20) and thoroughly rinsed in distilled water. Then, the samples were cut into segments and sterilized for 40 min in the solution of 200 μL 5% sodium hypochlorite (NaOCl) in 200 mL distilled water with a subsequent rinse in distilled water. The major sterilization was performed for 15 min in the solution consisting of 15 mL 5% sodium hypochlorite and 200 mL distilled water in a laminar flow cabinet. The samples were rinsed in sterile distilled water. The sterilized shoots were cut into 1.5–2 cm explants, each with a single axillary bud, and transferred into a Murashige and Skoog nutrient medium (MS0) [43]. All media formulations and plant growth regulators (PGR) were purchased from Duchefa Biochemie (Haarlem, The Netherlands).

The test tubes with the explants were placed in the growth chamber at +24 °C under LD 16:8 photoperiod, 5000 lx light intensity and 70% relative air humidity.

The following morphometric parameters were assessed during the cultivation period: microclone height, number of leaves, number of axillary shoots and roots and condition on a 5-point scale of visual assessment of microclones (where 1 point is unsatisfactory, 5 points is excellent). The proliferation of the obtained shoots was the next stage. For this purpose, well-developed microclones from the group treated with 1.5 $\mu\text{g/L}$ TiS_3 were transplanted into the growth media containing the following components: 0.2 mg/L benzylaminopurine (BAP), 0.1 mg/L indoleacetic acid (IAA) and 0.3 mg/L gibberellic acid (GA). The synergistic effect of the phytohormones and 1.5 $\mu\text{g/L}$ nanoparticles has also been analyzed. Histological examination was carried out by means of a VideoTesT-Morphology 4.0 hardware and software complex (Argus-BIO, St. Petersburg, Russia).

To assess the photosynthetic system of the plants, we measured the induction of variable fluorescence of chlorophyll-a in assimilatory tissues (F_v/F_m), which makes it possible to assess the activity of the photosystem-2 chlorophyll-containing tissues and can serve as a diagnostic indicator of the state of the photosynthetic apparatus of plants. The intensity of photosynthesis of chlorophyll-containing tissues was recorded using an IFSR-2 fluorimeter (Moscow State University, Moscow, Russia) according to the Genty method [44].

Biochemical analysis was performed using a PE-5400VI spectrophotometer (Ecoschim, St. Petersburg, Russia). The activity of catalase was determined by means of the spectrophotometric technique [45], as in the assessment of the velocity of the chemical reaction of decomposition of hydrogen peroxide with catalase. A measurement of 250 mg of raw plant material was ground in a cold porcelain mortar with 0.5 mL of extraction buffer solution (50 mM phosphate buffer, pH = 7.0). The obtained homogenate was centrifuged for 5 min at $12,000 \times g$ and cooled down to 4 °C. Amounts of 2.95 mL of 50 mM phosphate buffer (pH = 7.0), 30 µL of extract and 20 µL of 0.6 M hydrogen peroxide were put into the spectrophotometer cuvette. Amounts of 2.95 mL of 50 mM phosphate buffer (pH = 7.0) and 30 µL of extract were put into the control cuvette. Optical density was measured at the wavelength of 240 nm every second for 120 s. The activity of catalase in relative units as per 1 g of the dry weight was determined by means of the following formula:

$$A = (\Delta D \cdot V \cdot X) / (T \cdot L \cdot m \cdot \Delta m) \quad (1)$$

where A—enzyme activity; ΔD —change of optical density during the reaction T; V—total volume of the obtained extract, mL; X—correlation between the volume of the raw material put into the cuvette and the cuvette volume; T—time of reaction, s; L—optical path length of the cuvette, cm; m—weight of vegetable material, g, and Δm —the difference between the weight of dry vegetable material and the weight of raw vegetable material.

Rooting was the final stage of the assessment of TiS_3 effects on microclones. Well-formed regenerants (2–3 cm high) were isolated and transferred to the rooting $\frac{1}{2}$ woody plant medium (WPM) in culture bottles [46]. For rhizogenesis promotion, the nanomaterial was added at 1.5 µg per 1 L of growth medium.

All biological and biochemical parameters were measured in three biological and analytical repetitions. Each experimental and control group at each stage of the study contained 30 plants. Descriptive statistics methods included an estimate of the arithmetic mean (M) and standard deviation (S). For statistical data processing, Microsoft Excel 2010 (Descriptive Statistics software package) with one-way analysis of variance (ANOVA) at a 5% predetermined level of significance was used.

3. Results

3.1. TiS_3 Sample Analysis

SEM examination of the obtained TiS_3 sample (Figure 1A) showed that the material had the following mean dimensions: thickness less than 100 nm, length 1–10 µm and width 0.4–1 µm (Figure 1B). According to the atomic force microscopy data, the nanoribbons height varied within the 10–60 nm range (Figure 1C).

Raman spectroscopy (Figure 1D) showed prominent titanium trisulfide bands 22, thus proving that this phase was present in the sample.

3.2. Initiation Stage

Assessment of the effects of TiS_3 nanoribbons in the growth medium at the initiation stage showed that the material is an effective sterilizing agent. We observed that 100% of the poplar × aspen hybrid and birch microshoots were uncontaminated at all the concentrations above 0.75 µg/L, while in the control group the values were 80% and 75%, respectively (Figure 2A). At 1.5 and 3 µg/L TiS_3 , we observed a 100% survival rate of the explants (Figure 2B), though an increase in the concentration reduced the survival rate values. At 15 µg/L, the poplar × aspen hybrid displayed the minimal survival rate value

of 67% compared to 80% for the control, thus indicating the toxic effects of the nanomaterial against the poplar × aspen hybrid microclones.

Figure 1. TiS_3 nanoribbons synthesis and sample characterization: (A) reaction scheme, (B) SEM image, (C) AFM image, (D) Raman spectra.

Figure 2. Biomorphological parameters of the microclones at the initiation stage: (A) Mean number of sterile microclones; (B) Mean number of survival microclones; (C) Mean shoot height; (D) Mean number of leaves (hereinafter the height of the column corresponds to the mean value, bar—to the standard deviation, * is significant deviation from control).

Analysis of the titanium trisulfide nanoribbons effects on the microclones morphometric parameters revealed that medium concentrations of the nanomaterial (1.5 $\mu\text{g/L}$ and 3 $\mu\text{g/L}$) resulted in maximal values of the shoots length and number of leaves (Figure 2C,D). The height of poplar $\times$ aspen hybrid plants increased by 0.5–1.5 cm, while for birch, this value was 1.6 and 1.1 cm, respectively. However, an increase of TiS_3 content in the medium up to 6 $\mu\text{g/L}$ reduced these parameters to the control level, while at 15 $\mu\text{g/L}$, the microshoots development was significantly suppressed.

Medium concentrations of 1.5 and 3 $\mu\text{g/L}$ had a positive effect on other parameters of the microshoots as well. In these groups, the microclones developed axillary shoots. Poplar $\times$ aspen hybrid microclones had two axillary shoots, and birch microclones produced one axillary shoot each. Furthermore, poplar $\times$ aspen hybrid microclones developed roots.

As one can see from Figure 2, the concentrations of 6 and 15 $\mu\text{g/L}$ either had no effect on the morphological parameters or reduced them. Thus, at the initiation stage, 1.5 and 3 $\mu\text{g/L}$ of TiS_3 in the nutrient medium exposure causes a stimulating effect on the microshoots development.

3.3. Proliferation Stage

At the proliferation stage, cuttings were taken from the microclones from the group treated with 1.5 $\mu\text{g/L}$ TiS_3 and were transplanted into the media containing phytohormones and the nanomaterial at the same concentration. The study results are presented in Figure 3.

Figure 3. Biomorphological parameters of the microclones at the proliferation stage: (A) Mean number of the surviving microclones; (B) Mean shoot height.

As one can see from Figure 3A, the nanomaterial introduced into the growth medium increased the microclones survival rate. In the TiS_3 -treated groups, poplar and birch survival rate was 90%, exceeding the control values by 10% and 14%, respectively. In the nanomaterial-free variant, the survival rate was below the control values by 1–2%. In the nanomaterial-free medium, we observed the lowest height increment (Figure 3B). More specifically, the heights were 1.5 cm for both hybrid poplar and birch microclones, as compared to 3.5 and 2.3 cm in the control groups. When we added the nanomaterial, we still observed the suppressive impact on the hybrid poplar, though it was less evident than in the previous case. The height of birch microclones in the TiS_3 -treated group was not significantly different from the control.

The impact of titanium trisulfide on axillary shoots and leaves formation was diverse. During the assessment of the formation of additional shoots, their absence was noted in the control variants, both poplar $\times$ aspen and birch. When phytohormones were added to the medium, three shoots appeared in both cases. When the medium with hormones was supplemented with 1.5 $\mu\text{g/L}$ TiS_3 , the indicator in poplar $\times$ aspen microclones increased to four and decreased to two in birch.

The number of leaves in poplar $\times$ aspen averaged four, regardless of the composition of the medium; however, in the case of birch, the nanomaterial had an inhibitory effect again: two leaves compared with three in the control variant. The largest number of leaves

(four leaves) was in the group with phytohormones. Thus, the species-dependent effect of the nanomaterial is noted.

Histological examination of poplar × aspen hybrid microclones leaves (Figure 4) revealed an increase in the area and in degree of stomatal opening in the TiS_3 -treated group against lower stomatal area and density in the control group. Most probably, the observed increase in the stomatal opening area and degree of stomatal opening compensates for the lower number of stomata per unit area.

Figure 4. Stomata of the leaves of the studied plants. Hybrid poplar × aspen: (A) control, (B) TiS_3 ; Downy birch: (C) control, (D) TiS_3 .

Furthermore, the nanomaterial in the growth medium decreased the leaf lamina thickness and stem diameter and, as we have already mentioned, reduced the regenerants general condition values by one point.

Upon examination of the downy birch leaf lamina histological parameters, one can assume that the TiS_3 -treated plants are more stress resistant than the regenerants from other groups. This is evidenced by a comparatively high degree of stomatal pore opening coupled with high stomatal density per mm^2 (Table 1). In addition, TiS_3 nanoribbons in the growth medium increased the leaf lamina thickness and stem diameter; these are the indications of a better adaptive potential of the plants from this group.

The analysis of photosystem II and enzyme activity revealed no significant effects of titanium trisulfide upon the studied parameters in poplar × aspen hybrid microclones (Figure 5). At the same time, the birch regenerants displayed a large rise in photosystem II activity—0.55 units as compared to 0.34 units in the control group (Figure 5A)—and catalase activity—2.94 units/mg dry weight compared to 2.05 units/mg dry weight in control (Figure 5B).

Table 1. TiS₃ effects to the histomorphological parameters of the microclones at the proliferation stage.

Parameters	Hybrid Poplar × Aspen		Downy Birch	
	Control	Hormones + TiS ₃	Control	Hormones + TiS ₃
Stomatal pore area, μm^2	74.88 ± 5.1	86.06 ± 3.2	86.04 ± 5.32	142.94 ± 6.24
Stomatal area, μm^2	553.41 ± 16.4	499.88 ± 15.1	1208.93 ± 76.31	1151.28 ± 69.45
Stomatal density, pcs./mm ²	5.18 ± 0.9	3.99 ± 0.7	2.99 ± 0.5	2.59 ± 0.3
Degree of stomatal pore opening, S stom./S open	0.13 ± 0.01	0.17 ± 0.01	0.07 ± 0.005	0.12 ± 0.008
Leaf lamina thickness, μm	100.06 ± 9.6	89.71 ± 5.8	65.07 ± 4.46	71.89 ± 3.04
Stem diameter, μm	1206.38 ± 75.9	1193.50 ± 63.1	630.90 ± 28.18	810.13 ± 69.23

Figure 5. Microclones parameters: (A) photosystem II activity, (B) catalase activity (* is significant deviation from control).

The results of histological and biochemical examinations show that the TiS₃ nanoparticles in the growth medium had no significant effect on the poplar × aspen hybrid microclones. The noted lower stomatal number and area was compensated by an increase in the stomatal density and degree of their opening. In this case, slightly lower values of leaf lamina thickness and stem diameter were observed as well. The nanomaterial had no statistically significant effect on the measured biochemical parameters. On the other hand, both histological and biochemical parameters of the birch microclones were strongly affected by TiS₃ exposure. We observed a higher degree of stomatal pore opening coupled with high stomatal density per mm², the leaf lamina thickness and stem diameter values were higher, and the material had a pronounced stimulating effect on the photosystem II and catalase activity compared to the control group. All these could be indicative of a better stress resistance of the TiS₃-treated birch microclones.

3.4. Rooting Stage

For rooting stimulation, we added 1.5 $\mu\text{g/L}$ of TiS₃ per 1 L of nutrient medium. All the poplar × aspen hybrid microclones exposed to TiS₃ rooted within three weeks and developed, on average, four roots, while in the control group only 50% of all the microclones rooted, developing only one root each (Table 2). In the case of birch, rhizogenesis was observed in 98% of the microclones and the plantlets developed, on average, three roots. In the control group, only 35% of plantlets developed roots (on average, one root per explant). In the TiS₃-exposed group the microclones were in excellent general condition.

Table 2. TiS₃ effects at the rooting stage.

Variant	Number of Surviving Microclones, %		Number of Microclones with Roots, %		Number of Roots Per Explant		Microclones Condition According to a 5-Point Scale	
	Poplar × Aspen	Birch	Poplar × Aspen	Birch	Poplar × Aspen	Birch	Poplar × Aspen	Birch
Control	100	100	50 ± 3	35 ± 5	1	1	4	4
TiS ₃	100	100	100	98 ± 1	4	3	5	5

3.5. Proliferation Stage

To determine the mechanisms of the effect of nanoribbons on the roots of shoots, we analyzed the accumulation of elements in them by EDS (Figure 6A,B). We found no difference in the accumulation of elements in the control and experimental samples. The exception was the homogenates of the plant roots of both experimental groups, where we found the accumulation of sulfur, in contrast to the control. Figure 6B shows a map of the distribution of sulfur in the homogenate, from which it can be seen that sulfur is diffusely distributed. This means that it uptakes in the roots in an ionic form, separate from titanium, which was not detected in the samples.

Figure 6. (A) SEM image of birch root sample homogenate (1.5 µg/L TiS₃-exposed group), (B) EDS sulfur distribution map, (C) emission of hydrogen sulfide from titanium trisulfide nanoribbons in bidistilled water for 7 days.

To clarify the problem of hydrogen sulfide emission from the surface of titanium trisulfide nanoribbons into water, we carried out a model experiment, which showed that hydrogen sulfide is released into water with a slowly decreasing intensity (Figure 6C).

4. Discussion

Thus, our study has revealed that titanium trisulfide is a powerful sterilizing agent, because at the initiation stage, 100% of the microshoots were uncontaminated at all the concentrations above 0.75 µg/L, and in the 1.5 µg/L and 3 µg/L variants, we observed a 100% survival rate of the explants, while higher concentrations either had no impact or produced toxic effects. The sterilizing properties of TiS₃ can be connected with the antimicrobial activities of titanium dioxide [47,48], forming on the nanoribbons surfaces as a result of their oxidizing in aqueous media. It is known that the photocatalytic activity of titanium dioxide nanoparticles prevents the fungal colonization of wood treated with suspensions of nanoparticles for a long time compared to untreated wood [49].

The same medium concentrations stimulated the shoots growth and axillary shoots formation. One should also note that hybrid poplars formed roots at this stage. On the other hand, a further increase in the nanomaterial concentration produced an adverse effect: 15 µg/L TiS₃ significantly suppressed the microclones development. At the proliferation stage, exposure to TiS₃ either had no impact on the plant development or displayed a suppressing activity. At the rooting stage the nanoribbons stimulated rhizogenesis, increasing the number of roots in poplar × aspen hybrid microclones by twofold, while in downy birch microclones, we observed almost a threefold increase in the number of roots compared to the control values. The high tolerance of birch to the effects of nanomaterials can be associated with the high adaptability of these woody plants [50]. It is known that

birch belongs to pioneer plants, which, in particular, are the first to populate burned-out areas [51,52]. Taking into account the fact that forest fires are a natural source of nanoparticles, primarily carbon ones [53], it can be assumed that species such as birch have a pre-adaptation to the effects of nanoparticles in high concentrations in soils. Note that titanium trisulfide nanoribbons in size, shape and physicomechanical properties resemble graphene nanoribbons or carbon nanotubes. However, this hypothesis needs further research.

It was revealed that TiS_3 nanoribbons do not penetrate into the root system of microclones, but they are partially destroyed in the cultivation medium with the release of low-molecular sulfur compounds, which we found in the root homogenate. Probably the effect of sulfur compounds explains the observed stimulation of rhizogenesis, as well as the sterilizing effect, which is discussed in more detail below.

Furthermore, the nanomaterial improved the overall condition of the studied microplants. The phytostimulating properties of titanium-containing nanoparticles has been reported before [54,55]. It has been shown that TiO_2 nanoparticles can reduce oxidative damage via H_2O_2 content reduction and antioxidant enzymes activity stimulation [56]. It has also been shown that low concentrations of Ti enhance crop productivity by stimulating the activity of certain enzymes, increasing chlorophyll content and photosynthetic activity and boosting nutrient uptake and stress resistance [57]. Perhaps this explains the observed stimulation of photosystem II and an increase in catalase activity in birch.

The possible mechanism of the sterilizing effect of 2D transition metal trichalcogenides was proposed in our recent work [40]. We have explained the antibacterial effects of ZrS_3 nanosheets by the aqueous hydrolysis, which resulted in the formation of ZrOx on the surface of ZrS_3 particles and the release of toxic H_2S . The XPS analysis confirmed the formation of ZrOx , wherein toxicity in aqueous solutions may also increase over time due to hydrolysis and the formation of H_2S . Perhaps this mechanism can explain the sterilizing effect of TiS_3 . This version is supported by the slow release of hydrogen sulfide from titanium trisulfide nanoribbons into water and by the presence of diffuse sulfur in the roots of plants from the experimental groups, which we described in the previous section. Hydrogen sulfide plays a signaling role in higher plants, it mediates persulfidation, a post-translational modification and regulates wide range of physiological functions, including root growth and development [58–60], and the beneficial effects of H_2S are mainly caused by the stimulation of antioxidant systems [61]. Therefore, the protective and stimulating effects of titanium trisulfide as well as its toxicity in high concentrations can be explained by the release of hydrogen sulfide as a result of aqueous hydrolysis of nanoribbons and its effect on plants. The hydrogen sulfide and titanium dioxide nanoparticles formed during hydrolysis of nanoribbons likely have a combined effect. As a long-term source of slow emission of hydrogen sulfide into aqueous solutions, titanium trisulfide nanoribbons and other 2D transition metal trichalcogenides may be of interest for biotechnology, agriculture and biomedicine.

Thus, our study has shown through the example of poplar $\times$ aspen hybrid and downy birch microclones the effectiveness of the application of titanium trisulfide nanoribbons as a sterilizing and stimulating agent at the initiation stage and as a rhizogenesis-activating agent at the rooting stage. However, concentration-dependant toxic effects manifesting themselves through the microclones viability suppression have been discovered. We have identified specificity in the studied plants' reactions to TiS_3 exposure, i.e., downy birch is more tolerant than the poplar $\times$ aspen hybrid. The assessment of the biological effects produced by TiS_3 nanoribbons at further stages of ontogenetic development, as well as the identification of the mechanisms of their action, requires additional studies.

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