The use of biogenic nanoparticles of ferrihydrite in the propagation of horticultural crops by cutting

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Abstract. The effect of biogenic ferrihydrite nanoparticles on the rhizogenesis of garden plants (Salix ledebouriana, Philadelphus coronarius, Thuja occidentalis, and Cerasus fruticosa) was studied. It was shown that nanoparticles of biogenic ferrihydride (tested 4 modifications) are biologically active. It is assumed that the mechanism of their influence on the rhizogenesis of the tested plant species is general and is associated with the generation of reactive oxygen species in interaction with endogenous hydrogen peroxide.

1. Introduction

Studies of nanoparticles and nanomaterials, which have been actively conducted over the past 10 to 20 years, open up approaches to creating effective growth stimulants, the synthesis of biologically active substances, plant protection products, and to fundamentally new solutions in the field of agricultural biotechnology. The contact of plants with nanoparticles can lead to both positive and negative effects, depending on their nature, concentration, contact time and reaction properties of the nanoparticles. The value of the toxic or positively influencing plant growth concentration of nanoparticles depends, in turn, on the type of plant, stage of its development and state. Cyto- and phytotoxicities of nanoparticles are often explained by an increased level of reactive oxygen species (ROS) in the cells of living organisms [1, 2]. Under oxidative stress, ROS cause lipid peroxidation, membrane destruction, DNA and protein damage. In accordance with this hypothesis, the difference in the cytotoxicity of nanoparticles of different nature can be associated with their ability to decompose H₂O₂ and, accordingly, generate ROS. So, Auffan et al. [3] believe that the cytotoxicity of magnetite (Fe3O4) is due to the presence of Fe (II) ion in its structure, in contrast to the less toxic maghemite (γ-Fe2O3) and hematite (α-Fe2O3) that do not contain it. At the same time, ROS is involved in the regulation of the expression of a number of genes and control the root formation and growth of the aerial parts, the cell cycle and other processes of plant development [4; 5; 6; 7]. The greatest attention is paid to nanoparticles of Ag, oxides of Ti, Si, Al and significantly less to oxides of iron (magnetite, hematite). The effect of nanosized iron hydroxides on plants, in particular ferrihydrite, which is widely
present in soils, has not been practically studied. Along with this, the mechanisms of their influence on living objects are poorly studied and are still not clear. Studies conducted with nanoparticles of various natures and a wide range of plant species demonstrate both positive and negative effects of their interaction.

The purpose of the work is to analyze the effect on rhizogenesis of various plant species of nanoparticles of one class. A comparative study was conducted with nanoparticles of biogenic iron hydroxide - ferrihydrite (four modifications), green and lignified cuttings of various types of garden plants. Propagation of garden plants by stem cuttings is considered one of the most effective methods for solving this problem.

2. Objects and research methods

The objects of study were the lignified Ledebour Willow cuttings (Salix ledebouriana), green cuttings of the Mock orange (Philadelphus coronarius L.), Western thuja (Thuja occidentalis), and Steppe cherry (Cerasus fruticosa). Rooting was carried out by the traditional method [8], soaking the cuttings in auxin aqueous solutions (indolyl-3-acetic acid, 0.07%, control) and in the same solution with nanoparticles of biogenic ferrihydrite (“pure” or undoped, two modifications – Feh, Feh2 and ferrihydrite doped with aluminum (Feh_Al) or cobalt (Feh_Co). The concentration of nanoparticles in solutions was 1-2 mg/l. In solutions of each of these compositions, 5-6 cuttings were soaked. The exposure of the cuttings in solutions is 12 and 24 hours. The placing test variants of the cuttings in the soil were systematic. The planting pattern of cuttings was 7×7 cm. After treatment in solutions, the cuttings were planted in a greenhouse with translucent polycarbonate panels and microdrop irrigation into a prepared substrate. All experiments were repeated three times. The morphometric parameters of rooted cuttings were determined three months after planting in the ground. For growing, rooted cuttings were transferred to open ground. Assessment of the quality of seedlings was carried out in accordance with GOST R53135-2008 [9]. Statistical analysis of the data was performed using the program Statistica 6.0 software package (StatSoft, the USA).

Biogenic ferrihydrite nanoparticles were obtained in cultures of aerobic bacteria (Delftia tsuruhatensis) on mineral-salt media. After removal of bacteria and purification from extracellular metabolite products, nanoparticles were isolated in the form of a colloidal solution of 2- and 6-line ferrihydrite. The size of individual nanoparticles was 2–10 nm [10]. The obtained sols of nanoparticles retained sedimentation stability for several months.

3. Research results and discussion

In experiments with Ledebour willow [11], increased initiation of the formation of adventitious roots was observed during the treatment of lignified cuttings (252 pcs.) with nanoparticles of biogenic ferrihydrite. Compared with control options for processing cuttings, the positive effect of nanoparticles was observed both in the case of soaking them in solutions with indolyl-3-acetic acid (3×18 pcs.), and without growth phytohormones (18 pcs.) (Table 1). The data obtained characterize the initial period of root formation. The adnexal roots sprouted through hypertrophic lenticels. The observed effects are statistically significant with a probability of 95%.

Table 1. The effect of biogenic ferrihydrite nanoparticles at the initial stage of rooting of lignified cuttings of Ledebour willow.

| Cuttings processing options | Control period | The number of roots of the 1st branching order, pcs | The total length of the roots of the 1st branching order, cm |
|----------------------------|----------------|-----------------------------------------------|---------------------------------------------------------------|
|                            |                | Block 1 | 2  | 3 | Average | 1  | 2  | 3 | Average |
| IAA+Feh                    | 5 days         | 3.1     | 4.4 | 3.9 | 3.8     | 8.8 | 82.0 | 10.9 | 9.4     |
| IAA                        |                | 2.0     | 2.0 | 5.0 | 3.0     | 6.2 | 2.0  | 4.2  | 4.2     |
| Water                      |                | 0       | 4.0 | 2.0 | 2.0     | 0   | 2.0  | 6.0  | 4.0     |
The presence of free radicals (ROS) in plant tissues was recorded by electron paramagnetic resonance spectroscopy. The spectra were recorded on an X-band EPR spectrometer (Bruker ELEXYS E580) at a temperature of 90 K three days after the treatment of the cuttings with root-stimulating solutions. Tissues of hypertrophied (Feh.hl) and dormant lenticels (Feh.dl) cuttings treated with nanoparticles and IAA, hypertrophied and dormant lenticels of cuttings treated only with IAA (IAA.hl and IAA.dl, respectively), as well as a phloem (Feh.fl) were analyzed. The results of comparing the EPR spectra (intensity of the radical center lines) are shown in Figure 1. Hypertrophied lenticels indicate the active proliferation of plant tissue cells, including the formation primordia of adventitious roots. Visually, a morphological change in resting lenticels was not observed. However, the free radical line intensity (ROS) for this sample of lenticels (Feh.dl) was the highest. Obviously, they were in the initial stage of initiation of adventitious root growth. Note that ROS serve as signaling molecules of the formation of the adventitious and lateral roots [12].

![Figure 1](image_url)

**Figure 1.** The intensity of the free radical lines in the EPR spectra of plant tissues of lignified cuttings of Ledebour willow (1 - Feh.dl, 2 - Feh.hl, 3 - IAA.hl, 4 - IAA.dl).

The positive effect of short-term contact of stem cuttings with nanoparticles of biogenic ferrihydrite is observed in experiments with other garden plants. Table 2 shows the results of rooting of the green cuttings of the Mock orange, which were exposed in solutions for 12 hours. Control measurements of root formation parameters were carried out 3 months after processing the cuttings in stimulating solutions with IAA and a mixture of IAA with nanoparticles. Four types of nanoparticles were tested: “pure” ferrihydrite (two modifications), and doped with aluminum or cobalt. Modification of Feh2 nanoparticles was obtained by changing the procedure for their isolation from bacterial culture. Table 2 shows that all four types of nanoparticles stimulated the formation of adventitious roots. The total length of adventitious roots relative to control variant of cuttings processing increased by 12.4 - 120%. Compared to treating the cuttings only with auxin, the greatest increase was observed in the options for treating the cuttings with Feh nanoparticles (modification 1) and ferrihydrite doped with aluminum (Feh.Al).

In experiments with the Mock orange, an exceptionally high degree of stimulation of root formation was observed in the variants Feh (1) and Feh.Al. The formation of adventitious roots occurred not only in the nodes, but also along the interstitial longitudinal rows [13].
Table 2. Biometric indicators of adventitious roots of rooted green cuttings of the English dogwood.

| Cuttings processing options | The number of roots of the 1st branching order, pcs | The total length of the roots of the 1st branching order, cm |
|----------------------------|-----------------------------------------------|----------------------------------------------------------|
| H₂O                        | 11                                            | 29.8                                                     |
| IAA+Feh (1)                | 17                                            | 57.5                                                     |
| IAA+Feh (2)                | 10                                            | 33.5                                                     |
| IAA+Feh_Al                 | 10                                            | 65.5                                                     |
| IAA+Feh_Co                 | 12                                            | 27.7                                                     |

The quality of seedlings obtained from rooted cuttings after growing in open ground is shown in Table 3. The treatment of green cuttings of the Mock orange with nanoparticles reduced the proportion of non-standard seedlings by 12.6 - 7% and made it possible to obtain first-grade seedlings. Tests on Western thuja also showed a decrease in the yield of non-standard seedlings by 7.7 - 19.6% and the formation of seedlings of the first grade. Unlike the traditional method of rooting cuttings using plant growth hormone (IAA), all three types of nanoparticles tested made it possible to obtain seedlings of the first grade (up to 20%). Adding of citric acid into solutions with nanoparticles contributed to an increase in the effect of nanoparticles and root formation in general. Citric acid was used to reduce the aggregation of nanoparticles and stabilize colloidal solutions. However, it should be noted that simultaneously with the stabilization of nanoparticles in the colloidal state, citric acid influenced root growth as an organic substrate. In this experiment, the task of differentially determining the effect of nanoparticles and citric acid was not posed.

Table 3. The quality of the planting material of the Mock orange and Western thuja, obtained in the open ground.

| Cuttings processing options | Mock orange, % | Western thuja, % |
|----------------------------|----------------|------------------|
|                            | 1st variety    | 2nd variety      | non-standard     | 1st variety | 2nd variety | non-standard |
| IAA                        | нет            | 40.0             | 60.0             | нет         | 42.2        | 57.8         |
| IAA+Feh_Al+ca              | 13.0           | 37.0             | 50.0             | 9.4         | 50.6        | 40.0         |
| IAA+Feh                    | 4.5            | 42.45            | 53.0             | 10.6        | 39.3        | 50.1         |
| IAA+Feh+ca*                | 7.6            | 45.0             | 47.4             | 19.8        | 42.0        | 38.2         |

*ca – citric acid

In the experiment with steppe cherries, nanoparticles of biogenic ferrihydrite (Feh, Feh_Al) and the same nanoparticles in the presence of citric acid in colloidal solutions had a positive effect on rhizogenesis (table 4). The greatest effect was obtained with Feh nanoparticles (without citric acid). The root formation parameters of this variant exceeded the control by 5 times.

Table 4. Effect of biogenic ferrihydrite nanoparticles on the growth of the adventitious roots of green cuttings of steppe cherry.

| Cuttings processing options | The number of roots of the 1st branching order, pcs | Total length the roots, cm | Maximum branching order |
|----------------------------|-----------------------------------------------|---------------------------|------------------------|
| IAA                        | 3.2                                           | 20.2                      | 2                      |
| IAA+Feh                    | 17.0                                          | 98.6                      | 2                      |
| IAA+Feh+ca                 | 5.7                                           | 20.5                      | 2                      |
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tive effect on the plant. The method of synthesis of biogenic nanoparticles of biogenic ferrihydrite can affect other stages of plant development. The high activity 

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and participation in the regulation of the ROS level in plant tissues. Since ROS act 

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which appear in plant tissues as a result of the interaction of endogenous hydrogen peroxide with nanoparticles was the signal for the initiation of primordia. The level of ROS in the cells is controlled by an antioxidant system, which includes catalase, peroxidase, superoxide dismutase and other active agents. It is known that iron oxides and hydroxides (magnetite, goethite, etc.) exhibit peroxidase and catalase activity [14; 15; 16]. Active nanoparticles, which are capable of catalytically decomposing hydrogen peroxide, act as an exogenous factor in the regulation of the concentration of signaling molecules in the rhizogenesis (organogenesis) of plants. Under these conditions, the concentration of ROS can go beyond the limits (threshold level) set by the antioxidant system of plant organs and cells. The experimental data obtained in the work show that this can lead to significant stimulation of root formation. It is natural to assume that at high concentrations the nanoparticles of biogenic ferrihydrite will have a negative effect on the plant. The method of synthesis of biogenic nanoparticles under conditions of controlled bacterial culture is technologically convenient from the point of view of process scalability and the possibility of obtaining nanoparticles in a colloidal state.

4. Conclusion

The positive (or negative) effect of the impact of nanoparticles on the rhizogenesis of garden plants does not depend on the type of plant. The determining factor is the mechanism of influence of nanoparticles. In the case of biogenic ferrihydrite nanoparticles, it is associated with their high activity and participation in the regulation of the ROS level in plant tissues. Since ROS acts as a factor in the regulation of rhizogenesis and other stages of plant organogenesis, it can be assumed that nanoparticles of biogenic ferrihydrite can affect other stages of plant development. The high activity of biogenic ferrihydrite nanoparticles gives reason to consider them as a promising material for obtaining plant growth stimulants.

References

[1] Wang B, Yin J-J, Zhou X, Kurash I, Chai Z, Zhao Y and Feng W 2012 Physicochemical origin for free radical generation of iron oxide nanoparticles in biomicroenvironment: Catalytic activities mediated by surface chemical states. J Phys Chem C 117(1) 383-92
[2] Fu PP, Xia QS, Hwang HM, Ray PC and Yu HT 2014 Mechanisms of nanotoxicity: generation of reactive oxygen species J Food Drug Anal 22 64–75
[3] Auffan M, Rose J, Achouak W, Roncato MA, Chanéac C, Waite D T, Masion M, Woicik J C, Wiesner M R and Bottero J Y 2008 Relation between the redox state of iron-based nanoparticles and their cytotoxicity toward Escherichia coli. Environ Sci Technol 42 6730–35
[4] Foreman J, Demidchik V, Bothwell J H F, Mylona P, Miedema H, Torresk M A, Linstead P, Costa S, Brownlee C, Jonesk J D G, Davies J M and Dolan L 2003 Reactive oxygen species produced by NADPH oxidase regulate plant cell growth Nature 422 442–46
[5] Choudhury S, Panda P, Sahoo L and Panda S K 2013 Reactive oxygen species signaling in plants under abiotic stress Plant Signal Behav 8(4) e23681
[6] Mangano S, Juárez S P D and Estevez J M 2016 ROS regulation of polar growth in plant cells. Plant Physiol 171 1593

| IAA+Feh_Al  | 7.9  | 32.4  | 2    |
| IAA+Feh_Al+ca | 11.0 | 27.5  | 2    |

*ca – citric acid
[7] Orman-Ligeza B, Parizot B, de Rycke R, Fernandez A, Himschoot E, Van Breusegem F, Bennett M J, Périlleux C, Beeckman T and Draye X 2016 RBOH-mediated ROS production facilitates lateral root emergence in Arabidopsis. Development 143 3328-39

[8] Aladina O N 2013 Optimization of propagation technology of garden plants by herbaceous cuttings Izvestia of Timiryazev Agricultural Academy 4 5-22

[9] GOST R 53135-2008 Planting stock of fruit, berry, subtropical, nut, citrus crops and tea 2009 (Moscow: Standartinform)

[10] Teremova M I, Petkovskaya E A, Romanenko A S, Tuzikov F V, Gurevich Yu L, Tsibina O V, Yakubailik E K and Abhilash 2016 Ferritization of industrial waste water and microbial synthesis of iron-based magnetic nanomaterials from sediments Environ Prog Sustain Energy 35(5) 1407-14. DOI: 10.1002/ep.12368

[11] Bopp V L, Mistratova N A, Petakovskaya E A, Gurevich Yu L, Teremova M I and Khlebopros R G 2018 The influence of nanoparticles of biogenic ferrihydrite on the rooting of lignified cuttings of the Ledebour Willow. Biophysics 63(4) 621–28

[12] Manzano C, Pallero-Baena M, Casimiro I, De Rybel B, Orman-Ligeza B, Van Isterdael G, Beeckman T, Draye X, Casero P and del Pozo J C 2014 The Emerging Role of Reactive Oxygen Species Signaling during Lateral Root Development Plant Physiol 165 1105–19

[13] Bopp V L, Gurevich Yu L, Mistratova N A, Teremova M I and Khizhnyak S V 2017 Effectiveness of biogenic ferrihydrite nanoparticles in green cutting Mock orange Modern Science Success 5(1) 48-55

[14] Elizarova G L, Zhidomirov G M and Parmon V N 2000 Hydroxides of transition metals as artificial catalysts for oxidation of water to dioxygen Catalysis Today 58 71-88

[15] Pariona N, Herrera-Trejo M, Oliva and Martinez A I 2016 Peroxidase-like activity of ferrihydrite and hematite nanoparticles for the degradation of methylene blue J Nanomaterials Article ID 3427809

[16] Wei H, Wang E 2013 Nanomaterials with enzyme-like characteristics (nanozymes): next-generation artificial enzymes Chem Soc Rev 42(14) 6060