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IRON OXYHYDROXIDE EFFECT ON ROOTING OF CUTTINGS OF *RIBES NIGRUM* AND *RIBES RUBRUM*

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ABSTRACT: This work was aimed at studying the effect of a preparation containing nanoparticles of iron oxyhydroxide on rooting and development of the root system of cuttings of the two plant species sensitive to iron deficiency: *Ribes nigrum* and *Ribes rubrum* (Grossulariaceae). Research has shown that the iron oxyhydroxide has an inhibitory effect on the growth and development of the root system of cuttings of black and red currant. When concentration increases from 0.001% to 0.1%, rooting of cuttings decreases, and the Spearman rank correlation shows strong negative dependence of the number of roots and their length on the concentration of the preparation (*Rs* = -0.83 -0.94...) – the number of roots on cuttings decreases, while in case of the maximum concentration, root length significantly reduces. In the studied concentration, the preparation containing iron oxyhydroxide nanoparticles cannot be used as a stimulant of root formation for rooting of cuttings of black and red currant.

Keywords: Iron oxyhydroxide, rooting of cuttings, Black currant, Red currant, Ribes nigrum, Ribes rubrum, Grossulariaceae.

1. INTRODUCTION

Industrial cultivation of various groups of ornamental and edible shrubs in nurseries is aimed at improving the quality of the planting material and reducing production costs. For this purpose, modern methods of accelerated vegetative and improved seed reproduction of plants are used. Vegetative reproduction of plants allows preserving the varietal characteristics. The use of various stimulants significantly increases the percentage of cuttings rooting, and the quality and quantity of formed roots.

Currently, the use of nanomaterials in agriculture [1], [2] is actively researched. Many papers have been devoted to the effect of metal nanoparticles, including iron, on growth and development of agricultural plants [3]-[7]. Metal and metal oxide nanoparticles have various effects on various plant species. So, nanoparticles of Pt and Zn inhibit root development in cuttings of *Ribes nigrum* [8]; at the same time, Pt stimulates biomass increase in *Amaranthus cruenthus* and the yield of *Triticum aestivum* [9]. Iron is an essential element of mineral nutrition for plants, and is involved in many physiological processes: respiration, photosynthesis, nitrogen fixation, etc. [10]-[13]. Iron oxides and oxyhydroxide are widely spread in mineral rocks and soils, from which they enter into plants in dissolved form [14], [15].

Artesian water of the West-Siberian basin contains finely dispersed iron in the amount of 2-12 mg/l [16]. Thousands of tons of mineral of iron sediment formed on filters during water treatment by the aeration method at water treatment plants are discharged to the terrain, thus polluting the environment [17]. Meanwhile, these iron oxides and oxyhydroxide may be used for creating biological products with a stimulating effect on living organisms, simultaneously solving an environmental problem of disposing this kind of wastes. From iron oxyhydroxide, preparations have already been obtained that have been tested in medicine and agriculture: Ferrigel has wound-healing and bactericidal action on animals' tissues [18], [19]; a preparation for potatoes pre-treatment, which improves the yield and quality of potato tubers [20].

Cuttings of *Ribes nigrum* and *Ribes rubrum* root easily, but in the severe climatic conditions of Siberia, 5-22% [21] of cuttings die. In both currant species, the percentage of shoots that die in winter is different for various cultivars. The use of rooting stimulants improves the quality of the root system, and increases the percentage of the shoots surviving the winter.

This work was aimed at studying the effect of a preparation containing nanoparticles of iron oxyhydroxide on rooting and development of the root system of cuttings of the two plant species sensitive to iron deficiency: *Ribes nigrum* L. and *Ribes rubrum* L. (Grossulariaceae).

2. MATERIALS AND METHODS

The objects of testing in the research were black currant 'Lama' and red currant 'Ustina'. In the first week of July, 360 one-year 10-12 cm long cuttings with 2-3 internodes were cut from mother plants. Then the cuttings were divided into 4 groups of 90 cuttings for each variant of the experiment (30 pcs in 3 biological repetitions). In the reference group, the basal parts of the shoots were immersed 6-7 cm deep in water, and the other 3 groups of shoots were placed for 16 hours into solutions of the iron oxyhydroxide preparation. The following concentrations of oxyhydroxide iron were used: 0.001%, 0.01% and 0.1%. The experimental preparation was made at the Institute of Petroleum Chemistry of the Siberian Branch of RAS of the wastes from the artesian water deferrization station: the mineral water sludge of creamy consistency, containing 10% of dispersed particles of iron oxyhydroxide with the particle size of 30-50 nm, was treated with ultrasound for 60 minutes [22], since it had been shown that metal nanoparticles exhibited high biological activity if they were presented in the oxide or hydroxide forms and redispersed with ultrasound [3]. After cuttings incubation in solutions of the preparation, they were washed with water and planted for rooting into a greenhouse. The substrate for rooting had two layers: fertile soil underneath, and mixture of sand and peat in the of 1:1 ratio above. The microclimate in the greenhouse was maintained by means of automatic atomized spraying, watering and ventilation. Rooting of the cuttings occurred with the following microclimate parameters: air temperature during the day +22...+25°C, at night +16...+18°C; soil temperature +18...+20°C; humidity of the substrate 85%; air humidity 90%. After 30 days of rooting, the cuttings were carefully removed from the substrate, the number of rooted cuttings and the number of roots were counted, and their length was measured.

According to the obtained results, the average value of the trait (M), and standard deviation (SD) were calculated. To determine the nature of root length dependence on their number, the Pearson correlation coefficient (Rp) was calculated; the Spearman rank correlation coefficient (Rs) was calculated to determine the average length and number of roots dependence on the concentration of the preparation. The correlation was considered weakly positive with $R > 0.01 \leq 0.29$; moderately positive with $R \geq 0.30 \leq 0.69$; strongly positive with $R \geq 0.70 \leq 1$; weakly negative with $R > -0.01 \leq -0.29$; moderately negative with $R \geq -0.30 \leq -0.69$; and strongly negative with $R \geq -0.70 \leq -1$.

The statistical significance of length difference and the number of roots between the variants of the experiment and the reference group were determined by the Student's t-test, and by the Mann-Whitney u-test in small samples (percent of rooting).

3. RESULTS

The use of iron oxyhydroxide in the studied concentrations had a negative effect on growth and development of the root system (Figure 1). The percentage of rooted cuttings decreased with increasing concentration of the preparation. Compared to the reference group, the use of the highest concentrations of the preparation (0.1%) reduced the percentage of rooting by 18% in *R. nigrum* 'Lama' and by 9% in *R. rubrum* 'Ustina' (Table 1). The quality of the root system also reduced: the use of the preparation in the minimum concentration (0.001%) reduced the number of roots on the cuttings of *R. nigrum* 'Lama' by 26% and that of *R. rubrum* 'Ustina' by 29%, and in the highest concentration – by 37% and 34%, respectively. The Spearman rank correlation coefficient showed strong negative dependence of the number of roots on the concentration of the preparation: Rs = -0.92 for *R. nigrum* 'Lama' and Rs = -0.83 for *R. rubrum* 'Ustina'.

Root length in both variants of experiment with the minimum concentration of iron oxyhydroxide did not have statistically significant difference, but in case of using the preparation in the maximum concentration, root length significantly reduced by 34% for *R. nigrum* 'Lama and 42% for *R. rubrum* 'Ustina'. Same as in the case of the number of roots, there was strong significant negative dependence of root length on the concentration preparation: Rs = -0.93 and Rs = -0.94 for the first and the second grade, respectively. Lengthwise root growth was more inhibited for *R. rubrum* 'Ustina' compared to *R. nigrum* 'Lama' in all tested concentrations of iron oxyhydroxide.

In the reference groups, the shoots had well-developed root system and there was a significant positive correlation between the number and length of roots – moderate for *R. nigrum* 'Lama' (Rp = 0.36) and strong for *R. rubrum* 'Ustina' (Rp = 0.78). In the variants of experiment with the use of 0.001% and 0.01% of the iron oxyhydroxide preparation, correlation characteristic of the reference groups was disrupted, became weak, and at the concentration of 0.1% – moderately negative, i.e., roots of the cuttings, while fewer in the number, became slightly longer.

Fig.1 The root system of cuttings of *Ribes nigrum* 'Lama' after 30 days of rooting. Left to right: reference, 0.001%, 0.01% and 0.1% solutions of iron oxyhydroxide.

Table 1 Qualitative and quantitative characteristics of the root system of red and black currant cuttings when exposed to various concentrations of iron oxyhydroxide

Iron oxyhydroxide concentration	Rooting, %	Number of roots, pcs	Length of roots, cm	Dependence of the length of roots on their number (Pearson correlation), *Kp*
		Ribes nigrum 'Lama'		
Reference	84.8 ± 10.6	23.1 ± 5.5	9.7 ± 1.7	0.36*
0.001%	80.4 ± 7.4	17.0 ± 3.4*	9.3 ± 2.5	0.24
0.01%	78.5 ± 7.9	16.3 ± 3.8**	6.5 ± 1.5**	0.13
0.1%	69.6 ± 4.6*	14.4 ± 4.7**	6.4 ± 2.1**	-0.54
		Ribes rubrum 'Ustina'		
Reference	94.1 ± 5.7	17.9 ± 7.7	14.6 ± 3.2	0.78**
0.001%	93.7 ± 3.4	12.6 ± 2.3*	12.0 ± 2.8	0.19
0.01%	86.3 ± 5.6	11.5 ± 3.0*	8.3 ± 2.6**	-0.13
0.1%	85.2 ± 6.1	11.9 ± 2.5*	8.5 ± 4.0**	-0.57*

Note: Asterisks indicate significant differences: * p-value ≤ 0.05; ** p-value ≤ 0.01

4. DISCUSSION

Ribes nigrum and *Ribes rubrum* are valuable currant shrubs sensitive to iron deficiency. It is known that black currant accumulates Fe^{3+} in the leaves in the composition of globular protein ferritin, which is extracted unchanged during the preparation of aqueous extracts of the leaves, and may be used for treating diseases associated with low iron content [23].

Black and red currants are widely spread in the forest zone of Eurasia. Both species grow mainly at forest edges, on waterlogged soils along rivers [24], [25]. On waterlogged soils in the presence of easily oxidized organic substances and iron reducing agents, Fe^{3+} recovers to Fe^{2+}, which is bio-available for plants [26].

Despite the fact that iron is a vital element in mineral nutrition of plants, it has a negative effect in high concentrations. The toxic effect of iron may be both direct and indirect [27]. A wide variety of morphological and physiological changes in plants growing at high concentrations of iron has been described: slowed down growth, decreased length of shoots and size of the leaves, elongated roots, reduced branching of roots, destructed cell structures, inhibited translocation of phosphorus into the leaves, etc [13], [27]-[29].

The plants mainly absorb Fe^{+2}, which is obtained after recovery of Fe^{+3} on the surface of the roots [13], [30], [31]. However, excess of Fe^{+2} may have a negative effect even on plant species sensitive to lack of iron. For example, adding high concentrations of Fe^{+2} into nutrient media during the reproduction of *Ribes nigrum* in vitro reduces the height of the regenerants, their rooting, but increases the length of the roots [32].

Our study is consistent with the above data, and has shown that the preparation with oxyhydroxide nanoparticles as the source of Fe^{+3} has an inhibitory effect on growth and development of the root system of cuttings of red and black currants. This may be due to the fact that applying the preparation to the cutting of shoots has a toxic effect on the tissues, since in the absence of roots, Fe^{+3} does not recover to Fe^{+2}, which is biologically available for plants.

5. CONCLUSION

In the studied concentrations (0.001%, 0.01% and 0.1%) the preparation containing iron oxyhydroxide nanoparticles has an inhibitory effect on growth and development of the root system in cuttings of *Ribes nigrum* and *Ribes rubrum*, respectively, and cannot be used as a root formation stimulant.

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