Screening of different Fe (II) and Fe (III) complexes at the stage of rhizogenesis in vitro of gooseberry plants

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Abstract. During clonal micropropagation of the Pink-2 (Rosovy-2) gooseberry cultivar at the stage of rhizogenesis, the efficiency of modification of the media with mineral salts according to Quoirin-Lepoivre (QL) was shown by replacing iron, which is standardly used in the form of FeSO4·7H2O together with Na2EDTA, with chelated forms with carboxyl-containing ligands Fe(III)-EDTA and Fe(III)-DTPA and the organophosphate complexone Fe(II)-HEDP. On the 45th and 60th days of subculturing, the distribution in descending order of the impact of chelate iron compounds on the rooting rate of the studied gooseberry plants was as follows: Fe(III)-EDTA>Fe(III)-DTPA>Fe(II)-HEDP>Fe(III)-EDDHA>Fe(III)-HEDP. On the 60th day of subculturing in the best variants of the experiment, the rooting rate of gooseberry microcuttings of cultivar Pink-2 was 86.7-100% compared to 60% in the control variant.

1. Introduction

Optimizing the composition of media and the nature of their constituent elements for clonal micropropagation is a difficult task associated with the different nutritional requirements of various plant species and the numerous chemical interactions of nutrients. Despite the fact that mineral nutrition is one of the most important factors of plant clonal micropropagation, its effect on morphogenesis has been poorly studied [6, 15]. In this regard, when developing a suitable composition of media, plant growth regulators are usually modified without changing mineral nutrients [1, 7]. However, many species and cultivars of fruit crops that are slow growing or difficult to propagate in vitro do not lend themselves to the classical optimization approach by testing the hormonal components of plant growth and are difficult to grow on any of the standard media. [1, 4, 7, 12, 17]. In addition, metabolic disorders caused by nutritional imbalance affect the sensitivity of tissues and cells to growth regulators [15].

In the last decade, more and more information has appeared in the literature about the critical influence of the optimal values of meso- and microelements on the better regeneration of shoots and rooting of micro-seedlings of fruit crops by in vitro methods [2, 3, 5, 12, 16]. It should be noted that...
the lack of sufficient fundamental knowledge about the mechanisms of the availability and absorption of nutrients in plant biology, and even more so in tissue culture, serves as a serious obstacle to the development of progressive methods of obtaining nutrient solutions [15].

In addition to the concentrations of components and their ratios among themselves, the focus of attention should be on such factors as the chemical form of the component, ensuring its availability to the plants, chemical interactions between components in the media, the effects of antagonism and synergism in the absorption of elements by plant tissues [8].

In this regard, an interdisciplinary approach using knowledge of a related subject area can be very effective for solving these problems. Thus, for example, it is known that for plant nutrition, chelated forms of iron are preferred over its mineral salts. In this regard, iron is usually introduced into nutrient media for clonal micropropagation in the form of FeSO₄×7H₂O together with the disubstituted sodium salt of ethylenediaminetetraacetic acid (Na₂EDTA) [7, 10]. This technique assumes that such an introduction of the components further ensures the formation of an iron complex with EDTA in the medium. However, simple mixing of the components is insufficient for the complete course of the complexation process, which requires compliance with the necessary technological conditions: temperature, pH-medium and reaction time [9].

Thus, there is a high probability that the chelated form of iron is not formed in standard nutrient media and the absorption of this most important trace element is impaired. As a consequence, systemic explant diseases associated with iron deficiency occur, which has been noted in numerous publications on clonal micropropagation of fruit crops [11-13, 15-16].

The purpose of this work was to study the effect of introducing various forms of iron into the media according to the Quorin-Lepuavr (QL) protocol at the stage of rhizogenesis of gooseberry microplants, cultivar Pink-2.

2. Materials and methods
The experiments were carried out in the laboratory of clonal micropropagation of garden plants of the fruit growing laboratory of the Moscow State Agricultural Academy named after K.A. Timiryazev in 2020. Research objects: gooseberry microplants, cultivar Pink-2.

Various iron chelates were included in the experiment scheme:
1. FeSO₄×7H₂O + Na₂EDTA (control)
2. NaFe(III)EDTA - iron complex with ethylenediaminetetraacetic acid (Fe = 13.4%)
3. Na₂Fe(III)DTPA - iron complex with diethylenetriaminepentaacetic acid (Fe = 9.81%)
4. Fe(III)HEDP - iron complex with hydroxyethylidene diphosphonic acid (Fe = 0.65%)
5. Fe(III)EDDHA - iron complex with ethylenediamine-bis-hydroxyphenylacetic acid (Fe = 0.015%)
6. Fe(II)HEDP - a complex of iron from the class of bisphosphonates with exiethylidene difbasic acid (Fe = 0.52%)

Microplants of gooseberry cultivar Pink-2 were planted on a cultural medium with mineral salts according to the Quorin-Lepuavr (QL) protocol [14] for the stage of rhizogenesis, enriched with the following substances: (mg / l) thiamine hydrochloride (B1), pyridoxine hydrochloride (B6), nicotinamide (PP) - 0.5; meso-inositol - 100; glycine - 1000; 6-BAP - 0.3; sucrose - 30,000, agar-agar - 6,000.

Typically, in the nutrient medium according to the Quoirin-Lepoivre recipe, FeSO₄×7H₂O is used in an amount of 27.8 mg / l. Calculating the molecular weight of FeSO₄×7H₂O (278.05 amu) and knowing the molecular weight of Fe (55, 85 amu), we found that 27.8 mg of FeSO₄×7H₂O contains 5.58 mg of Fe.

Table 1. Experimental concentrations of iron complexes

| Complex                | Concentration of complexes |
|-----------------------|---------------------------|
|                       | Reduced (×0,5) | Standard (×1) | Increased (×1,5) | Doubled (×2) |
| NaFe EDTA (13,4%) mg/l| 0,02          | 0,04          | 0,06            | 0,08         |
| Na₂Fe DTPA (9,81%) mg/l| 0,03          | 0,06          | 0,09            | 0,12         |
Fe(III)-HEDP (0.65%) mg/l 0.43 0.86 1.29 1.72
Fe-EDDHA (0.015%) mg/l 18.6 37.2 55.8 74.4
Fe(II)-HEDP (0.52%) mg/l 0.54 1.07 1.61 2.15

Next, the amount of iron was calculated in the variants of the experiment. In the control FeSO₄·7H₂O + Na₂EDTA, the standard concentration of iron chelate (×1) was used; in the auxiliary control, its concentration was doubled (×2).

According to the experimental scheme, iron complexes were added to the nutrient media at the calculated concentrations: half reduced (×0.5), standard (×1), increased 1.5 times (×1.5), and doubled (×2) (table 1).

The pH of the culture medium was adjusted to 5.8. The culture medium, previously poured into culture bottles, was sterilized in an autoclave at a temperature of 120°C and a pressure of 0.1 MPa for 20 min. In a laminar flow hood, 5 microcuttings 2-3 nodes long were placed in each bottle. Then the cultures were incubated in a light room at an illumination intensity of 2500 lux, a 16-hour photoperiod and a temperature of 20-22 °C.

On the 45th and 60th days of subculturing, the morphometric parameters of microshoots were counted. The following were taken into account: necrosis rate, number of shoots, average length of shoots, total length of shoots, rooting rate of shoots, number of roots, total length of roots.

3. Results and discussion
The results showed that the effectiveness of gooseberry microshoots rooting was significantly influenced to varying degrees by such factors as the form of insertion (the introduced complexes, as such, had a more positive reaction compared to the control), the concentration of complexes and their combined effect.

On the 45th and 60th days of subculturing, the distribution in decreasing order of the effectiveness of the studied chelate iron compounds influence on the rooting rate of studied plants microcuttings was as follows: Fe(III)-EDTA>Fe(III)-DTPA>Fe(II)-HEDP>Fe(III)-EDDHA>Fe(III)-HEDP.

On the 45th day of subculturing, the average rooting rate of microplants in the variants with the introduction of a chelate form of iron with a carboxyl-containing ligand Fe(III)-EDTA into the nutrient medium was 80%. The best rooting rate of microcuttings was found in variants where the iron concentration was reduced by half (×0.5) and was standard (×1) - in which it was 86.7-100% compared to 13.3% in the control (×1).

Also on the 45th day of subculturing, the best rooting rate of microcuttings (86.7%) was revealed in experimental variants with the introduction of a chelated form of iron into the nutrient medium with a carboxyl-containing ligand Fe(III)-DTPA in standard (×1), increased by 1.5 times (×1.5) and doubled (×2) concentrations. As for the reliability of differences, ANOVA showed a significant effect of the form of iron (factor b) and concentration (factor a) and their interaction (ab) on the number and total length of roots in the variant with the standard concentration of Fe(III)-DTPA (×1) (table 2). It is also worth noting the presence of necrosis (13%) in Fe (III)-DTPA in standard concentration (×1).

| Ligand | Necrosis, % | Number of shoots, pc. | Average length of shoots, cm | Total length of shoots, cm | Rooting rate, % | Number of roots, pc. | Total length of roots, cm |
|--------|-------------|------------------------|-----------------------------|---------------------------|-----------------|---------------------|-------------------------|
| FeSO₄·7H₂O + Na₂EDTA (control)×1 | 0.0 | 1.3 | 2.2 | 2.7 | 13.3 | 0.2 | 0.2 |
| FeSO₄·7H₂O + Na₂EDTA (control)×2 | 0.0 | 1.0 | 0.9 | 0.9 | 0.0 | 0.0 | 0.0 |
Fe-EDDHA×0,5  6,7  1,1  1,6  2,2  46,7  0,7  0,2  
Fe-EDDHA×1,0  6,7  1,0  1,6  1,8  13,3  0,1  0,0  
Fe-EDDHA×1,5  0,0  1,0  1,3  1,4  33,3  0,6  0,2  
Fe-EDDHA×2,0  0,0  1,2  2,0  2,4  46,7  0,9  0,4  
Fe(III)-HEDP×0,5  0,0  1,4  1,6  2,2  33,3  0,5  0,1  
Fe(III)-HEDP×1,0  0,0  1,0  0,9  1,2  0,0  0,0  0,0  
Fe(III)-HEDP×1,5  6,7  0,7  0,4  0,9  0,0  0,0  0,0  
Fe(III)-HEDP×2,0  20,0  0,3  0,4  0,4  0,0  0,0  0,0  
Fe-EDTA×0,5  0,0  1,5  1,3  1,9  100,0  2,1  0,6  
Fe-EDTA×1,0  0,0  1,4  1,7  2,3  86,7  2,1  1,7  
Fe-EDTA×1,5  6,7  1,1  1,3  1,6  66,7  1,5  1,0  
Fe-EDTA×2,0  0,0  1,2  2,0  2,3  66,7  1,2  1,3  
Fe-DTPA×0,5  0,0  1,1  1,9  2,1  53,3  1,3  1,0  
Fe-DTPA×1,0  13,3  1,0  1,6  1,7  86,7  2,1  2,0  
Fe-DTPA×1,5  0,0  1,1  1,8  2,1  86,7  1,8  1,1  
Fe-DTPA×2,0  0,0  1,3  1,5  2,0  86,7  1,6  0,9  
Fe(II)-HEDP×0,5  0,0  1,5  1,4  1,9  66,7  1,4  0,7  
Fe(II)-HEDP×1,0  0,0  1,6  0,9  1,2  80,0  1,5  0,6  
Fe(II)-HEDP×1,5  0,0  2,0  1,0  1,9  40,0  0,5  0,2  
Fe(II)-HEDP×2,0  0,0  1,5  0,9  1,4  40,0  0,9  0,3  

Least significant difference P < 0.05 a - 0,26  0,19  0,43  16,0  0,41  0,39  
Least significant difference P < 0.05 b - 0,22  0,16  0,36  14,0  0,34  0,33  
Least significant difference P < 0.05 ab - 0,75  0,53  1,23  46,0  1,16  1,11  

On the 60th day of subculturing, the average rooting rate of microplants in variants with the introduction of a chelated form of iron with a carboxyl-containing ligand Fe(III)-EDTA into the nutrient medium was 83%. The advantage of the variants remained where the concentration of chelated iron was reduced by half (×0.5) and was standard (×1) - 86.7-100% compared to 60% in the control (×1).

In addition, the advantage of the experimental variants with the introduction of the chelated form of iron with the carboxyl-containing ligand Fe(III)-DTPA in the standard (×1), increased 1.5 times (×1.5) and doubled (×2) concentrations also remained, where the rooting rate of microshoots was 86.7-93.3% compared to 60% in the control (×1). Moreover, analysis of variance showed a significant effect of the form of iron (factor b) and concentration (factor a) and their interaction (ab) on the number and total length of roots.

As for the morphometric parameters of plant development, there remained a significant difference in the average number of roots between the variant with the introduction of the chelated form of iron Fe(III)-EDTA into the nutrient medium and the control (×1). Also, there was a significant difference in the total root length between the variants with Fe(III)-EDTA and Fe(III)-DTPA at standard
concentrations (×1) and control (×1). The percentage of necrosis on the 60th day of the rhizogenesis stage remained the same as on the 45th day.

It should be noted that on the 60th day of subculturing, the advantage of variants with a bivalent chelate form with the organophosphate complexone Fe(II)-HEDP was revealed in variants where the iron concentration was halved (×0.5) and was standard (×1), while the rooting rate of microshoots was 86.7-93.3% compared to 60% in the control (table 3). This fact provides a prospect for further research in this direction, since it is known that the bivalent form of Fe is energetically more preferable for the vital activity of most plants. The use of the Fe(III)-EDTA and Fe(II)-HEDP complexes in a cultural medium had a positive effect on the rooting of microshoots even at concentrations lower than the standard (×0.5) and (×1.0). At the same time, the range of effective action of iron concentrations in the nutrient medium with Fe (III) -DTPA was stably in the area of higher values (×1.0) and (×2.0).

Table 3. Influence of different iron sources and concentrations on the rooting rate and morphometric indicators of gooseberry shoots cultivar Pink-2 (The 60th day of subculturing)

| Ligand | Necrosis, % | Number of shoots, pc. | Average length of shoots, cm | Total length of shoots, cm | Rooting rate, % | Number of roots, pc. | Total length of roots, cm |
|--------|-------------|-----------------------|-------------------------------|---------------------------|----------------|---------------------|--------------------------|
| FeSO₄×7H₂O + Na₂EDTA (control) ×1 | 0,0 | 1,3 | 2,9 | 3,5 | 60,0 | 1,1 | 0,8 |
| FeSO₄×7H₂O + Na₂EDTA (control) ×2 | 0,0 | 1,1 | 1,5 | 1,6 | 6,7 | 0,1 | 0,1 |
| Fe-EDDHA ×0,5 | 6,7 | 1,3 | 1,7 | 2,3 | 53,3 | 0,6 | 0,2 |
| Fe-EDDHA ×1,0 | 6,7 | 1,1 | 1,7 | 2,1 | 26,7 | 0,4 | 0,1 |
| Fe-EDDHA ×1,5 | 0,0 | 1,1 | 1,4 | 1,6 | 53,3 | 0,9 | 0,3 |
| Fe-EDDHA ×2,0 | 0,0 | 1,7 | 2,1 | 3,3 | 53,3 | 1,1 | 0,5 |
| Fe(III)-HEDP ×0,5 | 0,0 | 1,8 | 1,7 | 2,8 | 60,0 | 1,3 | 0,3 |
| Fe(III)-HEDP ×1,0 | 0,0 | 1,2 | 1,0 | 1,4 | 0,0 | 0,0 | 0,0 |
| Fe(III)-HEDP ×1,5 | 6,7 | 0,9 | 0,5 | 0,9 | 0,0 | 0,0 | 0,0 |
| Fe(III)-HEDP ×2,0 | 20,0 | 0,7 | 0,5 | 0,6 | 6,7 | 0,1 | 0,0 |
| Fe-EDTA ×0,5 | 0,0 | 1,3 | 1,7 | 2,0 | 100,0 | 2,9 | 1,0 |
| Fe-EDTA ×1,0 | 0,0 | 1,5 | 2,2 | 3,1 | 86,7 | 2,2 | 2,7 |
| Fe-EDTA ×1,5 | 6,7 | 1,2 | 1,8 | 2,3 | 66,7 | 1,5 | 1,2 |
| Fe-EDTA ×2,0 | 0,0 | 1,3 | 2,1 | 2,6 | 80,0 | 1,6 | 1,9 |
| Fe-DTPA ×0,5 | 0,0 | 1,1 | 2,5 | 2,8 | 53,3 | 1,5 | 1,5 |
| Fe-DTPA ×1,0 | 13,3 | 1,1 | 2,1 | 2,4 | 86,7 | 2,1 | 2,5 |
| Fe-DTPA ×1,5 | 0,0 | 1,3 | 2,2 | 2,7 | 86,7 | 2,1 | 1,6 |
| Fe-DTPA ×2,0 | 0,0 | 1,6 | 1,8 | 2,8 | 93,3 | 2,1 | 1,7 |
| Fe(II)-HEDP ×0,5 | 0,0 | 1,6 | 1,5 | 2,2 | 86,7 | 2,2 | 1,5 |
| Fe(II)-HEDP ×1,0 | 0,0 | 2,1 | 1,0 | 1,7 | 93,3 | 2,1 | 1,2 |
4. Conclusion
As a result of research, has been shown the effectiveness of cultural medium modification with mineral salts according to the Quoirin-Lepoivre protocol by replacing the form of iron, which is standardly used in the form of FeSO₄·7H₂O together with Na₂EDTA, with chelated forms with the carboxyl-containing ligands Fe(III)-EDTA and Fe(III)-DTPA and the organophosphorus complexone Fe(II)- HEDP.

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