Different Fe(III) and Fe(II) complexes in clonal micropropagation of Gooseberry

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Abstract. Iron chlorosis and tissue necrotization are often problems of plant growth in sterile conditions. Therefore, the overall multiplication factor and the productiveness dramatically decrease. The aim of work paper was to study the effect of various forms of iron chelates, including the stable complex of iron (II) with phosphorus-containing organic ligand, 1-hydroxyethylidenediphosphonic acid (HEDP), to the nutrient medium on the development of micro-shoots of Pink-2 (Rosovy-2) gooseberry cultivar according to Quoirin & Lepoivre (QL). Seven types of media were used in the screening: FeSO₄×7H₂O + Na₂EDTA (two control variant); Fe-EDTA – a complex of iron with ethylenediaminetetraacetic acid as such; Fe-DTPA (ferric diethylenetriaminepentaacetate), Fe₃⁺ and Fe₂⁺ -HEDP (ferric (III,II) 1-hydroxy ethylidene-1,1-diphosphonate), Fe-EDDHA (ethylenediamine di-2-hydroxyphenyl acetate ferric). The influence of 4 concentration values of concentrations of introduced iron complexes were studied experimentally: reduced (∗0.5), standard (∗1), increased 1.5-fold (∗1.5) and double concentration (∗2). Results have shown that form of introduction of iron sources, the valence of the iron ion, the ligand types and their concentrations are important factors in shoot multiplication of gooseberry cultivar ‘Pink-2’.

1 Introduction

Clonal micropropagation offers wide opportunities for selection and rapid propagation of promising, virus-free and genetically resistant cultivars. One of the key factors for successful plant propagation in vitro is the optimum composition and formula of the nutritional medium. In this context, a special attention is paid to improvement of the iron nutrition of microplants. Iron chlorosis and tissue necrotization are often problems of plant growth in sterile conditions [1]. Therefore, the overall multiplication factor and the productiveness dramatically decrease.

Iron ion plays a very important role in the physiology of plants, in the synthesis of chlorophyll, photosynthesis, breathing, synthesis of hormones, osmoprotection, assimilation of sulfates, and in the synthesis and recovery of DNA [2-5]. There are two common elemental

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states of iron, ferrous iron (Fe$^{2+}$), which is relatively soluble but readily oxidized, and ferric iron (Fe$^{3+}$), which is less soluble [6,7]. To enhance its solubility, Fe$^{3+}$ has been used in chelated forms ethylenediamine tetra acetic acid (Fe-EDTA) in micropropagation medium. As a rule, introduced into nutriculture media in the form FeSO$_4$$\times$7H$_2$O in combination with sodium salt of ethylenediaminetetraacetic acid (Na$_2$EDTA) [8]. This technique presumes that such a combined introduction of components in the medium further insures the formation of the iron-EDTA complex. However, the simple mixing of components is insufficient for a complete complex formation process. Required technology conditions should are met: the temperature regime, the pH of the medium, and the duration of stay [9]. In such a way, there is a high probability that when breeding a pathogen-free material in vitro, the chelated form of iron is not formed. As consequence, there is a risk of iron starvation, and systematic diseases of explants occur [10,11].

Attempts to improve the iron nutrition during plants propagation in vitro have been made previously. In doing so, the nutrition medium modification technique by introducing a more stable iron complex Fe-EDDHA as such was used. For example, the one described by Van Der Salm (1994), for micropropagation of Rosa hybrida L. ‘Moneway’ [12]. More recent papers report the data about the influence of the substitution of FeSO$_4$$\times$7H$_2$O + Na$_2$EDTA composition with Fe-EDDHA in nutrition media on in vitro growth and development of various plants: papaya, blackberry [13], raspberry [14], hazelnut and walnut [15, 16], olive [17], baptisia [18], peach [17, 19, 20], radish [21] etc [22]. However, no serious studies of other iron sources, except for Fe-EDDHA, have been conducted. At the same time, other efficient chelate compounds of iron are also known, both with carboxyl-containing and phosphorus-containing chelants. More to that, organophosphorous complexes are able to form, under certain conditions, stable complexes with both Fe$^{3+}$ and Fe$^{2+}$, the latter of which is more energetically beneficial for plants [23]. As for carboxyl-containing ligands, such as EDTA, DTPA, EDDHA, etc., it is impossible to obtain stable Fe$^{2+}$ complexes based on them. Moreover, complexe of Fe(III)EDTA are rapidly photodegraded [24,25].

The purpose of this work was to study the effect from the introduction of different forms of iron, including stable Fe$^{2+}$ complex with phosphorus-containing organic ligand, 1-hydroxyethylidenediphosphonic acid (HEDP), to the nutrient medium on the development of micro-shoots of Pink-2 (Rosovy-2) gooseberry cultivar.

2 Methods

Biological material for experiments were in vitro shoots of gooseberry cultivar ‘Pink-2’ (Rosovy-2) grown on Quoirin & Lepoivre (QL) culture medium [26] supplemented with following substances: (mg/l) thiamine hydrochloride (B1), pyridoxine hydrochloride (B6), nicotinamide (PP) – 0.5; mesoinoside – 100; glycine – 1,000; 6-benzylaminopurine – 0.3; saccharose – 30,000; agar-agar – 6,000.

Seven types of media were tested for shoot multiplication: QL medium [26] and its modification. The composition of all culture media is exactly the same with exception of iron source. Two QL medium (control variant 1 and 2) contains iron compound FeSO$_4$$\times$7H$_2$O + Na$_2$EDTA; modification №1 QL media (M1) contains Fe-EDTA complex as such (ferric sodium ethylenediaminetetraacetate) as iron source; modification №2 QL media (M2) contains Fe-DTPA (ferric diethylenetriaminepentaacetate); modification №3 QL media (M3) contains Fe$^{3+}$-HEDP (ferric (III) 1-hydroxy ethylidene-1,1-diphosphonate); modification №4 QL media (M4) contains Fe$^{2+}$-HEDP (ferric (II) 1-hydroxy ethylidene-1,1-diphosphonate) and modification №5 QL media (M5) contains Fe-EDDHA (ethylenediamine di-2-hydroxyphenyl acetate ferric). We tested the effect of modification QL media by iron chelates and their different concentrations on shoot multiplication on cultivar ‘Pink-2’.
The influence of 4 concentration values of concentrations of introduced iron complexes were studied experimentally: reduced (×0.5), standard (×1), increased 1.5-fold (×1.5) and double concentration (×2). On the 60th day of subcultivation, morphometric indicators of micro-shoots were recorded. Table 1 shows the studied sources of and their concentrations.

Table 1. Experimental concentrations of iron complexes

| Complex                  | Concentration of complexes | Reduced (×0.5) | Standard (×1) | Increased (×1.5) | Double (×2) |
|--------------------------|----------------------------|----------------|---------------|-----------------|-------------|
| C1 Control-1 (FeSO₄×7H₂O + Na₂EDTA) |                           |                |               |                 |             |
| C2 Control-2 (FeSO₄×7H₂O + Na₂EDTA) | +                         |                |               |                 |             |
| M1 Fe-EDTA (13,4%) mg/l   | 0.02                      | 0.04           | 0.06          | 0.08            |             |
| M2 Fe-DTPA (9,81%) mg/l   | 0.03                      | 0.06           | 0.09          | 0.12            |             |
| M3 Fe⁺⁺-HEDP (0,65%) mg/l | 0.43                      | 0.86           | 1.29          | 1.72            |             |
| M4 Fe⁺⁺⁺-HEDP (0,52%) mg/l| 0.54                      | 1.07           | 1.61          | 2.15            |             |
| M5 Fe-EDDHA (0,015%) mg/l | 18.6                      | 37.2           | 55.8          | 74.4            |             |

The culture medium, previously poured into culture vessels, was sterilized in an autoclave for 20 min at a temperature of 120°C and with a pressure of 0.1 MPa. In the laminar box, 5 micro-gears with a length of 2-3 nodes were placed in each vessel. Next, the cultures were incubated in a growth chamber at a lighting intensity of 2500 Lux, a 16-hour photoperiod, and a temperature of 20-22 °C.

3 Results and Discussion

Results have shown that form of introduction of iron sources, the valence of the iron ion, the nature of the ligand and their concentrations are important factors in shoot multiplication of gooseberry cultivar ‘Pink-2’. The introduction of iron chelate complexes directly into the nutrient media as such showed higher results compared to the standard version (Table 2.)

The general ranking of Fe-chelat effectiveness was:
Fe⁺⁺⁻⁻-HEDP>Fe-DTPA>Fe⁺⁺⁺⁻⁻-HEDP>Fe-EDTA>Fe-EDDHA

Table 2. Influence of different iron sources and concentrations on multiplication and of morphometric indicators cultivar of gooseberry shoots Pink-2

| Ligand | Necrosis % | Average number of shoots, pc | Average length of shoots, cm | Total length of shoots, cm | Rooting, % | Average number of roots, pc |
|--------|------------|-------------------------------|------------------------------|----------------------------|------------|-----------------------------|
| Control C1 | 0,0        | 1,1                           | 1,1                          | 1,2                        | 80,0       | 1,5                         |
| Control C2 | 26,7       | 0,9                           | 0,5                          | 0,6                        | 40,0       | 0,9                         |
| EDTA 0,5 | 0,0        | 1,3                           | 0,9                          | 1,1                        | 86,7       | 1,7                         |
| EDTA 1,0 | 0,0        | 1,0                           | 1,2                          | 1,2                        | 93,3       | 1,3                         |
| EDTA 1,5 | 0,0        | 1,2                           | 1,4                          | 1,6                        | 80,0       | 1,8                         |
| EDTA 2,0 | 0,0        | 1,0                           | 0,9                          | 0,9                        | 53,3       | 0,9                         |
| DTPA 0,5 | 0,0        | 1,2                           | 1,0                          | 1,2                        | 60,0       | 0,9                         |
| DTPA 1,0 | 0,0        | 1,1                           | 1,5                          | 1,6                        | 60,0       | 1,1                         |
Fig. 1. The effect of different iron type sources and concentration on shoot multiplication factor of gooseberry cultivar ‘Pink-2’ (Rosovy-2).

Fe^{2+}-HEDP showed maximum multiplication factor values of 2 and 1.8 were obtained at reduced and double concentrations (0.5-fold and 2-fold, respectively). For Fe-DTPA, best values of the multiplication factor, 1.3 and 1.7, were obtained in reduced and double concentrations (0.5-fold and 2-fold); for Fe^{3+}-HEDP, 1.5 and 1.3 values were obtained in increased and double concentrations, for Fe-EDTA 1.3 and 1.4 values were obtained in
reduced and increased concentrations. Complex Fe-EDDHA showed a slight improvement (Fig.1)

The excess of the multiplication factor when using different sources of iron was from 6% to 107% as compared to control variants. Here, it should be mentioned that during the experiment, the manifestation of retardant properties of HEDP were observed. Thus, during spontaneous risogenesis in micro-shoots of gooseberry, more radices were formed, but they were shorter.

4 Conclusions

In such a way, results of the comparative screening conducted allow to make the conclusion that the modification of the nutritional medium with iron complexes as such is more preferable. The substitution of the commonly used compound FeSO$_4$$\times$7H$_2$O + Na$_2$EDTA with the divalent complex of iron with oxyethylenediphosphonic acid demonstrated high effect on the process of proliferation of Rosovy-2 gooseberry breed explants, which in general opens new prospects for the enhancement of the efficiency of the cloned micropropagation technology. And Fe$^{2+}$- HEDP is worth broader studies and testing on various plants.

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