Aaftereffect of long-term deposition of raspberry micro-plants in a light room on nutrient media with the addition of Superstim drug modifications on their rhizogenesis and adaptation

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Abstract. When the clonal micropropagation of raspberry remontant the sorts of Briliantovay and Hercules) at the stages of in vitro rhizogenesis and adaptation to non-sterile conditions, the aftereffect of depositing micro-plants in a light room was evaluated when adding Superstim-1 and Superstim-2 preparations in small and ultra-small doses to the nutrient medium. Analysis of effective concentrations during deposition and aftereffect studies at all further stages of clonal micropropagation showed the advantage of the preparation Superstim-1 in concentration for the diamond variety 1×10-9 %, for the Heracles variety 1×10-6 %. When you Deposit in the culture room, micro plants remain viable without signs of necrosis and chlorosis for 10-12 months, during the remediation of the propagation coefficient is 3-5 times higher than those of control, on the rhizogenesis 20-30 days reduce the duration of the period of subcultivation, and the best options 100% accustomed at the stage of adaptation to non-sterile conditions in 2 - 2.5 times superior to the control indicators.

1. Introduction

After long-term deposition, regenerating plants need restoration procedures before the next stages of clonal micropropagation [1, 6]. The stage of rhizogenesis is an important stage of clonal micropropagation, which should ensure the development of a functional root system, with the help of which the micro-plant will be able to fully grow and develop during the transition to adaptation in non-sterile conditions. In most cases, the duration of the period of subcultivation of micro - shoots on a nutrient medium for rhizogenesis is variety- and species-specific. The rooting capacity of micro-plants increases with increasing passage duration, but the survival rate at the stage of adaptation of
such plants in General becomes lower. Efficiency of clonal micropropagation of plants of the genus Rubus L. it is largely determined by genotypic diversity, which is manifested in the ability to grow meristems, reproduction and rooting [9, 13, 15, 18].

With any rooting methods, the process of adventive root formation goes through 3-4 stages: induction, initiation, and the appearance of roots outside the stem part. The first two stages take 15 days, while the last one takes 10 or more days to complete, depending on the type of initial micro-gear, since at this time the cells acquire the ability to form meristematic foci, in which the synthesis of root-specific proteins begins, and then the appearance and growth of roots begins. Root meristems in cuttings are most often formed at the intersection of the cambium and phloem by core rays, while root primordia are laid in close proximity to vascular tissues [5].

For rooting micro-shoots of plants of the genus Rubus L. in vitro, a nutrient medium diluted twice in the content of macronutrients according to the Murashige and Skuga (MS) recipe with a double content of iron chelate is used [5, 11]. Traditionally, BMI, IUC auxins and, more rarely, NUC are used for the induction of rhizogenesis. It is considered optimal to use BCI at a concentration of 0.2-1.0 mg/l [12]. An important role is played by the duration of the micro-plants on the nutrient medium with auxins. Auxins necessary for the initiation of root rudiments are required only for the first 7-10 days, and the subsequent action of auxins inhibits root growth and promotes the development of callus [2, 16]. The roots of micro-plants are often devoid of root hairs during in vitro cultivation, which is associated with a lack of oxygen [2] and makes it difficult to absorb water and mineral salts [14]. In this regard, it is necessary to study the effect of new biologically active substances on rhizogenesis in vitro and develop techniques that reduce the duration of subcultivation at the stage of rhizogenesis.

Adaptation to non-sterile conditions is the main stage that characterizes successful micropropagation of plants in vitro. At this stage, it is necessary to create such conditions for regenerating plants that they will be able to continue their growth and development in new ex vitro conditions. Often at this stage, there is a stop in growth, leaf fall and death of more than 50% of micro-plants. Therefore, it is necessary to develop special techniques for transplanting test tube plants in non-sterile conditions [7, 17].

The adaptation period includes at least 4 parallel processes: adaptation of the assimilating apparatus to low air humidity and to a new infectious load, adaptation of adventitious roots to the substrate and soil microflora. The main task is to achieve the functionality of the root system while maintaining air humidity close to 100% in the area of the aboveground part with relative sterility of the substrate. The main criterion for the survival of micro-plants is the beginning of growth of the aboveground system, which indicates the adaptation of the root system to the conditions of the new substrate, which usually takes 2-3 weeks [4].

Our previous research has shown the feasibility of adding the preparations Superstim-1 and Superstim-2 to the nutrient medium in small and ultra-small doses for depositing raspberry repair micro-plants for 12 months in a light room, while recultivation after depositing experimental micro-plants in terms of reproduction coefficient is 3-5 times higher than the control indicators without depositing. Further, it was important to study the aftereffect on rooting in vitro and survival of experimental micro-plants at the stage of adaptation.

Preparations Superstim-1 and Superstim-2 (the originator of NNPP "NEST M"), are an extract from potato apexes, a complex multicomponent system of biologically active substances with high physiological activity, which is determined by the presence of vitamins, enzymes, organic and nucleic acids, as well as a full set of growth-stimulating phytohormones that regulate the synthesis of their own phytohormones in treated plants and increase their yield and resistance to diseases. The preparations are distinguished by the presence of diatoms in the preparation Superstim-2, the cells of which are distinguished by the presence of silicon dioxide [8].

The aim of the research is to study the aftereffect on rooting in vitro and survival at the stage of adaptation to non-sterile conditions of micro-plants of raspberry remontant after deposition in a light room on a nutrient medium with the addition of preparations Superstim-1 and Superstim-2.
2. Methods and materials

Objects of research: varieties of raspberries remontant Breliantovay and Hercules.

When deposited in a light room, experimental micro-plants were planted on a nutrient medium with mineral salts according to the Murashige and Skuga (MS) recipe, to which, according to the experiment scheme, Superstim-1 and Superstim-2 preparations were added in the concentration range from 1\times10^{-2} to 1\times10^{-18}\% without the use of synthetic cytokinins. Next, the cultures were deposited in a light room for 12 months at a light intensity of 2500 Lux, a 16-hour photoperiod, and a temperature of 20-22 °C. After that, with the preservation of the variants, two consecutive passages were performed on the nutrient medium for the animation stage according to the MS prescription, with the addition of 6-BAP (0.25 and 0.5 mg/l), the duration of subcultivation was 60 days.

Further, to study the aftereffect, experimental plants were planted at the stage of rooting in vitro in a nutrient medium for rhizogenesis according to MS prescription, diluted twice in the content of macronutrients with a double content of iron chelate, enriched with the following substances: (mg/l) thiamine-hydrochloride (B1), pyridoxine-hydrochloride (B6), nicotinamide (PP) - 0.5; BMI - 0.2; sucrose - 15,000, agar-agar - 6000. In a laminar box, 10 micro-gears with a length of 2-3 nodes were placed in each vessel.

On day 30 of subcultivation, micro-plants were planted at the stage of adaptation to non-sterile conditions in a substrate consisting of transitional enriched peat and perlite in a ratio of 3:1. 24 hours before micro-plants were planted, the substrate was saturated with water, and then spilled with a solution of the Previcur fungicide at a concentration of 2 ml/l. After 20 and 35 days, the survival rate of ex vitro plants, the length of shoots, the number and length of roots were taken into account. The repeatability of experiments is twofold with 7 plants in one repetition.

3. Results and discussion

After two passages at the stage of animation of raspberry remontant Brilliantovay experimental plants were planted on the stage of rhizogenesis in vitro. On day 30 of subcultivation in all variants with the study of the aftereffect of the drug Superstim-1, regardless of the concentration of 6-BAP, with the exception of the 1\times10^{-2}\% variant, the rooting rate of micro-plants was 100% compared to 70.0-71.4% in controls. When using the drug Super stim-2 only in one variant - 1\times10^{-9}\%, the rooting rate of micro-plants was 100% (table 1).

Taking into account the survival rate and development of ex vitro plants on day 35 after transplantation in non-sterile conditions showed that in the variants with the study of the aftereffect of the drug Superstim-1, with the exception of 1\times10^{-2} \% and 1\times10^{-15} \%, the survival rate was 100% against 85.7\% in the control. As for the development of plants, there were significant differences with the control for all the indicators taken into account. However, the best results were obtained in variants 1\times10^{-3}\% and 1\times10^{-9}\%, where on the 35th day of adaptation, the total length of shoots was 88.6-102.8 cm against 64.7 cm in the control, and the leaf surface area was 40.3-46.2 against 36.4 cm².

As for the preparation Superstim-2, on the 35th day of adaptation, only in two variants 1\times10^{-6} \% and 1\times10^{-9} \%, the survival rate of plants was 100% against 85.7\% in the control. The best results of development indicators were obtained in variant 1\times10^{-9}\%, where on the 35th day of adaptation, the total length of shoots was 88.6 cm against 64.7 cm in the control, and the leaf surface area was 40.3 against 34.6 cm².
Table 1. Aftereffect of depositing raspberry remontant micro-plants in a light room (Billiantovaya variety) with the introduction of Superstim-1 and Superstim-2 preparations into the nutrient medium for rooting at the stage of rhizogenesis in vitro

| Concentration (mg/l) | Rooting, % | Average number of roots, pieces | Rooting, % | Average number of roots, pieces |
|---------------------|------------|----------------------------------|------------|----------------------------------|
|                     | BMI control 0.2 | Superstim-1 (b) | Superstim-2 (b) |                     | at the stage of multiplication 6-BAP 0.25 mg/l |
|                     | 71.4 | 0.9 | 71.4 | 0.9 |
| 1×10⁻² %           | 100  | 2.6 | 71.4 | 1.0 |
| 1×10⁻³ %           | 260  | 2.0 | 100.0 | 1.4 |
| 1×10⁻⁶ %           | 100  | 1.7 | 85.7 | 1.6 |
| 1×10⁻⁹ %           | 100  | 2.1 | 71.4 | 0.7 |
| SSD₀.₀₅ a           | -     | 0.15 | - | 0.15 |
| SSD₀.₀₅ b           | -     | 0.05 | - | 0.05 |
| SSD₀.₀₅ ab          | -     | 0.24 | - | 0.24 |
|                     | BMI control 0.2 | Superstim-1 (b) | Superstim-2 (b) |                     | at the stage of multiplication 6-BAP 0.5 mg/l |
|                     | 70.0 | 0.9 | 70.0 | 0.9 |
| 1×10⁻² %           | 100  | 2.7 | 71.4 | 1.0 |
| 1×10⁻³ %           | 100  | 1.7 | 57.1 | 0.9 |
| 1×10⁻⁶ %           | 100  | 2.0 | 100.0 | 1.4 |
| 1×10⁻⁹ %           | 100  | 2.1 | 57.1 | 0.7 |
| SSD₀.₀₅ a           | -     | 0.13 | - | 0.13 |
| SSD₀.₀₅ b           | -     | 0.04 | - | 0.04 |
| SSD₀.₀₅ ab          | -     | 0.21 | - | 0.21 |

The repair raspberry variety Hercules is difficult to root, requires a long period of rhizogenesis (more than 60 days) and usually takes root no more than 40-50%. On day 30 after transplantation for rhizogenesis in vitro, the advantage of variants with the study of the aftereffect of adding the Superstim-1 drug to the nutrient medium was revealed, since in the variants: 1×10⁻⁶ % and 1×10⁻⁹ % rootability of micro-plants was 85.7-100% against 42.9 % in controls. When using the drug Superstim-2 only in one variant - 1=10-3%, the rooting rate of micro-plants was 71.4% (table 2).

Taking into account the survival and development of ex vitro plants on day 35 of the adaptation stage when studying the aftereffect of the drug Superstim-1 in variants 1×10⁻⁶%, 1×10⁻⁹%, 1×10⁻¹⁵%, 1×10⁻¹⁸% the survival rate of regenerants was 100% against 85.7% in the control. As for plant development indicators, the best results were obtained in the following variants on the 35th day of adaptation 1×10⁻⁶%, 1×10⁻⁹%, 1×10⁻¹⁸%, where the total length of shoots was 57.8 -64.7 cm, against 29.5 cm in the control, and the leaf surface area was 33.7 -37.3 against 20.4 cm².

As for the preparation Superstim-2, significant differences with the control in survival and plant development indicators were noted only in the variant 1×10⁻⁶ %, where the total length of shoots was 57.8 - against 29.5 cm in the control, and the leaf surface area was 33.7 - against 20.4 cm².
Table 2. Aftereffect of depositing in a light room of micro-plants of raspberry remontant (variety Hercules) when introducing Superstim-1 and Superstim-2 preparations into the nutrient medium for rooting at the stage of rhizogenesis in vitro

| Concentration (mg/l) | Rooting, % | Average number of roots, pieces | Rooting, % | Average number of roots, pieces |
|---------------------|------------|---------------------------------|------------|---------------------------------|
|                     | Superstim-1 (b) |                                | Superstim-2 (b) |                                |
| at the stage of multiplication 6-BAP 0.25 mg/l |
| BMI control 0.2 | 42.9 | 1.2 | 42.9 | 1.2 |
| 1×10⁻² % | 71.4 | 2.0 | 28.6 | 0.6 |
| 1×10⁻³ % | 42.9 | 0.6 | 71.4 | 1.0 |
| 1×10⁻⁶ % | 100.0 | 2.4 | 57.1 | 1.4 |
| 1×10⁻⁹ % | 85.7 | 1.8 | 57.1 | 0.6 |
| 1×10⁻¹² % | 57.1 | 1.2 | 42.9 | 0.6 |
| 1×10⁻¹⁵ % | 71.4 | 1.6 | 57.1 | 0.8 |
| 1×10⁻¹⁸ % | 57.1 | 1.6 | 42.9 | 0.8 |
| SSD sub a | 0.73 | 0.11 | 0.73 | 0.11 |
| SSD sub b | 0.24 | 0.04 | 0.24 | 0.04 |
| SSD sub ab | 1.15 | 0.18 | 1.15 | 0.18 |

| at the stage of multiplication 6-BAP 0.5 mg/l |
| BMI control 0.2 | 42.9 | 1.0 | 42.9 | 1.0 |
| 1×10⁻² % | 71.4 | 1.6 | 28.6 | 0.4 |
| 1×10⁻³ % | 42.9 | 0.6 | 71.4 | 1.0 |
| 1×10⁻⁶ % | 100.0 | 2.4 | 57.1 | 1.6 |
| 1×10⁻⁹ % | 85.7 | 1.6 | 57.1 | 0.8 |
| 1×10⁻¹² % | 57.1 | 1.0 | 42.9 | 0.6 |
| 1×10⁻¹⁵ % | 71.4 | 1.4 | 57.1 | 1.0 |
| 1×10⁻¹⁸ % | 57.1 | 1.6 | 42.9 | 1.2 |
| SSD sub a | 0.73 | 0.14 | 0.73 | 0.14 |
| SSD sub b | 0.24 | 0.04 | 0.24 | 0.04 |
| SSD sub ab | 1.15 | 0.21 | 1.15 | 0.21 |

Analysis of effective concentrations during deposition and aftereffect at all further stages of clonal micropropagation showed the advantage of the preparation Superstim-1 in concentration for the diamond variety 1×10⁻⁹ %, for the Hercules variety 1×10⁻⁶ %. Probably, the variety-specific reaction is due to the fact that the studied varieties have a different ratio of initiators of regeneration and inhibitors of plant growth, which explains their different ability to activate the potential of the plant organism to reproduce. The variety of raspberry repair Hercules is difficult to propagate, and therefore, to activate the regenerative potential, it requires a higher dose of the studied drug Superstim-1 1×10⁻⁶ %. For the raspberry variety remontantnaya brilliant effective is a lower concentration of 1×10⁻⁹ %, since it is medium-multiplicable.

4. Conclusion
When assessing the aftereffect of depositing micro-plants of raspberry remontant (varieties brilliant and Hercules) in the culture room at the stage of rhizogenesis in the best cases, when using the drug Superstim-1, the duration of the rooting period was reduced by 20-30 days. The survival rate at the stage of adaptation was 100%, and in terms of development, the experimental ex vitro plants were 2-2.5 times higher than the control ones.
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