The use of Superstim modifications in low and ultra-low doses for long term deposition of micro-plants of perpetual raspberry in the culture room conditions

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Abstract. A method for long-term deposition of micro-plants in the culture room conditions when adding Superstim-1 and Superstim-2 products in low doses to the nutrient medium has been developed for clonal micro-propagation of perpetual raspberry (Diamond, Gerakl varieties). This method does not require any special equipment and ensures viability sustaining of micro-plants without signs of necrosis and chlorosis for 10-12 months without additional transfers to a fresh nutrient medium. When depositing regenerants of the Brilliantovaya variety for 12 months in the culture room conditions, the advantage of adding Superstim-1 in concentration of $1 \times 10^{-9}$ %, $1 \times 10^{-15}$ %, $1 \times 10^{-18}$ % to the nutrient medium composition was revealed. When depositing the Gerakl variety, it was found that storing of micro-plants of the Gerakl variety for more than 10 months is impractical, since at the 12-th month of sub-culturing the viability of micro-plants did not exceed 30% even in the best variants of the experiment. At the 10th month of sub-culturing, the advantage of adding the Superstim-1 product $1 \times 10^{-6}$ %, $1 \times 10^{-9}$ %, $1 \times 10^{-15}$ %, $1 \times 10^{-18}$ % to the nutrient medium composition of the Superstim-1 product was revealed.

Plants of Rubus L. kind are valuable berry crops, popular not only in Russia, but also in many countries of the world. The analysis of the current state of nursery production shows that it is difficult to obtain domestic planting material of the highest quality categories in the shortest possible time with the existing material and technical base [1]. In this regard, it is promising to improve the technology of clonal micro-propagation as a modern intensive method of mass reproduction of plants [2,3,5].

In clonal micro-propagation, mericlones require frequent transfers to a fresh nutrient medium, exposing plants to stress, loss of physiological and genetic stability [8, 9]. The number of passages at the stage of multiplication should not exceed 10-15, since with increasing their number, the probability of somaclonal variability in regenerating plants increases, reducing the regenerative potential, rooting ability and survival when transferred to non-sterile conditions [2, 3, 6, 11, 13, 14].

Optimization of the technological process, in which part of the plants introduced into the culture \textit{in vitro}, are sent for mass reproduction, after which all the micro-plants are adapted to non-sterile
conditions may be promising. And the other part of the sterile culture is deposited until the next production cycle.

By now the literature sources contain extensive experimental material regarding the methods of conservation and reproduction of plants in vitro with a detailed description of a large number of techniques and methods. In the end, all the methods of maintaining collections in vitro can be divided into two categories: transplant collections, in which the storage of plant material occurs under normal growth conditions, and deposited collections, in which plants are in a state of slow growth [20].

There are many ways to deposit plant tissue cultures in vitro: deep freezing to the temperature of liquid nitrogen (-196°C), depositing the collection at low positive temperatures through selecting the appropriate composition of the gas medium and the spectral composition of light, changing the concentration of carbohydrates in the nutrient medium, using retardants, hypoxia, etc. [7, 15, 17, 18, 19, 21, 22, 23, 24].

In most cases, the ability of plants to genetically stable regeneration is significantly higher in cultures with slow growth, so the development of new and improving the existing methods of storage of plants in a state of slow growth is a promising direction for preserving the gene pool of garden plants [2].

One of the ways to increase the production of environmentally safe products is the use of physiologically active substances in low and ultra-low doses [12]. In this regard, it is promising to study the effectiveness and develop regulations for the use of a new generation of Superstim-1 product from “NEST M” Company, which can become a support for modernization of the elements of the clonal micro-propagation technology of garden plants.

Superstim-1 and Superstim-2 products (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 crop capacity and resistance to diseases. The products differ in the presence of diatoms in the Superstim-2 product, whose cells differ in the presence of silicon dioxide [10].

Objective of research - Develop a method for long-term deposition of micro-plants of perpetual raspberry in the culture room conditions when adding Superstim-1 and Superstim-2 products to the nutrient medium in low and ultra-low doses.

Research technique.

Subjects of research: Brilliantovaya and Gerakl varieties of perpetual raspberry.

When depositing in the culture room conditions, micro-plants were planted on a nutrient medium with mineral salts according to the Murashige & Skoog (MS) inscription, enriched with the following substances: (mg/l) thiamine-hydrochloride (B1), pyridoxine-hydrochloride (B6), nicotinamide (PP) - 0.5; mesoinosite - 100; glycine - 1000; sucrose – 30000, agar-agar - 6000. According to the experimental design, the Superstim-1 and Superstim-2 products were added in the concentration range from 1×10^{-2} to 1×10^{-18} %, without application of synthetic cytokinins. As a control, we used a nutrient medium for rhizogenesis with mineral micro- and ½ macro-salts according to MS inscription, with addition of indole butyric acid (IBA) (0.3 mg/l), auxiliary controls - osmotic inhibitors Mannitol and Sorbitol by 4 g/l. The technology of preparation of experimental solutions of the studied products provided for sequential dilution of the initial mother liquor. To prepare the initial solution with a concentration of 1×10^{-2}%, the studied drugs in an amount of 100 mg were dissolved in 1000 ml of the finished nutrient medium. Then 100 ml of the solution with a concentration of 1×10^{-2} % was brought to 1000 ml with a nutrient medium and a solution of 1×10^{-3} % was obtained. Similarly, we performed successive dilution to lower concentrations. In the laminar box 10 micro-cuttings with a length of 2-3 nodes were placed in each vessel. Next, the cultures were incubated in a light room at a lighting intensity of 2500 Lux, a 16-hour photoperiod, and a temperature of 20-22°C. On the 30, 60 and 120 days of sub-culturing, morphometric indicators of micro-shoots were taken into account, and then the
viability of experimental micro-plants was taken into account once every 2 months. The repetition of experiments when depositing is three times for 5 plants in one repetition.

**Results and discussion**

When studying the dynamics of changes in morphometric indicators of micro-shoots of perpetual raspberry of the Brilliantovaya variety on the 30-th day of sub-culturing, it was found that in general, regardless of the products and their concentration, the experimental micro-plants differed slightly from the plants under control. However, on the 60-th day of sub-culturing in experienced options the indicators of the number of roots and length of shoots exceeded the control indicators (IBA) in 1.5-3 times and the total root length – in 2 - 5 times.

After four months of sub-culturing, due to the increase in the length and number of micro-shoots and roots, it was no longer possible to take detailed morphometric measurements of regenerants, so during the next 8 months, the viability of regenerants was recorded at intervals of 60 days.

As a result, it was found that starting from the sixth month of depositing in the control with IBA (0.3 mg/l), the viability of micro-plants deteriorated sharply and amounted to 53.3%, and by the 12th month of depositing it is decreased to 10%. In the variants with the use of osmotic inhibitors mannitol and sorbitol, the viability of regenerants was 73.3% in the sixth month of sub-culturing, then it is decreased evenly, and by the 12-th month of storage it was only 23.0-30.2%. In the experimental variants with the use of Superstim-1 $1 \times 10^{-9}$ %, $1 \times 10^{-15}$ %, $1 \times 10^{-18}$ % at the sixth month of sub-culturing, the viability of explants amounted to 90.0-93.3%, and by the 12-th month of sub-culturing – 80-86.5%.

As for application of the Superstim-2 product in variants $1 \times 10^{-9}$ %...$1 \times 10^{-18}$ % for the sixth month of sub-culturing, the viability of explants amounted to 86.7-100%, and by the 12-th month - in variants $1 \times 10^{-9}$ %, $1 \times 10^{-15}$ %,$1 \times 10^{-18}$ % only 48.4-55.9%, the advantage was preserved only by one variant $1 \times 10^{-12}$ % where the viability was 65.4% (figure 1).

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**Figure 1.** Dynamics of maintaining the viability of micro-plants when introducing modifications of the Superstim product into the nutrient medium during 12 months of depositing (Brilliantovaya variety).

When studying the dynamics of changes in morphometric indicators of micro-shoots of perpetual raspberry of the Gerakl variety on the 30-th day of sub-culturing, it was also revealed that the experimental micro-plants differed slightly from the plants in the control variants. On the 60-th day of sub-culturing in the experimental variants the root number indicators exceeded the IBA control indicators by 2-3. 5 times, with the length of shoots by 2 times, and the total length of roots by 2 - 3 times. In general, the advantage of the Superstim-1 product is also preserved.

As for the dynamics of maintaining the viability of micro-plants, it was found that since the sixth month of depositing in the control with IBA, the viability deteriorated sharply and amounted to 36.2%...
and by the twelfth month of storage amounted to 15.6% only. In the variants with the use of osmotic inhibitors mannitol and sorbitol, the viability of regenerants was 53.3-58.4% for the sixth month of sub-culturing, then it decreased evenly, and by the 12-th month of storage it was 15.6%.

In the experimental versions with the use of the Superstim-1 product in concentration of $1 \times 10^{-6}$ %, $1 \times 10^{-9}$ %, $1 \times 10^{-15}$ %, $1 \times 10^{-18}$ % at the sixth month of sub-culturing, the viability of explants amounted to 76.7-85.2%, and by the 10th month of sub-culturing it was 66.7-80%, but by the 12th month it fell down to the control level – 6.7-20.2%. When using the Superstim-2 product in the experimental variants for the sixth month of sub-culturing, the viability of explants amounted to 46.7-93.3%, but by the 10th month only in two experimental variants of $1 \times 10^{-2}$ %, $1 \times 10^{-12}$ %, the viability amounted to 60.0-80.0%, and by the 12th month of sub-culturing it decreased to 15.6-28.9% (figure 2).

Figure 2. Dynamics of maintaining the viability of micro-plants when introducing modifications of the Superstim product into the nutrient medium during 10 months of deposition (Gerakl variety).

Thus, the expediency of adding Superstim-1 and Superstim-2 products to the nutrient medium for depositing of micro-plants of perpetual raspberry in the culture room conditions was revealed. This is probably due to the fact that the growth of plants, where one of the defining processes is cell division, is regulated by several hormones, so we can assume that this reaction of plants is the result of the interaction of pathways for transmitting hormonal signals. The effects of auxins and cytokinins on the cellular multiplication processes have been studied in sufficient detail, while the value of ethylene and abscisic acid (ABA) remains the subject of intense discussion, and data on the interaction of signal pathways of these hormones in connection with cell division is not available.

It is known that during long-term sub-culturing of micro-plants at the stage of multiplication, the amount of ethylene increases in the culture vessel over time, which, acting on specific receptors of regenerating plant cells, causes necrotizing of tissues and even the death of micro-plants. Presumably, when Superstim-1 and Superstim-2 are added to the nutrient medium, the hormonal composition of the medium changes, which blocks the synthesis of ethylene or, penetrating into the micro-growth reduces the susceptibility to ethylene of specific cell receptors [16].

Further research in this direction with expansion of possible research objects, not only of the genus Rubus L., but also of the other economically valuable garden plants, is of great practical importance. Research on the possible duration of deposition in the culture room conditions is also promising.

Conclusion
1. When depositing in the culture room conditions with a light intensity of 2500 Lux, a 16-hour photoperiod and a temperature of 20-22° the advantage of adding the Superstim-1 product to the nutrient medium for multiplication based on MS inscription was revealed in micro-plants of perpetual raspberry.
2. When depositing the Brilliantovaya variety, the advantage of adding the Superstim-1 product to the nutrient medium was revealed, since in the variants of $1 \times 10^{-9}\%$, $1 \times 10^{-15}\%$, $1 \times 10^{-18}\%$ at the sixth month of sub-culturing, the viability of explants was 90.0-93.3%, and by the 12th month of sub-culturing – 80-86.5% against 10.0-30.2% in control variants.

3. When depositing the Gerakl variety, it was found that storing micro-plants of the Gerakl variety for more than 10 months is impractical, since for the 12-th month of sub-culturing, even in the best variants of the experiment, the viability of micro-plants did not exceed 30%. At the 10-th month of sub-culturing in variants with the use of Superstim-1 $1 \times 10^{-6}\%$, $1 \times 10^{-9}\%$, $1 \times 10^{-15}\%$, $1 \times 10^{-18}\%$ preservation of regenerants amounted to 66.7-80%.

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