Adaptation of microclones of blackberries to in vivo conditions

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Abstract. The results of studying the effect of mineral fertilizing on rhizogenesis and the development of aboveground organs of regenerant plants of blackberry thornless adaptable to in vivo conditions in the laboratory of selection, vegetable growing and horticulture, cloning "UNITS" Agrotechnopark "of Belgorod State Agrarian University are presented. Regenerated plants of thornless blackberry cultivar Agavam were adapted to in vivo conditions in a peat-perlite mixture with the addition of microelements and growth regulator root 16 days earlier than in the control. An active growth of the aboveground part and roots of regenerated plants of thornless blackberry was noted on the 21st day, in the control - on the 42nd day after the start of adaptation. By the end of the rooting stage on the 24th day, the regenerant plants formed an aerial part of two pairs of leaves 22 mm high and a developed root system - 37 mm. The mineral and hormonal composition of nutrient media for the cultivation of thornless blackberries has been optimized, an effective combination of physical and chemical factors at different stages of micropropagation has been determined, which enhance the proliferation of shoots and roots, and the dependence of the efficiency of adaptation of regenerated plants to in vivo conditions has been established. Along with traditional breeding methods, new opportunities for solving the problem of thorn-free blackberry varieties are provided, along with traditional breeding methods, which make it possible to accelerate the process of obtaining valuable planting material to provide the population and the processing industry with valuable berry products.

1. Introduction
Taking into account the complexity and high science intensity of genetic engineering work at the first stage of biotechnology development in agricultural scientific institutions, cellulated technologies have been greatly developed and made it possible to create insignificant volumes of somaclonal plant variants based on suspension and cell samples, haploid and autodiploid variants of anther and pollen crops, regenerants from germs formed in callus tissues obtained from immature germs and other plant parts. For the successful cultivation of isolated plant cells and tissues it is necessary to observe certain growing conditions, where phytohormones are the main tools for controlling the processes of callus formation, differentiation, growth and development of regenerative plants [1].

Blackberries relatively recently began to appear in the gardens of residents of Russia. While this culture is not widespread enough. The thornless blackberry (Rubus fruticosus) is a relatively rare berry in the Russian Federation and it is of interest for the food, perfume and pharmaceutical industries. Its specific features are high yields, thornless scions and drought resistance. Blackberries contain a wide range of vitamins and minerals [2, 3]. The crop has a high potential - it is less fastidious plant than...
Raspberries, it bares drought more easily and has a strong plant immunity to diseases; its plant performance is higher compared with raspberries one.

The popular Agavam variety is distinguished by small berries weighing up to 4 grams, they are characterized by a black tint and shine, which is found in all blackberry varieties. After full ripening, the fruits have an exquisite taste, are very sweet and can be easily removed from the stalk. Its yield is constant and rather high, about 10 kg from one bush. This variety is resistant to low temperatures, so the bush can withstand frosts down to -30 degrees without shelter. Thanks to such features, this variety compares favorably with others and is often used for planting in northern regions without additional shelter.

The main problems are insufficient knowledge in the conditions of the Russian Federation, the limited availability of domestic varieties and the inaccessibility of foreign ones [4]. A key element of successful blackberry cultivation is the use of virus-free certified planting material.

To meet the need for planting blackberries material one uses a vegetative propagation. But to accelerate this process there are other ways, which include microclonal reproduction, which allows you to get a large amount of blackberry planting material.

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Adaptation is an important part in micropropagation technology and it is the second most difficult technological process in micropropagation. At this stage a lot of attention should be paid to climate control and sterility of adaptation boxes. The choice of substrate and its parameters depend on the biological needs of crop requirement [5, 6, 7].

In plants cultivated in vitro, physiological processes vary within a fairly wide range, depending on the genotype, composition, and substrate of the medium (agar-agar, perlite, etc.). Physiological processes also change under the influence of growth regulators and cultivation conditions. At the same time, an urgent task is to research the effect of mineral fertilizing on rhizogenesis and the development of aboveground organs of regenerated thornless blackberry in vivo. The rate of development of the root system at the stage of rhizogenesis largely determines the intensity of development of plants when they are transplanted into the soil.

2. Materials and methods

The researches for effect examination of mineral fertilizing on rhizogenesis and the development of aboveground organs of adaptable regenerants of thornless blackberry of Agavam varieties were carried out in the "Agrotechnopark” - laboratory of selection, vegetable growing, horticulture and cloning at the Belgorod State Agricultural University.

At the stage of rooting a modified nutrient medium based on the Murashige and Skoog medium was a substrate for the regenerants (Table 1).

Cytokinins and auxins fulfill their functions only in combination with each other. Their effect depends on the ratio of components and the crop on which this complex is applied. The cytokinin concentration was 0.1 mg / l, the auxin concentration was 0.5 mg / l. Cytokinins stimulate cell fission, activates branch buds, removes the effect of apical dominance and in excess concentrations it stimulates the callus formation. Auxin can be synthesized by plant itself and it serves to induce rhizogenesis and causes apical dominance [8].

The concentration of vitamins B was 0.25 mg / l, vitamin PP - 0.15 mg / l, vitamin C - 0.30 mg / l.
Table 1. The composition of nutrient medium for rooting

| Component                  | Concentration, mg / l |
|----------------------------|-----------------------|
| NH$_4$NO$_3$               | 825.0                 |
| KNO$_3$                    | 950.0                 |
| KH$_2$PO$_4$               | 85.0                  |
| CaCl$_2$ * 2 H$_2$O        | 220.0                 |
| MgSO$_4$ * 7 H$_2$O        | 185.0                 |
| Na$_2$EDTA                 | 18.7                  |
| FeSO$_4$ * 7 H$_2$O        | 13.9                  |
| MnSO$_4$ * 4 H$_2$O        | 11.2                  |
| H$_3$BO$_3$                | 3.1                   |
| ZnSO$_4$ * 4 H$_2$O        | 4.3                   |
| KI                         | 0.42                  |
| Na$_2$MoO$_4$ * 2 H$_2$O   | 0.125                 |
| CuSO$_4$ *5 H$_2$O         | 0.0125                |
| CoCl$_2$ *5 H$_2$O         | 0.0125                |
| 6-benzylaminopurine        | 0.1                   |
| indolebutyric acid         | 0.5                   |

For rooting explants the containers of 0.5 l were used, the substrate was a peat-perlite mixture. After transplanting the containers with seedlings were closed hermetically with transparent covers and placed in a phytotron. The temperature was constantly kept at + 23° C for seven days. In the next 12 days the temperature was kept at +21 ° C in the daytime and at +17 ° C – in the night-time. Airing of containers with seedlings was started on the 5th day. On the 14th day of adaptation the cover was completely removed from the containers. The relative air humidity in the phytotron throughout the entire adaptation period was 90%.

On the 14th day one applies the fertilizing with nutrients adding root channel to the nutrient solution (Table 2). The control element is water.

Table 2. Composition of fertilizing solution

| Component                  | Concentration, mg / l |
|----------------------------|-----------------------|
| NH$_4$NO$_3$               | 206.3                 |
| KNO$_3$                    | 237.5                 |
| KH$_2$PO$_4$               | 21.3                  |
| CaCl$_2$ * 2 H$_2$O        | 55.0                  |
| MgSO$_4$ * 7 H$_2$O        | 46.3                  |
| Kornevin                   | 100.0                 |

On a peat-perlite substrate one can regularly apply fertilizing, which leads to less stress caused by changes in growing conditions and an increase in the regenerants establishment.

Biometric measurements were performed every 7 days, visual control was taken every 24 hours.

3. Results

The adaptation is successful when the regenerants start to grow. This means the plants have overcome stress of changing conditions in vitro to in vivo ones.

By the end of rooting phase (24 days) the regenerants have formed a root system and developed aboveground part. Blackberry plants formed 2 pairs of leaves and 4.1 roots (Table 3). The length of aboveground part of the regenerants averaged 22 mm and 37 mm of the roots.
Table 3. Linear measurements

| Indicators                        | 14 days control | 14 days fertilizing | 21 days control | 21 days fertilizing |
|----------------------------------|-----------------|---------------------|-----------------|---------------------|
| Height of aboveground part, mm    | 22              | 22                  | 22              | 24.1                |
| Number of leaves, parts          | 4               | 4                   | 4               | 4.2                 |
| Root length, mm                  | 23.4            | 23.5                | 24.2            | 27.3                |
| Number of roots, parts           | 3               | 4.1                 | 4.6             | 5.1                 |

During the first week of fertilizing applying (14 days), the linear measurements of the regenerants did not differ from the control ones. The height of the explants was 22 mm, the number of leaves was 2 pairs.

A more intense root formation was noted in comparison with the control one when applying fertilizers and stimulants. On the 21st day the regenerated blackberry plants started to grow while no signs of growth were noted in the control.

Blackberry plants formed an average of 5.1 parts of roots, which is 1.4 parts more than in the control group. The root length was 23 mm. The growth of the aboveground organs was 2.1 mm and the roots growth was 4.3 mm on average.

The active growth of the aboveground part of plants in the control group was noted on the 42nd day of adaptation.

According to our research fertilizing applying with a growth stimulator "Kornevin" allowed the regenerated plants to overcome stress, which led to early regrowth (on day 21) of the above-ground organs and roots compared to the control ones.

4. Conclusion

As a result of studies carried out in the laboratory of selection, vegetable growing and horticulture, cloning "UNITS" Agrotechnopark "Belgorod State Agrarian University, the mineral and hormonal composition of nutrient media for the cultivation of thornless blackberries was optimized, an effective combination of physical and chemical factors at different stages of micropropagation was determined, which enhance the proliferation of shoots and roots, the dependence of the efficiency of adaptation of regenerated plants to in vivo conditions was established. The results obtained make it possible to increase the practical significance of the used biotechnological methods as in the system of production of planting material for blackberry thornless.

References

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