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Article



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THE USE OF EXTRA RAYS OF ULTRAVIOLET EXCILAMPS IN SYMBIOSIS BY ENRICHING THE SOIL WITH HUMIC FERTILIZERS TO SOLVE PROBLEMS WITH INFERTILE SOIL IN ORDER TO ACCELERATE THE FORTIFICATION OF FORESTS FROM SEEDLINGS TO LARGE INDIVIDUALS OF CONIFEROUS AND DECIDUOUS TREES: BETA PROTOCOL

Abstract: The relevance of the topic. Currently, along with traditional methods of reproduction of valuable forms and varieties of forest woody plants, the method of culture of isolated organs and tissues (clonal micro-propagation of plants) is used. This problem is even more urgent in the years of accelerated reforestation of mountain forests with deciduous tree species. The advantages of this method include: the speed of production, the exclusion of viral diseases, the need for a small number of initial explants and limited space, the possibility of year-round production of planting material, its long-term preservation with minimal volumes of cold storage, the production of a large amount of planting material per year. The need for mass reproduction of genetically improved forms of woody plants with the help of tissue culture to improve the qualitative composition of forest plantations by obtaining clone plants resistant to diseases and pests, stress and man-made factors can accelerate the reproduction of forest resources, will allow obtaining genetically improved material much earlier than under normal conditions.

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Introduction

The first works on the organogenesis of *Pinus sylvestris* and *Picea abies* in Russia were carried out on the basis of the use of hypocotyl explants or seeds at the Russian State Agricultural University (Timiryazev Moscow Agricultural Academy) and the Institute of Bioorganic Chemistry of the Russian Academy of Sciences [13, 14, 24].

Currently, during the joint work of the Institute of Bioorganic Chemistry and SPbNIIH,

organogenesis based on the use of the buds of plus trees has been performed, recommendations have been developed for the adaptation of the obtained microregenerants in peat substrate under conditions of film greenhouses [31, 34]. The planting material grown in this way includes several experimental plots of forest crops and their growth is monitored, control is provided by crop variants using two-year-old seedlings from the seedling department of the nursery,

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having approximately the same biometric parameters with micro-gear seedlings at the time of planting.



Picture 1.

In the forest zone of Russia, simplified technologies widely used in practice for laying and growing forest crops, as a rule, do not give them growth advantages compared to young plants of natural origin and do not even stop the process of replacing spruce and pine with aspen and birch. In contrast to such plantings, spruce and pine crops planted using intensive agrotechnical techniques grow according to II - 1b bonus classes, i.e. on average 1-3 times higher in productivity than conventional crops. The accelerated high growth rate of such crops is achieved due to a combination of certain factors and conditions. Among the most significant factors that have a huge potential for improvement is the use of selection-improved planting material. However, the development of methods of clonal micro-propagation of conifers in the practice of forestry in Russia is still taking only the first steps.

Of the traits used in population forest genetics, molecular (including biochemical) markers of genes differ in many useful properties. They do not require

many years of inheritance analysis in a series of generations, their manifestation does not depend on the modifying action of the environment. Molecular markers, unlike morphological features in coniferous species, have a monogenic nature of inheritance and therefore are objective genetic markers of the degree of differences between populations and taxa. Chronologically, the first molecular markers were allozymes (isoenzymes) - hereditary forms of enzyme proteins. Despite the rapid development of DNA analysis methods, isoenzymes remain very useful genetic markers, since they can be used to obtain reliable and complete genetic information [22]. To date, the results of genetic studies using the method of isoenzyme analysis have allowed us to solve complex issues of taxonomy of pine species [22], spruce [6, 10, 12, 23], larches [20]. The method of molecular labeling is widely used for genetic certification of clonal forest-seed plantations and archives of softwood clones

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**Picture 2.**

At 15 years of age, the average height of regenerants is greater than that of seedlings by 1.9 m. Since $G_f >$ there is no doubt about the reliability of the indicators found. The strength of the influence of the studied factor is not high $\eta^2 = 18\%$. In the conditions of sandy loam forest soils of the Horse-Well forestry in 15 years, the average height of birch trees in seedlings is 12.7 ± 0.35 m, in regenerants - 14.6 ± 0.38 .

Hanging birch cultures, grown hanging birch cultures, grown from regenerants in vitro: age 14 years of seedlings; age 14 years

Figure 1 - Hanging birch cultures created by regenerants in vitro and

The selection of individual birch biotypes and clones for fiber length may be important for the creation of special-purpose plantation crops (for the production of cellulose raw materials). Long-fiber forms are considered the most valuable. But in general, wood with a libriform length of 0.8 mm or more is suitable (Poluboyarinov, 1976; Gavrilova, 1971, etc.). The use of birch wood is very diverse: from small home crafts to use in construction and the pulp industry.

The length, wall thickness and diameter of the wood libriform were studied. The results of the study show that regenerants have higher indicators than seedlings (Table 5).

Table 5 - Characteristics of the wood libriform of regenerants and seedlings

Tree origin Libriform length, mm Libriform width, mm libriform wall thickness, mm

Birch regenerant 0.88 ± 0.019 22.3 ± 0.06 4.0 ± 0.02

Birch seedlings 0.79 ± 0.016 21.4 ± 0.05 3.8 ± 0.01

5. Cultivation of planting material and creation of alder crops

Black alder grows on waterlogged soils in D4.s conditions, where no other tree species can grow. With the right technology for creating crops, alder stands give high productivity indicators.

To preserve valuable biotypes of black alder (*Alnus glutinosa* (L.) Gaertn.) and gray (*Alnus incana* L.), a method of microclonal reproduction has been developed, which is promising for the creation of clone archives, forest seed plantations and plantation crops.

Plantation cultures of black and gray alder from regenerants and seedlings have been created in the Novousmanskyy forestry. Comparison of the growth of regenerants in the experiment with a closed and open root system showed significantly better growth in the first case ($F=2.75 > t_{cr}=2.04$).

But it is not so easy to exclude GMOs from our diet: even if we find "Non-GMO" on the package, there is not enough confidence that this is really the case. The issue of food safety is not considered for plants, such as trees or cotton, which are a food source. But, the same genetic transformation of cedar pine was carried out (*Pinus Sibirica*), at the Siberian Institute of Plant Physiology and Biochemistry (based

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on a gene isolated from corn) in 1999 And cedar is the breadwinner of birds, animals and humans. Biotechnological experiments were also conducted with birch. And birch buds in any phytoapteca are sold as an environmentally friendly product, a recognized remedy in medicine. The green foliage of trees (birches, aspens, etc.) is harvested for animal feed (brooms for goats), conifers in winter give food to moose, hares gnaw the bark of aspens. In general, there is still cause for concern in terms of the safety of transgenic trees for animals and humans. Currently, in the forestry of Russia, methods of biotechnology they are used for the cultivation of planting material, the production of biological forest protection products, the creation of new forms of woody plants with specified characteristics. The most widely used methods of clonal micro-propagation of plants (various birch clones). Intensive work is underway on the culture of hybrid willows, poplars, aspen, birch, etc. The actual task is to develop an effective system of clonal micropropagation for conifers, (including *Pinus sibirica* – cedar). The technology of the next decade is the transgenesis (genetic modification) of woody plants. Increasing the growth rate of the main forest species, increasing their stability and improving the properties of wood are priority tasks of modern forestry science. Creation of new forms of forest breeds by traditional methods of breeding. Genetic transformation makes it possible to modify the properties of woody plants in a short time. The complex of methods of genetic transformation makes it possible to modify individual plant traits point-by-point: to give stability to herbicides, to increase productivity, etc., i.e. to create forms of target purpose. This direction of forest biotechnology orientiis focused exclusively on the plantation method of forest cultivation. More than 150 field polygons have been registered in the world to study the growth of transgenic forest species. Most of them are carried out on the territory of the USA (103), China (9), Canada (7), Finland (5). In Russia, work on the genetic modification of tree species was started in 1999 . At the Siberian Institute of Plant Physiology and Biochemistry. The work was carried out in order to increase the growth rate of aspen, poplar and cedar pine based on the use of the ugt gene isolated from corn. Currently, the problem of reducing the biological diversity of the plant world, including woody plants, is noticeably revealed. Therefore, scientists consider the methods of genetic transformation, molecular labeling, and clonal micro-reproduction of the most valuable tree forms (birch, pine, aspen, and some others) justified. But, despite the fact that good results have been achieved in various areas of forest biotechnology, their widespread introduction into practice has not yet been observed. This is a matter of the future. So, the benefits of biotechnology in forestry are obvious: they consist in an economic advantage for the industry, in

order to obtain "fast" wood during plantation reforestation, increased productivity, lower prices for consumers, easier processing of wood. But biotechnological innovations raise such issues of biosafety and the impact of transgenic plants on the natural ecosystem, especially in the issue of genetic exchange between genetically modified trees and wild populations. And there are no convincing answers to these questions yet, and there cannot be. Biotechnology in forestry is a very young science, only time will help scientists to identify and correctly calculate, not only everything Gray alder, No. 6, seedling 48 52 - -

Analysis of the final stage of microspore formation showed that in regenerants and seedlings of black and gray alder, the process of development of the male generative sphere proceeds without significant deviations and ends with the formation of high-quality, equalized pollen.

The characterization of quantitative indicators in cones showed that the differences in the shape of cones were not significant. The cone shape coefficient averaged 0.50 - 0.64. The length of cones in seedlings averaged from 1.6±0.03 to 1.8±0.06 cm, in regenerants - from 1.4± 0.04 to 1.7±0.04 cm. The differences in the length of the cones are significant at (=0.05. The width of the cones in seedlings and regenerants ranged from 0.8 ± 0.04 to 0.9± 0.02 cm. There are no differences in the width of cones in seedlings and regenerants. Laboratory germination of seeds obtained from regenerants and seedlings does not differ and is 85%.

6 Poplar plantation crops

6.1 Poplar crops in the cuttings of the VGLTA Educational and experimental forestry

Plantation crops of the best varieties of poplars were laid in the spring of 1994 during the development of an innovative project for accelerated cultivation of tree species. Fresh aspen cutting with an area of 3.6 hectares was prepared according to the VGLTA technology, type of forest growing conditions C2. The soil is dark gray sandy loam forest, the type of forest growing conditions is C2 (C3), the number of stumps is 650-750 pcs / ha.

Table 8 — Poplar growth in height and diameter in 14 years

The name of the poplar WED. height, m (Him) Coefficient of variation, % Accuracy of experience, % Cp. diameter, cm (D±t) Coefficient of variation, % Accuracy of experience, %

Brabantika №56 18,6±0,54 18,5 3,0 19,0±0,62 23,5 3,5

ES-38 19,6±0,43 10,7 0,8 19,4±0,58 19,6 2,5

And-45/51 19,6±0,95 16,8 1,5 18,9±0,30 15,8

ZD

I-16 17,6±0,99 19,4 2,7 18,6±0,37 24,4 3,5

Regenerate №90 21,7±0,89 14,1 2,3 18,5±0,85 19,0 2,0

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Black hybrid 16,1±0,57 13,8 3,0 15,7±0,36 17,4 3,7

The Euro-American Regenerate poplar is distinguished by the best indicators at the age of 14, the average height is 21.7 ± 0.89 m, a pairwise comparison according to the Student's criterion with other varieties, the differences are significant at $p = 0.05$; the differences in diameter are not significant. We believe that in 15-20 years, in winter or early spring, it is possible to cut poplars. The remaining stumps will give root offspring and shoots from the stump, which will form a new planting of poplars.

6.2 Creation of poplar crops in areas that have been released from agricultural use

In 1983, an experimental variety testing site of polyploid and diploid poplars was created in the Semiluksky nursery of TSNILGIS, this is an example of the creation of poplar plantation crops in open areas. The soil is degraded chernozem, its preparation was carried out according to the black steam system, seating 4 x 4 m.; the experience is laid down in three repetitions, a widespread diploid - balsamic poplar is presented as a control. For comparison, other triploid and diploid poplars have been introduced. Test crops from fast-growing breeds allow you to get commercial wood in a short time. Experience shows that the Hopersky poplar variety 1 can give an increase of up to 25-30 m³/ha per year (Table 9). Table 9 - Indicators of growth and productivity of poplar cultivars at the age of 26 at the test site in the Semiluksky nursery__

Poplar name Height, m (H±ha) Diameter, cm (D + t) Stock, m³/ha

Balsamic	18.3±0.40	18.0±0.61	91
Robusta - 236	22.5±0.33	26.5 ±0.83	284
E.S.-38	22.8±0.26	23.8±0.72	227
Balsamic Po No. 1	21.5±0.15	24.8±0.91	232
Balsamic Po No.2	22.5±0.17	23.5 ±0.74	243
Khopersky 1	27.2±0.43	37.3±1.15	715

The table shows that at the age of felling (26 years) the Hopersky poplar 1 exceeds the balsamic poplar (control) in diameter by 19 cm, according to height at 9 m.

To clarify the role of the genotype on growth in diameter, we performed a one-factor analysis of variance. Mathematical processing of the experimental results confirms the validity of the differences: $F_{1f} > F_s$, (3.38 > 3.1) at $P = 0.05$. The null hypothesis is rejected: the difference in the influence of varietal affiliation on growth in diameter turns out to be statistically significant, the strength of the influence is $\eta^2 = 59\%$. When studying the growth in diameter of clones and varieties of poplar at the test site of the Semiluksky nursery, it can be noted that the Hopersky poplar 1 exceeds the diameter of other samples by 11-19 cm.

A one-factor analysis of the variance of the influence of the poplar genotype on height growth also confirms the validity of the differences: $F_p > F_{SI}$ (3.38 > 3.1) at $P = 0.05$. Since $F_{r/1} > F_s$, the null

hypothesis is rejected: it is highly reliable that individual cultivars with different strengths affect the growth in height of trees: the strength of the influence is $\eta^2 = 16\%$.

6.3 Creation of poplar plantation crops by regenerants in vitro

Another plot of poplar plantation crops Khopersky 1 is located in block 26 of the Kony-Kolodezsky forestry of the Lipetsk region. These are the first in the Central Chernozem region test plantation poplar crops from regenerants in vitro, cuttings and seedlings laid in the spring of 1996 on an area of about 7 hectares. The plot under the crops is flat (uprooted felling of pine affected by root sponge), the soils are gray forest sandy loam, forest growing conditions C2.z. Soil preparation was carried out by furrows with a PKL-70 plow. The crops were created by ordinary planting after 6 m., in a row after 3 m., a shrub of pemphigus was introduced as a sealer. Planting regenerants manually under Kolesov's sword and shovel, cuttings - under Kolesov's sword. Regenerates of Hopersky poplar 1 were obtained in the NILGIS genetics laboratory, for comparison, cuttings of triploid balsamic poplar obtained by E.M. Gulyaeva in the same laboratory were used. Cuttings of balsamic poplar are harvested from a mother plantation with a length of 25 - 30 cm. The survival rate of regenerants was 100%, cuttings took root by 93%.

The plants are monitored annually (Figure 3). The analysis of the obtained data showed that the first three years of the regenerates showed better growth than cuttings of balsamic poplar seedlings. Since 1999, there has been a faster growth of balsamic poplar, and in 2002 the Hopersky poplar 1 lags behind in growth by 88 cm. In 2004, the average height of the Hopersky poplar 1 was 9.3 m, balsamic poplar - 8.8 m, that is, regenerates exceed cuttings by 0.5 m. By 2012, that is, at the age of 16, the heights of the studied poplars are 21.0± 0.45 - 19.4±0.39 m.

The diameter of the trees has a greater influence on the stock of wood. At the age of 16, the average diameter of regenerants of the Hopersky poplar 1 is 21.4± 0.51 cm, balsamic triploid poplar 21.8± 0.47 cm.

— ♦ — balsamic poplar Zp — v — Hepersky poplar 1

Figure 3 - Dynamics of growth in height of regenerants of Hopersky poplar 1 and cuttings of triploid balsamic poplar seedlings

Taking into account the condition (viability) at the age of 16 showed that 96% of the trees of the Hopersky poplar are in good condition and 4% are satisfactory, and all the trees of the balsamic poplar are in good condition.

In the Right-bank forestry on dark gray loam, regenerants of the Hopersky poplar 1b 16 years old reach a height of 18.0 ± 0.58 m. and a diameter of 33.0 ± 0.85 cm.

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Sixteen-year observations of experimental crops show that it is quite possible to propagate and create plantation poplar crops with regenerants in vitro. Most of them have normal upright growth and good condition.

7. Economic studies of the effectiveness of growing seedlings and regenerants in vitro of hanging birch

To determine and compare the costs of growing 1 hectare in the open ground of the nursery of seedlings and regenerants in vitro of the hanging birch, we calculate technological maps.

It was found that the total cost of growing regenerants in vitro in the open ground of the hanging birch nursery is 31953.8 rubles, seedlings -33548.4 rubles. The cost of growing one plant regenerant in vitro and seedling differ slightly. The advantage of the ways of biotechnology is that in a short time (about 2 years) it is possible to obtain a large amount of genetically homogeneous planting material, significantly speeding up the breeding process. In addition, it is extremely important that at the same time there is a recovery of the planting material. By the method of tissue cultures, it is possible to propagate plants that hardly or do not reproduce vegetatively at all.

For the perspective and relevance of the research topic of the dissertation work, we have conducted research on the commercialization of biotechnology in vitro and profit from sales of regenerants of the hanging birch, and also developed a financial plan for the organization of a small enterprise.

The calculation data showed that net discounted income at a discount rate of 10% for the 3rd year of the project and after income taxes will amount to 1,125 million rubles. subject to the sale of 100 thousand pieces of regenerants. The project requires investments of about 1.9 million rubles. These investments are necessary for production costs (about 1.2 million rubles) and salary payments (0.7 million rubles). For 3 years, with the sale of 50 thousand pieces of regenerants, it is predicted to recoup all costs and make a profit. These calculations are given with an estimated share of planting material sales in the Voronezh region market of about 0.3%.

As a result of the conducted research on the use of microclonal reproduction to create plantation crops of fast-growing tree species in the forest-steppe, the following conclusions and recommendations can be made:

1. The most valuable fast-growing species for plantation afforestation in the forest-steppe are: hanging birch, the best varieties of poplar and black alder. In vitro biotechnology is of fundamental importance for the reproduction of economically valuable forms and varieties of woody plants that are difficult to propagate by seeds and cuttings.

2. Dispersion analysis has established the influence of the origin of planting material (in vitro

regenerates, seedlings) of hanging birch on growth in height and diameter. On the gray sandy loam forest soils of the Kon-Kolodez forestry, the stock of 14-year-old plantation birch crops created by in vitro regenerates is 192.6 m³/ha, seedlings - 85.6 m³/ha, but the quality of the trunks of seedlings is better than that of regenerants.

3. The experience of creating plantation crops of Karelian birch from regenerants in vitro has shown the great importance of the latter in obtaining special sorts valuable for furniture production.

4. It has been experimentally established that in black and gray alder cultures, differences in height and diameter of in vitro regenerants and seedlings aged 8 years on dark gray loamy moist soils of the Tavrovka River floodplain of the Novousmansky forestry are not significant. For alder, in vitro biotechnology is important when creating clone archives of economically valuable forms, varieties; clonal forest-seed plantations.

5. It is shown that the fruiting of clonal alder seed plantations created by regenerants in vitro begins earlier - from 3-4 years, than in seedlings - from 5-6 years. The viability of pollen ranges from 95 - 98%. The quantitative composition of the emerging pollen in microclonal plants has 22-23% in black alder and up to 52% in gray alder of large pollen (more than 31 microns.). According to the coefficient of the shape of cones, the differences in seedlings and regenerants in vitro are not significant. Laboratory germination of seeds reaches 80%.

6. Plantation crops of poplar of the subgenus Eupopulus Dode, laid by winter cuttings on aspen felling with lowering of stumps and disking of root offspring showed the possibility of obtaining wood for construction parts and pressing in a short time. At the age of 14, the average height of trees ranged from 16.1 ± 0.57 m in the Black Hybrid to 21.7 ± 0.89 m in Regenerate No. 90, the average diameter ranged from 15.7 ± 0.36 cm. in the Black hybrid up to 19.4 ± 0.58 cm in the poplar E.S. - 38.

7. In the Central forest-steppe, 16 years of experience has been accumulated in the creation of plantation crops of a hard-to-root triploid variety of poplar graying Hopersky 1 with in vitro regenerants on gray forest sandy loam soils of the Kony-Kolodez forestry and on dark gray forest loamy soils of the Right-Bank forestry. At the age of 16, the average height of the poplar trees of Khopersky 1 on sandy loam was 21.0 ± 0.45 m, the average diameter was 21.4 ± 1.36 cm; on loam, respectively, 21.0 ± 0.58 m and 33.0 ± 0.85 cm.

The relevance of the topic. Currently, along with traditional methods for breeding valuable forms and varieties of forest woody plants, the method of culture of isolated organs and tissues (clonal micro-propagation of plants) is used. This problem is even more urgent in the years of accelerated reforestation of mountain forests with deciduous tree species. The

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advantages of this method include: speed, exclusion of viral diseases, the need for a small number of initial explants and limited areas, the possibility of year-round production of planting material, its long-term preservation with minimal volumes of refrigerating chambers, the production of a large amount of planting material per year.

The need for mass reproduction of genetically improved forms of woody plants with the help of tissue culture to increase the qualitative composition of forest plantations by obtaining clone plants resistant to diseases and pests, stress and man-made factors can accelerate the reproduction of forest resources, will allow obtaining genetically improved material much earlier than under normal conditions.

In recent years, plantation crops of birch, alder, poplar with a short rotation period for obtaining small-scale wood for pressing purposes, construction parts have become particularly important due to the high efficiency of its use.

The relevance of the research topic is currently increasing, due to the need to use selectively improved forms of birch, alder and poplar for plantation afforestation.

The dissertation was carried out in accordance with grant No. 14213 Participant of the youth scientific and innovative competition, topic No. 16 "Development of biotechnology in vitro for growing planting material and creating plantation crops of fast-growing tree species in the conditions of the Central Forest", as well as in accordance with the direction of research work of the Voronezh State Forestry Academy - included in the project 5.4108.2011 "Forestry-ecological foundations of accelerated reforestation of mountain forests" of the Ministry of Education and Science of the Russian Federation.

The aim of the work was to study and analyze the growth and stability of crops of fast-growing tree species using microclonal reproduction. Development

of recommendations for the creation of plantation crops of birch, alder and poplar; the use of in vitro biotechnology for the creation of clone seed plantations.

To achieve this goal, it is necessary to solve the following tasks:

1. Select valuable biotypes in stands and crops for cloning.

2. To identify the condition and productivity of plantation crops of birch, alder and gray poplar, created by seedlings, cuttings and regenerants in vitro.

3. To study the qualitative characteristics of the wood libriform of regenerants, seedlings and cuttings of seedlings of the studied species.

4. To give a comparative economic assessment of forest crops created from in vitro regenerants and seedlings.

5. To consider the application of biotechnology in vitro in the creation of clonal forest-seed plantations of alder and birch.

6. To develop a technology for creating plantation crops of birch, alder and poplar using microclonal reproduction.

The following provisions are submitted for protection:

1. Characteristics of valuable biotypes of birch, alder and poplar for obtaining planting material in vitro;

2. Condition, productivity and growth dynamics of birch, alder and poplar plantation crops created by in vitro regenerants and seedlings;

3. Comparative characteristics of regenerant wood, seedlings and cuttings;

4. The use of in vitro biotechnology in the creation of clonal alder seed plantations;

5. Economic and economic assessment of plantation crops created from in vitro regenerants and seedlings.

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