1. Introduction

Over the past two decades, the Russian forest industry has faced many challenges. The combination of growing competition from imports, digitalization of the economy, competition from substitute materials, environmental problems and recent economic sanctions have led to a decrease in investment activity in the innovative development of the forest industry. The growing realization of the ecological properties of wood encourages governments and private enterprises to invest resources in research and development of innovative materials and products derived from this material [1]. Innovation in the forest sector plays a key role towards a green economy and a sustainable future where most products are produced from renewable resources and materials are selected according to their overall environmental impact [2]. Achievements of modern forest biotechnology enable in vitro cloning of economically valuable aspen genotypes and obtain planting material for plantation forest growing. The study of the manifestation features of valuable traits and genetic stability of in vitro clones propagated in the field (ex vitro) is important for the development of effective and reliable propagation methods that guarantee the preservation of the genetic and economic value of parent trees for their sustainable and targeted reproduction [3].

Common aspen (Populus tremula L.) is a rapidly growing species that can reduce the time of maturation of wood by half, and can be effectively used as a source of thermal energy. This species is distributed throughout Russian Federation. Aspen is adapted to forest conditions and grows on medium-fertile soils, and is resistant to frost. Aspen wood has good physical and mechanical properties – it is soft, light, in the dry state is very strong. Unfortunately, aspen has a tendency to be affected by fungal diseases that cause core rot of the trunks, which in turn leads to a decrease in the quality of the wood. However, highly productive clones resistant to fungal diseases are found in the Republic. Currently, cellular biotechnology method is a promising alternative to traditional breeding methods [4].

Microclonal reproduction of common aspen (Populus tremula L.) genotypes in the Republic of Tatarstan

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Abstract. The research presents the results of an experiment on propagation of core rot-resistant aspen clones (Populus tremula L.) of diploid and triploid genotypes using microclonal propagation method and their introduction into forestry production in the Republic of Tatarstan. The expediency of using this method in the conditions of the Republic for obtaining healthy aspen planting material is proved, and the prospects of reproduction of clone No.35 with a triploid genotype are shown. The reliable difference of test tube regenerating plants of triploid forms in the height of the shoot and in the development of root systems is proved.
In order to propagate aspen in industrial scale and preserve its biodiversity, clonal micropropagation (in vitro) methods are used, which can improve the quality of tree plant planting material by improving it and selectively propagating only the best genotypes [5]. It is very important to use valuable genotypes as source material. Methods of shoot culture can be used for reproduction of many tree species, including aspen. Aspen grows in vitro twice as fast as normal and is more resistant to fungal diseases [6,7]. In this regard, the formation of productive and stable aspen biogeocenoses becomes relevant.

The aim of this study is to reproduce aspen by methods of cell biotechnology in the Republic of Tatarstan and to compare statistically of the studied variables between two clones.

2. Methods and materials
The studies were conducted from 2007 to 2019. As the primary explant for introduction into the culture, vegetative buds of shoots of aspen clones No.34 and No.35 that are not subject to core rot were used, grown by the Kostroma Forest Experiment Station located in the Kostroma region (Russia). Clone No.34 has a diploid genotype (f2), clone No.35 has a triploid genotype (f11). For growing aspen regenerants of both genotypes, the Murasiga and Skuga nutrient medium was used, which included exogenous growth stimulants – α-naphthylacetic acid (NAA) and 6-benzylaminopurine (BAP) in various concentrations and ratios as additives. The introduction of aspen buds into the culture was carried out from December to March. [8]. Before starting the process of growing aspen regenerants, the buds were sterilized. Then the scales were removed from them and the leaves adjacent to the meristem were removed. The resulting meristem with a small portion of the base was ready for further work. After the introduction in culture of all plant material, the flasks were placed in a light room where the light intensity of 1250 Lux and a temperature of 23 °C. The experiment was carried out in 3 repetitions. Under these conditions, microshoots of aspen were formed from isolated buds during one passage, which were further propagated of graftage. A nutrient composition with β-indolyl-3-butyric acid (IBA) at a concentration of 2 mg/L was used as a root formation accelerator (table 1). The average reproduction rate was 5-6 microshoots from one explant introduced into the culture.

Table 1. Changes in the composition of the nutrient medium at different stages of obtaining aspen regenerating plants.

| Stages of reproduction | Composition of the nutrient medium |
|------------------------|-----------------------------------|
|                        | MS | BAP, mg/L | NAA, mg/L | IBA, mg/L |
| Introduction of the meristem in culture | + | 0.5 | 0.02 | - |
| Grafting | + | 0.1 | - | - |
| Rooting | + | - | - | 2.0 |

Transplanted regenerants were grown in a light room for 30 days under the same light and temperature conditions. During this time, they took root and were ready to be planted in the greenhouse. For further acclimatization of test tube plants, they were transplanted into a greenhouse, where all necessary conditions were created – watering, fertilizing, comfortable humidity for plants, as well as measures of agrotechnical care. Regenerant plants were grown in transplant bags or transferred directly to the soil. Plant nutrition was carried out daily with the mineral fertilizer ‘Kemira Lux’ with the addition of chalk or lime. Fertilizer ‘Kemira lux’ has a stimulating effect on the growth and formation of strong shoots of plants.

3. Results and discussion
In general, the formation of microbeads on the primary explant occurred within 1 month. The beginning of growth of meristem of triploid aspen was noted two days earlier than diploid aspen. In the course of statistical processing of the obtained data, the \( \chi^2 \) method (Pearson Criterion) was used for nonparametric communication indicators. The presence of a significant difference between the compared forms of meristem survival and shoot formation was proved using the student's criterion.
The ability of meristem to form shoots already at the initial stage of cultivation, characterizes clone No.35 as a form that has more significant morphogenetic resources (90%) than clone No.34 (70%). During one month of cultivation, micro-shoots of triploid forms reached a height of 3 cm, and diploid - only 1 cm.

The micro-shoots formed in this way with 5-6 leaves were ready for the grafting. Micro-shoots of triploid aspen were characterized by more intensive growth compared to diploid plants, so there were more cuttings from such forms. Prepared cuttings were transplanted to a fresh nutrient medium. Transplanting micro-gears to a medium containing a root-forming stimulator allowed to obtain plants with a developed root system. This process took place the period of 1.5 months. Plants were considered formed and ready to adapt to soil conditions at the time when they formed 2-3 leaves and developed a root system (figure 1).

![Figure 1. Aspen sprout obtained from vegetative buds of aspen clone No.35, grown at the experimental station of the Kostroma Forest Experiment Station located in the Kostroma region (Russia).](image)

Plants-regenerants of triploid forms had a shoot height 2.5 times than regenerants of diploid forms and almost 20% more difference in the development of root systems (table 2).

### Table 2. Growth indicators of the research aspen regenerants.

| Plants-regenerants | Average height (H), cm | Number of plants with roots, % |
|--------------------|------------------------|--------------------------------|
| Clone №34 (f2)    | 1.5 ±0.4               | 76.0 ±0.2                     |
| Clone №35 (f11)   | 3.7 ± 0.2              | 95.0 ±0.3                     |

The differences are valid. By the height of test tube plants t=5>3, by the number of plants with roots t=52.3>3.

Adaptation of test tube plants to external conditions took place in the greenhouse. As our observations have shown, the survival rate of test tube plants was affected by the period of their transplantation. The most favorable time was the period – spring or early summer. In average, the survival rate of plants in vivo was 47.5%. Weather conditions had an adverse effect on the adaptation
process of regenerating plants after planting, the air temperature was high. Table 3 shows the results of statistical analysis of biometric indicators of aspen regenerants.

Table 3. Statistical analysis of biometric indicators of aspen regenerant plants Genotypic forms of aspen.

| Statistical indicators | Diploid forms | Triploid forms |
|------------------------|--------------|----------------|
| Height, cm             | 1.8          | 3.9            |
| Standard deviation, σ  | 0.6          | 1              |
| Standard error, mx     | 0.02         | 0.05           |
| Significance level (P), % | 1.1       | 1.3            |
| Variation (V), %       | 31           | 25.6           |
| Height (H)             | 2.7          | 4.2            |
| Standard deviation, σ  | 0.6          | 1              |
| Standard error, mx     | 0.03         | 0.06           |
| Significance level (P), % | 1.1     | 1.4            |
| Variation (V), %       | 20.1         | 23.8           |

The research showed that the successful adaptation of regenerates to the external environment is influenced by the following factors: the genotype, the height of the regenerants, length of the root system, the timing of planting, method of transfer of regenerants of the tubes in the substrate, found that aspen with a triploid genotype had a better survival rate (65%) than aspen with diploid genotype (30%) [7]. The aspen plants adapted to the external conditions were transferred to the forest area.

The aspen plants adapted to external conditions were moved to the forest-cultivated area. For this purpose, the first mother aspen plantation was created on the territory of the arboretum garden of the Sabinsky forest. The growth dynamics of the studied genotypes is shown in figure 2.

During the entire study period, since landing in 2007, the average height of triploid clones is on average 2 times higher than diploid genotypes. Differences in the student's criterion (t) are significant. At the age of 12 years, the height and diameter of the triploid clone by 1.2 and 1.7 times, respectively, also predominate diploid. This led to an increase in the average trunk volume by 3 times (table 4).

Similar results of the predominance of giant aspen are found in the literature [5, 9-13].

Figure 2. Comparative growth dynamics of diverse aspen clones.
Table 4. Biometric growth indicators of different-type aspen clones, 2019.

| Form            | Diameter, cm | Height, m  | Volume, m³ |
|-----------------|--------------|------------|------------|
| Clone No.34 (f2)| 7.55±0.37    | 11.25±0.55 | 0.032      |
| Clone No.35 (f11)| 13.2±0.51    | 14.6±0.36  | 0.102      |

Speed of aspen trees growth and their resistance to core rot are related. Fast-growing aspen forms are most rot-resistant [9]. Fruit bodies of tinder fungi of the false aspen tinder *Phellinus tremulae Bond* were not found on aspen trunks. However, on the plantation there are isolated instances of aspen affected by black cancer (*Hypoxylonholwayi Ell.*). Cancers covered with a black sooty coating of the pathogen are found only trees previously damaged by hares. It is known that even with a wide spread and frequent occurrence of the disease, no more than 5-10% of aspen is usually affected. The incidence of aspen on the plantation under study is within the specified limits (figure 3).

![Figure 3. Mother plantation of triploid aspen (Arboretum garden of Sabinsky forestry, 2019).](image)

4. Conclusion

The methods of microclonal reproduction allow obtaining the necessary amount of planting material resistant to core rot clones of aspen [12]. In the Republic of Tatarstan is the most promising turned out to be a clone, having a triploid genotype. Triploid forms of aspen have a high morphogenetic potential (90%) in comparison with diploid forms (70%) in forming microshoots already at the first stage of cultivation. Triploid aspens are characterized by increased adaptive capacity (65%) compared to diploid forms (30%). In continuation of these studies, highly productive, rot-resistant triploid genotypes of aspen in the forests of Tatarstan were selected in the republic, followed by their laboratory molecular genetic identification [10] and experimental plantations of fast-growing tree species were created.
References

[1] Fetischeva Z I 2007 Ways of development of the wood industry to modern conditions. Forestry Bulletin 3 163 [In Russian]

[2] Golovina E Yu and Dykusova A G 2020 Financing of innovative projects in the forest industry. IOP Conf. Ser.: Earth Environ. Sci. 459 062030 doi: 10.1088/1755-1315/459/6/062030

[3] Mashkina O S, Shabanova E A, Varivodina I N and Grodetskaia T A 2019 Field trials of in vitro propagated aspen clones (Populus tremula L.): growth, productivity, wood quality, and genetic stability. Lesnoy Zhurnal (Russian Forestry Journal) 6(372) 25 doi: 10.17238/issn0536-1036.2019.6.25 [In Russian]

[4] Campbell M M, Brunner A M, Jones H M and Strauss S H 2003 Forestry’s fertile crescent: the application of biotechnology to forest trees. Plant Biotechnol. J. 1(3) 141 doi: 10.1046/j.1467-7652.2003.00020.x

[5] Zhigunov A V, Shabunin D A and Butenko O Yu 2014 Forest plantations of triploid aspen created by planting material in vitro. Vesting of Volga State University of Technology. Series: Forest. Ecology. Nature Management 4(24) 21 [In Russian]

[6] Shabunin D A 2014 Studies on micropropagation of forest species in the St. Petersburg Forestry Research Institute. Proceedings of the Saint Petersburg research Institute of forestry 2 32 [In Russian]

[7] Schulzke R 1988 Die Anwendung von vitro kultutehniken bei Waldbanmen. Osterr. Fortzfg. 99(3) 66

[8] Khattab S 2011 Effect of different media and growth regulators on the in vitro shoot proliferation of aspen, hybrid aspen and white poplar male tree and molecular analysis of variants in micropropagated plants. Life Sci. J. 8(1) 177

[9] Bagaev S N, Korenev I A, Bagaev S S and Umbrellas D N 2013 Features of the formation of fast growing clones in the genetics reserves of Populus tremula gigas. Forestry 2 26 [In Russian]

[10] Ulrich K and Ewald D 2014 Breeding triploid aspen and poplar clones for biomass production. Silvae Gene. 63(1-2) 47 doi: 10.1515/sg-2014-0008

[11] Tullus A, Rytter L, Tullus T, Weih M and Tullus H 2012 Short-rotation forestry with hybrid aspen (Populus tremula L. × P. tremuloides Michx.) in Northern Europe. Scand. J. Forest Res. 27(1) 10 doi: 10.1080/02827581.2011.628949

[12] Greer B T, Still C, Cullinan G L, Brooks J R and Meinzer F C 2018 Polyploidy influences plant-environment interactions in quaking aspen (Populus tremuloides Michx.). Tree Physiology 38(4) 630 doi: 10.1093/treephys/txs120

[13] Pozdnyakov I, Azarova A and Shestibratov K 2016 Effect of the volume of production of planting material on the basis of clonal micropropagation on the cost price of invitro-rooted birch and aspen microplants. IJESE. 11(18) 12031