

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

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ABSTRACT

The influence of the volume of invitro-rooted microplants of the forest crops (birch and aspen) production in the range from a few hundred to a few tens of thousands on the cost and efficiency of the production has been studied. A wide range of genotypes of aspen and birch has been studied, 15 genotypes for each of the families. The influence of the volume of produced goods on the cost and efficiency of the microplants obtained during the multiplication stage has been well established. The equations, reflecting the theoretical relationship between the volume of production of the rooted microplants and their cost, have been calculated. Theoretical calculations using regression equations has allowed to establish the degree of influence of the batch volume in the range from 1 000 to 100 000 units. It turned out to be such that for a tenfold increase in the volume of production the cost of microplants of the Birch increases per 8,66 roubles and one plant of the Aspen increases per 3.14 roubles, which reflects the effect of the genotype on the level of genus of the propagated plant. It has been found that the linear dependence of the equation cannot fully describe the impact of the volume of produced microplants and the regularities observed under the regular occurrence. Along with that, the graph of the power function has turned out to be closer to the objectively observable results. The degree of dependence has made it possible to predict that the cost price of the Birch under the adopted technology can overcome the level of 10.00 roubles with an increase in the production volume of more than 111 260 microplants. For the Aspen such level of cost price may be achieved at the level of production of at least 560,850 units of microplants. One of the reasons that influenced the reduction in the cost price and which has been discovered in the experiment appeared to be more effective use of plants resulting from the multiplication stage.

KEYWORDS

Micropropagation, invitro-rooted birch, aspen microplants

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Introduction

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One of the most important trends of the last two decades in the field of renewal of forest stands is the use of vegetative or clonal planting material during the laying of forest plantation crops. Without the use of vegetative propagation the widespread use of overwhelming majority of highly productive forms and hybrids of forest plants is not currently possible because of the high level of inherent heterozygosity, as well as due to the fact that many highly productive forms of arboreal species are sterile polyploids or mixoploids (Zhigunov, Shabunin & Butenko, 2014; Mashkina & Isakov, 2002). Many highly productive form of arboreal plants are interspecific hybrids (Saya et al., 2008). The most common way of producing clonal planting material is cutting. Mass vegetative reproduction of cultivars of such genera as *Eucalyptus*, *Populus*, *Tectonia*, *Coffea* is realized in this way (Bonal & Monteuiis 1997; Gangopadhyay et al., 2003; Ducos, Lambot & Pétiard, 2007; Shestibratov, Lebedev & Miroshnikov, 2008).

Clonal micropropagation is used as one of the very promising breeding methods in the process of production of seedlings of arboreal plants used in forestry. This method of reproduction has already proven itself as commercially independent for the production of a number of agricultural and ornamental crops such as banana, pineapple, orchids, rhododendrons, ornamental plants of the Araceae family (Khan, Nasib & Saeed, 2004; Rout, Mohapatra & Mohan, 2006; Viegas et al., 2007). Without the use of clonal propagation it is practically impossible to create virus-free plants of all without exception agricultural crops. Despite the undeniable success of clonal propagation in the field of agriculture, its use in the creation of clonal forestry plantations is much more modest.

Despite the fact that the clonal micropropagation is used for arboreal forest plants for more than 50 years (Park, Bonga & Moon, 2016), it is almost never used for forest arboreal plants on the territory of the Russian Federation due to the general economic situation in the industry. This method is generally applicable only to lay the scientific and experimental plantations, but not for commercial forest plantations. To begin the creation of forest plantations with the seedlings obtained using clonal propagation, a comprehensive assessment of its cost effectiveness is certainly needed. One of the possible and obvious barriers to creating forest plantations, on the basis of the seedlings obtained in vitro, becomes the higher cost of production, compared to the seedlings conventionally used for laying forest plantations. A similar pattern is observed for the number of crops for which this method of reproduction has long been used and justified for a number of reasons, such as banana. Its clonal reproduction is explained by the fact that the varieties of banana are triploid forms and that it allows to avoid the transmission of disease, but in the areas with low-tech production efficiency of production the efficiency of plantations, laid on the basis of the invitro seedlings, is slightly lower than the efficiency of the plantations based on cutting (Hanumantharaya et al., 2008). Thus, based on the study of the proposals on the market, the price of spruce seedlings produced in different regions of the Russian Federation is in the range of 2.00 - 3.00 roubles. It should be noted that the difference of the price prevailing on the seedlings in other countries may be substantial. The price of the seedlings of forest species may be very different depending on their belonging to this or that variety. Thus, according to the catalogue of the Forest Service of Northern California for 2017 (www.buynctrees.com), the price of annual seedlings of loblolly pine is US \$0.12 (about 7-8 roubles), the price of the black birch seedlings is US \$0.33 (20 roubles). So, according to some researchers, the full cost of the seedlings obtained using clonal micropropagation was fluctuating in the range of 20-30 roubles (Mashkina et al., 2016).

It is obvious that the clonal plants cannot compete with such a low price of seedlings. A comprehensive assessment of the economic efficiency of operation of forest plantations on the basis of seedlings obtained by clonal micropropagation will help to

reduce costs and compare the costs and increase profits from the use of this type of planting material. For example, the productivity of selected, vegetatively propagated forms of Eucalyptus may be almost 2 times higher the productivity of the plants obtained from seedlings (Park, Bonga & Moon, 2016). It must be remembered that the economic viability is affected by other genotypic characteristics, such as resistance to pests, as well as the fact that the clonal propagation method significantly reduces the risk of the spread of disease (Fenning & Gershenzon, 2002). Another important reason for the use of clonal micropropagation is the loss by many arboreal plants of the ability for the successful vegetative reproduction and the recovery of this ability can only be achieved by using cultivation under invitro (Ballester & Vieitez, 2012).

It is widely known that the effectiveness of production as well as the amount of the production cost are dependent on the volume of production. It is logical to assume that the increase in the scale of production of arboreal plants by clonal micropropagation to a certain level will significantly reduce their costs and possibly make their cost effectiveness comparable with the cost effectiveness of seedlings. To assess whether how substantial the effect of the production volume at the cost of microplants rooted invitro may be, the experience of large-scale production of the Birch and Aspen was founded. The work was conducted as the part of laying the forest plantations from the selected genotypes of arboreal crops, under the agreement of 22.08.2014 № 14.607.21.0044 with the Ministry of Education and Science of the Russian Federation.

Our first experiment involved the large-scale propagation of selected clones of arboreal plants of the Aspen (*Populustremula* i*P.tremuloides*x*P.tremula*) and Birch (*Betulapendula* and *Betulapubescens*) and evaluated the costs that were spent on their production. The main objective of the study was to establish the degree of influence of the production volume on the cost price of the microplants rooted invitro. We have assumed that a sufficient increase in the volume of production may lead to the cost price of a microplant in the amount of 10 roubles. In addition, there was a task to determine whether the cost structure is influenced by the volume of production.

Materials and Methods

Used Genotypes of Arboreal Crops

The objects of the study were the selected clones and selected mutants with different levels of ploidy of aspen and birch. In total, this experiment involved 15 genotypes of Birch, belonging to two varieties (*Betulapendula* and *Betulapubescens*) and 15 genotypes of Aspen, belonging to the variety *Populustremula* and hybrids *P.tremuloides*x*P.tremula*.

The birch genotypes included such forms as: bp3f-1, bp4a, bp1b, bb31, bb4b, tr1, tr2, drv-1, kc06, bc-1, kb76, 66-150/10, kb81, 30b1 and one control clone of the silver birch. These birch clones were selected on the basis of ploidy (tri-, tetraploid, mixoploidy) and an increased rate of growth in forest plantations, in comparison to the baseline genotypes.

The aspen was represented by the following genotypes in the experiment: 47-1, 23, ptv22, L-4, C-control, pt2, mutant 4, 47-1-1-27, 47-1-1-31, mutant 4, 47-1-1-22, mutant 21, 47-1-2-53, f2, mutant 21 that had such characteristics as an increased ploidy (tri-, tetraploid, mixoploidy), resistance to pathogens *Phellinustremulae* *Phellinusignarius*, causing cenral rot, higher productivity and growth rate.

It is important to note that under the invitro all these genotypes of each of the genera possessed approximately the same growth characteristics and morphology. Plants reached readiness for transplanting, in the case of both aspen and birch, every 3



weeks (20-21 days). The multiplication factor for all genotypes of birch averaged 4.64, the multiplication factor of aspen was slightly lower and amounted to an average of 4.27. Rooting of the clones were not lower than 80% and ranged from 80 to 100%. Inclusion into the group with a similar volume of production of genotypes with different characteristics in growth, multiplication and rooting rate made it initially possible to neutralize the effect of the genotype within the same culture.

Physical Factors of Cultivation

The cultivation and transplanting of plants, during both multiplication rooting stages, occurred in laboratory rooms that were the compartments of the equipment complex with the air filtration through HEPA filters and the creation of clean air overpressure. After transplantation the culture containers with plants were moved to the shelves where they were cultured under the following conditions. The culture containers were placed on the shelves in two tiers, which could be provided due to the fact that in order to cover the cultivation containers transparent glass covers were used. Above the surface of the shelf the lighting devices were placed that provided the illumination of 2-2.5 klx of cool white light on the shelf level for 14 hours. The air temperature was maintained constant at 21 ± 3 °C through a system of air conditioning and ventilation. Cleanliness air was maintained at Class 6 of the ISO standard (up to 35,000 particles of the diameter of 0.5 microns in 1 m³) (Fig. 1). The range of cultivation conditions differed depending on the culture stage.



a



b

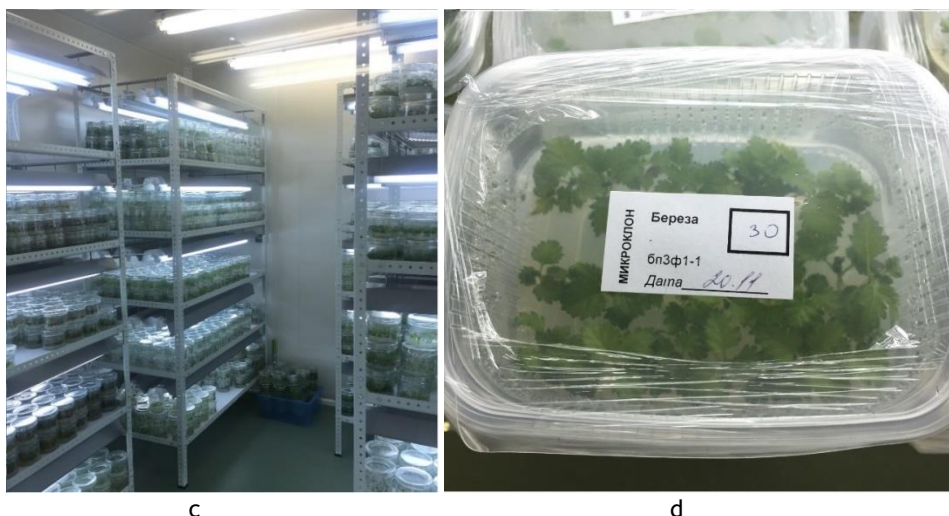


Figure 1. The cultivation conditions of arboreal crops microplants in areas the with HEPA filtration and shelves for the placement of clonal micropropagation of arboreal crops. a - cultivation container with the microplants of Aspen; b - location of the containers with the microplants on shelves; c - a room with a HEPA air filtration; d - Polypropylene container with the rooted microplants of Birch

At the stage of multiplication the plants were placed in glass jars with a volume of 330 ml in the number of 18 units in each. The jars were covered with glass lids and sealed with a plastic "stretch" film. The jars with the newly planted crops were marked with a permanent marker on the walls of jars (Fig. 2, 3).

Cultivation Methods and Media Used on Multiplication and Rooting Stages

The cultivation methodology was based on the method previously developed by the group of forest biotechnology of the Branch of the Institute of Bioorganic Chemistry, Russian Academy of Sciences (Lebedev & Shestibratov 2010; 2016), which has been modified for the genotypes of Aspen and Birch (Shestibratov et al., 2015; Azarova, Lebedev & Shestibratov, 2016). During the multiplication of the Birch microplants a method based on the suppression of apical dominance was used in which, under the influence of N6-benzyladenine, added to the medium, there was inhibition of growth of the top buds, and the axillary buds of the explant sprout formed side sprouts which were then separated and transplanted to the fresh growth medium.

For the reproduction of Aspen the microcutting method used, where the primary explant representing a microcutting with 2-3 nodes formed a new sprout from one (rarely two) upper bud. Next, a new sprout with multiple nodes was redivided into fragments with 2-3 nodes. For such a reproduction process it was not required to add regulatory substances in the nutrient medium.



Figure 2. Appearance of microplants of Birch on the multiplication stage (a - immediately after transplanting on the fresh medium, b - after 3 weeks from the date of transplanting)



Figure 3. Appearance of the jars with the Aspen microplants on the multiplication stage on shelf racks for sterile crops

At the rooting stage plants were transplanted on fertile ground in plastic polypropylene containers with the volume 250 ml., which were sealed by a plastic "stretch" film. Each container included 30 microcuttings. Within 15-20 days the microcuttings transplanted into fertile ground formed adventitious roots. Plants, of both Aspen and Birch, even at the stage of multiplication, were capable of spontaneous rooting, so the rooting culture medium was used without the addition of growth regulators.

For the cultivation of microplants of Aspen and Birch, both at the stage of animation and the stage of rooting, the G. Lloyd (1980) culture medium was used (WPM), supplemented with sucrose in the amount of 30 g/ l. As a hardening agent Bacto Agar of the American type was used in an amount of 7 mg/l. The volume of culture medium in the cultivation containers both during the multiplication and rooting

stages was 50 ml. Growth regulators and vitamins were added separately after sterilization during the filling of the medium to the cultivation containers. Sterilization of regulatory substance solutions and vitamins was performed by filtration through membrane filters. With this method of preparation of media and number of plants in a container of 2,8-3 litres of the culture medium were needed per 100 plants at the multiplication stage, and 1.5-1.7 litres at the rooting stage. Sterilization of media was carried out in 1-litre flasks, to each of which 0.8 l of medium was added. In case of necessity the medium from flasks was used for filling the cultivation containers. The cultivation containers were sterilized separately.

Microplants Production Process Organization

Transplantations of sterile crops were conducted at a fixed rate - 1200 explants per shift. During the working day the employees involved in sterile cultures transplantation also performed the following activities:

- heating of the flasks with a nutrient medium;
- adding solutions of vitamins and regulatory agents (if needed);
- transplanting plants on fresh medium;
- labelling of cultivation containers and placing them on the shelves;
- heating of the glass jars and lids in the hot-air sterilizer.

Production of rooted microplants took place in the conditions that imitated the production ones as much as possible. Batches of microplants were produced in accordance with the order of manufacturers of industrial partners, who were the ultimate consumers of microplants of Aspen and Birch. Under the conditions of this experiment, marketable products were the rooted invitro microplants, which were transferred to industrial partners. When the invitro-rooted plants became available, they checked for compliance with the quality parameters, which were carried out by the supervisor. The marketable products included only the plants that had passed the inspection by the supervisor, they were sent to the industrial partner and adopted by them. The microplants were transferred to partners in the containers on a nutrient medium, which were packed in the shipping containers and transported by automobile vehicles to the partner's region (Fig. 4). Industrial partners, in turn, under the agreement conducted the adaptation of plants to non-sterile conditions and used the adapted regenerants for laying the forest plantations.

The production process was strictly regulated, allowing the most accurate account of all costs associated with the production of regenerants. In general, the production cycle can be expressed as a scheme (Fig. 5). Volumes of transplanted plants were recorded daily, both during multiplication and the rooting stages. Recording the amounts of transplanted microplants, culling of defective batches, the number of rooted microcuttings was carried out in a specialized programme. The data according to which the cost efficiency evaluation was conducted was being recorded for two years in 2015 and 2016.

genotype alone equals to one group), 15 of them were the Birch genotypes and 15 were the Aspen genotypes. Each of these groups was propagating separately and the individual record of the volume of production was kept for each of them. Each group was being produced in a different amount, though not arbitrarily, but according to the received orders from industrial partners. Thus, we can say that the choice of the volume of production of each group (genotype) was not dependent on the will of the experimenter, but was an incoming condition. At the end of the two-year production cycle the expense calculation took place which was used in the process of reproduction of each genotype, and the cost calculation was performed in each group individually and of all groups together. On the basis of the received cost price value, the evaluation of the effect of the volume of the produced output of each group on the cost of the plants in this group was conducted.

Methods of Statistical Processing

The methods of correlation and regression analysis were used for the data analysis and testing the prepared assumptions. Using correlation analysis established the extent and accuracy of the influence of the studied factors (lot size) on the parameters (cost effectiveness and cost structure). Regression analysis as a tool used for the mathematical evaluation of the alleged factor influence on the studied parameters was used to predict the scope and the way the change of production volume affected the cost price. The Statistica 6.1 (StatSoft, USA) software was used to carry out the mathematical analysis.

Results and Discussions

The amount of 993 573 units of rooted microplants of Birch and Aspen were produced during the experiment, 850,830 units of which were transferred to industrial partners in the form of commercial products. Thus, the proportion of wastage and unclaimed products was 14.37%. In order to produce a specified number of rooted in vitro microshoots at the stage of multiplication 654,425 plants were obtained. The overall production indices of rooted microplants of Aspen and Birch are shown in Table 1.

Table 1. The number of in vitro produced microplants of all genotypes of Aspen and Birch received during two years of pilot production.

Type of woody plant	Received amount of plants					
	In year 2015			In year 2016		
	At the stage of multiplication	At the stage of rooting	Transferred to the partners	At the stage of multiplication	At the stage of rooting	Transferred to the partners
Birch	78 829	162 118		215 232	156 185	
Aspen	119 056	310 187		241 308	365 083	
Total:	197 885	472 305	417 836	456 540	521 268	432 994

The cost estimation used in the production process of the total number of Aspen and Birch, produced within the research, has shown that the production cost price of rooted in vitro microplants of Birch made 12.54 rubles and 13.12 rubles of Aspen. The cost price difference is quite agreed with the biological features of these plants, in particular, with the fact that the multiplication factor of Aspen is slightly lower than that of Birch (Table 2).



The results of the cost price calculation of plants with different production volume showed the presence of significant differences in cost price within the plants with different volume. Thus, the maximum cost price was observed in one of Birch groups and reached the number of 26.40 rubles. The minimum cost price was also observed in one of Birch groups — 10.62 rubles. The costs price of different groups of Aspen plants already ranged from 17.54 to 11.14 rubles. This is probably due to the fact that the variation in production volumes of different groups of Birch was significantly greater (from 872 up to 127 111 units) than those of Aspen (from 8849 to 95 395).

The correlation analysis suggests that during the plants production through clonal micropropagation, there is a noticeable correlation tightness between the production volume and the cost price. The correlation coefficient between the production volume of Birch and the cost price of this group plants made — 0.602, and for Aspen groups it made — 0.536. Product release of Birch plants group, with the approximate volume in 100 thousand plants, allowed to reach the cost price of a single plant by 10 000 rubles, but practically this value was not overcome in the experiment. It is possible due to the fact that the amount of 100 000 units was exceeded only by one group of Birches.

Table 2. The volumes of produced microplants of different Birch and Aspen groups (genotypes) and their cost price.

Type of woody plant	Group (genotypes)	The volume of produced products, amount.	The cost price, rubles.
Birch	bp3f1	127 111	10,62
Birch	bp4a	64 198	11,42
Birch	bp16	45 800	12,68
Birch	bb31	40 493	12,12
Birch	4	35 845	12,89
Birch	bb4b	34 738	11,00
Birch	tr1	23 747	11,92
Birch	drv-1	16 671	12,52
Birch	kc06	12 715	12,53
Birch	64-1	4 226	21,82
Birch	tr2	3 030	13,07
Birch	kb76	3 012	25,14
Birch	66-150.10	2 728	21,58
Birch	kb81	2 550	26,40
Birch	30b1	972	25,78
Total:	-	417 836	-
Aspen	47-1	95 395	12,07
Aspen	23	74 478	11,14
Aspen	ptv22	47 182	12,63
Aspen	L-4	46 469	11,99
Aspen	C-control	35 152	13,00
Aspen	pt2	22 638	13,2
Aspen	mutant 4	20 943	14,57
Aspen	47-1-1-27	14 908	13,36
Aspen	47-1-1-31	12 467	11,80
Aspen	mutant 14	11 994	13,07
Aspen	47-1-1-22	11 124	13,39
Aspen	mutant 2	11 116	17,54
Aspen	47-1-2-53	10 503	12,70
Aspen	f2	9 776	13,92
Aspen	mutant 21	8 849	16,67
Total:	-	432 944	-

With the help of a regression analysis the test of assumption was conducted, showing that starting from a certain amount of produced microplants, the cost price of the plant could drop below 10.00 rubles. The regression analysis was carried out separately for many Birch groups, and for many Aspen groups. As the result there were two equations that reflect the relationship between the produced volume of microplants within the same group (genotype) and the cost price of one microplant. The dependence equation of the form $y = \beta_0 + \beta_1 x$ for Birch groups was as follows:

$$y = 19,11 - 0,000108x,$$

where y — the cost price of microplants group (rubles), x — the produced volume of microplants within the same group, 19,11 and -0.000108 — the coefficients β_0 and β_1 , respectively.

The received function has obvious disadvantages which are connected with the characteristics of all linear functions, firstly — the coordinate taking of a zero value. In addition, the actual data, as well as the estimation of the average approximation error ($\bar{A}, \%$) indicates that the cost price, within the large or small production volumes, should not change linearly. The cost price rate of drawdown with an increase in the production volume should decrease. For this reason, in order to predict the dependence of microplant cost price on the volume of produced party, a power function of the form $Y = 10^a \cdot X^{-b}$ was chosen. The coefficients a and b were obtained using a linearization procedure of variables and the subsequent potentiation of equation of the form $Y = a - bX$.

The received power equation for Birch groups acquired the following form:

$$Y = 10^{1,9583} \cdot X^{-0,1890},$$

where Y — the cost price of microplants group (rubles), X — the produced volume of microplants within the same group, 1,9583 and 0,1890 — the coefficients a and b , respectively.

For the cost price dependence on the production volume of different Aspen groups was received the following equation:

$$Y = 10^{1,4990} \cdot X^{-0,08680},$$

where Y — the cost price of microplants group (rubles), X — the produced volume of microplants within the same group, 1,4990 and 0,0868 — the coefficients a and b , respectively. In accordance with these equations the graphs were constructed, predicting the behavior of microplants cost price depending on the volume of produced goods (Fig. 6 and 7).

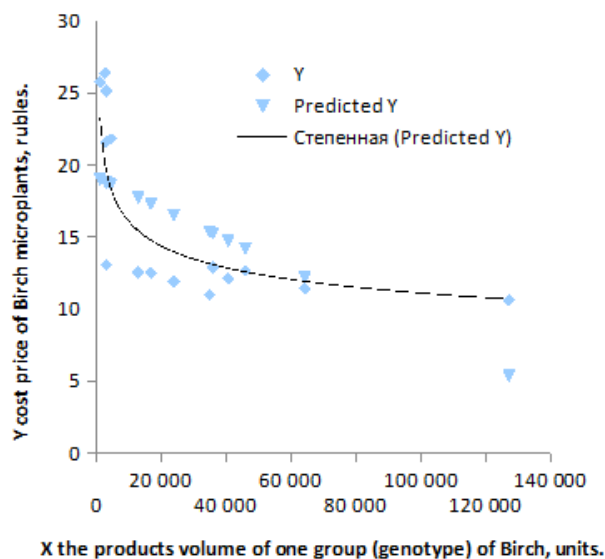


Figure 6. The Graph of the regression equation predicting the dependence of Birch microplants cost price on the volume of produced plants, built on the basis of experimental data. $\blacklozenge$ Y - the graph points, the coordinates of which correspond to the size of a particular group (genotype) and its plant cost price. $\blacktriangledown$ predicted Y - the graph points, the coordinates of which correspond to the predicted size of the cost price for a particular production volume of a group (genotype)

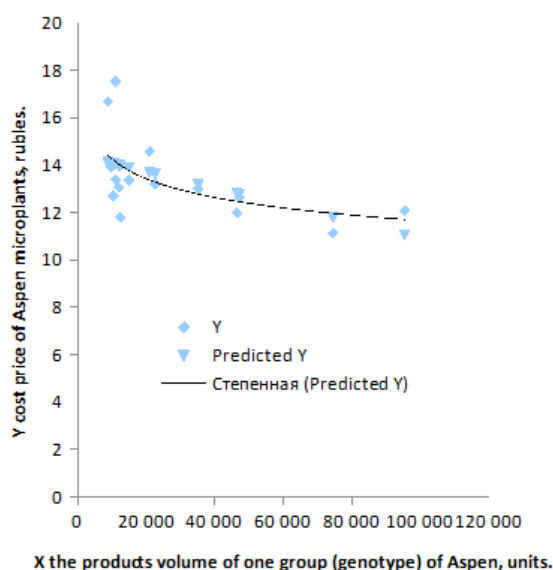


Figure 7. The Graph of the regression equation predicting the dependence of Aspen microplants cost price on the volume of produced plants, built on the basis of experimental data. $\blacklozenge$ Y - the graph points, the coordinates of which correspond to the size of a particular group (genotype) and its plant cost price. $\blacktriangledown$ predicted Y - the graph points, the coordinates of which correspond to the predicted size of the cost price for a particular production volume of a group (genotype)

The difference in the equation coefficients reflects the impact of the species specificity on the regularity between the production volume and the total cost price.

Thus, a greater impact of production volume was observed for birch than for aspen. The increase in volume of produced Birch microplants by 10 times (from 1 000 to 10 000 units) can potentially reduce the cost price by 8.66 rubles. The influence of produced volume is much weaker for Aspen, the same 10-fold increase of the production volume in the range from 1 000 to 10 000 units lead to decrease of the cost price by 3.14 rubles.

Using the equation of power regression, you can theoretically predict what size of the party shall be to ensure that the cost price of one microplant fell below a certain level of cost price. For example, in order to low down the Birch microplants cost price below 10.00 rubles, there should be produced at least 111 260 plants during 2 years. Thus, it is possible to calculate the party size necessary to generate for any randomly selected size of the cost price (Table 3).

Table 3. Theoretically calculated number of plants necessary to be produced in order to achieve the cost price level of interest, units.

Type of woody plant	To achieve cost price by 15,00 rubles.	To achieve cost price by 10,00 rubles.
Birch	13 151	111 260
Aspen	5 249	560 850

Comparing the graphs of the cost price dependence on the volume of produced amount for two types of woody plant, it can be seen that the cost price of birch plants is under more influence than the cost price of aspen ones. It is also shown by the value of the coefficient b , which in the case of Birch plants took a value by 2.18 times greater than the value of the corresponding coefficient for Aspen. It is possible that the value of this coefficient could be affected by the difference in the reproduction constant of Birch and Aspen, which for Birch was somewhat higher than that for Aspen.

The assessment of the cost structure showed that the largest share of them took labor costs (over 48%) and energy costs (over 26%) (Figure 8). With regard of this it should be noted that such feature is characteristic for the majority of plant production on the basis of clonal micropropagation (Mashkina et al., 2016). It is clear that any measures aimed at increasing the efficiency of the production on the basis of clonal micropropagation, as one of its objectives must have the task of reducing the share of manual labor in the total amount of expenses. One of the purposes of this study – to establish whether there is a relationship between the volume of produced products and the cost structure, in particular, whether the production volume effects the usage efficiency of such resource as labor costs. Comparing the share of labor costs in the total cost of groups of different volume of Aspen did not reveal a significant impact of the group volume on the share of labor costs and on average, they made about 47.32% of the cost price of Birch plants and 48.04% of the cost price of Aspen plants (Table 4). But as for Birch groups there was a significant dependence between the production volume and the share of labor costs. Moreover, with the increase in volume of produced microplants, the share of labor costs even slightly increased.

The quite obvious assumption, stating that the production volume would lead, along with the costs reduction in general, to a reduction of one of the prevalent types of costs, does not look so obvious with a closer look at it. In general, the increase in the effective usage of labor costs is always associated with the modernization of production (the automation of manual processes, the introduction of new equipment, materials), or with a change in the labour organization (the change of the technological scheme). Carrying out this experiment did not plan such changes, and the same technology was used during receiving the parties of different volumes.



Table 4. The labour costs share of total cost price for commercial products of Birch and Aspen microplants of different groups (genotypes)

Type of woody plant	Group (genotype).	The cost price share, %.
Birch	bp3f1	49,31
Birch	bp4a	47,63
Birch	bp1b	48,27
Birch	bb31	48,22
Birch	4	48,18
Birch	bb4b	49,31
Birch	tr1	49,25
Birch	drv-1	47,09
Birch	kc06	47,65
Birch	bc-1	47,74
Birch	tr2	46,62
Birch	kb76	45,71
Birch	66-150.10	47,09
Birch	kb81	44,61
Birch	30b1	43,07
Total:	-	-
Aspen	47-1	48,36
Aspen	23	48,77
Aspen	ptv22	48,11
Aspen	L-4	48,45
Aspen	C-control	48,26
Aspen	pt2	47,96
Aspen	mutant 4	47,54
Aspen	47-1-1-27	48,16
Aspen	47-1-1-31	48,79
Aspen	mutant 14	48,21
Aspen	47-1-1-22	47,97
Aspen	mutant 2	47,77
Aspen	47-1-2-53	48,21
Aspen	f2	47,21
Aspen	mutant 21	46,76
Total:	-	-

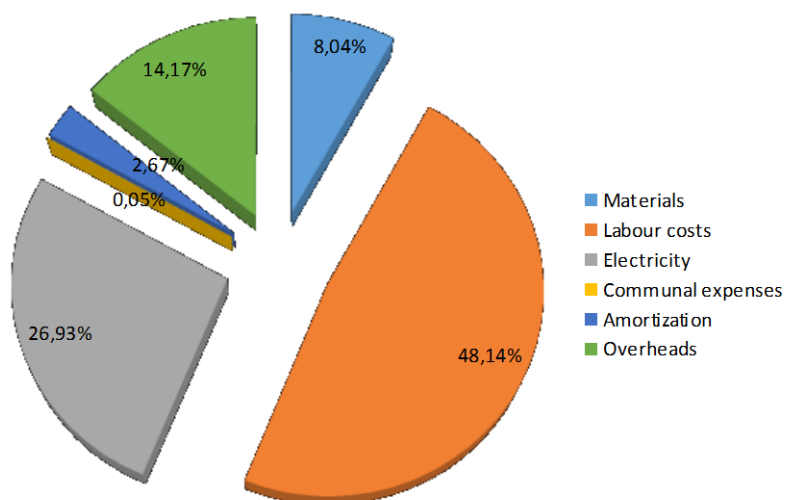


Figure 8. Costs structure during the production of 15 Aspen genotypes over the years 2015 -16

Trying to explain the reason of reducing labor costs due to increased volume production in our experience has led to the fact that another regularity was discovered. The analysis of the received volume of microplants at the stage of multiplication allowed to establish the following regularity – with the increase in the production volume of one group for the production of one rooted microplant it took fewer plants at the stage of multiplication. In other words, with the increase in the production volume, more efficient usage of plants obtained at the stage of multiplication was observed. The number of plants needed to obtain at the stage of multiplication (N_{multi}) in order to receive a single plant at the rooting stage, were calculated as the quotient between the number of produced commercial microplants ($k_{prod.}$) within the same group and the number of microplants of this group ($k_{multi.}$), obtained at the stage of multiplication:

$$N_{multi.} = k_{prod.} / k_{multi.}$$

The obtained values $N_{multi.}$, associated with the volume of produced goods allowed to establish that there is a correlation between them. The regression analysis showed a regularity similar to that observed between the production of the microplants and the cost price. With the increase in the production volume, the number of microplants at the stage of multiplication required for the production of rooted microplants was decreasing. The regression equation obtained from the analysis made it possible to make the graphs of dependence between the production volume within the $N_{multi.}$ group index (Fig. 9).

It is likely that the dependence that reflects the impact of the production volume on the efficiency of usage of multiplication phase plants, as well as with the dependence of cost price on the production volume should not be linear. The linear dependence predicts that for the production volume of 103 790 units of Birch, that will be required at the stage of multiplication to ensure the production of one commercial microplant, should be taken a negative value. However, in reality this is not observed. Nevertheless, the predicted trend is possible and allows, for example, for Birches to note a regularity – the party increase by 3 times leads to almost two-fold reduction in the number of plants that will be required at the stage of multiplication in order to produce one rooted microplant. The nature of the interaction described by a power function is closer to the apparent influence of the produced goods volume on the cost price. Therefore, as the trend lines, reflecting the



regularity of influence of the production volume on the cost price, the graphs shows the lines of power function instead of a linear function.

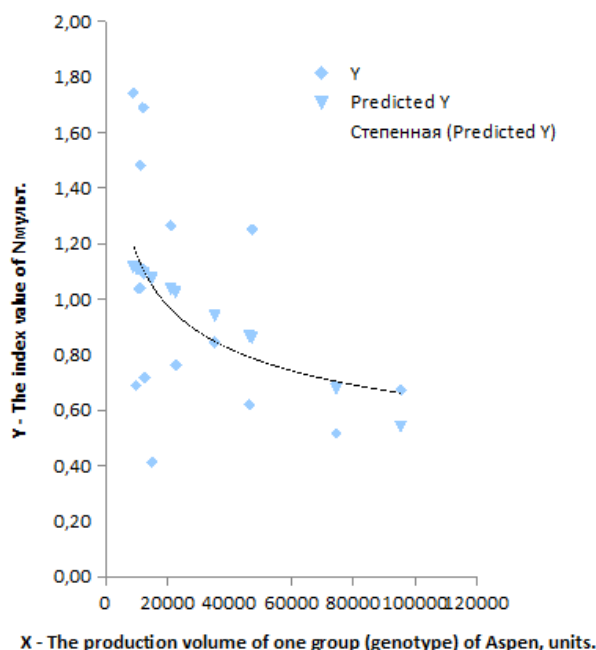


Figure 9. The graph of the regression equation predicting the dependence of the microplants needed to produce one rooted microplant of Aspen on the volume of produced plants. ♦Y - the graph points, the coordinates of which correspond to the size of a particular group (genotype) and the plants amount at the stage of multiplication needed to produce one rooted microplant. ■predicted Y - the graph points, the coordinates of which correspond to the predicted size of the plants amount at the stage of multiplication for a particular production volume of a group (genotype). The calculated power equation of regression of the form $Y = 10a \cdot X^{-b}$ for Birch groups had the form: $Y = 101,5785 \cdot X^{-0,3800}$, and for Aspen groups: $Y = 100,9002 \cdot X^{-0,2188}$

Conclusion

The installed impact of the production volume on the usage efficiency of microplants obtained at the stage of multiplication, can assure that exactly this factor influenced the cost price reduction in response to the increase in production volumes. The main mechanism that underlies this regulation – is to decrease domestic technological losses.

The correlation analysis confirmed the presence of significant interactions, but the impact of the volume of produced goods on the cost price was not linear, since the graph of the power function more accurately reflects the dependence observed in the experiment than the linear function. The research results have shown that if to accept that the nature of the interaction is close to the linear, the cost price of 10 rubles per one rooted microplant can be achieved for Birches with the volume production of more than 84,352 units, and for Aspens with the volume production of 126 286 units and more. Since the nature of the interaction is closer to the power function, the party volume value for Birch should reach 111,260 units, while for Aspen – 560 850. The analysis of the received functions showed that the dependence can not be linear. In the case of Aspen the cost price level of 10 rubles for one rooted microplant can be achieved without changing in the production technology only with a relatively large production volumes (more than 560 thousand units of plants). Of course, achieving the

competitive price in 5.00 rubles and less can be practically obtained only with the production volumes, measured in millions of units (for birch – not less than 4, 000, 000 units), in the case that the production technology will remain the same during the process of scaling. The application of the technology of receiving microplants from the somatic embryos, designed, for example, for such woody plant such as Hevea (Carron et al., 1995) and for a number of gymnosperms (Fenning & Gershenzon, 2002, Fenning & Park, 2012), or the cultivation with the usage of bioreactors (Yoeup & Chakrabarty, 2003), for reproduction of Birch and Aspen, may open the way to further reduction of cost price. The large-scale production, ranging from several thousand to several tens of thousands, demonstrated the importance of the influence of genotypic differences between Aspen and Birch, but found no significant effect between the genotypes of the same breed.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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