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Optimization of microclonal propagation conditions for increasing the multiplication factor of poplar microshoots

Abstract: Representatives of the genus poplar – *Populus L.* (kind *Salicaceae*) are widely used for landscape gardening residential areas and creating different types of protective plantings. Difficulties of propagation for some types of poplars by traditional methods is their weak rooting, as well as a high level of bacterial and fungal infection rate. Therefore using such methods as clonal micropropagation of plants in aseptic conditions on artificial nutrient medium is relevant for valuable forms of poplar. The purpose of this work is optimization of the conditions of microclonal propagation for increasing the coefficient of propagation of microshoots of *Populus alba L.* and *Populus bolleana L.* from axillary shoots introduced into an *in vitro* culture. The main objective set for solving this purpose were the regeneration of plants on the basis of direct proliferation of axillary meristem, their rooting and multiplication of the microshoots. High regeneration of axillary basic shoots of two types of poplars occurred on a WPM nutrient medium with the addition of the hormones BA (0.5 mg/l) and GA 0.2 mg/l. The WPM nutrient medium with the addition of BA hormones of 0.2 mg/l and 0.2 mg/l appropriate for increasing the quantity of shoots from axillary shoots. More optimal for rooting and growth of microshoots of *Populus alba L.* and *Populus bolleana L.* is S WPM nutrient medium for woody cultural with the addition of the hormone IBA 0.01 mg/l. Thereby, conditions of microclonal propagation was been optimized for increasing the coefficient of propagation of poplar microshoots.

Keywords: Populus alba L., Populus bolleana L., axillary shoods, cultivation medium, in vitro.

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Abbreviations: WPM – Woody plant medium; BA – 6-benzylaminopurine; IBA – indole-3-butyric acid; GA – gibberellic acid; NA – nicotinic acid; medium MS.

Introduction. The success of landscape gardening in large amount depends on the correct selection of decorative trees. Knowledge of the morphological and decorative features of woody plants and their relationship to the adverse conditions to the city environment allows to improve the landscape of the city [1].

The genus *Populus* become an important component of the world's potential renewable resources for the XXI century. The genus *Populus* has many commercially important components like hybrids and species, and is the most highly distributed woody plant worldwide [2, 3]. Poplar one of the fastest growing species used in plantation cultivation for various aims [4]. In altogether, here are around 110 poplar speciesin the world that widely distributedin the northern hemisphere [5].

In the list of the Royal Botanic Garden Kew were numbered 199 scientific names of *Populus* genus, which only 87 were recognized as specific names [6].

Forgardening the cities and region with severeclimatic conditions, landscapers provide planting and reproduction of *Populus alba L.* and *Populus bolleana L.* [7]. Valuable quality of two types of decorative poplar is sufficient stability against smoke and gas, the ability to enrich the air with volatile production and kill pathogenic microbes. They are winterhardy, relatively drought and gas-resistant, light-requiring, heat-resistant, and wind-resistant [8].

Despite the advantage of two types of poplar, there are limitations when cultivation for gardening ecologically unfavorable territories [9]. There exist a problem, which connects with surviving percentage of *Populus alba L.,* which relateswith effect of fungal and viral diseases *Populus bolleana L.* rooting process is difficult [10]. For reproduction of *Populus alba L.* and *Populus bolleana L.*with the most appropriate and optimal method is microclonal reproduction by axillary buds, since it achieves the highest genetic stability and uniformity [11].

By the researchers were selected and optimized nutrient medium, determined the significance of auxins and cytokines for shoot growth, to induce root formation under conditions *in vitro* [12, 13, 14].

Poplars were one of the first objectives of propagation trials, where Cambial tissues were use dasbasicmaterial [15, 16]. Initially, callus improvement, following shoot or root development was generated on the callus surface. Sometimes it occurred without any outside effect. The link between the regenerated shoots and roots was sometimes unclear. Gathered opinions established the vegetative propagation founded from a single bud and different originated callus based plant regeneration [17].

Several researchers' micropropagated sterile bud originated plantlets of poplars for the first time [18, 19]. They presented in their results about difficulties of culture establishment and genetically determined differences between the species. It was identified that the success of establishment also depends onthe age of the mother plants [17].

Another research aimed to develop micropropagation methods of poplars for commercial purposes [20]. Thus, that research improved the micropropagation method of the tetraploid clone 'Ta – 10' for the substitution of vegetative propagation by grafting. Research around the development of the micropropagation procedure for different poplar species continues today [21]. Many scientists summarized the multiplication procedure of several poplar species that were inoculated or not inoculated with ectomycorrhizal fungus for different experimental purposes [13, 22, 23].

The aim of the study was to optimize the conditions of micropropagation to increase the multiplication factor of poplar microshoots of *Populus alba L.* and *Populus bolleana L.* from axillary shoots introduced into the culture *in vitro*.

Material and Methods

Sterilization of shoots . The cut annual shoots of poplar about 6 cm in size were thoroughly washed with running war water and sterilized. Sterilization consisted of two stages: 1 – preliminary sterilization, which took place in non-sterile conditions (surface sterilization), 2 – basic sterilization in aseptic conditions (in laminar-flow box conditions).

Introduction of axillary buds into in vitro culture

In laminar-box in axillary buds removed cover scales and leaves, leaving the two most deeply located leaves, which were isolated and placed in a nutrient medium WPM (Woody plant medium) with the addition of 0.5 mg/l BA (6-benzylaminopurine), 0.2 mg/l GA (gibberellic acid) for the growth of axillary buds. Axillary shoots were cultivated in the climatic chamber "BINDER KBWF 720" with a 16-hour light mode, illumination – 2-3 kilolux, temperature 24-26 ° C, the humidity level of 70%.

Regeneration and rooting of test-tube plants. Regeneration of meristem plants consisted of the following stages – induction of shoot formation, their elongation and rooting. To increase the number of shoots of poplar species of *Populus alba L.* and *Populus bolleana L.*, nutrient media WPM and MS with BA growth hormones in concentration - 0.2 mg/l; 0.5 mg/l; 1.0 mg/l and GA – 0.2 mg/l were used. Rooting of *in vitro* shoots of two species of poplar was conducted on WPM medium with half the content of macrosalts (S WPM), growth hormone IBA (indole-3-butyric acid) 0.01 mg/l; 0.5 mg/l or nutrient medium free of hormones.

Results and discussion

Regeneration of poplar axillary shoots. The regeneration of axillary shoots is a particularly important step in the development of poplar micropropagation technology. The main purpose of this stage is the regeneration of plants on the basis of direct proliferation of axillary meristems, their rooting and animation of the obtained microshoots. Annual non-woody shoots of *Populus alba L* and *Populus bolleana L.* poplar with axillary vegetative buds were used to regenerate the main axillary shoots. The researchers of poplar cultivation using MS and WPM with the addition of phytohormones, such as 6-benzylaminopurine (BA) [21].

We optimized the composition of the nutrient medium WPM benefits for the growth and development of axillary shoots of two species of poplars from explant. The influence of phytohormones (BA and GA) was studied. Following variants of the WPM medium were studied: 1) BA 0.2 mg/l,

GA 0.2 mg/l; 2) BA 0.5 mg/l, GA 0.2 mg/l. Sterilized shoots were cut under aseptic conditions into segments of size 0.5–2 cm with one axillary bud (Figure 1).

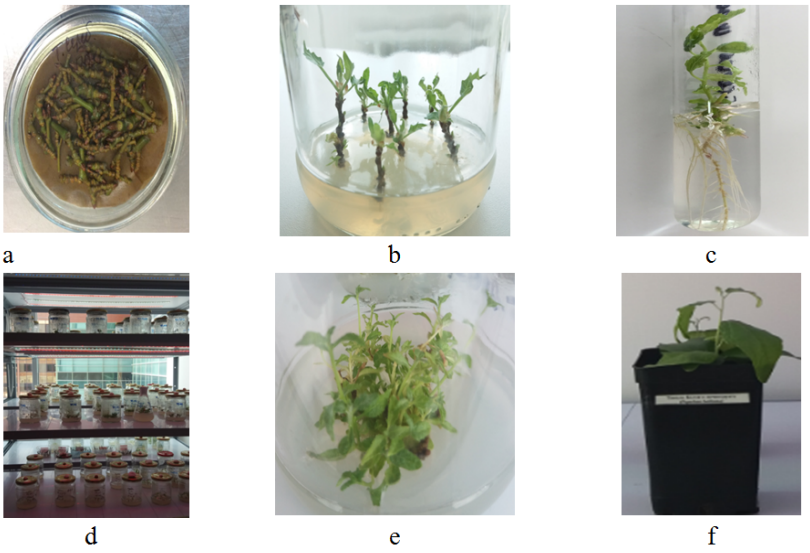


Figure 1 – **Obtaining poplar seedlings through micropropagation**
a) sterile axillary shoots of *Populus alba L*; b) development of the main axillary shoot; c) rooted of microshoots; d) growing microshoots in light room; e) reproduction of induced shoots of *Populus alba L;* f) microshoots planted in the soil

After 30 days of cultivation, the results of the experiment were analyzed and it was found that high regeneration of axillary main shoots of two poplar species occurred in the second variant of the nutrient medium WPM with the addition of hormones (BA 0.5 mg/l and GA 0.2 mg/l). The highest percentage of well-developed axillary main shoots was obtained by cultivating explants on the nutrient medium WPM with BA 0.5 mg/l and GA 0.2 mg/l in *Populus alba L* – 70.2%, in *Populus bolleana L.*– 57.3% (Figure 2).

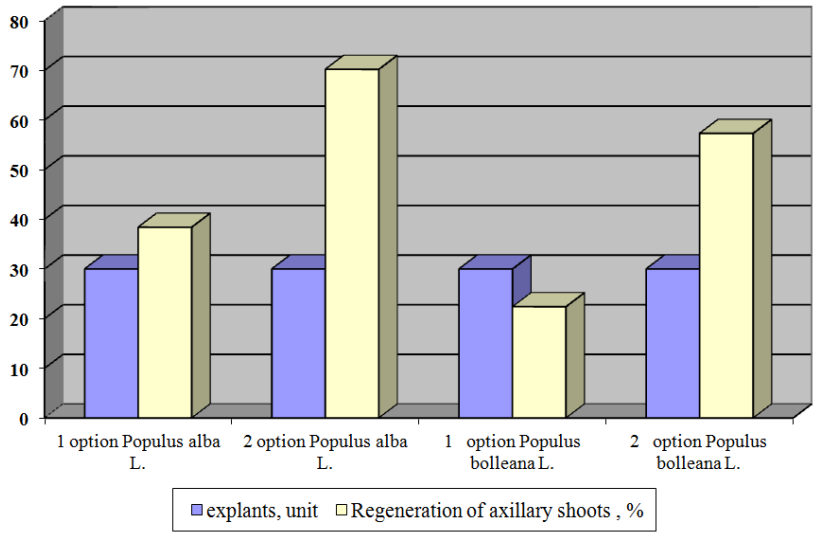


Figure 2 – **Regeneration of axillary shoots depending onthe hormonal medium composition WPM**

The percentage of regeneration of axillary shoots in *Populus alba L.* is higher than that of *Populus bolleana L.* This is due to the fact that *Populus alba L.* has a high intensity of vital and rapid growth in culture *in vitro* and natural conditions, compared with *Populus bolleana L.*[19]. Thus, for regeneration of axillary shoot of *Populus alba L.* and *Populus bolleana L.* the environment of WPM with the addition of hormones BA 0,5 mg/l and GA 0,2 mg/l is better suited.

Clonal micropropagation of poplar microshoots

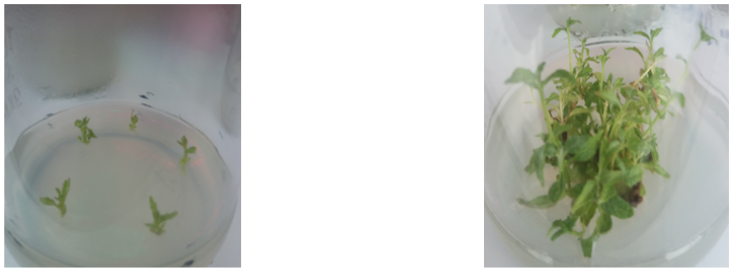
In the literature, there are data on the use of different nutrient media with hormones to increase the number of induced shoots [13, 14, 25]. It is shown that the best growth in the proliferation of shoots in poplar was on the medium WPM for tree crops with BA in the concentration of 0.1 and 0.2 mg/l, and the increase in the concentration of BA did not have a positive effect on the increase of the shoots number [14].

We have optimized the composition of the nutrient medium to increase the number of induced shoots. The effect of concentration of BA and GA phytohormones were studied. The following WPM medium variants were tested: 1) BA 1.0 mg/l, GA 0.2 mg/l; 2) BA 0.5 mg/l, GA 0.2 mg/l; 3) BA 0.2 mg/l, GA 0.2 mg/l; 4) BA 0.5 mg/l; MS medium: 5) BA 1.0 mg/l, GA 0.2 mg/l; 6) BA 0.5 mg/l, GA 0.2 mg/l; 7) BA 0.2 mg/l, GA 0.2 mg/l; 8) BA 0.5 mg/l (Figure 3, table 2). Experiments were carried out on *Populus alba L.* the primary explant with a cut axillary shoot was used.

Table 1 – Indicators of increase in the number of induced shoots in *Populus alba L.*

Options	1 day		15 days		30 days		50 days	
	Shoots units	Shoot length (cm)	Shoots units	Shoot length (cm)	Shoots units	Shoot length (cm)	Shoots units	Shoot length (cm)
I - WPM with BA 1,0 and GA 0,2 mg/l	1	1.5±0.1	2.0±0.1	1.8±0.1	3.0±0.2	2.1±0.1	5.4±0.3	2.3±0.1
II - WPM with BA 0,5 and GA 0,2 mg/l	1	2.2±0.1	2.0±0.1	2.4±0.1	3.2±0.2	2.5±0.1	5.4±0.3	2.7±0.1
III - WPM with BA 0,2 andGA 0,2 mg/l	1	3.2±0.2	3.2±0.2	3.4±0.2	7.0±0.4	5.3±0.3	11.2±0.6	5.5±0.3
IV - WPM with BA 0,5 mg/l	1	1.4±0.1	1.2±0.1	1.5±0.1	2.2±0.1	1.7±0.1	3.6±0.2	1.8±0.1
V – MCwith BA 1,0 and GA 0,2 mg/l	1	1.3±0.1	1.0±0.1	1.5±0.1	1.2±0.1	1.6±0.1	1.8±0.1	1.7±0.1
VI – MCwith BA 0,5 and GA 0,2 mg/l	1	1.8±0.1	1.0±0.1	2.1±0.1	1.0±0.1	2.1±0.1	1.4±0.1	2.1±0.1
VII – MCwith BA 0,2 and GA 0,2 mg/l	1	2.2±0.1	2.6±0.1	2.4±0.1	5.0±0.3	3.2±0.2	7.8±0.4	3.3±0.2
VIII - MCwith BA 0,5 mg/l	1	1.6±0.1	1.8±0.1	1.8±0.1	3.0±0.2	20±0.1	5.4±0.3	2.6±0.1

After 15, 30 and 50 days of cultivation, the results of the experiment were analyzed and it was found that the high number of poplar shoots occurred in the third options of the nutrient medium



a b

Figure 3 – **Propagation of induced shoots of poplar**
induced shoots on the WPM medium with BA 0.2 mg/l, GA 0.2 mg/l;
b) propagation of induced shoots after 30 days

WPM with the addition of hormones (BA 0.2 mg/l and GA 0.2 mg/l). Thus, the average number of shoots of white species was 11.2 units; shoots length – 5.5 cm.

The slowest development of shoots was observed in the sixth variant of the medium (MS with BA 0.5 and GA 0.2 mg/l): the average number of shoots wasonly 1.4 units, the length of shoots – 2.1cm. There was no positive effect of BA 1.0 mg/l and GA 0.2 mg/l on the increase in the number of shoots. Thus, to increase the number of shoots from axillary shoots solid medium MS with of hormones the addition BA 0.2 mg/l and GA 0.2 mg/l was more applicable.

Rooting of poplar microshoots. One of the important stages of obtaining seedlings in the culture of *in vitro* male specimens of *Populus alba L.* and *Populus bolleana L.* is the induction of root formation. For obtaining poplar seedlings of *Populus alba L.* and *Populus bolleana L.* from the test-tube, it is initially necessary to choose a nutrient medium with hormones, there will be normal growth and development of the roots. In the literature as the medium for induction of root formation microshoots four poplar species used nutrient medium Schenk Hildebrandt (SH) and Woody Plant Medium (WPM) supplemented with 5 mg/l nicotinic acid (NA) WPM or SWPM (with a reduced content of macrosalts) without hormones [26]. WPM with the auxin, indole-3-butyric acid (IBA) 0.5 mg/l.

We have optimized the composition of the nutrient medium WPM for the induction of root formation and rooting of microshoots of two types of poplars. It was studied the influence of without hormonal environment and WPM supplemented with auxin is indole-3-butyric acid (IBA). Tested the following mediums WPM: 1) no hormone; 2) SWPM (reduced content of macrosalts); 3) SWPM, IBA 0.5 mg/l; 4) SWPM, IBA 0.01 mg/l was added auxin, IBA at a concentration of 0.01 mg/l and 0.5 mg/l in the medium to determine the required amount of exogenous hormones to receive microshootson the basis of proliferation of axillary meristems (direct distillation axillary shoots) and the induction of root formation, except for the stage of callus formation. The increased concentration of auxins in the medium contributes to the formation of callus tissue, which is confirmed by literature data [13, 25].The experiments were carried out on two types of poplar, 30 test-tube microshoots were analyzed for each variant. To obtain whole microshoots, the formed shoots were isolated and transferred to the rooting medium (Table 2).

Table 2 – Induction of roots formation of microshoots in culture *in vitro*

Genotype	Option of mediums	Amount of microshoots			Length of shoots, cm	Callus induction
		total	rooted	%		
Populus alba L.	1 – WPM	30±1.5	16±0.8	53.3±2.7	4.1±0.2	–
	2 – S WPM	30±1.5	20±1.0	66.7±3.3	6.8±0.3	–
	3 – S WPM + IBA 0,5	30±1.5	9±0.5	30.0±1.5	5.1±0.3	+

	4 –S WPM + IBA 0,01	30±1.5	24±1.2	80.0±3.2	7.2±0.4	–
Populus bolleana L.	1 – WPM	30±1.5	10±0.5	33.3±1.7	5.0±0.3	–
	2 – S WPM	30±1.5	16±0.8	53.3±2.7	7.7±0.4	–
	3 – S WPM + IBA 0,5	30±1.5	8±0.4	26.7±1.3	5.6±0.3	+
	4 – S WPM + IBA 0,01	30±1.5	17±0.9	56.7±2.8	6.4±0.3	–
Notes: 1 – «+» induced callus; 2 – «–» callus is not formed						

After 30 days of cultivation, the results of the experiment were analyzed and it was found that the best rooting of poplar microshoots occurred in the fourth variant with a reduced content of macrosalts – SWPM with the addition of IBA 0.01 mg/l, which was 80% for *Populus alba L.* 56.7% for *Populus bolleana L.* Also, the best results were obtained in the second option of the medium - SWPM without hormone, *Populus alba L.* - 66.7%, *Populus bolleana L.* - 53.3%. Two types of poplars at the option of SWPM medium with auxin, IBA 0.5 mg/l was observed the formation of callus tissue.

The percentage of rooted plants in two poplar species is different, this is due to different levels and a set of own hormones, because of this, they can exhibit different morphogeneticactivity and the ability to regenerate whole plants in vitro culture (Figure 4). By rooting microshoots obtained rapid growth in their height. After a month of cultivation, shoots reached a length of 4.1 to 7.7 cm.

Figure 4 – **Rooting of microshoots of two species of poplars**
a) rooting of *Populus alba L.* microshoots; b) induced roots of *Populus bolleana L.* on nutrient medium SWPM; c) induction of callus formation of microshoots on nutrient medium SWPM with IBA 0.5 mg/l

Consequently, the nutrient medium SWPM for woody crops with the addition of the hormone IBA 0.01 mg/l is the most optimal for rooting and growth of microshoots of *Populus alba L.* and *Populus bolleana L.* Thus, the optimized conditions of micropropagation in tractable traditional method of *Populus alba L.* and *Populus bolleana L.* for mass production and receiving improved planting material.

Conclusion. Research of the conditions clonal micropropagation of plants in aseptic conditions on artificial nutrient medium which are difficult to root by the traditional method of *Populus alba L.* and *Populus bolleana L.* showed that the use of this method is optimal at all stages of the production of regenerated plants. High-level regeneration of the axillary main shoots of two species of poplars occurred on a WPM nutrient mediumwith the addition of the hormones BA 0.5 mg/l and GA 0.2 mg/l. To increase the quantity of shoots from axillary shoots better suits the WPM nutrient medium with the addition of hormones BA 0.2 mg/l and GA 0.2 mg/l. The most optimal for rooting and growth of microshoots of *Populus alba L.* and *Populus bolleana L.* is S WPM nutrient medium

for woody plant culture with the addition of the hormone IBA 0.01 mg/l. Thereby, the conditions for isolating explants, sterilization modes, cultivation conditions and composition of nutrient mediumwere selected. The obtained microplants of *Populus alba L.* and *Populus bolleana L.* are suitable for further industrial use in urban landscape gardening.

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Теректің микро өркедерін көбейтудің коэффициенттерін артыру үшін микроклонды көтейтудің жағдайларын оңтайландыру

Аңдатпа *Populus L.* (тұқымдас. *Salicaceae*) терек тұқымдастарның өкілдері елді мекендерді көгалдандырмен әртүрлі типтегі қорғалатын көшеттерді жасау мақсатында кеңінен қолданылады. Кейбір терек түрлерін дәстүрлі жолмен көбейтудің қиындықтары, олардың әлсіз тамырлануы, сондай-ақ бактериялық және саңырауқұлақ жұқпаларының жоғары деңгейде жұғуы болып табылады. Сол себепті, асептикалық жағдайда жасанды қоректік ортада клоналды микро көбейтудің осы әдістерін қолдану, теректің бағалы түрлері үшін өзекті болып табылады. Жұмыстың негізгі мақсаты-культураға енгізілген қолтық бүршіктерден шыққан ақ және Болле теректердің микроөркендерінің көбейту коэффициентін жоғарлату үшін микроклонды көбейтудің жағдайларын оңтайландыру болып табылады. Берілген мақсаттың шешімі үшін өсімдік регенерациясы қолтық бүршіктердің тура пролиферациясы негізінде алынған микроөркендердің мультипликациясы және оның тамырлануы басты міндет болып табылады. Теректің екі түрінің негізгі өркендерінің жоғары регенерациясы WPM қоректік ортасына БАП 0,5 мг/л және ГК 0,2 мг/л гормондары қосылған ортасында болды. Өркендер санын арттыруда қолтық бүршіктер үшін БАП (BA) 0,2 мг/л және ГК(GA) 0,2 мг/л гормондары қосылған WPM қоректік ортасыда өсірген өте оңтайлы. Ағаш өсімдіктері үшін ақ және Болле теректерінің микроөркендерінің өсуімен тамырлануында (ИМҚ) 0,01 мг/л гормоны қосылған S WPM қоректік ортасы оңтайлы болып табылады. Осылайша, теректің микроөркендерінің көбейту коэффициентін жоғарлатуда микроклоналды көбейтудің жағдайлары оңтайландырылды.

Түйін сөздер: Ақ терек, Болле терегі, қолтық бүршік, қоректік орта, *in vitro.*

Қысқартулар: WPM – *Woody plant medium*; БАП – 6-бензиламинопурин; ИМҚ – индолил май қышқылы; ГҚ – гибберелин қышқылы; НҚ – никотин қышқылы; МС – Мурасиге-Скуг қоректік ортасы.

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Оптимизация условий микроклонального размножения для повышения коэффициента размножения микропобегов тополя

Аннотация: Представители рода тополь – *Populus L.* (сем. *Salicaceae*) широко используются для озеленения населенных мест и создания различного типа защитных насаждений. Трудностью размножения некоторых видов тополей традиционными способами является их слабая укореняемость, а также высокий уровень зараженности бактериальной и грибной инфекцией. Поэтому применение такого метода как клональное микроразмножение растений в асептических условиях на искусственных питательных средах является актуальным для ценных форм тополя. Целью настоящей работы является оптимизация условий микроклонального размножения для повышения коэффициента размножения микропобегов тополя серебристого и тополя Болле из пазушных почек, введенных в культуру *in vitro.* Основными задачами, поставленными для решения данной цели, были регенерация растений на основе прямой пролиферации пазушных меристем, их укоренение и мультипликация полученных микропобегов. Высокая регенерация основных пазушных побегов двух видов тополей происходило на питательной среде WPM с добавлением гормонов БАП 0,5 мг/л и ГК 0,2 мг/л.Для увеличения количества побегов из пазушных побегов лучше подходит питательная среда WPM с добавлением гормонов БАП 0,2 мг/л и ГК 0,2 мг/л. Наиболее оптимальной для укоренения и роста микропобегов тополя серебристого и тополя Болле является питательная среда S WPM для древесных культур с добавлением гормона ИМК 0,01 мг/л. Таким образом, оптимизированы условия микроклонального размножения для повышения коэффициента размножения микропобегов тополя.

Ключевые слова: тополь серебристый, тополь Болле, пазушные почки, питательная среда, *in vitro.*

Сокращения и обозначения: WPM – Woody plant medium; БАП – 6-бензиламинопурин; ИМК – индолилмасляная кислота; ГК – гиббереллловая кислота; НК – никотиновая кислота; МС – среда Мурасиге-Скуга

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