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Features of Rooting Paulownia *in vitro*

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THE presented materials deal with the topical problem of rhizogenesis induction and rooting of genus paulownia plants obtained by in vitro method. According to the research results, the optimal composition of the nutrient medium for the regenerants cultivation on the stage of rhizogenesis induction has been defined, the technologically optimal age of the regenerants for the in vitro - ex vitro transition, the depth of the seedlings, the number of post-septic nipples has been established. The obtained results allow to improve the technological process at the stages of rhizogenesis induction and plants rooting during the post-septic adaptation.

Keywords: Paulownia, In vitro, Rhizogenesis, Auxins, Post-septic adaptation.

Introduction

In recent years, Paulownia genus trees have gained popularity in Ukraine. This ancient in its area crop grows at temperatures ranging from -25 to +45 ° C, so it can be one of the species of trees that can survive and develop in extreme conditions. Massive drying of coniferous forest plantations in Ukraine have been reported more and more often recently. According to scientists [1], this occurs "due to a significant decrease in groundwater levels and a large number of pests and diseases - known and completely new ones, which enter the territory of the state with imported ornamental plants. Climate change affects the process significantly as well. "In Ukraine, Paulownia is a new crop characterized as fast-growing and quick to fill up with high quality wood with light weight, low water-absorbing properties and high heat output. Given its advantages, it can be argued that this plant is promising for industrial and decorative use as well as an energy culture. Despite this, Paulownia remains a rare plant on the territory of Ukraine, primarily due to difficulties in its reproduction. It is difficult to get seeds in our climatic conditions, and new hybrids are can be reproduced successfully only *in vitro*. The forms producing seeds can be invasive.

The issue of obtaining *in vitro* a healthy, well-developed root system of planting material of various species and hybrids of the Paulownia genus was studied by the foreign scientists B.A. Bergmann and R. Whetten, Zayova E., LB Magar, Chunchucov and Yancheva, Doina Clapa during 1997-2015. [2-6]. This technology of growing Paulownia has also been developed in Ukraine in the last decade by domestic scientists, in particular, by O.V.Matskevich, M.M. Lisovyi [7, 8]. Technological methods of introduction into aseptic conditions and *in vitro* regeneration are worked out. However, the study needs further development of a part of the technological process at the stage of rhizogenesis induction and plants rooting in post-septic conditions.

The aim of the research is to study the influence of the main factors of root formation

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on the stages of rhizogenesis and post-septic adaptation to optimize the technological process of commercial MKR.

The research tasks are to determine the optimum composition of the nutrient medium and temperature for cultivating the regenerants at the stage of rhizogenesis induction, to establish the technologically optimal age of the regenerants for the their transition *in vitro-ex vitro,* the medium, depth of planting, the number of post-septic engraftments.

Experimental

The object of the research is the plants of the Paulownia, namely *Paulownia tomentosa×Paulownia elongata* hybrid. Studies were conducted under standard laboratory conditions [9]. Sample number - 60 plants. The sequence of experiments is as follows: the best version of the previous experiment was taken as a control in the next experiment. The extraordinary fragility of the regenerants stem in the root cervix area and its pubescence, which requires cautious irrigation, were taken into account while planted in a humid chamber on a peat substrate (substrate Alonet [10]). Bellson 20W LED lamps were used as light source and placed parallel to the rows above the plants with the a lamp power of 20W and the light flux of 1780 Lm (analog to LB-36). Lighting increased gradually for two weeks from 1500 lux to 3000 lux. Previkur® Energy 840 SL fungicide tested for post-extraction of other cultures was used to protect the plants against fungi infection [11].

Results and Discussion

Induction of plant rhizogenesis in vitro is an important step in obtaining a viable plant material *in vitro*. Auxins are known to be of vital importance at this stage. Thus, Zayova E. with co-authors found that the optimum concentration for Paulownia rhizogenesis of 0.5 mg/l IBC against the background of 1/2MS medium [5]. In similar studies, L. B. Magar and co-authors determined the optimal concentration of 2 mg/l BAP added to the MS medium [4]. The comparison of NAA and IBC auxins as rhizogenesis inducers in different concentrations in our studies found that the best option was to add 3.0-4.0 mg / l IBC - the length of the root system 30 days after the cultivation was 64-171 mm (Table. 1). Adding this amount of NAA stimulated intense callus formation in the basal part of the shoots. A similar problem with callus formation was observed in the studies by Doina Clapa with co-authors under applying 0.5 mg / l BAP [6].

In our studies, roots formation was observed under addition of NAA in amounts of 2 and 3 mg/l, but the first 3-5 mm long roots were noted on the 22-30th days with relatively slow development of the above-ground part of the regenerants.

Adding of 0.5 mg/l of NAA and 4.0 mg/l of IBA to the nutrient medium resulted in the intensive development of the root system almost without shoot formation (Fig. 1).

We believe that this feature of the root development can be used in the studies on obtaining a powerful Paulownia root system as a reproductive organ. Similar techniques are successfully used to get microtubers in potato reproduction. It gives a certain amount of time to accumulate vegetative material, to enter it into dormant state and, if necessary, to awaken. As we have previously established [12], the *in vitro* introduction of regenerants improves the post-septic adaptation of potato plants as well as the hosts.

Under simultaneous adding 2.0 mg/l of both auxins to the medium, bunches of short roots (20 and more) were formed. The shoot began to develop on the 20-30th days of cultivation. More than 80% of such regenerants died after transplantation into post-septic conditions.

It is important to obtain rooted and adapted plants for the full commercial use of microcollonal reproduction. It was established that for The comparison of investigated media with the addition of 4.0 mg / l IBC revealed that QL medium was the optimal one (Table 2).

The regenerants formed a larger number of roots on the BDS medium, but they yielded to QL in length. In our opinion, one of the reasons for the better development of the regenerants in the QL medium is the high content of potassium and nitrogen in the KNO_3form.

We have compared the development of regenerants for their cultivation in two illuminated rooms with temperatures of +24 and +32°C. Rhizogenesis began on the 11th days of cultivation at higher temperatures, whereas at the temperature of 24°C the first roots formed on the 14th day. Also, the root system had visually had a larger number of branches and root hairs.

TABLE 1. Auxins impact on Paulownia rhizogenesis *in vitro 30 days after cultivating**

Concentration, mg/l	Control**		Naphtylacetic acid		Indole butyric acid	
	roots length, mm	roots number, ps.	roots length, mm	roots number, ps.	roots length, mm	roots number, ps.
0.1			60	2.0	59	1.9
0.5			15	2.3	71	2.1
1.0			14	2.5	70	2.9
2.0	59	1.8	3	22	82	3.2
3.0			5	30	164	3.2
4.0			-	-	171	6.3
5.0			-	-	33	16.5

Note: * Murashige and Skoog medium; ** no auxins added on the control.

Fig. 1. Features of Paulownia shoots and root *in vitro* development under adding 4.0 mg/l IBA and 0.5 mg/l NAA to the medium.

TABLE 2. Rhizogenesis of Paulownia regenerants on different nutrient media for 30 days of cultivation

Rhizogenesis indices	WPM	MS	QL	BDS
roots length, mm	111	174	186	64
roots number, ps.	3.7	6.3	9.2	13.0

With further improvement, we selected the optimal concentrations of activated carbon (Table 3). Since we have achieved rhizogenesis in a period of less than two weeks, the records in this comparison were taken on the 15th day of cultivation. The optimal amount of activated carbon in the nutrient medium was 2.0-2.5 mg/l for the compared concentrations. Variants with a lower concentration yielded both to the root formation beginning and to the roots length and number. An increase in concentration up to 3.0 mg/l resulted in the signs of phytotoxic influence on the general condition of the regenerants, including the shoot height, growth rate and root formation.

Adding $AgNO_3$ to the nutrient medium revealed its positive effect on the regenerants. Visually the plants had larger and intensely colored leaves, the stems were did not extracted in length. Also, the roots of the second order increased in size.

In order to select the optimal age of *in vitro* plants for planting in a greenhouse on a peat substrate, the effectiveness of the use of

TABLE 3. Activated carbon impact on Paulownia *in vitro* rhizogenesis on the 15th day of cultivation

Rhizogenesis indices	Concentration, g/l						
	0 (control)	0.5	1.0	1.5	2.0	2.5	3.0
Root formation beginning	11	11	10	10	6	6	14
roots length, mm	6.1	6.4	6.6	6.9	8.3	8.6	4.1
roots number, ps.	147	146	149	168	173	166	112

TABLE 4. Features of Paulownia plants post-septic adaptation depending on the age

Adaptation indices		Age, days			
		15	20	30	40
Establishment, %		75	77	73	52
Plants height in a greenhouse, mm	15th day of the experiment	157	161	198	146
	30th day of the experiment	340	342	361	219

TABLE 5. E.ffect of planting depth on the number of living plants

in vitro planting depth, mm	Live plants for observation date, %		
	5 day	10 day	15 day
0 mm (on the substrate surface)	50	32	30
2-3 mm (control)	83	79	77
5-6	80	57	41
8-10	71	54	33

Fig. 2. Changes in Paulownia plants in vitro (right) after 15 days of adaptation in the greenhouse (left).

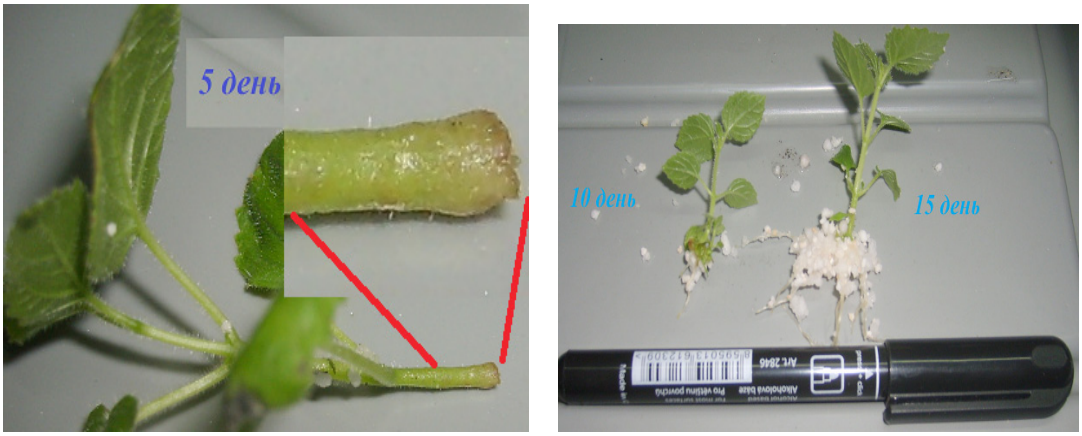

Fig. 3. Paulownia *in vitro* rhizogenesis (the first post-septic grafting).

Fig. 4. Paulownia grafts rooting on the 6th day for the second post-septic grafting.

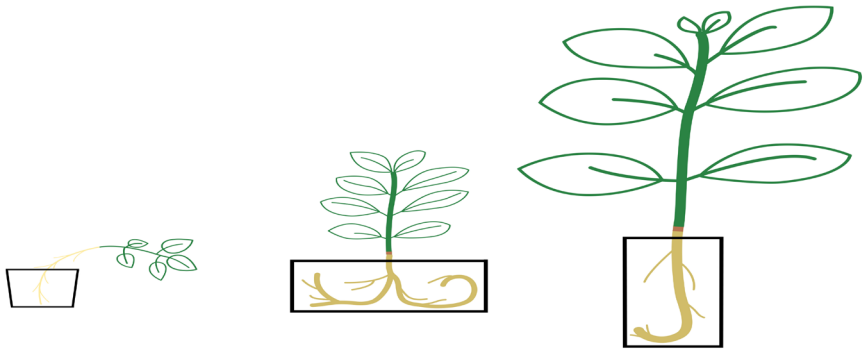

Fig. 5. Difference between plants depending on the shape and volume of the cell / pot: a - 0.1 l, b - 0.5 l shallow, b - 0.5 l deep.

TABLE 6. Influence of cassette cell shape and size on Paulownia plants survivability and morphometric indices

Index	Volume and form		
	cassette cell 0.1 l	0.5 l pot	0.5 l pot deep
Establishment, %	64	5	98
Plants height, mm	19	168	341
Leaflet plate size, mm	26	45	78
Stem diameter in the radical area, mm	0.8	2.7	4.6

regenerants of the following ages was compared: 15 days; 20 days; 30 days; 40 days (Table 4).

It was established that the vitality did not differ significantly in 15, 20, 30 days variants and remained within 73-77%. In older regenerates (40 days), half of the *in vitro* planted plants established (52%). A break in the root collar area of the shoots was often observed in such regenerants. In most plants, the root system formed as early as *in vitro* almost did not developed. Instead, a slow laying of new roots in the substrate was observed, which was one of the reasons for older regenerants growth delay. The first three variants also did not differ in height. Therefore, you can use 15-day regenerants and thus save the resources (Figure 2).

The negative influence of the residual agar medium on the root system is established. For example, the establishment of the regenerants planted without washing the medium from 77% to 31%. Its organic remains showed a toxic effect on plants. In our opinion, they may be fermentation products. In re-planting the plants previously washed on the substrate, where non-washed plants died, 40-50% of new plants died. Since agar washing in large industrial volumes requires considerable expenses of time and labor, we have tested a non-agar medium, with the agar replaced with vermiculite at the stage of rhizogenesis. The research results reveal no difference in establishment of plants grown on the agar medium and washed away from it. Also, the number of injured plants decreased.

The rooting and establishment of the regenerants was also influenced by planting depth (Table 5). It was established that the largest number- 77-83% - of live plants was observed for the depths of 2-3 mm. An in-depth plantinfg negatively affected the establishment. For this planting, anaerobic conditions, favorable for the pathogenic microflora, were created. The lowest rates - 30-50% - were observed in the variant of planting on the substrate surface.

Rapid growth rates of both the shoots and the root system are typical for the post-septic adaptation of the Paulownia, as well as for natural conditions (Fig. 3). If the influx formation is noted in the basal part on the fifth day, several roots have already formed on the 10th day.

In two weeks, the regenerants are suitable for their transplanting in larger containers or in open soil, provided drip irrigation and mulching.

Paulownia plants *in vitro* juvenilization is inherited for several generations under post-septic grafting. After planting the regenerants grown “on agar”, they can be re-grafted 2 or 3 times (Fig. 4). After 4-5 graftings, juvenility and therefore the regenerative properties including and the formation of adventitious roots are lost.

There has been established the influence on rhizogenesis and morphometric parameters of size and shape of cassette cell / pot used for plants adaptation (Fig. 5).

Thus, planting plants in small cells volumed 0.1 l negatively affected the rhizogenesis, and, as a consequence, their growth and vitality (Table 6). Plants formed a low, thin stem, the leaflet plate was poorly developed, the establishment was 64%. There was a dropout (bumping) of plants from the cassette cells.

Planting in deep pots contributed to a well-developed root system formation, the plants establishment was the best - 98%. The plants formed a thickened stem with three internodes (on average) and well-developed leaf plates. Plants in shallow pots of the same volume, yielded for their morphometric indices and establishment. According to our observations, in contrast to the previous version (with deep pots), these plants had a larger number of truncated internodes (5 pcs.), their stem stiffening and the auxiliary buds awakening took shorter time. Hereby, it can be assumed on the induction of the synthesis of

abscisic acid under adverse conditions (the root system can not grow deep) for the development of the root system, since under natural conditions, Paulownia forms the root system of 6-9 m depth.

Conclusions

It was established that in order to improve the technological process at the stages of rhizogenesis induction and post-septic adaptation of the Paulownia it is necessary:

1. To use 3.0-4.0 mg / l IBA as rhizogenesis inducer.
2. In commercial use, the use of the QL medium, cultivation in a light room at + 32°C, adding activated carbon in the amount of 2.0-2.5 mg /l and $AgNO_3$ (3-5 mg/l) are optimal.
3. To use vermiculite in a nutrient medium instead of agar at the stage of rhizogenesis.
4. To use deep pots of 0.5 l at the stage of planting in a greenhouse on a peat substrate, plant 15-day old regenerants to a depth of 2-3 mm. An in-depth landing negatively affected the plants establishment.

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