Scientific Bulletin. Series F. Biotechnologies, Vol. XXIV, No. 1, 2020
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

BIOTECHNOLOGICAL APPROACHES TO *Paulownia IN VITRO* PROPAGATION AND *IN VIVO* ADAPTATION

Anastasya FOKINA[1], Tetiana SATAROVA[1, 2], Kateryna DENYSIUK[2], Mykola KHARYTONOV[3], Mykhaylo BABENKO[3], Iryna RULA[3]

[1]Ukrainian State University of Chemistry and Technology, Gagarin av. 5, 49008, Dnipro, Ukraine
[2]Institute of Grain Crops of NAASU, V. Vernadskyst., 14, 49027, Dnipro, Ukraine
[3]Dnipro State Agrarian and Economic University, S. Yefremova 25, 49600, Dnipro, Ukraine

Corresponding author email: kharytonov.m.m@dsau.dp.ua

Abstract

Different species and hybrids of Paulownia are valuable technical, bioenergetic, medicinal and decorative crops cultivated all over the world. We investigated in vitro microclonal propagation of hybrid Paulownia elongata × Paulownia fortunei via auxiliary buds activation. The phytohormone regulation of the growth and development of cuttings was studied on the base of modified MS medium with different concentrations of 6-benzylaminopurine with and without 0.2 mg/l 3-indole-acetic acid. The addition of 2.0 mg/l alone appeared to be optimal for new shoot formation. The best for rooting was ½MS supplemented with 1.0 mg/l indole-3-butyric acid. The mass loss of wood at the stage of cellulose decomposition was ranged from 57.3 to 63.9% in two versions of soil profiles containing water retention layer. The initial results allow estimating P. elongata × P. fortunei as a plant which demonstrated stable adaption potential as bioenergy tree.

Key words*: adaptation, biochar, microclones, phytohormones, rhizogenesis.*

INTRODUCTION

The biomass of woody plants, which is predominantly wood, can provide alternative energy raw materials and simultaneously reduce the emissions of toxic gases that accompany the combustion of hydrocarbons (Lemus and Lal, 2005). *Paulownia* is an important energy, decorative, landscaping plant that is becoming more widespread in the world as well as in Ukraine. It is a fast-growing woody energy plant, which makes it possible to create high-productive plantations with a long service life. Last decade *Paulownia* tree has been considered as "magic" because of its fast growth rate and the high amount of the wood generated in a short time period. Each *Paulownia* tree aged 5-7 years old can generate 1 m^3 timber in the plantation with density of 2000 plants/ha, offering a total production of 330 t/ha. In the areas planted with a smaller number of plants *per* area unit can reach a production of 150 t/ha (Icka et al., 2016). However, the investigations of the possibility of *Paulownia* cultivation on different types of soil and in specific climatic conditions to preserve its properties as a bioenergy crop remains relevant. *Paulownia* is actively investigated as a medicinal plant for humans (Zhang et al., 2019; Wang et al., 2019) and animals (Yang et al., 2019). Molecular and genetic characteristics of this genus are being intensively studied in connection with problems of taxonomy, phylogeny and systematics (Li et al., 2020), the nature of gene expression and its regulation by biotic and abiotic factors (Fan et al., 2015; Wang et al., 2019). At the present stage of production this woody, vegetative propagating crop needs a sufficient amount of high quality planting materials. The technology of the plant microclonal propagation via the activation of axillary buds *in vitro* allows obtaining quickly the required number of cuttings of a particular genotype. In general, this technology consists of three main steps. The first one is the sterilization of plant material and its introduction into culture *in vitro*. The second one is the sterile multiplication, most often via activation of axillary buds with the formation of newly formed shoots and their following 5-7-fold cutting. The third step is the rooting of the obtained microclones *in vitro*. The next technological operation is the transplantation of rooted microclones into the soil, their adaptation and *in vivo* cultivation according to the

requirements of a crop (Cherevchenko et al., 2008). For many species of *Paulownia*, the choice of the type of microclonal propagation and the optimization of all its stages is an urgent task. The solution of this task is in the plane of the use of phytohormones and other biologically active substances both in the nutrient media at the stages of cutting and rooting *in vitro* as well as at the stage of regenerated plantlets adaptation in the soil.

For species *P. tomentosa*, *P. elongata*, a hybrid *P. tomentosa* × *P. fortunei*, there is contradictory information regarding the efficiency of representatives of main classes of phytohormones such as auxins, cytokinins and their combinations in microclonal propagation *in vitro* (Bahri and Bettaieb, 2013; Rahman et al., 2013; Pozoga et al., 2019). The study of other biologically active substances which mechanism differs from classical phytohormonal regulation is especially interesting at the stage of rooting and plantlets adaptation in the soil. Different techniques and methods were used for this purpose. Thus, positive effect of bacteria *Bacillus meganterium* ONU 500 on adaptation of *P. tomentosa* microclones in soil after *in vitro* conditions was shown (Tesliuk and Avramovich, 2019). For intensification of *in vitro* rooting *P. tomentosa* × *P. elongata*, vermiculite instead of agar was used and the addition of 2.0-2.5 g/l activated charcoal was effective (Filipova et al., 2019). In this regard the use of biochar as an admixture to a nutrient medium or substrate for *in vivo* plant growth and development is of significant interest and was not previously been studied for microclonal propagation of *Paulownia*. Biochar (biocoal) is a substance derived from biomass carbonation. It can be used to bind carbon from the atmosphere (Mulabagala et al., 2015). Biochar can be added to soils to improve substrate function. There is growing interest in the use of biochar to mitigate the effects of global warming and increase plant bioproductivity (Wang et al., 2012). It has been established that biochar can accelerate plant growth by improving the chemical, physical and biological properties of soil (Glaser et al., 2002; Lehmann and Rondon, 2006). The positive effect of biochar is related to neutralization of pH of acidic soils and improving their physical properties - the ability to retain water, preserve nutrients, and reduce nutrient loss under leaching (Lehmann et al., 2003; Lehmann, 2007). The negative impact of biochar on plant bioproductivity was recorded at a deficiency of microelements distorted by high pH values of biochar (Mikan and Abrams, 1995). Differences in the plant response on biochar depend on the properties of a sample, soil type, plant species (Chan et. al., 2007; Rondon et. al., 2007; Majeed et al., 2018).

Thus, the study of the peculiarities of the influence of biologically active substances on the efficiency of microclonal propagation by activation of auxiliary buds for industrial cultivars of *Paulownia* is actual. In this regard, the purposes of this work were to investigate the effect of phytohormones of the class of auxins and cytokinins as well as their combinations at the cutting stage and the effect of biochar at the rooting stage for optimization of *Paulownia* microclonal propagation *in vitro*. We also aimed to investigate the special bioenergy properties of *Paulownia* microclones after cultivation *in vivo* on different types of soil substrate.

MATERIALS AND METHODS

Hybrid *Paulownia elongate* × *Paulownia fortunei*, the industrial cultivar, was used as the material of the investigations. The donor plants were grown in the vegetative vessels of 20 liters in volume in the substrate consisted of 9 black soil: 1 sand, moistened twice a week. The donor annual plant represented itself a tree of 1 m height with one erect main shoot, which housed non-lignified branches with opposite leaves. In the sinus of each leaf one auxiliary bud was situated. Cuttings of 1 cm length were isolated from donor plants, directly from young, non-lignified branches of 2-3 months old for further sterilization and explantation onto the nutrient medium. Leaves were removed from the selected cuttings before explantation to eliminate superfluous, undesirable contamination. Thus, the cuttings included a region of a stem and two buds located into the nodes. These cuttings were sterilized in a saturated solution of calcium hypochlorite for 10 minutes and washed five times with sterile distilled water. Sterile cuttings were implanted on a nutrient medium for induction of shoot development via activation of axillary buds.

This medium contained macro-, microcomponents MS (Murashige and Skoog, 1962), 2.5 mg/l lysine, 30 g/l sucrose, 7 g/l agar but did not contained phytohormones. Cuttings were cultivated for 20 days at a temperature of 25°C, 16 h photoperiod and the light intensity of nearly 1500 luxes. After 20 days, as soon as the newly formed shoots had been developed from buds the first sterile cutting was made on the medium of the same composition - MS with 30 g/l sucrose, 7 g/l agar, without phytohormones. After 20 days in culture the newly formed shoots had grown, and these ones were cut ones more. Received cuttings consisted of a stem region of 1.0 cm in length and two opposite auxiliary buds with the residues of leaf scapes (Figure 1).

Figure 1. Cuttings of *P. elongate* × *P. fortunei* explanted on the nutrient media for microclonal propagation

They were used as the material in the first experiment on the effect of phytohormones on the effectiveness of the next (the second) cycle of sterile cutting. This experiment was carried out in the base of the control medium for cutting contained macro-, microcomponents and vitamins MS, 2.5 mg/l lysine, 30 g/l sucrose and 7 g/l agar. To study the potential of the multiplication, cytokinin 6-benzylaminopurine (BAP) in concentrations of 0.5, 1.0, 1.5, 2.0 and 2.5 mg/l was added to the control medium as well as the combinations of these concentrations of BAP with 0.2 mg/l auxin 3-indole-acetic acid (IAA) - Table 1.

Table 1. The content of phytohormones in the medium for cutting of *P. elongate* × *P. fortunei* as the scheme of experiment

Trial	IAA (mg/l)	BAP (mg/l)	Trial	IAA (mg/l)	BAP (mg/l)
1 (Control)	0.0	0.0	7	0.2	0.0
2		0.5	8		0.5
3		1.0	9		1.0
4		1.5	10		1.5
5		2.0	11		2.0
6		2.5	12		2.5

Note: IAA = 3-indole-acetic acid, BAP = 6-benzylaminopurine

Cuttings in the second sterile cycle were cultivated at a temperature of 25°C, 16-hour photoperiod and the light intensity of nearly 1500 luxes. In the experiment on the effect of phytohormones on the multiplication of *P. elongate* × *P. fortunei* shoots 50 cuttings were explanted *per* a variant. The analysis of the results was carried out on the 30th day of cultivation counting from the explantation of cuttings in the second sterile cutting cycle.

The effects of phytohormones on the growth and development of cuttings were estimated on the following traits:

- the shoot formation frequency, % - percentage ratio of the number of cuttings with at least 1 newly formed shoot to the total number of cuttings explanted;
- a number of newly formed shoots *per* 1 explanted cutting, pcs. - ratio of total number of newly formed shoots to total number of cuttings explanted;
- a number of internodes *per* 1 newly formed shoot, pcs. - the ratio of the total number of internodes on newly formed shoots to the total number of newly formed shoots;
- a number of internodes of newly formed shoots *per* 1 explanted cutting, pcs. - the ratio of the total number of internodes on the newly formed shoots to the total number of cuttings explanted. This trait is integral and conbines both the number of newly formed shoots *per* a cutting and the number of internodes *per* a newly formed shoot.
- the length of newly formed shoots, cm;
- the frequency of rooting,% - the percentage ratio of the number of rooted cuttings to the total number of cuttings explanted.

In the second experiment, the effect of biochar on root formation of *P. elongate* × *P. fortunei* cuttings *in vitro* was studied in the next, third cycle of sterile cutting. The control medium for

rhisogenesis contained reduced twice concentration of macro-, microcomponents and vitamins MS, 2.5 mg/l lysine, 20 g/l sucrose, 1.0 mg/l indole-3-butyric acid (IBA) and 7 g/l agar. The experimental medium included all the components of the control medium added with 5 g/l biochar. Based on sunflower husk biochar was obtained from the private company in Odessa city.

Cuttings in the third cycle were also cultivated at a temperature of 25°C, 16 hours photoperiod and the light intensity of nearly 1500 luxes. In the experiment on rhizogenesis of *P. elongate* × *P. fortunei* 100 cuttings were explanted *per* a variant. The analysis of the results was carried out on the 30th day of cultivation counting from the explantation on rooting media.

To evaluate the results of the second experiment the following traits were estimated:

- the frequency of rooting, % - the percentage ratio of the number of rooted cuttings to the total number of cuttings explanted;
- the length of the root system, cm;
-the density of the root system, points. Density assessment was performed on a point system in the range from 1 to 10 points: the most developed root system was evaluated at 10 points and the least developed - at 1 point;
-the length of newly formed shoots, cm.

The testing of *P. elongate* × *P. fortunei* adaptation to six artificial soil profiles was carried out at Pokrov land reclamation station of Dnipro State Agrarian and Economic University, which is situated in Dnipropetrovsk region, south-east of Ukraine. The following models of technogenic edaphotops were used to test the growth of *P. elongate* × *P. fortunei* plants as following:

1. 100 cm of loamy like loam and red brown clay mix (LLL+RBC);
2. 40 cm black soil + 60 cm sand (BS+Sand);
3. 40 cm black soil + 60 cm gray-green clay (BS+GGC);
4. 100 cm red-brown clay (RBC);
5. 100 cm black soil (BS);
6. 40 cm black soil +10 cm sand + 50 cm red-brown clay (BS+Sand+RBC).

A comparative thermogravimetric analysis of branches of one year age trees of *Paulownia elongate* × *Paulownia fortunei* grown on different artificial soil profiles was carried out to obtain the wood thermal stability information. The analysis was performed using the derivatograph Q-1500D of the "F. Paulik-J. Paulik-L.Erdey" system (Kharytonov et al., 2017). Differential mass loss and heating effects were recorded. The results of the measurements were processed with the software package supplied with the device. Samples of annual wood were analyzed dynamically at a heating rate of 10°C/min in air atmosphere. The mass of each sample was 100 mg. Aluminum oxide was used as a reference substance.

The reliability of all the results was evaluated at a significance level of 0.05 using the Student's test.

RESULTS AND DISCUSSIONS

The stage of sterile cutting needs the optimization of the processes that ensure the activation of auxiliary buds of *P. elongate* × *P. fortunei*, the formation of as many as possible shoots from axillary buds of an explant and internodes on newly formed shoots to increase the rate of multiplication, rapid growth of new shoots in length and reduction or absence of untimely, spontaneous rooting. The shoot formation frequency of *P. elongate* × *P. fortunei* on all investigated nutrient media for cutting was 100%, so for all cuttings explanted the activation of at least one axillary bud of each cutting had taken place (Figure 2).

Figure 2. *P. elongate* × *P. fortunei* newly formed shoots after sterile cutting and 30 days cultivation *in vitro*

The number of newly formed shoots *per* 1 explanted cutting for IAA-free media averaged 1.71 pc., but under 0.2 mg/l IAA it tended to decrease and amounted to 1.57 pcs. (Table 2).

Table 2.The influence of phytohormone composition of the nutrient medium for cutting *P. elongate* × *P. fortunei* on the development of newly formed shoots

Phytohormones in medium for cutting	Number of newly formed shoots *per* 1 explanted cutting, pcs.	Number of internodes *per* 1 newly formed shoot, pcs.
Control (hormone free)	1.3 ± 0.2	3.3 ± 0.3
0.5 mg/l BAP	1.6 ± 0.2	3.2 ± 0.4
1.0 mg/l BAP	1.6 ± 0.2	3.6 ± 0.4
1.5 mg/l BAP	1.9 ± 0.2	3.5 ± 0.4
2.0 mg/l BAP	2.0 ± 0.2	3.5 ± 0.3
2.5 mg/l BAP	1.9 ± 0.2	3.1 ± 0.3
0.2 mg/l IAA	1.02 ± 0.04	3.5 ± 0.3
0.2 mg/l IAA + 0.5 mg/l BAP	1.7 ± 0.3	2.4 ± 0.3
0.2 mg/l IAA + 1.0 mg/l BAP	1.7±0.2	2.7 ± 0.3
0.2 mg/l IAA + 1.5 mg/l BAP	1.6±0.2	2.9 ± 0.3
0.2 mg/l IAA + 2.0 mg/l BAP	1.8±0.2	3.2 ± 0.2
0.2 mg/l IAA + 2.5 mg/l BAP	1.6±0.2	3.1±0.2

The highest quantitative value of the trait in this experiment was observed under the influence of 2.0 mg/l BAP, which significantly differed from the control and the medium with 0.2 mg/l IAA and tended to exceed the values of the other variants. The number of internodes *per* 1 newly formed shoot of *P. elongate* × *P. fortunei* varied without auxin treatment from 3.1 to 3.6 pcs., on average 3.4 pcs., but under IAA it varied from 2.4 to 3.5 pcs., in average 3.0 pcs., that is, tended to decrease under the influence of auxin load. No significant differences for this trait were observed between media of on the non-auxin background. Otherwise, under 0.2 mg/l IAA the addition of low BAP amounts significantly reduced the number of internodes *per* 1 newly formed shoot. At the same time increased BAP contents ensured the development of the trait at a level that did not significantly differ from that on the BAP-free medium with 0.2 mg/l IAA. Therefore, the variant of the phytohormone composition of the medium for sterile cutting with 2.0 mg/l BAP was the most favourable both for a number of newly formed shoots *per* 1 explanted cutting as well as for a number of internodes *per* 1 newly formed shoot of *P. elongate* × *P. fortunei*.

The number of internodes of newly formed shoots *per* 1 explanted cutting (Figure 3) on the average on a non-auxin background was 5.73 units, and on the same variants of concentrations of BAP, but under 0.2 mg/l IAA showed a tendency to decrease and amounted to 4.53 pcs.

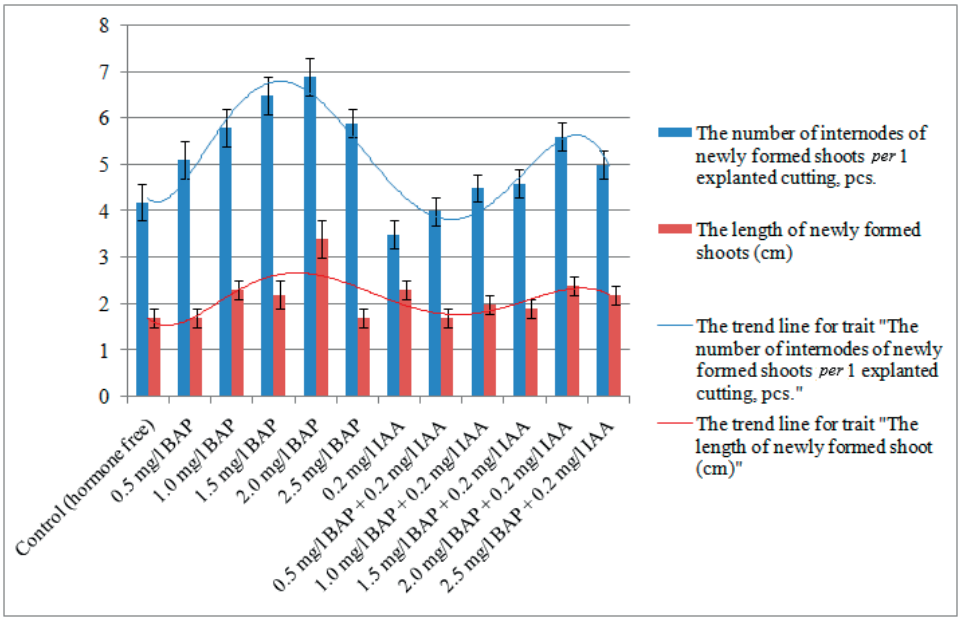

Figure 3. Effect of phytohormone composition of the medium for cutting on the number of internodes of newly formed shoots *per* 1 explanted cutting and the length of newly formed shoot of *P. elongate* × *P. fortunei in vitro*

On the non-auxin background, the highest value (6.9 pcs.) was provided with the addition of 2.0 mg/l BAP. However, comparing the values on MS + 2.0 mg/l BAP and MS + 0.2 mg/l IAA + 2.0 mg/l BAP shows that the addition of IAA reduces the positive effect of BAP on the number of internodes of newly formed shoots *per* explant. The best phytohormonal composition for sterile *P. elongate* × *P. fortunei* cutting when estimating the integral value of internodes number of newly formed shoots *per* 1 explanted cutting was MS + 2.0 mg/l BAP. Figure 3 clearly shows the trend line for this integral trait, which shows the tendency of the enhancement of this integral indicator according to the growth of BAP contents. There is a similar trend line for this trait under auxin background, which was formed with 2.0 mg/l IAA, but at a slightly lower level of values. Likewise, the trend line is positioned to indicate the length of newly formed shoots.

The length of new shoots of *P. elongate* × *P. fortunei* which were formed due to the activation of auxiliary buds *in vitro* varied on different media from 1.7 cm in control up to 3.4 cm for medium with 2.0 mg/l BAP.

There were significant differences in comparison with control for media with 1.0, 1.5 and 2.0 mg/l BAP (Figure 3). Variants with 0.5 or 2.5 mg/l BAP did not differ from control significally. The addition of 0.2 mg/l IAA in the medium for cutting contributed to a significant increase in the length of newly formed shoots compared to the control. The simultaneous application of 0.2 mg/l IAA and BAP did not significantly affect the shoot length compared to the control, but reflected a certain downward trend. The best option for the phytohormonal composition to increase the length of newly formed shoots of *P. elongate* × *P. fortunei* was 2.0 mg/l BAP in a medium for cutting. At the stage of sterile cutting of *P. elongate* × *P. fortunei in vitro* as well as in microclonal propagation of other plant species, it is important to avoid spontaneous rooting, as this can redistribute nutrients in a plantlet and reduce the yield of cuttings *per* an explant. As rooting at the stage of cutting is a rare event, the size of the samples used in our study was some insufficient and the differences between media were not very noticeable. However the observations show that the highest frequency of spontaneous root formation was observed for media without BAP - the control medium without phytohormones (66%) and the medium with 0.2 mg/l IAA (69%). Addition of BAP at the studied concentrations inhibited spontaneous root formation compared to control media to 12-30% under non-auxin background and to 6-53% under 0.2 mg/l IAA. Phytohormone composition in medium for cutting, which was the best in the analysis of the previous traits, 2.0 mg/l BAP, provided a significant reduction in spontaneous rooting to control and to all variants on auxin background, while did not differ significantly from the rest concentrations of BAP under non-auxin background.

Therefore, the given study revealed a clear pattern of the influence of BAP in the concentration range from 0.5 mg/l to 2.5 mg/l on the growth and development of *P. elongate* × *P. fortunei* cuttings *in vitro* both on non-auxin background and under the influence of auxin IAA at a concentration of 0.2 mg/l. This pattern means an increase of the positive effect of BAP in the range of concentrations from 0.5 mg/l to 2.0 mg/l and a tendency to inhibit the development of cuttings while increasing the concentration of BAP up to 2.5 mg/l. The positive effect of BAP on growth and development of *P. elongate* × *P. fortunei* cuttings is somewhat offset with the inclusion into the nutrient medium of auxin IAA, but nevertheless prevents a significant inhibitory effect of IAA. BAP also restrains spontaneous rooting of cuttings. Thus, it should be recommended to use 2.0 mg/l 6-benzylaminopurine at the stage of sterile cutting of *P. elongate* × *P. fortunei in vitro* to ensure maximum number of cuttings *per* an explant for the next cycle of sterile cutting and prevent unwanted spontaneous rooting.

It is somewhat difficult to compare the results of the effect on the microcutting of *Paulownia*, since the information available relates to different species, their hybrids and cultivars. Thus, in the investigation of Bahri and Bettaieb (2013) for *P. tomentosa* on 6-benzylaminopurine, indole-3-butyric acid and their combinations it was found that on MS medium with 30 g/l sucrose the best shoot multiplication was taken place at 1.0 mg/l IBA while rooting - at 0.5 mg/l IBA. Rahman et al. (2013) for the same species *P. tomentosa*

observed the most effective shoot multiplication on MS added with 30 g/l sucrose, 2.5 mg/l BAP, 0.5 mg/l naphthylacetic acid, but root formation - on ½ MS with 0.5 mg/l IBA. Ipekci and Gozukimizi (2003) in studies of somatic embryogenesis of *P. elongata* for the development of artificial seeds production technology have found that for MS + 30 g/l sucrose + 500 mg/l casein hydrolyzate thidiazuron (10 mg/l) was better than BAP, IAA, naphthylacetic acid, kinetin. For cuttings of hybrid *P. tomentosa* × *P. fortunei*. M. Pozoga et al. (2019) considered the most effective for microclonal propagation the supplementation of ½MS with 20 g/l sucrose, 0.5 mg/l BAP, whereas under 0.2 mg/l BAP the growth of plants became slow, and at 1.0 mg/l BAP callusogenesis was observed. For root formation on cuttings these authors recommended to add 1.0 mg/l IBA to ½MS + 20 g/l sucrose. In our study, the influence of phytohormones at the stage of sterile cutting of *P. elongata*×*P. fortunei* showed the most significant positive effect with the 2.0 mg/l BAP while the concentrations of BAP 0.0, 0.5, 1.0, 1.5, 2.5 mg/l both on the non-auxin background and under 0.2 mg/l IAA provided lower cutting efficiency and shorter length of shoots.

Such differences in the response of plant material to the same phytohormone concentrations can be explained by investigations of different species of *Paulownia* as well as basic nutrient media varied in the content of macro-, microelements, vitamins and sucrose. The certain contribution to the variation of results was made by different conditions of plant growth *in vitro*, in particular temperature and light regimes, especially in the investigation of Pozoga et al. (2019). This approach proves once again the need to test nutrient media for specific species or industrial cultivars of *P. elongate* × *P. fortunei* as well as for specific cultivation conditions.

In the following experiment, the effectiveness of biochar (5 g/l) in the medium for rhizogenesis of *P. elongate* × *P. fortunei* was studied in order to obtain the most developed root system of cuttings at the rooting stage *in vitro* for further transplantation into soil (Figures 4, Figure 5a and Figure 5b).

As it was mentioned in "materials and methods", the control medium for rhizogenesis contained reduced twice contents of macro-, microsalts and vitamins MS, 2.5 mg/l lysine, 20 g/l sucrose, 1.0 mg/l IBA and 7 g/l agar. The rooting frequency on both media was 100%. That is the development and rooting of all explanted cuttings occurred. As it was shown by the experiment, the addition of biochar at some rate inhibited the development of the root system of cuttings at the stage of rooting *in vitro*. Thus,under the influence of biochar the length of the root system decreased by 36.5% compared to control, its density decreased by 31.4%, while the length of the newly formed shoots remained at the level of control.

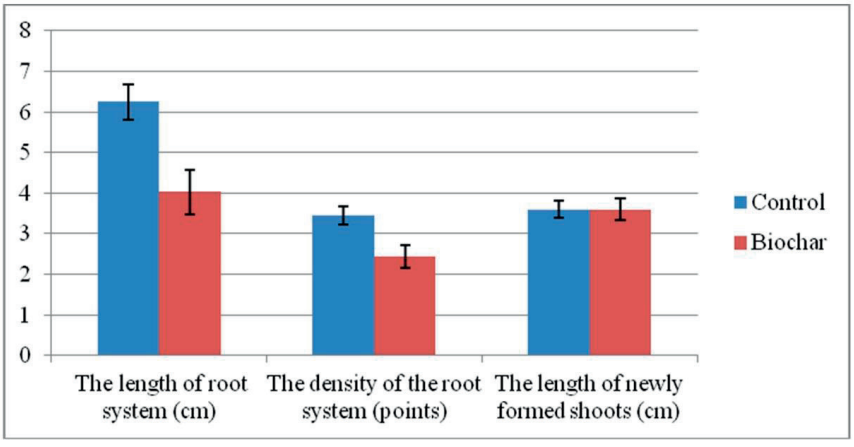

Figure 4. Effect of biochar (5 g/l) in the medium for rhizogenesis at the rooting of cuttings of *P. elongate* × *P. fortunei in vitro*

Figure 5a. *In vitro* formed plantlets of *P. elongate* × *P. fortunei* on control

Figure 5b. *In vitro* formed plantlets of *P. elongate* × *P. fortunei* on control + 5 g/l biochar media for rhizogenesis

In general, we performed the five-fold cutting of sterile *P. elongate* × *P. fortunei* plants in isolated culture. However, there was a progressive decrease in auxiliary buds activation, shoot growth and rooting frequency in generations, so the fourth and fifth cycles of cutting were conducted on MS medium supplemented with 30 g/l sucrose, 2.5 mg/l BAP, 4.0 mg/l GA_3, 0.5 mg/l IAA, 2.5 mg/l adenine and 0.05 mg/l kinetin. On this medium in the late cycles of microclonal propagation the shoot formation on *P. elongate* × *P. fortunei* cuttings occurred at a frequency of about 97%. The use of a nutrient medium with half the content of macro-, microelements and vitamins MS, 15 g/l sucrose, 1.0 mg/l BAP and 0.05 mg/l IAA allowed to root 100% of cuttings obtained *in vitro*.

After transferring to the soil regenerated plants were adapted well enough and grown first in 100 cm^3 soil cells and then in 1000 cm^3 soil vegetation vessels.

The cultivation of regenerated plants in the soil at models of technogenic edaphotops made it possible to evaluate the wood characteristics of there regenerant plants after cultivation. The thermolysis process of wood biomass of *P. elongate* × *P. fortunei* consisted of two phases (Figure 6 and Figure 7).

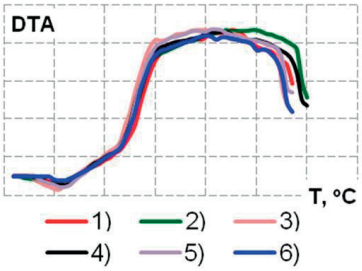

Figure 6. Difference Thermo Analysis of curves of the thermal decomposition of wood samples of *P. elongate* × *P. fortune:*
1 - LLL+RBC; 2 - BS+Sand; 3 - BS+GGC; 4 - RBC; 5 - BS; 6 - BS+Sand+RBC

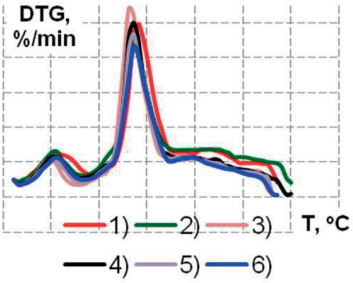

Figure 7. Differential termogravimetry graphic the total mass change % of *P. elongate* × *P. fortunei* wood samples total mass change, %:
1 - LLL+RBC; 2 - BS+Sand; 3 - BS+GGC; 4 - RBC; 5 - BS; 6 - BS+Sand+RBC

The first period included the processes of evaporation of water, volatile compounds and decomposition of the main components of the wood. The second phase, in turn, included the destruction of hemicelluloses and cellulose (stage 1), the decomposition of lignin and the formation of a non-combustible residue (stage 2). The first phase began in the temperature range of 50-60ºC and ended in the range of 160-190ºC. This process was characterized mainly by endothermic reactions. *P. elongate* × *P. fortunei* wood contained a small amount of volatile components. The process speed was also low. One peak was observed at a temperature of 100-110ºC. The highest rate of decomposition was observed at a temperature of

90ºC in the samples of the profile no. 3 (BS+GGC). The loss of mass was also the greatest - 6.1%. Decomposition of hemicelluloses and cellulose in profiles no. 1 (LLL+RBC) and no. 2 (BS+Sand) occurred in the temperature range 170-180°C ÷ 380-400°C. In other variants, this process took place in the region of lower temperatures 160ºC ÷ 350-370ºC. Exothermic reactions took place at this stage. Their destruction was delayed due to the specific composition of hemicelluloses in *Paulownia* wood. Therefore, the peak of hemicellulose decomposition is overlapped by the peak of cellulose decomposition (Figure 6). The mass loss at this stage was the greatest and ranged from 57.3% (profile no. 6, BS+Sand+RBC) to 63.9% (profile no. 2, BS+Sand) comparative to other experiment profiles. The lowest temperature (260°C) of the maximum mass loss rate was recorded for the profile no. 3 (BS+GGC). The last stage of thermolysis was characterized by the greatest exothermic effect in the range of 410-450°C. It was observed less pronounced in the region of lower temperatures (380-390°C) in the profile no. 3 (BS+GGC). The most pronounced and prolonged exothermic effect was in profile no. 2 (BS+Sand) (Figure 7). The rate of lignin decomposition was low, with no pronounced peaks. The mass loss ranged from 25.4% (profile no. 2, BS+Sand) to 31.3% (profile no. 6, BS+Sand+RBC). It should be noted that *Paulownia* wood has low ash content. The proportion of non-combustible residue did not exceed 5-7%. Thus, the results allow estimating *P. elongate* × *P. fortunei* as a plant which demonstrated stable adaption potential as bioenergy tree.

CONCLUSIONS

Microclonal propagation of *Paulownia elongate* × *Paulownia fortunei* at the stage of *in vitro* auxiliary buds activation and sterile cutting has been investigated to choose the optimal phytohormones combination among 6-benzylaminopurine concentrations in the range of 0-2.5 mg/l with or without 0.2 mg/l 3-indol-acetyc acid. The most effective one occurred the addition of 2.0 mg/l 6-benzylaminopurine into the medium MS with 2.5 mg/l lysine, 30 g/l sucrose and 7 g/l agar. This approach at the 30th day of cultivation *in vitro* allowed reaching 100% shoot formation frequency, in average 6.9 internodes of newly formed shoots *per* 1 explanted cutting and new shoots of 3.4 cm in length. At the stage of *in vitro* root formation of *Paulownia elongate* × *Paulownia fortunei* microclones the most suitable medium was ½MS supplemented with 2.5 mg/l lysine, 20 g/l sucrose, 1.0 mg/l indole-3-butyric acid and 7 g/l agar while the addition of 5 g/l biochar inhibited the root system development.

The thermal stability of the wood of plants grown on profile (40 cm black soil + 60 cm green gray clay) was slightly lower than in other versions of the experiment. The mass loss at the stage of cellulose decomposition was the greatest and ranged from 57.3 to 63.9% in two profiles with water retention layer comparative to other experiment trials.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Ministry of Education and Science of Ukraine.

REFERENCES

Bahri, N. B., & Bettaieb, T. (2013). *In vitro* propagation of a forest tree *Paulownia tomentosa* (Thumb.) Steud. A valuable medicinal tree species. *Albanian Journal of Agricultural Sciences*, 12(1), 37–42.

Chan, K. Y., Van Zwieten, L., Meszaros, I., Downie, A., & Joseph, S. (2007). Agronomic values of green waste biochar as a soil amendment. *Australian Journal of Soil Research,* 45(8), 629–634.

Cherevchenko, T. M., Lavrentyeva, A. N., Ivannikov, R. V. (2008). *Biotechnology of tropical and subtropical plants in vitro*. Kyiv: Naukova Dumka.

Fan, G., Cao, X., Niu, S., Deng, M., Zhao, Z., & Dong, Y. (2015). Transriptome, microRNA, and degradome analyses of the gene expression of *Paulownia* with phytoplasma. *BMC Genomics*, 16, 896. doi: 10.1186/s12864-015-2074-3.

Filipova, L. M., Matskevych, V. V., Karpuk, L. M., Stadnyk, A. P., Andriievsky, V. V., Vrublevsky, A. T., Krupa, N. M., & Pavlichenko, A. A. (2019). Features of rooting Paulownia *in vitro*. *Egyptian Journal of Chemistry.* 57–63. doi: 10.21608/EJCHEM.2019.18333.2127.

Glaser, B., Lehmann, J., & Zech, W. (2002). Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal. *Biology and Fertility of Soils*, 35, 219–230.

Icka, P., Damo, R., & Icka, E. (2016). *Paulownia tomentosa*, a fast growing timber. The annals of "Valahia" University of Targoviste, 6. doi: 10.1515/agr-2016-0003.

Ipekci, Z., & Gozukimizi, N. (2003). Direct somatic embryogenesis and synthetic seed production from *Paulownia elongata. Plant Cell Reports*, 22(1), 16–24. doi: 10.1007/ s00299-003-0650-5.

Kharytonov, M., Babenko, M., Martynova, N., Rula, I., Sbytna, M., & Fuchilo, Y. (2017). The poplar saplings survival in reclaimed mineland depending on clone and root treatment. *Agriculture and Forestry*, 63(4), 141–151. doi:10.17707/AgricultForest.63.4.16.

Lehmann, J. (2007). Bio-energy in the black. Concepts and Questions. *Frontiers in Ecology and the Environment*, 5(7), 381–387.

Lehmann, J., da Silva, Jr. J .P., Steiner, C., Nehls, T., Zech, W., & Glaser, B. (2003). Nutrient availability and leaching in an archaeological anthrosol and a ferralsol of the Central Amazon basin: fertilizer, manure and charcoal amendments. *Plant and Soil*, 249, 343–357.

Lehmann, J., Rondon, M. (2006). Biochar soil management on highly weathered soils in the humid tropics. In: N. Uphoff (Ed.), *Biological Approaches to Sustainable Soil Systems* (pp. 517–529). Boca Ration, London, New York: Taylor & Francis Group.

Lemus, R. W., & Lal, R. (2005). Bioenergy Crops and Carbon Sequestration. *Critical Reviews in Plant Sciences*, 24(1), 1–21.

Li, P., Lou, G., Cai, X., Zhang, B., Cheng, Y., & Wang, H. (2020). Comparison of the complete plastomes and the phylogenetic analysis of Paulownia species. *Scientific Reports*, 10(1), 2225. doi: 10.1038/s41598-020-59204.

Majeed, A. J., Dikici, H., & Demir, Ö. F. (2018). Effect of biochar and nitrogen applications on growth of corn (*Zea mays* L.) plants. *Turkish Journal of Agriculture - Food Science and Technology*, 6(3), 346–351, doi: 10.24925/turjaf.v6i3.346-351.1746.

Mikan, C. J., & Abrams, M. D. (1995). Altered forest composition and soil properties of historic charcoal hearths in southeastern Pennsylvania. *Canadian Journal of Forest Research*, 25, 687–6.

Mulabagala, V., Baaha, D. A., Nosa, O., Egiebor, N. O., & Chen, W.Y. (2015). Biochar from biomass: a strategy for carbon dioxide sequestration, soil amendment, power generation, and CO_2 utilization. *In Handbook of Climate Change Mitigation and Adaptation*, 1–31. doi: 10.1007/978-1-4614-6431-080-1.

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant*, 15(3), 473–497. doi: 10.1111/j.1399-3054.1962.tb08052.x.

Pozoga, M., Olewnicki, D., & Jablonska, L. (2019). *In vitro* propagation protocols and variable cost comparison in commercial production for *Paulownia tomentosa* × *Paulownia fortunei* hybrid as a renewable energy source. *Applied Sciences*, 9, 2272. doi: 10.3390/app9112272.

Rahman, M. A., Rahman, F., & Rahmantullah, M. (2013). *In vitro* regeneration of *Paulownia tomentosa* Steud. plants trough shoots in explants derived from selected mature trees, by studing the effect of different plant growth regulators. *American-Eurasian Journal of Sustainable Agriculture*, 7(4), 259–268.

Rondon, M. A., Lehmann, J., Ramirez, J., & Hurtado, M. (2007). Biological nitrogen fixation by common beans (*Phaseolus vulgaris* L.) increases with biocharadditions. *Biology and Fertility of Soils*, 43, 699–708.

Tesliuk, N. I., & Avramovych, N. I. (2019). Improving of adaptation methods of *Pawlownia tomentosa* microclones to conditions *in vivo* with use of bacteria of *Bacillus megatrium* ONU50. *Microbiology & Biotechnology*, 3, 92–102. doi:10.18524/2307-4663.2019.3(47).182814.

Wang, J., Wang, H., Deng, T., Liu, Z., & Wang, X. (2019). Time-coursed transcriptome analysis identifies key expressional regulation in growth cessation and dormancy induced by short days in Paulownia. *Scientific Reports*, 9(1), 16602. doi: 10.1038/s41598-019-53283-2.

Wang, J. Y., Pan, X. J., Liu, Y. L., Zhang, X. L., & Xiong, Z. Q. (2012). Effects of biochar amendment in two soils on greenhouse gas emissions and crop production. *Plant Soil*, 360, 287–298.

Wang, Y. A., Guo, X., Jia, X. H., Xue, J., Du, H. F., Du, C. L., Tang, W. Z., Wang, X. J., & Zhao, Y. X. (2019). Undescribed C-geranyl flavonoids isolated from the fruit peel of *Paulownia catalpifolia* T. Gong ex D. Y. Hong with their protection on human umbilical vein endothelial cells injury induced by hydrogen peroxide. *Phytochemistry*, 158, 126–134. doi: 10.1016/j.phytochem.2018.11.010.

Yang, H., Zhang, P., Xu, X., Chen, X., Liu, Q., & Jiang, C. (2019). The enhanced immunological activity *of Paulownia tomentosa* flower polysaccharide on Newcastle disease vaccine in chicken. *Bioscience Reports*, 39(5), BSR20190224. doi: 10.1042/BSR20190224.

Zhang, J. K., Li, M., Du, K., Jinjin, Lv., Zhang, Z. G., Zheng, X. K., & Feng, W. S. (2019). Four C-geranyl flavonoids from the flowers of *Paulownia fortunei* and their anti-inflammatory activity. *Natural Product Research*. doi: 10.1080/14786419.2018.1556263.