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CLONAL MICROPROPAGATION OF PAULOWNIA *IN VITRO*

MANUSHKINA T.M. – Candydate of Agricultural Sciences, Associate Professor
orcid.org/0000-0001-5843-271X
Mykolaiv National Agrarian University
KOVALENKO O.A. – Doctor of Agricultural Sciences, Associate Professor
orcid.org/0000-0002-2724-3614
Mykolaiv National Agrarian University
KHOMUT V.P. – Director
orcid.org/0009-0002-4448-3019
FE "Agrolife" of the Mykolaiv region
KOLOMIIETS N.P. – Student of Higher Education
orcid.org/0009-0000-8462-4950
Mykolayiv National Agrarian University

Formulation of the problem. In modern conditions, Paulownia is gaining popularity, the prospects of which are considered in several aspects, namely as a valuable wood, bioenergy and soil protection crops [1; 2]. Another promising area of use of paulownia is its cultivation as an ornamental plant in various landscaping and urban landscaping. It is well suited for creating alley plantations and as a tapeworm. The plant has large leaves that give a lot of shade, absorb xenobiotics and emit a lot of oxygen, and in the spring the trees bloom very well [3].

An important stage in the introduction of plants is the provision of quality planting material and the study of adaptive capabilities in specific environmental conditions. The most effective method of vegetative propagation is clonal micropropagation in vitro, which provides a high reproduction rate, genetic identity of the source material and recovery of plants from pathogens. Some aspects of clonal micropropagation of Paulownia have been studied in [4–11], but it is necessary to optimize some techniques in connection with the influence of genotype and level of processability. No scientific publications have been identified on the cultivation of Paulownia in the Southern Steppe of Ukraine, so research on the adaptive capacity of the plant in specific climatic and environmental conditions is relevant.

Analysis of recent research and publications. Clonal micropropagation of paulownia is relevant, due to the advantages of the method, such as a high reproduction rate, genetic stability, and improvement of planting material. In work [4], buds and one-year shoots from paulownia plants growing in the field were used to introduce plant material into in vitro culture. The selection was carried out in the winter-spring period. To activate the buds, the shoots were cut into segments with three buds each, washed with running water with household soap and soaked in a 2% solution of quinosol. After that, the shoots were kept in a 0.2% solution of IAA and put for germination at a temperature of 25–30°C. After 7–20 days, material was taken from the shoots for further work [4]. A similar approach was used in the studies presented in works [2, 5–7], but it was noted that the optimal period for the selection of plant material is December-January, but it is also possible to select in August-October, while the buds must be treated with gibberellic acid to wake up. According to the results of research [8], it is shown that the best for the height of regenerants and the number of microshoots in the conglomerate is a weakly acidic environment with a pH of 5.6–5.8.

The best conditions for the growth of regenerants were found when combining kinetin 0.8 mg/l + BAP 2.0 mg/l. It has been shown that for the rapid accumulation of a significant number of plants by direct morphogenesis, it is possible to use one of the ways of grafting - division of a conglomerate of microshoots or single-node cuttings [9].

In [10] it was shown that QL nutrient medium with the addition of 4.0 mg/l NAA is optimal for rhizogenesis in vitro. B. A. Bergman, H. K. Moon [11] showed that the variability of paulownia clones even within the same genotype is significant, which determines the need to adjust the breeding technology. In this regard, it is relevant to study the morphogenesis of paulownia *in vitro* in order to optimize the stages of clonal micropropagation.

The purpose of the article – to develop technological methods of clonal micropropagation of paulownia *in vitro*.

Research materials and methods. Paulownia plants *Paulownia* Sieb. et Zucc. were the material for the research.

Paulownia Clone in Vitro 112® is an artificially bred and cloned tree that is able to survive and thrive in extreme conditions (from -25–27 to +45°C). Registered in 2007 at the Institute of Plant Species (official body of the EU). It has international recognition, a European passport, a European quality certificate and an international trade permit. Listed in the Register of plant varieties recommended for cultivation in Ukraine.

Paulownia 9501 is a hybrid between *P. tomentosa* and *P. fortunei*. Climate zone 7 (-17.5°C to -12.5°C). Propagated vegetatively and by seed. It is characterized by cold resistance and rapid growth, straight stem and narrow shape of the tree crown.

Place of research. The research was conducted in the conditions of FE "Agrolife" and of the Department of Agriculture, Geodesy and Land Management of Mykolayiv National Agrarian University, during 2018-2023.

Clonal micropropagation. In the course of research, generally accepted methods in plant biotechnology were used [12; 13]. Aseptic work was performed in the laminar box KPG-1. Murashige and Skuga (MS) were used as the basic nutrient medium for the cultivation of isolated buds and micropods [14].

Mathematical processing of research results was carried out according to the method [15].

Research results. The method of clonal micropropagation based on the culture of isolated apical meristems provides a high reproduction rate, maximum genetic stability and recovery of the resulting regenerating plants.

Sterilization of plant material. To select paulownia explants from mother plants, annual branches were cut and germinated in water, and the buds were treated with gibberellic acid GK3. Young shoots formed during germination were treated with a soap solution, then washed under running water and distilled water. Axillary buds from germinated shoots were used as explants. Sterility and viability of explants were determined on the 10th day of cultivation. Stepwise sterilization using 70% ethanol solution and 1% sodium hypochlorite solution proved to be the most effective, which ensured the yield of sterile explants at the level of 92.5–95.0%. The viability of the kidneys was 90.0–97.5%.

In vitro culture and initiation of isolated kidney development. To initiate the development of isolated paulownia buds used nutrient medium MS. The basic nutrient medium was supplemented with cytokinins or combined with GA. The frequency of regeneration of isolated kidneys was quite high in all variants of the nutrient medium – from 75 to 100%. However, microsaliva formed on nutrient media with different hormone composition differed in biometric parameters, which ultimately affected the reproduction rate. The multiplication factor was calculated as the sum of single-node cuttings of the main shoot and additional shoots that can be used for subculturing. This parameter collectively shows the effectiveness of the selection of cultivation factors at the stage of introduction of explant in in vitro culture. The highest reproduction rate in Paulownia was observed on nutrient medium supplemented with kinetin 1.0 ml + 0.5 ml GA, in Clone in Vitro 112 – 8.7, in hybrid 9501 – 9.2.

Own micropropagation. At the stage of proper micropropagation, the main task is the proliferation of shoots in vitro, which for several cycles can be subcultured to nutrient media for further production of new shoots from its axillary buds or adventitious shoots. In the second stage, to obtain explants, the main shoot of microplants was divided into single-node micro-cuttings 4–6 mm long or separated additional shoots. 6 microlivers were cultured in 250 ml vessels with a nutrient medium volume of 30 ml. The dependence of the growth and development of paulownia microplants in in vitro culture at the second stage of clonal micropropagation on the composition of the nutrient medium was established (Table 1). The frequency of regeneration of shoots at this stage was high in all tested variants of the nutrient medium and was 85.0–100.0%. The peculiarity of the development was the formation of additional shoots that formed a beam with a height of 38.3–85.6 mm, depending on the composition of the nutrient medium (Fig. 1).

The reproduction rate in the second stage of clonal micropropagation was calculated similarly to the first stage. The highest reproduction rate was found on nutrient medium supplemented with kinetin and GA – in Clone in Vitro 112 – 10.3, in hybrid 9501 – 12.0.

Fig. 1. Paulownia microplants at the stage of proper micropropagation

Rooting of micro shoots in vitro. To form the roots, the shoots were separated and planted on a nutrient medium 1/2 MS, supplemented with auxins (Fig. 2).

The study of the influence of the hormonal composition of the nutrient medium on the rooting of paulownia micropods in in vitro culture showed that the genotypes

Table 1

Development of paulownia microplants in in vitro culture in the second stage of clonal micropropagation (30 days of cultivation)

Concentration of hormones, mg / l	Frequency of regeneration, %	Height of shoots, mm	Reproduction rate
Clone in Vitro 112®			
BAP 0,5	95,0±5,0	38,3±3,8	4,7±0,4
BAP1,0	90,0±10,0	42,8±4,1	6,0±0,5
BAP 1,0+ GA 0,5	95,0±5,0	57,4±5,5	8,0±0,7
Kinetin 0,5	100,0±0,0	45,8±4,1	6,2±0,6
Kinetin 1,0	95,0±5,0	58,9±6,2	7,2±0,6
Kinetin 1,0+ GA 0,5	100,0±0,0	75,3±7,1	10,3±1,1
9501			
BAP 0,5	95,0±5,0	48,0±4,5	6,5±0,5
BAP 1,0	85,0±10,0	50,2±5,1	8,8±0,8
BAP 1,0+ GA 0,5	90,0±10,0	61,9±6,3	9,8±0,8
Kinetin 0,5	95,0±5,0	48,9±4,5	6,0±0,5
Kinetin 1,0	100,0±10,0	62,4±6,0	8,5±0,7
Kinetin 1,0+ GA 0,5	95,0±5,0	85,6±8,6	12,0±1,1

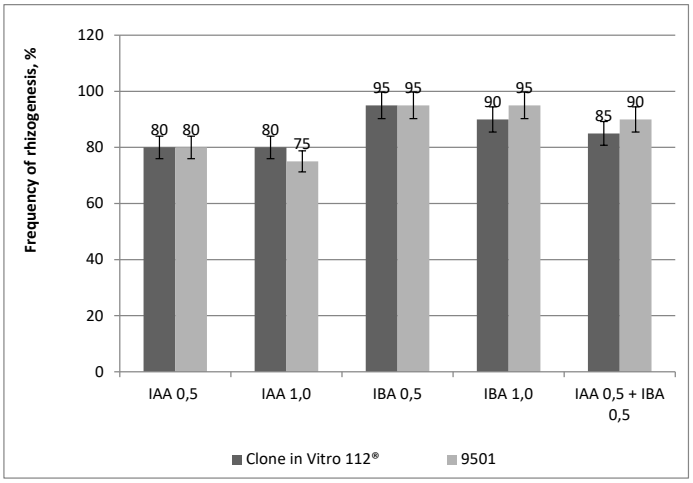

Fig. 2. Frequency of rhizogenesis in Paulownia microplants depending on the composition and concentration (mg / l) of auxins,% (30 days of cultivation)

Fig. 3. Primary adaptation of Paulownia to in vivo conditions

studied were characterized by a high frequency of root regeneration – 75.0–95.0%. However, the most optimal at this stage was a nutrient medium of ½ MS, supplemented with a IBA of 0.5 mg/l, in which the frequency of rhizogenesis was 95.0% and formed 5.3–7.8 roots with a length of 78.5–95.2 mm.

Adaptation of microplants to in vivo conditions. For the stage of adaptation to in vivo conditions, plants with a well-developed root system were selected, washed from the remnants of the nutrient medium and treated with biological products with fungicidal activity. Planted in cassettes with a substrate consisting of peat and perlite in a ratio of 3:1. Adaptation was carried out in stages. During the initial adaptation, plant cassettes were placed in climatic chambers (Fig. 3).

2-3 pairs of leaves were formed in 20 days. During the period of secondary adaptation, plant cassettes were moved to specially equipped tables with artificial fog and climate control systems and plants were cultivated in a climate chamber for another 20 days, after which they were grown in a film greenhouse. Gradual adaptation of plants to in vivo conditions for 40 days in climatic chambers and growing in a greenhouse provided the yield of seedlings at the level of 85.0–95.0%. The adapted plants had morphological features typical of the original plants.

Conclusions. On the basis of the carried-out experimental researches methods of clonal micropropagation in vitro are developed and adaptive possibilities and prospects of use of paulownia in gardening of the city of Mykolaiv are studied.

1. The most effective scheme of sterilization of paulownia is stepwise treatment of plant material with 70% ethanol solution and 1% sodium hypochlorite solution. The yield of sterile explants was 92.5–95.0%, viability – 90.0–97.5%.

2. At the stage of introduction into in vitro culture, the optimal nutrient medium is MS, supplemented with kinetin 1.0 mg/l and GA 0.5 mg/l, at which the reproduction rate was the highest and was 8.7–9.2.

3. At the stage of proper micropropagation, the most intensive proliferation of shoots also occurred on the nutrient medium MS, supplemented with kinetin 1.0 mg/l and GA 0.5 mg/l, which provided the highest reproduction rate – 10.3–12.0.

4. At the stage of rooting of micro-shoots the most optimal was the nutrient medium ½ MS, supplemented with a IBA of 0.5 mg/l, at which the frequency of rhizogenesis was 95.0% and formed 5.3–7.8 roots with a length of 78.5–95.2 mm.

5. Stepwise adaptation of plants to in vivo conditions for 40 days in climatic chambers and growing in a greenhouse ensured the yield of seedlings at the level of 85.0–95.0%.

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Key words: sterilization, own micropropagation, highest reproduction, rhizogenesis, adaptation.

Манушкіна Т.М., Коваленко О.А., Хомут В.П., Коломієць Н.П. Клональне мікророзмноження павловнії *in vitro*

Мета – розробити технологічні прийоми клонального мікророзмноження павловнії *in vitro.* **Методи.** Метод клонального мікророзмноження на основі культури ізольованих апікальних меристем забезпечує високу швидкість розмноження, максимальну генетичну стабільність і відновлення отриманих рослин-регенерантів. Рослини павловнії *Paulownia* Sieb. et Zucc. були матеріалом для дослідження. У ході проведення досліджень застосовували загальноприйняті у біотехнології рослин методи. Асептичну роботу проводили в ламінарному боксі КПГ-1. Для культивування ізольованих бруньок та мікроживців використовували як базове живильне середовище Мурасиге і Скуга (МС). **Результати.** Найбільш ефективною схемою стерилізації павловнії є ступінчаста обробка рослинного матеріалу 70%-ним розчином етанолу та 1%-ним розчином гіпохлориту натрію. Вихід стерильних експлантів становив 92,5–95,0%, життєздатність – 90,0–97,5%. На етапі введення в культуру *in vitro* оптимальним є живильне середовище МС, доповнене кінетином 1,0 мг/л та ГК 0,5 мг/л, на якому коефіцієнт розмноження був найвищим і становив 8,7–9,2. На етапі власне мікророзмноження найбільш інтенсивна проліферація пагонів відбувалася також на живильному середовищі МС, доповненому кінетином 1,0 мг/л та ГК 0,5 мг/л, що забезпечувало найвищий коефіцієнт розмноження – 10,3–12,0. На етапі укорінення мікропагонів найбільш оптимальним виявилося живильне середовище 1/2 МС, доповнене ІМК 0,5 мг/л, на якому частота ризогенезу становила 95,0% та формувалися 5,3–7,8 коренів довжиною 78,5–95,2 мм. Ступінчаста адаптація рослин до умов *in vivo* упродовж 40 діб у кліматичних камерах та дорощування у теплиці забезпечувала вихід саджанців на рівні 85,0–95,0%. **Висновки.** На основі проведених експериментальних досліджень розроблено прийоми клонального мікророзмноження павловнії *in vitro,* які доцільно застосовувати для масового виробництва генетично однорідного оздоровленого садивного матеріалу.

Ключові слова: стерилізація, власне мікророзмноження, коефіцієнт розмноження, ризогенез, адаптація.