

Induction of Morphogenesis of Adventitious Bud Explants of Fast-Growing Woody Plants of the Genus *Paulownia*

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Abstract. Recently, the interest of the scientific society has focused on the study of various forms and hybrids of *Paulownia*. The Shan Tong hybrid has been introduced in the Chechen Republic and work is underway on the introduction of Pao Tong Z07, both hybrids are frost-resistant -20 ...-30 0C. It is known that the induction of morphogenesis in most cultures is a difficult task, since it is accompanied by problems of selecting the optimal composition of the nutrient medium, as well as the type of explant being initiated, which takes a lot of time. The paper presents the results of the research of the laboratory "Biotechnology of agricultural plants for regenerative agriculture" for 2021-2022 on the cultivation in vitro of fast-growing plants with high sequestration potential from the genus *Paulownia*. The results of experiments to study the effectiveness of sterilizing substances – hypochlorites on the survival of explants initiated from the axillary kidneys are presented. A positive effect was noted in the induction of morphogenesis of adventitious kidneys from axillary kidneys in *Paulownia* Shan Tong and *Paulownia* Pao Tong Z07. The optimal protocol of the nutrient medium for the introduction and multiplication of micro-plants is given, the concentrations of plant growth regulators 6-BAP and BCI to increase the reproduction coefficient of plants *Paulownia* Shan Tong and *Paulownia* Pao Tong Z07 are also studied.

1 Introduction

Climate change has led to a rapid expansion of research to reduce carbon dioxide (CO₂) emissions. One of the forms of reducing CO₂ emissions into the atmosphere is the use of alternative energy sources, including the cultivation of fast-growing crops with a high binding potential [1,2,3]. As one of the fastest growing tree species in the world, the genus *Paulownia* has generated great interest in science and industry in recent years [6,7]. The great demand for *Paulownia* has led to the development of various hybrids, the most famous of which are Clone in vitro 112, Shan Tong, Sundsu 11 and Cotevisa [8].

In addition, plants of the genus *Paulownia* are important for the production of wood, and their flowers are seasonal by-products traditionally used in medicines. The phytochemistry and pharmacology of *Paulownia* flowers makes them economically viable for use in medicines, food, animal feed and cosmetics. The chemical composition of *Paulownia* flowers

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mainly consists of flavonoids, phenylpropanoids, terpenoids, volatile components, polysaccharides, lignans and iridoids, which have various beneficial properties, such as antioxidant, anti-inflammatory, antibacterial, antiviral, antitumor, hypoglycemic, lipid-lowering, neuroprotective and immunoregulatory activity. The safety of extracts of Paulownia flowers for animals has also been proven. They contribute to the development of new products and technologies using Paulownia flowers with intellectual property rights [9].

In connection with the above, the Shan Tong hybrid has been introduced in the Chechen Republic and work is underway to introduce Pao Tong Z07, both hybrids are frost-resistant -20 ... -30 0C, research is underway to study the sequestration potential of CO₂.

The aim of the studies was to establish optimal conditions for induction of morphogenesis in the plants Shan Tong and Pao Tong Z07 under conditions of in vitro tissue culture.

2 Research Methodology

The research was carried out in the laboratory of "Biotechnology of agricultural plants for regenerative agriculture" in 2021-2022. In the work with cell culture, axillary buds of cuttings of plants of the genus paulownia Shan Tong and Pao Tong Z07 were used, germinated in the conditions of the MEMMERT HPP climatic chamber with the following parameters: light 7 thousand liters, temperature 27 0C, humidity 77%, ventilation 100%, photoperiod was 8/16 for 1 month.

In the first experiment, hypochlorite Ca and hypochlorite Na were used for sterilization of the initial plant explants in various variants for processing time of 3 min, 5 min, 7 min and 10 min., test tubes with a size of 16x1.5 cm were used for planting explants. The treatment of shoots when introduced into the culture in vitro was carried out as follows: washing the selected shoots with running water for 30 minutes with the gradual introduction of green soap to remove sticky substances and pathogenic microorganisms from the surface of the explanted samples, washing with distilled water once. Then, in laminar conditions, explants were treated with sodium hypochlorite / calcium hypochlorite for 3-10 minutes, then they were subjected to 3-fold treatment with distilled water for 5 minutes. Explants were cultured on the MS nutrient medium with the addition of the antibiotic ceftriaxone – 100 mg/l and 6-BAP-1.5mg/l.

In the second experiment, MS, DKW and WPM nutrient media were used to stimulate the proliferation of micro-plants, and we looked at which variant of the plant would show a high reproduction coefficient. At this stage, 6-BAP and IBA were added in 3 concentration variants: 0.2mg/l+0.01mg/l, 0.5mg/l+0.5mg/l, 2.0 mg/l+0.5mg/l for each medium, the classic composition of MS components without regulators was taken as a control variant. At the same time, the first 3 passages of plants were taken into account, the data are given in the average value, the experiment was repeated 3 times.

The studies were carried out according to generally accepted methods when working with cell and tissue culture [10,11], mathematical and statistical processing was carried out using the programs Listomer, Biostat and Microsoft Excel.

3 Research Results

The degree of success of the introduction of isolated explants into the culture in vitro depends on a relatively large number of factors, starting with the choice of the explanted tissue, ending with the selection of the appropriate composition of the nutrient medium. Each component of the nutrient medium is important, some of them enhance the activity and digestibility of each other, the rest, on the contrary, can inhibit the processes of cell metabolism in a certain

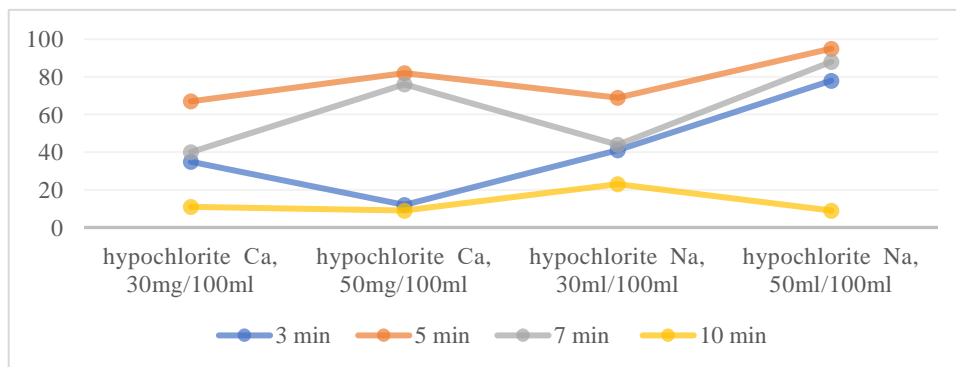
combination. This pattern of synergism and antagonism of a combination of substances of an endogenous type of effect on plants is especially pronounced in plant growth regulators.

In the experiment, axillary buds Shan Tong and Pao Tong Z07, obtained in a climate chamber in early December, were used for introduction into a sterile culture. It is important to note that hypochlorite Ca was used in powder form, and hypochlorite Na in liquid form.

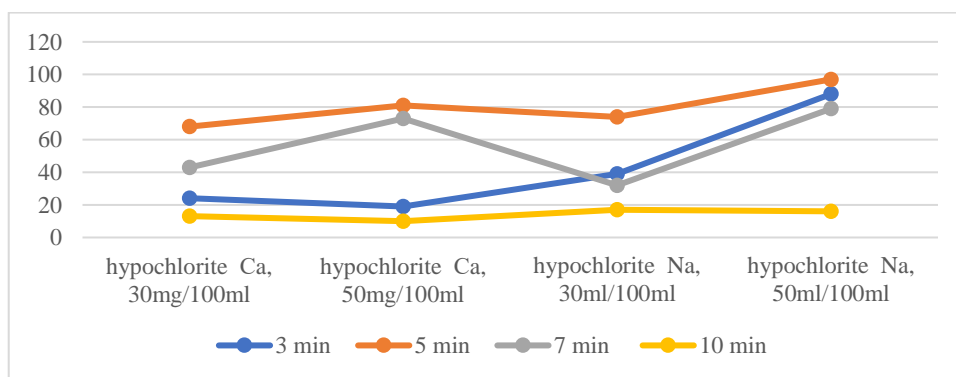
Table 1. The effect of sterilizing substances on the survival rate of plants of the genus Paulownia, n=20.

| Processing time, min | Ca(ClO) ₂ , 30mg/100ml | Ca(ClO) ₂ , 50mg/100ml | NaClO, 30ml/100ml | NaClO, 50ml/100ml |
|----------------------|-----------------------------------|-----------------------------------|-------------------|-------------------|
| 3 min | 3 | 12 | 7 | 15 |
| 5 min | 8 | 19 | 10 | 20 |
| 7 min | 13 | 14 | 14 | 17 |
| 10 min | 10 | 9 | 11 | 10 |

According to the data of the first stage of cultivation of plants of the genus Paulownia under in vitro conditions – Table 1, we can talk about high efficiency in terms of the number of plants that have taken root on variants using sodium hypochlorite 50ml/100ml and calcium hypochlorite 50mg/100ml when processing explants for 5 min.



a



b

Fig. 1. Survival rate during in vitro growth initiation within the genus Paulownia (a- Shan Tong; b- Pao Tong Z07), in %

It can be seen from the diagram in Figure 1 that the survival rate of Pao Tong Z07 explants was higher, so the best option was noted when treated with sodium hypochlorite at a concentration of 50ml/100ml for 5 minutes with a survival rate of 97%. Thus, already at the

4th week of initiation, explants began to induce morphogenesis, and by the end of the 2nd month, micro-plants could be passivated to the updated composition of the nutrient medium for animation (Figure 2).

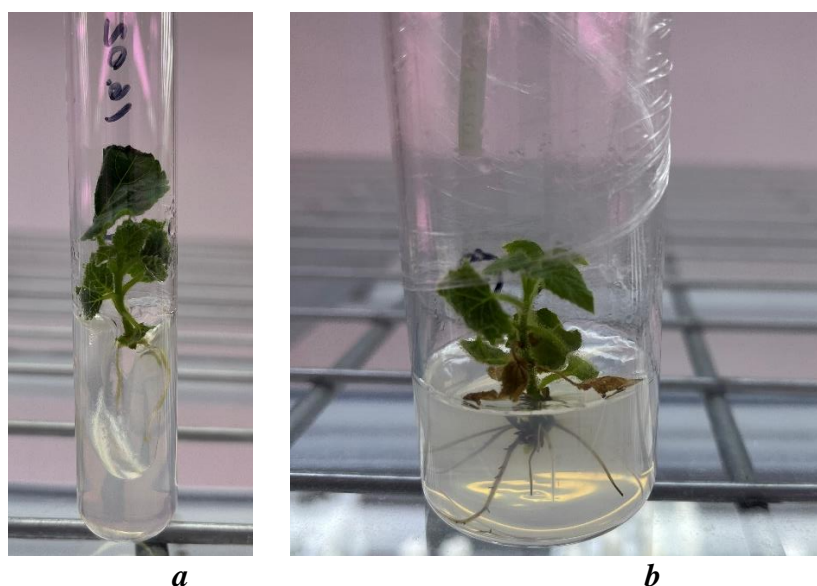


Fig. 2. Plants of the genus Paulownia introduced into culture in vitro (*a*- Shan Tong; *b*- Pao Tong Z07)

Also, in order to increase the multiplication rate of Shan Tong and Pao Tong Z07 plants, we tested various variants of nutrient media during the multiplication phase (Table 2).

Table 2. The effect of plant growth regulators on the reproduction coefficient on media according to various prescriptions in plants of the genus Paulownia, $n=20$

| № | Nutrient medium | Reproduction rate | |
|----|---------------------------------------|-------------------|--------------|
| | | Shan Tong | Pao Tong Z07 |
| 1. | Control - MS | 6,4 | 6,1 |
| 2. | DKW-6-BAP + IBA 0,2mg/l+0,01 mg/l | 6,5 | 6,2 |
| 3. | WPM-6-BAP + IBA 0,2 mg/l+0,01 mg/l | 8,2 | 7,9 |
| 4. | DKW-6-BAP + IBA 0,5 mg/l+0,5 mg/l | 7,9 | 8,4 |
| 5. | WPM-6-BAP + IBA 0,5 mg/l+0,5 mg/l | 7,6 | 7,3 |
| 6. | DKW-6-BAP + IBA 2,0 mg/l+0,5 mg/l | 10,1 | 8,8 |
| 7. | WPM-6-BAP + IBA 2,0 mg/l+0,5 mg/l | 9,0 | 7,1 |

According to Table 2, it can be seen that good multiplication results and a high reproduction coefficient show the variants of the nutrient medium the third - WPM-6-BAP + IBA 0.2mg/l + 0.01mg/l, the sixth - DKW-6-BAP + IBA 2.0 mg/l + 0.5mg/l and the seventh - WPM-6-BAP + IBA 2.0 mg/l + 0.5mg/l. It should be noted that both Paulownia samples showed the best reproduction coefficient on the variant using the Kunjuki Driver medium with a concentration of plant growth regulators of 6-BAP - 2mg/l and IBA - 0.5 mg/l, where in Shan Tong this indicator was 10.1 on average per plant, in Pao Tong Z07 - 8.8,

respectively. At the same time, the average height of plants reached 17 cm (cultivation was carried out in test tubes of 20 x 2 cm and in some cases micro-plants developed into 2 or more shoots).

4 Conclusions

Thus, there is no doubt that it is necessary to increase the yield of healthy Paulownia seedlings in vitro conditions due to biotechnological methods. Recent studies indicate an ever-growing demand for plants of the genus Paulownia due to their sequestration properties, which are still being studied, including in the conditions of the Chechen Republic.

Theoretically, many plants are easily amenable to morphogenesis in vitro, but with each species it is necessary to work out some elements for the successful initiation of micro-plants. The data obtained during the research indicate:

-about the high survival rate of plants of the genus Paulownia when used at the stage of introduction into sterile culture of sodium hypochlorite at a concentration of 50ml / 100ml for 5 minutes - 97%;

- on the effectiveness of the use of MS nutrient medium with the addition of ceftriaxone as an antibiotic at a concentration of 100 mg/l for the introduction into culture in vitro;

- on the positive effect on the induction of adventitious kidney morphogenesis from axillary kidneys in Paulownia Shan Tong and Paulownia Pao Tong Z07;

- on the effectiveness of the use of DKW and WPM nutrient media for the microclonal reproduction of Paulownia Shan Tong and Paulownia Pao Tong Z07;

- on the increase in the reproduction coefficient of microplants when using plant growth regulators in the ratio of 6-BAP + IBA at concentrations of 2 mg/l + 0.5mg/l, respectively.

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