

# Obtaining aseptic *in vitro* culture of *Hydrangea paniculata* 'Samarskaya Lydia'

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**Abstract.** Techniques for obtaining sterile culture *in vitro* *Hydrangea paniculata* 'Samarskaya Lydia' have been developed. The highest number of viable (92%) explants with a minimum of infected and necrotic ones was obtained by sequential treatment of apical and axillary buds from spring regrowth shoots in 3% hydrogen peroxide solution for 3 minutes and 7% sodium hypochlorite solution for 4 minutes. The selected Anderson nutrient medium containing BAP at a concentration of 5.0 mg/l and IAA – 1.0 mg/l is the most preferable for regeneration of shoots with a propagation coefficient of 6.

## 1 Introduction

*Hydrangea* belongs to the *Hydrangeaceae* family. This genus unites over 80 species of hydrangeas. The life form is represented by trees, shrubs, and lianas. Most of these plants grow in East and South Asia, North America, and the Far East. The work of selectionists has managed to adapt hydrangea to the harsh Central European climate. Especially frost-resistant are arborescent and paniculate hydrangeas.

*Hydrangea paniculata* 'Samarskaya Lydia' is a delightfully beautiful hydrangea variety. The size of the plant is average, it is 1.2 meters high and 1.1 meters wide. A shrub with strong erect red-maroon shoots with dark green relief, rough leaves forming a dense crown [1]. Very beautiful in color in autumn, the leaves become even more beautiful, turning burgundy.

Paniculate hydrangeas always bloom on the shoots of this year. The inflorescences are represented by thick, lush panicles 15 cm long. Characteristic of 'Samarskaya Lydia' is long flowering in summer and autumn. The inflorescence in the shape of a cone consists of tightly attached large flowers that change color during flowering from white, pink to red, ruby [2]. In July, flowering begins and the petals have a milky white shade with light greenery. The color changes when blooming to light pink, by August it is saturated to red. In September, at the end of the season, the petals of the flowers darken and become thicker in color. *Hydrangea* 'Samarskaya Lydia' is frost-resistant, withstands minus 34°C in winter without shelter [3].

The aim of our work was to obtain a sterile culture with good growth of *Hydrangea paniculata* 'Samarskaya Lydia' shoots.

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## 2 Materials and methods

For clonal propagation of *hydrangea paniculata*, the method of culture of plant tissues and organs was used in this work [4].

The object of propagation was *Hydrangea paniculata* 'Samarskaya Lydia', which grows in the collection of the botanical garden of Ufa. The explants for *in vitro* culture were axillary and apical buds from spring growing shoots. The work was carried out in aseptic laboratory conditions, the nutrient media and planting material were sterilized according to the method given in the literature [5, 6].

Shoots with buds were washed in a soap solution for 20 minutes and rinsed with running water. In the laminar box, the objects were sterilized with solutions of 70% ethanol, 0.1% diacid, 3% hydrogen peroxide, 7% sodium hypochlorite. The objects were sterilized by immersion in the above solutions for a period of 1 to 7 minutes, followed by three times rinsing in distilled sterile water for 45 minutes.

In experiments on the cultivation of *hydrangea* 'Samarskaya Lydia', the mineral bases of the nutrient media Murashige & Skoog (MS) [7] and Anderson (An) [8] were tested. The content of agar in the nutrient medium was 0.6%.

To trigger morphogenetic processes, growth regulators were: 6-benzylaminopurine (BAP) in concentrations from 0.2 to 5.0 mg/l; indolyl-3-acetic acid (IAA) – from 0.05 to 1.0 mg/l. The growth of active substances was added to the medium before bringing the pH of the medium to 5.4 and 5.6. Autoclaving of the nutrient medium was carried out at 120 °C for 20 minutes.

The cultivation of the objects was carried out in flasks and jars with a volume of 50-100 ml, at a 16-hour photoperiod, in fluorescent lighting of 3000 lux, at a temperature of 26 °C, and air humidity of 70%.

## 3 Results and discussion

Starting work in *in vitro* culture with the selected object, it was necessary to pre-select the conditions for its sterilization. At the stage of administration of isolated buds, it is necessary to obtain aseptic explants with active growth [9]. We achieved the production of sterile plant tissue by applying stepwise sterilization of objects, alternately dipping them in disinfectant solutions. The exposure time of sterilizing agents was selected so that they did not burn buds tissues, did not inhibit their further growth *in vitro* and maintained maximum sterility of the cultivated plants.

The success of sterilization of the injected material *in vitro* was assessed by the following indicators: the number of infected and viable explants during sterilization. According to the results of the experiments (Table 1) it was possible to obtain the maximum number of viable (92%) kidneys with a minimum number of necrotized (2%) by alternately immersing them in a 3% solution of hydrogen peroxide for 3 minutes and a 7% solution of sodium hypochlorite for 4 minutes.

For the sustainable support of a proliferating culture, the composition of the nutrient medium has the greatest influence of all factors [10]. So, to identify the ability of *hydrangea* explants of the studied variety to shoot, the nutrient media Murashige & Skoog (MS), Murashige & Skoog modified by macro salts – the concentration of ammonium nitrate reduced by 2 times and calcium nitrate increased to 1 mg/l, Anderson (An), containing modified concentrations of hormonal substances BAP and IAA (Table 2).

**Table 1.** The effect of a combination of sterilizing solutions on infection and viability of *Hydrangea paniculata* 'Samarskaya Lydia' buds culture *in vitro*.

Sterilization solutions		Percentage of explants, %		
Concentration	Exposure, min	Infected	Viable	Necrotic
70% ethanol	1	28.0	14.0	58.0
0.1% diacid	5			
70% ethanol	1	9.0	37.0	54.0
0.1% diacid	7			
3% hydrogen peroxide	3	2.0	58.0	40.0
7% sodium hypochlorite	7			
3% hydrogen peroxide	3	6.0	92.0	2.0
7% sodium hypochlorite	4			

**Table 2.** The influence of the nutrient medium on the numerical characteristics of the shoots of *Hydrangea paniculata* 'Samarskaya Lydia' *in vitro*.

Nutrient medium and growth regulators, mg/l	The average length of the shoot, mm	Number of leaves, pcs. per 1 shoot	The average number of shoots per explant, pcs.
MS; BAP 0,3	3.7±0.33	4.9±0.25	1.1
MS; BAP 0.5; IAA 0.1	4.4±0.23	4.6±0.16	1.4
MS modif. by macro-salts; BAP 0.2; IAA 0.05	11.2±0.14	6.1±0.31	2.3
An; BAP 5.0; IAA 1.0	23.4±0.16	12.4±0.22	6.1

In Table 2, it can be seen that the morphometric indicators of the average length of the shoot and the number of leaves on it are higher on the Anderson medium containing BAP at a concentration of 5.0 mg/l and IAA – 1.0 mg/l. The macrosol-modified Murashige & Skoog medium containing BAP 0.2 mg/l and IAA 0.05 mg/l is twice as inferior in terms of the listed indicators.

More active formation of axillary shoots of *Hydrangea paniculata* 'Samarskaja Lydia' is also noted on the Anderson medium, 3 times more than on the Murashige & Skoog medium.

We assume that the lower acidity of the Anderson medium had a positive effect on plant growth and development, since the variety is sensitive to the acidity of the substrate on which it is cultivated, as well as higher availability of macro- and microelements at a pH value of 5.4.

### 3 Conclusion

As a result of our work, we found that when introducing apical and axillary buds from growing shoots of *Hydrangea paniculata* 'Samarskaya Lydia' into aseptic culture, the use of sodium hypochlorite and hydrogen peroxide was effective compared with other options. A high number of viable sterile explants (92%) was obtained by treating them with a 3% solution of hydrogen peroxide for 3 minutes and a 7% solution of sodium hypochlorite for 4 minutes. Buds from spring shoots successfully regenerated shoots on used nutrient media: modified Murashige & Skoog containing 0.2 mg/l BAP and 0.05 mg/l IAA and Anderson containing 5.0 mg/l BAP and 1.0 mg/l IAA. The use of Anderson medium for further growth proved to be the most successful for the regeneration of hydrangea with better morphometric

indicators: the average length of the shoot is 23.4 mm; the number of leaves on the shoot is 12.4 pcs./shoot and the multiplication factor is 6.1 pcs./explant.

The use of the clonal micropropagation method is promising for propagation of a new variety *Hydrangea paniculata* 'Samarskaya Lydia' in the presence of a limited amount of starting material. *In vitro* culture allows you to get healthy, high-quality planting material by the planned date in the required volume.

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