

Optimization of the culture medium for the in vitro micropropagation of *Hydrangea L.* plants

M. Osipov, V. Polishchuk, A. Brovdi*, L. Kunpan and I. Kozachenko

Uman National University, 1 Instytutska Str., UA20305 Uman, Ukraine

*Correspondence: abrovdi@ukr.net

Received: May 1st, 2026; Accepted: October 5th, 2026; Published: October 16th, 2026

Abstract. Species of the genus *Hydrangea L.* are valuable ornamental plants widely used in landscaping, creating a demand for large quantities of genetically uniform planting material. Traditional vegetative propagation may be insufficiently effective for producing the required quantities of planting material, whereas in vitro micropropagation enables the rapid production of large numbers of regenerated plants under controlled conditions. However, the efficiency of hydrangea micropropagation depends considerably on genotype, the balance of plant growth regulators, the carbohydrate source, and mineral nutrition. Therefore, determining optimal conditions for their in vitro cultivation is of considerable importance. The aim of the study was to investigate and optimize the composition of the culture medium for the micropropagation of *Hydrangea L.* species to obtain high-quality, genetically uniform plant material for ornamental horticulture. The study was conducted in vitro during 2020–2024 using 1.0–1.5 cm long shoots containing apical meristems of *H. macrophylla* ‘Nikko Blue’, *H. paniculata* ‘Grandiflora’ and *H. arborescens* ‘Annabelle’. The explants were cultured for shoot multiplication on a medium based on the Murashige and Skoog (MS) formulation, supplemented with plant growth regulators, iron, and carbohydrates at various concentrations. Statistical analysis of the results was performed using analysis of variance (ANOVA) and Fisher’s F-test to assess the statistical significance of the effects of the factors under study. Differences between the means were evaluated using the least significant difference (*LSD*₀₅) at a significance level of $p < 0.05$. The use of MS medium supplemented with 6-benzylaminopurine in combination with auxins and gibberellin suppresses apical dominance and stimulates the growth of axillary buds in *Hydrangea* explants. The highest efficiency of micropropagation was achieved through repeated subculturing and the cultivation of shoot clusters without division during the early stages, which contributed to an increase in the multiplication rate from 0.0 in the first passage to 5.75 in the fourth passage. The differences observed exceeded the *LSD*₀₅ value (0.31). The highest multiplication rate, with an average of 5.75 across the *Hydrangea* species and cultivars studied, was obtained on MS-35 medium, whereas the lowest rate, 1.14, was recorded on MS-42 medium. The highest multiplication rate for an individual genotype was obtained for *H. arborescens* on MS-35 medium, reaching 5.93. The highest number of shoots was obtained with the addition of 3.0 mg L⁻¹ glucose and 3.0 mg L⁻¹ sucrose to the culture medium, reaching 6.3 and 6.1 shoots per explant, respectively. The maximum shoot length (7.8 cm) was recorded on MS-35 medium supplemented with 3.0 mg L⁻¹ sucrose. The addition of FeSO₄ to MS-35 medium significantly increased the number and length of shoots mg L⁻¹, with 8.36 shoots per explant and a shoot length of 4.62 cm, whereas the corresponding values in the control were 3.8 shoots per explant and 1.95 cm, respectively. The differences between the treatments exceeded the *LSD*₀₅ values, which were 0.31 for the number

of shoots and 0.19 for shoot length ($p < 0.05$). Thus, optimisation of the culture medium composition can increase the efficiency of micropropagation of *Hydrangea* species and provides a basis for the standardised production of large quantities of plant material *in vitro*. The results can be used to develop technological protocols for the accelerated clonal propagation of the studied genotypes.

Key words: micropropagation, culture medium, phytohormones, carbohydrate sources, explant.

INTRODUCTION

Micropropagation methods are widely used in the commercial production of planting material of various plant species and are considered among the most widely used approaches for the propagation of ornamental plants (Mehbub et al., 2022; Majumder et al., 2025). *In vitro* propagation offers a number of advantages over traditional plant propagation methods, including high efficiency and cost-effectiveness (Hasnain et al., 2022).

At the same time, the effectiveness of the method depends on addressing a number of challenges, including the selection of explants and culture media, optimisation of hormone concentrations and incubation conditions, as well as the control of microbial contamination (Sharma et al., 2023).

Hydrangeas are valuable flowering plants widely used both as cut flowers and in landscape design. Approximately one hundred billion dollars in annual sales are attributed to hydrangeas in the United States, while in East Asia, hydrangeas are among the major ornamental flower crops (Bak & Han, 2024). Despite their economic value, the commercial propagation of hydrangeas remains limited due to environmental constraints and difficulties in obtaining sufficient quantities of plant material using traditional propagation methods. Micropropagation can help overcome these limitations by enabling rapid multiplication and the production of uniform, high-quality plant material (Bak & Han, 2026). However, despite the promising potential of genetic engineering technologies, their application to hydrangeas remains limited, and there is insufficient systematically organised information to fully characterise the propagation process in hydrangeas (Wang et al., 2021). Therefore, sterilization conditions for the initial plant material, appropriate explant types, and culture medium composition for different micropropagation protocols need to be optimised individually for each genotype, while individual stages of the biotechnological process should also be further improved.

The earliest studies on the propagation of *Hydrangea* species focused on *H. quercifolia*, from which viable explants were obtained from leaf callus, and the induction of axillary bud development was achieved (Sebastian & Heuser, 1987; Ledbetter & Preece, 2004). However, the number of publications by international researchers on the micropropagation of hydrangea remains limited, while such studies in Ukraine are almost absent, with the exception of several works by G. N. Nestorov and O. Yu. Chorgobrov (Nesterova & Chornobrov, 2015; Nesterova & Chornobrov, 2016) devoted to the propagation of *H. macrophylla*. These studies developed approaches for the introduction of *H. macrophylla* explants into *in vitro* culture, including effective methods for their sterilisation.

During the preparation for micropropagation, the primary objective is to select the most suitable explants and prepare them for subsequent propagation. Explants for *in vitro* culture can be obtained from various plant organs, including roots, shoots, leaves, and meristems (Labidi et al., 2023; Redhwan et al., 2023). Since vegetative propagation is intended to produce genetically uniform material, ensuring the genetic stability of plants during the early stages of *in vitro* culture is essential. Effective control of contamination in the initial explant through surface sterilisation is essential, as microbial contamination is considered one of the major limitations of plant tissue culture and can negatively affect *in vitro* plant propagation (Babu et al., 2022; Permadi et al., 2023).

The efficiency of individual stages of *Hydrangea* micropropagation is largely determined by the composition of the culture medium and the concentrations of plant growth regulators. For example, Ruffoni et al. (2012) reported a high rooting efficiency of *Hydrangea* shoots *in vitro*: following proliferation, 100% of the shoots rooted on a medium containing NAA at a concentration of 0.5 mg L⁻¹. In contrast, direct *ex vitro* rooting after dipping microshoots in a K-IBA solution at a concentration of 5,000 ppm resulted in a rooting rate of 95%. These results demonstrate that both conventional *in vitro* rooting and direct *ex vitro* rooting of microshoots may be promising approaches for *Hydrangea*, potentially reducing certain stages of the propagation process.

The varietal specificity of plant responses to plant growth regulators is also of considerable importance. Khaing et al. (2018), in their study of *H. macrophylla*, found that the optimal BAP concentrations for the proliferation of the cultivars ‘Blaumeise’ and ‘Vanilla Sky’ differed. Furthermore, increasing the IBA concentration from 0 to 1.0 mg L⁻¹ resulted in a significant increase in the number of roots. However, the rooting response differed significantly between the cultivars. Thus, the results of this study indicate that a single protocol cannot be directly applied to different genotypes of *H. macrophylla* and highlight the need to select growth regulator concentrations according to cultivar-specific characteristics.

A study by Arafa et al. (2017) aimed at developing a protocol for the large-scale *in vitro* micropropagation of *H. macrophylla* showed that the efficiency of micropropagation depends on the type of explant, the sterilisation method, the composition of the culture medium, and the conditions for rooting and acclimatisation. The highest explant survival rate was achieved following sterilisation with 1.0% or 1.5% NaOCl for 20 minutes, while Murashige and Skoog (MS) medium supplemented with 1.0 mg L⁻¹ BA and 2.0 mg L⁻¹ Kin proved to be the most effective for micropropagation.

The study by Amrutha et al. (2025) also confirms that the morphogenesis of *H. macrophylla* depends on the concentration of plant growth regulators. The authors demonstrated the effect of BAP concentration on shoot proliferation. As the concentration of 6-benzylaminopurine (BAP) in Murashige and Skoog (MS) medium increased from 2.5 ppm to 3.5 ppm, the number of shoots per explant increased from 2.30 to 3.82, while the number of leaves increased from 8.37 to 16.26. The highest rooting percentage of regenerated shoots (92.53%) was obtained on MS medium containing 0.5 ppm naphthaleneacetic acid (NAA). These results confirm the need to optimise growth regulator concentrations according to the cultivation stage to improve the efficiency of *hydrangea* micropropagation.

Despite significant progress in developing protocols for the micropropagation of *Hydrangea*, a number of issues remain insufficiently studied. First and foremost, the

responses of different *Hydrangea* genotypes to the composition of the culture medium, particularly the effects of plant growth regulators, carbon sources, and mineral components, require further investigation. The scale-up of micropropagation also requires further optimisation while ensuring high regeneration capacity, genetic stability, and the quality of the resulting plant material. Therefore, there is a need to develop reproducible, genotype-specific *in vitro* protocols aimed at improving the efficiency of mass propagation and obtaining high-quality, genetically uniform planting material from promising *Hydrangea* genotypes suitable for use in urban landscaping.

The aim of this study was to investigate the effects of culture medium components on the efficiency of *in vitro* micropropagation of *Hydrangea L.* species for the production of high-quality plant material from ornamentally valuable genotypes.

MATERIALS AND METHODS

Laboratory studies were conducted during 2020–2024 at Uman National University of the ministry of Education and Science of Ukraine and at the National Dendrological Park ‘Sofiyivka’ of the National Academy of Sciences of Ukraine.

Research conditions

The initial plant material for *in vitro* culture consisted of shoots with apical meristems (1.0–1.5 cm in length) collected from 3–5-year-old plants.

Before to sterilization, the explants were rinsed under running water and then immersed in a 0.1% HgCl₂ solution for 1–1.5 min. One to two drops of Tween-80 were added to the solutions to enhance sterilization efficiency. After sterilisation, the explants were rinsed three times with sterile distilled water for 5 min each to remove any residual sterilising agents. All sterile and viable explants were used in subsequent micropropagation studies. All work involving HgCl₂ was performed in accordance with the safety requirements for handling toxic mercury-containing compounds. The solutions and materials used were collected separately for subsequent disposal in accordance with applicable hazardous waste disposal procedures.

Following sterilisation and rinsing, the explants were cultured on an appropriate modified Murashige–Skoog (MS) medium. Cultivation was carried out at an air temperature of 25 ± 1 °C, with a 16 h photoperiod, a light intensity of 3 klx, and a relative humidity of 75%.

To stimulate shoot formation, cytokinins and auxins were added to the culture medium at appropriate ratios, enabling the regulation of plant growth and development *in vitro* towards the production of regenerant plants via direct organogenesis, i.e. the formation of shoots or roots. Given the importance of the auxin-to-cytokinin ratio in the culture medium for controlled morphogenesis, 48 combinations of these growth regulators were evaluated (Table 1). The cytokinin concentrations were 0, 0.1, 0.5, 1.0, 1.5, 2.0, 2.5, and 5.0 mg L⁻¹, while

Table 1. Auxin-to-cytokinin ratios, mg L⁻¹

Auxins	Cytokinins							
	0	0.1	0.5	1.0	1.5	2.0	2.5	5.0
0	1	2	3	4	5	6	7	8
0.01	9	10	11	12	13	14	15	16
0.05	17	18	19	20	21	22	23	24
0.1	25	26	27	28	29	30	31	32
0.25	33	34	35	36	37	38	39	40
0.5	41	42	43	44	45	46	47	48

the auxin concentrations were 0, 0.01, 0.05, 0.1, 0.25, and 0.5 mg L⁻¹. The 48 treatments represented all possible combinations of the concentrations under study. The numbers 1–48 in the table correspond to individual culture medium treatments, each defined by a different combination of concentrations of these plant growth regulators.

The next stage of micropropagation involved suppressing apical dominance in *Hydrangea* explants and stimulating the growth of axillary buds. For this purpose, Murashige–Skoog (MS) medium containing 0.7 mg L⁻¹ agar and 3.0 mg L⁻¹ sucrose was supplemented with 6-benzylaminopurine (6-BAP) in combination with other plant growth regulators, namely β -indole-3-butyric acid (β -IBA) and gibberellic acid (GA₃), at the concentrations shown in Table 2. The effectiveness of the culture media was assessed, and the multiplication coefficient was calculated accordingly, after the fourth passage. The capacity for adventitious regeneration of *Hydrangea* species was studied using experimentally selected culture media that ensured a multiplication coefficient greater than two.

The explants were transferred to fresh culture medium once a month under aseptic conditions in a laminar-flow cabinet. To increase the multiplication coefficient during the initial passages, clusters of buds and shoots were not separated into individual units but were transferred as larger clumps.

The morphogenetic response of the explants was assessed based on the proportion of explants that formed new shoots or exhibited other signs of organogenesis. To characterise the dynamics of morphogenesis, assessments were performed on days 5, 10, 15, and 20.

The multiplication coefficient was determined after the fourth passage as the ratio of the number of newly formed shoots to the number of initial explants:

Micropropagation coefficient (MC) = number of induced adventitious / total number of explants.

To determine the effect of carbohydrate sources on explant morphogenesis, a modified MS-35 medium was used. Sucrose, glucose, fructose, and maltose were tested at concentrations of 1.0, 2.0, 3.0, and 4.0%.

To investigate the effect of iron (Fe) on the growth and development of *H. macrophylla* explants, iron was added to the MS-35 medium at concentrations of 2.25, 2.60, and 2.95 mg L⁻¹. The source of Fe was non-chelated iron sulphate (FeSO₄). The pH of the medium was 4.70.

The number of explants varied among the experimental treatments because only the sterile and viable explants actually obtained were used for subsequent cultivation. Tables 6 and 7 present the total number of explants used for each treatment across three replicates, while the number and length of shoots are presented as mean values across the replicates.

Table 2. Phytohormones content in modified nutrient media, mg L⁻¹

Nutrient medium	6-BAP	β -IBA	Gibberellin (A ₃)
MS-26	0.45	0.09	0.07
MS-32	1.11	0.01	0.00
MS-22	1.53	0.01	0.00
MS-35	2.24	0.48	0.00
MS-46	2.62	0.12	0.00
MS-42	5.21	0.12	0.00

Plant material

The study was conducted using plants of *Hydrangea macrophylla* ‘Nikko Blue’, *Hydrangea arborescens* ‘Annabelle’ and *Hydrangea paniculata* ‘Grandiflora’.

Statistical analysis

Statistical analysis of the experimental data was performed using the STATISTICA 6 software package (StatSoft, Inc.), employing analysis of variance, correlation analysis, and regression analysis in accordance with the methodological recommendations of Ermantraut et al. (2007) and the approach described by Fisher (2006). A p -value of < 0.05 was considered statistically significant.

Analysis of variance (ANOVA) was used to assess the effects of culture medium composition and the concentrations of its components on morphogenesis parameters. The statistical significance of the effects of the factors studied was assessed using Fisher’s F-test. When statistically significant differences were identified among treatments, the least significant difference (LSD_{05}) was calculated to compare mean values at the 5% significance level.

Each experimental treatment was established in three independent replicates. Statistical analysis was performed using the mean values of the parameters obtained for each replicate. For morphometric parameters, mean values and measures of variability were calculated. Differences were considered statistically significant at $p < 0.05$. Mean values denoted by different letters differed significantly from one another, whereas values denoted by the same letters did not differ significantly.

RESULTS

During the cultivation of *Hydrangea* meristematic apices and buds used as explants on artificial culture media, a gradual increase in the proportion of explants showing signs of development was observed (Table 3). No signs of development were detected during the first 5 days. The first signs of morphogenesis were recorded on day 10 of cultivation, when 35% of the explants showed signs of development (Fig. 1). This indicates the completion of the initial adaptation period of the isolated meristematic structures to in vitro conditions and the onset of active organogenesis. Mass development of meristematic buds was observed on day 15, when 78% of the explants showed signs of development, representing a 43 percentage-point increase compared with day 10. Continued cultivation resulted in a further increase in the proportion of explants showing signs of development, reaching 92% on day 20, i.e. a further 14 percentage-point increase compared with day 15.

Statistical analysis showed that the LSD_{05} for the proportion of explants showing signs of development was 4.2%. The differences between the values recorded on days

Table 3. Dynamics of *Hydrangea* meristematic bud development in vitro over the cultivation period

Cultivation period, days	Percentage of explants showing signs of development, %
5	0
10	35
15	78
20	92
LSD_{05}	4.2

10, 15, and 20 exceeded the LSD_{05} value, indicating a statistically significant increase in the proportion of explants showing signs of development over this period ($p < 0.05$). The greatest difference was observed between days 10 and 15, amounting to 43 percentage points, which is 10.2 times greater than the LSD_{05} value. The results indicate that the most pronounced increase in the proportion of *Hydrangea* meristematic explants showing signs of development occurred between days 10 and 15 of cultivation, whereas by day 20, signs of development were observed in the majority of explants (92%).

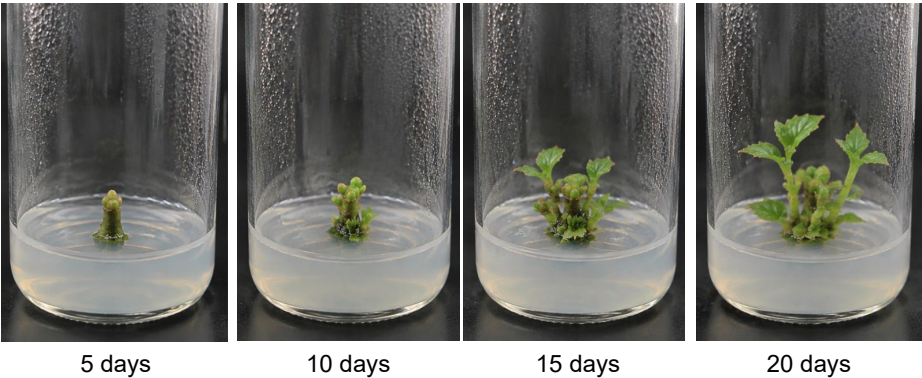


Figure 1. *Hydrangea* meristematic in vitro bud development over the cultivation period (Scale bar – 1 cm).

Explants were subcultured onto fresh culture medium once a month under aseptic conditions.

In the first passage, the multiplication rate was zero, indicating the absence of a morphogenetic response in the explants, whereas in the second passage, the initiation of adventitious bud formation was observed (Table 4). Subsequent passages were characterized by a marked increase in the multiplication rate, indicating the adaptation of the explants to in vitro conditions. In the third passage, the micropropagation coefficient was 3.6, which was 1.8 units, or twice that observed in the second passage. The highest value of this parameter was recorded in the fourth passage, at 5.75. Statistical analysis showed that the LSD_{05} for the micropropagation coefficient was 0.31. The differences between the values of this parameter in consecutive passages were 1.8, 1.8, and 2.15 units, respectively, corresponding to 5.8–6.9 times the LSD_{05} value. Thus, the increase in the micropropagation coefficient from the second to the fourth passage was statistically significant ($p < 0.05$).

Among the selected culture media, the lowest micropropagation coefficient, 1.14, was observed on MS-42, on average across all the *Hydrangea* species studied (Fig. 2). The highest micropropagation coefficient, 5.75, was achieved on the MS-35. On MS-26,

Table 4. Changes in the reproduction rate of *Hydrangea* depending on the passage

Passage	Reproduction rate
I	0.0
II	1.8
III	3.6
IV	5.75
LSD_{05}	0.31

the micropropagation coefficient was almost twice as high as that on MS-42. The MS-22 medium also resulted in a high micropropagation coefficient, although it was 0.65 lower than the highest value.

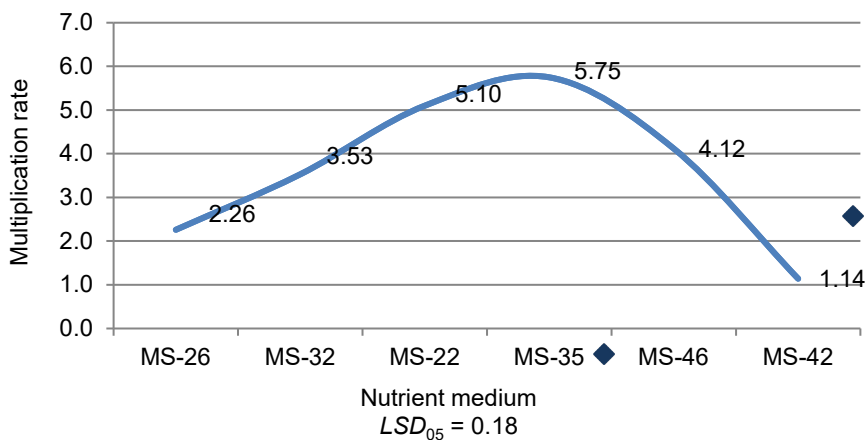


Figure 2. Multiplication rate of *Hydrangea* species depending on phytohormone content (mean value).

An analysis of the composition of the modified culture media (Table 2) showed that the micropropagation coefficient of *Hydrangea* depended on the concentrations and ratios of plant growth regulators in the medium. As the concentration of 6-benzylaminopurine (6-BAP) increased from 0.45 to 2.24 mg L⁻¹, the micropropagation coefficient increased progressively from 2.26 to 5.75. The highest value, 5.75, was recorded on MS-35 medium containing 2.24 mg L⁻¹ 6-BAP and 0.48 mg L⁻¹ β -indole-3-butyric acid (β -IBA). A further increase in the concentration of 6-BAP resulted in a decrease in the micropropagation coefficient. Thus, the results indicate the existence of an optimal level of hormonal regulation that ensures maximum shoot formation. However, this relationship cannot be attributed solely to changes in the concentration of 6-BAP, as the composition of the media under study also differed in their contents of β -IBA and gibberellic acid (GA₃). In particular, the maximum micropropagation coefficient on MS-35 medium was achieved with a combination of 2.24 mg L⁻¹ 6-BAP and 0.48 mg L⁻¹ β -IBA, which may indicate a favourable balance between cytokinins and auxins for plant development.

Analysis of the effectiveness of culture media showed that the micropropagation coefficient depended on the biological characteristics of *Hydrangea* species, indicating that culture media need to be selected individually for each species. The highest micropropagation coefficient for all the hydrangea species studied was observed on MS-35 medium, in particular, in *H. arborescens* this value reached a maximum of 5.93. On MS-22 medium, the micropropagation coefficient for this species was 0.12 units lower, whereas the lowest values were recorded on MS-26 and MS-42 media (Table 5).

Table 5. Phytohormones content in modified nutrient media, mg L⁻¹

Type, variety	Micropropagation coefficient					
	MS-26	MS-32	MS-22	MS-35	MS-46	MS-42
<i>H. macrophylla</i> ‘Nikko Blue’	2.59	3.65	4.58	5.61	5.11	1.18
<i>H. paniculata</i> ‘Grandiflora’	2.38	3.77	4.89	5.72	5.27	1.13
<i>H. arborescens</i> ‘Annabelle’	1.81	3.18	5.81	5.93	4.99	1.10

A high micropropagation coefficient for *H. paniculata* was obtained on both the MS-35 and MS-46 media, reaching 5.27. On the other media, this value was significantly lower. For *H. macrophylla*, high micropropagation coefficients were recorded on MS-35 and MS-46 media, at 5.61 and 5.11, respectively.

To further increase the micropropagation coefficient, studies were conducted to improve the culture media by supplementing MS-35 with carbohydrates and iron. Sucrose, glucose, fructose, and maltose were added to the modified MS-35 medium at concentrations ranging from 10 to 40 g L⁻¹. The greatest number of shoots was obtained when 3.0 mg L⁻¹ glucose and sucrose were added to the culture medium (Table 6).

When sucrose was used, the number of shoots ranged from 4.1 at a concentration of 1.0 mg L⁻¹ to 6.1 at 3.0 mg L⁻¹. As the sucrose concentration increased to 4.0 mg L⁻¹, the number of shoots decreased to 1.9. Taking into account the *LSD*₀₅ value of 0.22 for the number of shoots, differences between treatments were considered statistically significant when the difference between the means exceeded the corresponding *LSD*₀₅ value. When glucose was used, the number of shoots increased from 3.4 at 1.0 mg L⁻¹ to a maximum of 6.3 at 3.0 mg L⁻¹, whereas at 4.0 mg L⁻¹ it decreased to 4.2. The *LSD*₀₅ for glucose was 0.24, indicating statistically significant differences among the concentration treatments. The lowest number of shoots (1.1) was obtained when 4.0 mg L⁻¹ fructose was added to the culture medium. The analysis revealed a statistically significant effect

Table 6. Morphogenesis of *Hydrangea* explants depending on the type of carbohydrate added to the modified MS-35 nutrient medium (average values)

Source of carbohydrates and their content, mg L ⁻¹	Number of explants, pcs.	Average number of shoots, pcs.	Average shoot length, cm
Sucrose			
1.0	62	4.1 ^c	1.6 ^d
2.0	75	5.4 ^b	4.2 ^c
3.0	68	6.1 ^a	7.8 ^a
4.0	71	1.9 ^d	7.1 ^b
Glucose			
1.0	65	3.4 ^d	0.7 ^d
2.0	72	5.5 ^b	4.1 ^c
3.0	65	6.3 ^a	6.6 ^a
4.0	69	4.2 ^c	5.5 ^b
Fructose			
1.0	60	2.2 ^c	2.3 ^d
2.0	68	4.5 ^b	3.4 ^c
3.0	65	5.2 ^a	5.7 ^a
4.0	70	1.1 ^d	4.3 ^b
Maltose			
1.0	45	3.3 ^c	2.5 ^d
2.0	60	4.6 ^b	4.1 ^c
3.0	50	4.7 ^b	7.0 ^a
4.0	65	5.7 ^a	4.9 ^b
<i>LSD</i> ₀₅ sucrose		0.22	0.26
<i>LSD</i> ₀₅ glucose		0.24	0.21
<i>LSD</i> ₀₅ fructose		0.16	0.19
<i>LSD</i> ₀₅ maltose		0.22	0.23

Mean values within each carbohydrate source, denoted by different letters, differed significantly at *p* < 0.05. The *LSD*₀₅ was calculated separately for each carbohydrate source.

of fructose concentration on the number of shoots ($F(3, 8) = 44.36$; $p = 0.001$) and their mean length ($F(3, 8) = 12.42$; $p = 0.002$). The highest number of shoots (5.7) was obtained when 4.0 mg L^{-1} maltose was added to the culture medium, whereas at concentrations of 2.0 mg L^{-1} and 3 mg L^{-1} , the number of shoots was lower and nearly identical. For maltose, the effect of concentration was statistically significant for both the number of shoots ($F(3, 8) = 11.63$; $p = 0.003$) and shoot length ($F(3, 8) = 21.02$; $p < 0.001$). The results indicate that the effectiveness of carbohydrates in in vitro culture is determined not only by their presence as an energy source but also by the type of carbohydrate and its concentration.

The number of shoots is a more important parameter for in vitro plantlets than shoot length. However, shoot length was also assessed to evaluate the physiological effects of different carbohydrate sources on the growth and development of *Hydrangea* species in in vitro culture. Across all experimental treatments, shoot length was greatest at a concentration of 3.0 mg L^{-1} , regardless of the carbohydrate source. The longest shoots, measuring 7.8 cm, were obtained on MS-35 medium supplemented with 3.0 mg L^{-1} sucrose. At lower sucrose concentrations (1.0 mg L^{-1} and 2.0 mg L^{-1}) and at the higher concentration, shoot length was lower, measuring 1.6, 4.2, and 7.1 cm, respectively. The differences between treatments were statistically significant ($LSD_{05} = 0.26 \text{ cm}$; $p < 0.05$).

A similar trend was observed for the other carbohydrate sources. For example, at a glucose concentration of 1.0 mg L^{-1} , the mean shoot length was only 0.7 cm, whereas increasing the concentration to 2.0 mg L^{-1} resulted in an increase to 4.1 cm. The maximum shoot length of 6.6 cm was obtained at a concentration of 3.0 mg L^{-1} . With a further increase in the glucose concentration to 4.0 mg L^{-1} , shoot length decreased to 5.5 cm. With an LSD_{05} of 0.21 cm ($p < 0.05$), all values differed significantly from one another.

To investigate the effect of iron (Fe) on the growth and development of *H. macrophylla* explants, iron was added to the culture medium at concentrations ranging from 2.25 to 2.95 mg L^{-1} (Table 7). The source of Fe was non-chelated iron sulfate (FeSO_4). The concentrations listed are expressed as elemental Fe and refer to Fe added to the culture medium in the form of FeSO_4 . The pH of the medium was 4.70.

The studies revealed an effect of iron concentration in the MS-35 culture medium on the morphogenesis of *H. macrophylla* explants. Compared with the control, the number of shoots in plants grown on MS-35 medium supplemented with FeSO_4 was

Table 7. Morphogenesis of explants of representatives of the species *H. macrophylla* depending on the iron content in the modified nutrient medium MS-35 (average data)

Iron concentration in the medium, mg L^{-1}	Number of explants pcs.	Average number of shoots, pcs.	Average shoot length, cm
Without iron - control	45	3.8	1.95
2.25	55	6.27	3.95
2.60	60	8.36	4.62
2.95	57	6.76	4.55
LSD_{05}		0.31	0.19

Values denoted by different letters differ significantly according to the LSD_{05} criterion. Values denoted by different letters within the same parameter differ significantly at $p < 0.05$, whereas values denoted by the same letter do not differ significantly.

1.65–2.2 times greater than that in the treatment without FeSO_4 (Fig. 3). The mean number of shoots increased from 6.27 to 8.36 as the iron concentration increased from 2.25 to 2.60 mg L^{-1} , i.e. by 2.09 shoots, or 33.3%. The difference between the treatments was statistically significant ($LSD_{05} = 0.31$; $p < 0.05$).

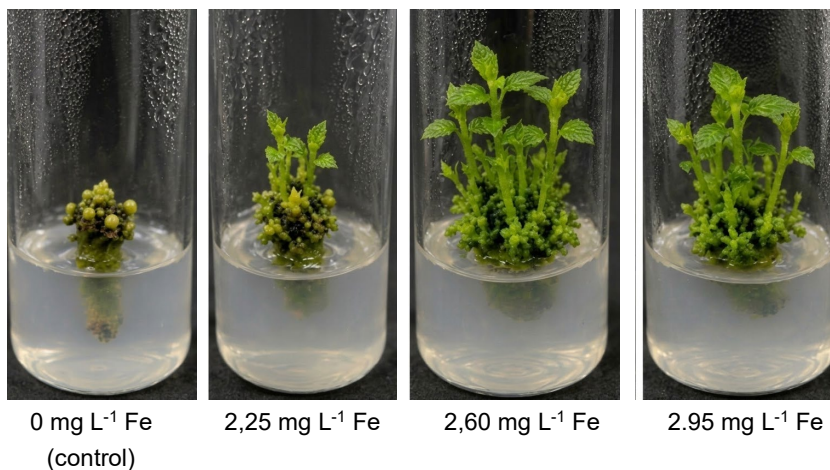


Figure 3. Morphogenesis of *H. macrophylla* explants depending on the iron content in the modified nutrient medium MS-35 (Scale bar – 1 cm).

The highest number of shoots, 8.36, was obtained when iron was added to the medium at a concentration of 2.60 mg L^{-1} , which exceeded the control value by 4.56 shoots. A further increase in the Fe concentration to 2.95 mg L^{-1} , however, was accompanied by a decrease in the mean number of shoots to 6.76. Compared with the concentration of 2.60 mg L^{-1} , this value decreased by 1.60 shoots, or 19.1%. This indicates the existence of an optimal level of iron supply for the expression of the morphogenetic potential of *H. macrophylla* explants.

A similar, albeit less pronounced, pattern was observed for shoot length. In the control treatment, the mean shoot length was 1.95 cm, whereas at iron concentrations of 2.25 and 2.60 mg L^{-1} , it increased to 3.95 and 4.62 cm, respectively. When the iron concentration was increased to 2.95 mg L^{-1} , shoot length was 4.55 cm, which was 0.07 cm shorter than that at a concentration of 2.60 mg L^{-1} . Since the difference between these values was less than the LSD_{05} of 0.19 cm, it was not statistically significant ($p > 0.05$). Thus, unlike the number of shoots, for which an increase in Fe concentration from 2.60 to 2.95 mg L^{-1} resulted in a statistically significant decrease, shoot length remained statistically unchanged at these concentrations. This indicates that an excessive increase in iron concentration primarily affected shoot formation, whereas the growth of already formed shoots was not significantly affected.

The analysis revealed a statistically significant effect of iron concentration on both the mean number of shoots ($F(3, 8) = 32.84$; $p < 0.001$) and their mean length ($F(3, 8) = 28.67$; $p < 0.001$).

DISCUSSION

All culture media contain compounds belonging to several classes in varying amounts and ratios (Greenway et al., 2012; Chauhan & Jindal, 2020). Culture media for in vitro plant cultivation generally contain macronutrients, micronutrients, vitamins, and organic compounds (Mehbub et al., 2022).

The presence of plant growth regulators – auxins, cytokinins, and gibberellins – is a key factor controlling the differentiation and morphogenesis of isolated tissues. By adjusting the ratios and concentrations of these substances, it is possible to regulate the organogenesis of explants in a targeted manner. The most commonly used auxins include α -naphthaleneacetic acid (NAA) at concentrations of 0.1–2.0 mg L⁻¹, indole-3-butyric acid (IBA) at 0.1–5.0 mg L⁻¹, indole-3-acetic acid (IAA) at 1.0–30.0 mg L⁻¹, and 2,4-dichlorophenoxyacetic acid (2,4-D) at 1.0–10.0 mg L⁻¹ (Lysak, 2023). Cytokinins alleviate apical dominance and induce the development of axillary buds, thereby stimulating the growth of dormant organs. Exogenous cytokinins are required for morphogenetic responses in isolated tissues, promoting differentiation in callus cultures and the formation of shoot buds and shoots. These compounds also regulate somatic embryogenesis and plant development, delay organ senescence, and enhance resistance to adverse environmental conditions. Gibberellins, particularly gibberellic acid (GA₃), promote stem and leaf elongation, induce seed germination, and break dormancy; in micropropagation, they are typically applied at concentrations of 0.1–0.5 mg L⁻¹ (Kushnir & Sarnatska, 2005).

Evaluation of the effectiveness of growth regulators in different regeneration processes, including axillary shoot formation and root induction, showed that the highest axillary shoot proliferation coefficient, 10.7, was achieved on Murashige and Skoog (MS) medium supplemented with 2.0 mg L⁻¹ 6-benzyladenine (6-BA), 0.2 mg L⁻¹ indole-3-butyric acid (IBA), and 1.0 mg L⁻¹ gibberellic acid (GA₃) (Ledbetter & Preece, 2004).

Díaz García et al. reported that the best proliferation rates (1.8–2.0 shoots per explant) for *H. macrophylla* were achieved by adding 1–3 mg L⁻¹ BAP to the culture medium without auxin, with an increase in the number of shoots observed from the third subculture onwards. Our study also revealed a gradual increase in the micropropagation coefficient with increasing numbers of passages, from 0.0 in the first passage to 5.75 in the fourth passage. This trend may be associated with the transition of the culture from the initial establishment phase to stable axillary bud proliferation and the accumulation of morphogenetically active material.

The results obtained are comparable to those reported by Hamama et al. (2011), who investigated the effect of maltose on the regeneration of *H. macrophylla* under different light conditions. The use of 110 mM maltose (approximately 37.6 g L⁻¹) in combination with low light intensity (17 μ mol m⁻²·s⁻¹) promoted regeneration processes. This is consistent with the findings of the present study regarding the effectiveness of maltose as a carbohydrate source, although direct comparison of the results requires consideration of differences in carbohydrate concentrations, light conditions, and cultivation protocols. Recent research by Druege and Chamas (2026) also indicates the complex physiological role of carbohydrates in *H. macrophylla*. They found that glucose, fructose, and sucrose are involved in regeneration processes, and that their accumulation and utilisation depend on genotype and cultivation conditions. At the same

time, glucose may have not only a metabolic but also a signalling function, influencing physiological processes in plants. Furthermore, the additional supply of sugars did not affect different *H. macrophylla* genotypes equally, confirming the complexity of the regulation of regeneration processes and indicating that a specific carbohydrate concentration should not be considered universally applicable to all genotypes.

In a study by Xiao et al. (2021) investigating the effects of carbohydrate source, Fe concentration, and medium acidity on photosynthesis and growth of *H. macrophylla*, plants grown with the addition of FeSO_4 at a final Fe concentration of 2.78 mg L^{-1} and a medium pH of 4.70 had the largest leaves and the greatest number of leaves. Our results are generally consistent with these findings, as an increase in the Fe concentration in the culture medium was also accompanied by improved growth parameters in *Hydrangea*. However, the optimal Fe concentration in our study was 2.60 mg L^{-1} , which was slightly lower than that reported by Xiao et al. (2021). This difference may be related to the composition of the basal culture medium, plant genotype, cultivation conditions, and evaluation criteria.

From a practical standpoint, the results indicate that increasing the micropropagation coefficient of *Hydrangea* requires comprehensive optimisation of the micropropagation protocol. Simply selecting an appropriate concentration of 6-BAP is not sufficient. Proliferation efficiency is determined by the interaction between growth regulators, the duration of subculturing, the carbon source, and mineral nutrition. The most promising approach identified in this study was the combination of MS-35 medium with sequential subculturing, an optimised carbon source, and the addition of FeSO_4 . At the same time, differences between genotypes indicate that this protocol requires adaptation depending on the *Hydrangea* species or cultivar.

CONCLUSION

It was determined that during micropropagation of *Hydrangea* species, the lowest micropropagation coefficient, averaged across cultivars, was observed on the MS-42 medium and was 1.14, whereas the highest value, 5.75, was recorded on MS-35 medium.

The micropropagation coefficient was found to depend on the biological characteristics of the *Hydrangea* species. The highest value was recorded for *H. arborescens*, reaching 5.93. A high micropropagation coefficient for *H. paniculata* was obtained on both MS-35 and MS-46 media, with values of 5.72 and 5.27, respectively. To assess the effect of culture medium modifications on micropropagation efficiency, carbohydrates and iron were added to the MS-35 medium. The highest number of shoots, 6.3 per explant, was obtained with the addition of 3.0 mg L^{-1} glucose. A nearly identical number of shoots was obtained with the addition of 3.0 mg L^{-1} sucrose. The lowest number of shoots (1.1 per explant) was recorded with the addition of 4.0 mg L^{-1} fructose. The addition of FeSO_4 to the culture medium had a positive effect on shoot number and shoot length. The number of shoots was 1.65–2.2 times greater in plants treated with FeSO_4 than in those grown without its addition. The highest number of shoots, 8.36 per explant, was obtained when 2.60 mg L^{-1} Fe was added to the medium, which was higher than the values obtained with the addition of carbohydrates. The greatest shoot length was also observed at this Fe concentration, reaching 4.62 cm. Therefore, the addition of iron in a plant-available form, combined with a medium pH

of 4.70, provides optimal conditions for the growth and development of *H. macrophylla* plants cultivated in vitro. Overall, the results demonstrate that the efficiency of hydrangea micropropagation depends on the composition of the culture medium and the concentrations of its components; however, different genotypes may exhibit specific morphophysiological responses to changes in medium composition. Therefore, to ensure efficient micropropagation, the composition of the culture medium should be selected and optimised with consideration of the species- and cultivar-specific characteristics of the plants. The studies conducted have made it possible to identify optimal culture medium compositions and concentrations of their components under specific cultivation conditions; however, these findings should not be considered universal for all hydrangea genotypes. The observed differences in the responses of different plant species to culture medium composition indicate the need for further research to determine the optimal concentration ratios of medium components for specific plant genotypes. Further testing of the developed protocols for in vitro propagation of Hydrangea, followed by assessment of the regenerative capacity and establishment of the resulting plant material, would be a promising direction for future research.

REFERENCES

- Arafa, A.M.S., Nower, A.A., Helme, S.S. & Abd-Elaty, H.A. 2017. Large Scales of Hydrangea macrophylla Using Tissue Culture Technique. *International Journal of Current Microbiology and Applied Sciences* **6**(5), 776-778. <https://doi.org/10.20546/ijcmas.2017.605.087>
- Amrutha, N.P., Kaberi, M., Palai, S.K., Sashikala, B., Jagadev, P.N., Pradyumna, T. & Abhiram, D. 2025. Standardization of an efficient protocol for rapid in vitro micropropagation of hydrangea (*Hydrangea macrophylla* Thunb. Ser). *Plant Science Today* **12**(4). <https://doi.org/10.14719/pst.11685>
- Babu, G.A., Mosa Christas, K., Kowsalya, E., Ramesh, M., Sohn, S.I. & Pandian, S. 2022. Improved sterilization techniques for successful in vitro micropropagation. In: Gupta, S., Chaturvedi, P. (eds) *Commercial Scale Tissue Culture for Horticulture and Plantation Crops*. Springer, Singapore. https://doi.org/10.1007/978-981-19-0055-6_1
- Bak, S. & Han, T.H. 2024. Evaluation of genetic diversity using simple sequence repeat markers and analysis of cross compatibility in hydrangeas. *Horticulture, Environment, and Biotechnology* **65**, 1043–1052. <https://doi.org/10.1007/s13580-024-00629-z>
- Bak, S. & Han, T.H. 2026. Evaluation of Culture Media for In Vitro Propagation of *Hydrangea arborescens* Based on Quantitative and Qualitative Assessment. *Horticultrae* **12**(5), 599. doi: <https://doi.org/10.3390/horticultrae12050599>
- Chauhan, A. & Jindal, T. 2020. Microbiological culture media: types, role and composition. In: *Microbiological Methods for Environment, Food and Pharmaceutical Analysis*. Springer, Cham. https://doi.org/10.1007/978-3-030-52024-3_3
- Greenway, M.B., Phillips, I.C., Lloyd, M.N., Hubstenberger, J.F. & Phillips, G.C. 2012. A nutrient medium for diverse applications and tissue growth of plant species in vitro. *In Vitro Cellular & Developmental Biology-Plant* **48**, 403–410. <https://doi.org/10.1007/s11627-012-9452-1>
- Hamama, L., Voisine, L., Peltier, D. & Boccon-Gibod, J. 2011. Shoot regeneration and genetic transformation by *Agrobacterium tumefaciens* of *Hydrangea macrophylla* Ser. leaf discs. *Scientia horticultrae* **127**(3), 378-387. doi: <https://doi.org/10.1016/j.scienta.2010.09.021>
- Hasnain, A., Naqvi, S.A.H., Ayesha, S.I., Khalid, F., Ellahi, M., Iqbal, S., Hassan, M.Z., Abbas, A., Adamski, R., Markowska, D., Baazeem, A., Mustafa, G., Moustafa, M., Hasan, M.E. & Abdelhamid, M.M. 2022. Plants in vitro propagation with its applications in food, pharmaceuticals and cosmetic industries; current scenario and future approaches. *Frontiers in Plant Science* **13**, 1009395. <https://doi.org/10.3389/fpls.2022.1009395>

- Khaing, M.T., Jung, H.J. & Han, T.H. 2018. Effect of plant growth regulators on regeneration and proliferation of *Hydrangea macrophylla* cultivars. *Flower Research Journal* **26**(3), 84–89. <https://doi.org/10.11623/frj.2018.26.3.01>
- Kushnir, H.P., Sarnatska, V.V. 2005. *Microclonal propagation of plants*. Kyiv: Naukova Dumka, 271 pp.
- Ledbetter, D.I. & Preece, J.E. 2004. Thidiazuron stimulates adventitious shoot production from *Hydrangea quercifolia* Bartr. leaf explants. *Scientia Horticulturae* **101**(1–2), 121–126. <https://doi.org/10.1016/j.scienta.2003.09.014>
- Labidi, R., Bayoudh, C., Majdoub, A., Lopez-Corrales, M. & Mars, M. 2023. In vitro propagation of caprifig and figs (*Ficus carica* L.) through various explants. *Journal of Applied Horticulture* **25**(3), 263–268. doi: <https://doi.org/10.37855/jah.2023.v25i03.47>
- Lysak, Yu.S. 2023. Features of the technology of clonal propagation of hazelnuts by cuttings in vitro and ways to increase its efficiency. *Scientific Bulletin of UNFU* **33**(6), 33–47. <https://doi.org/10.36930/40330605>
- Majumder, S., Igamberdiev, A.U. & Debnath, S.C. 2025. Somaclonal variation and clonal fidelity in commercial micropropagation: challenges and perspectives. *Agronomy* **15**(6), 1489. <https://doi.org/10.3390/agronomy15061489>
- Mehbub, H., Akter, A., Akter, M.A., Mandal, M.S.H., Hoque, M.A., Tuleja, M. & Mehraj, H. 2022. Tissue culture in ornamentals: cultivation factors, propagation techniques, and its application. *Plants* **11**(23), 3208. <https://doi.org/10.3390/plants11233208>
- Nesterova, N.H. & Chornobrov, O.Yu. 2015. Regenerative capacity of explants of *Hydrangea macrophylla* L. plants in vitro. *Biology, achievements and hopes: materials of the IV All-Ukrainian scientific and practical conference of students, postgraduates and young scientists*. Kyiv, 66–77.
- Nesterova, N.H. & Chornobrov, O.Yu. 2016. Peculiarities of introducing explants of *Hydrangea macrophylla* L. plants into in vitro conditions. *Bulletin of the KhNAU* **2**, 139–145.
- Permadi, N., Nurzaman, M., Alhasnawi, A.N., Doni, F. & Julacha, E. 2023. Managing lethal browning and microbial contamination in *Musa* spp. tissue culture: Synthesis and perspectives. *Horticulturae* **9**(4), 453. <https://doi.org/10.3390/horticulturae9040453>
- Redhwan, A., Acemi, A. & Ozen, F. 2023. Effects of plant growth regulators on in vitro seed germination, organ development and callogenesis in *Pancreaticum maritimum* L. *Plant Cell, Tissue and Organ Culture* **154**, 97–110. <https://doi.org/10.1007/s11240-023-02514-6>
- Ruffoni, B., Sacco, E. & Savona, M. 2012. In Vitro Propagation of *Hydrangea* spp. In: Lambardi, M., Ozudogru, E., Jain, S. (eds) *Protocols for Micropropagation of Selected Economically-Important Horticultural Plants. Methods in Molecular Biology* **994**. Humana Press, Totowa, NJ. https://doi.org/10.1007/978-1-62703-074-8_18
- Sebastian, T.K. & Heuser, C.W. 1987. In vitro propagation of *Hydrangea quercifolia* Bartr. *Scientia Horticulturae* **31**(3–4), 303–309. [https://doi.org/10.1016/0304-4238\(87\)90056](https://doi.org/10.1016/0304-4238(87)90056)
- Sharma, N., Kumar, N., James, J., Kalia, S. & Joshi, S. 2023. Strategies for successful acclimatization and hardening of in vitro regenerated plants: Challenges and innovations in micropropagation techniques. *Plant Science Today* **10**(2), 90–97. <https://doi.org/10.14719/pst.2376>
- Wang, S., Yan, J., Ha, B. & Bai, Y. 2021. An Efficient Plant Regeneration System of *Hydrangea bretschneideri* Dipp via Stem Segments as Explants. *Phyton-International Journal of Experimental Botany* **90**(2), 595–604. doi: <https://doi.org/10.32604/phyton.2021.013693>
- Xiao, J., Guo, G., Jeong, B.R. 2021. Iron Supplement-Enhanced Growth and Development of *Hydrangea macrophylla* In Vitro under Normal and High pH. *Cells* **10**(11), 3151. <https://doi.org/10.3390/cells10113151>