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RESEARCH PAPER

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DIFFERENTIAL EFFECTS OF PLANT GROWTH REGULATORS AND CARBOHYDRATES ON *in vitro* PROPAGATION OF *Scutellaria barbata* D. DON

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ABSTRACT

Experiments were conducted to establish the procedure for sterilizing single-node explants from the mother plant of barbed skullcap *Scutellaria barbata* L7 line (characterized by high scutellarin content) grown in the greenhouse and to induce organogenesis. The effect of different PGRs and carbohydrates on shoot number and shoot length was investigated. The largest number of shoots per explant (16.4) was formed after treatment with 3 mg·dm^{-3} BAP. Shoot multiplication occurred most intensively on medium with the combination of 0.09 M sucrose and 1 mg·dm^{-3} KIN (9.8 shoots per explant), and their elongation on the medium with 1 mg·dm^{-3} GA_3 (10.0 cm). The rhizogenesis process was intensified by using 2 mg·dm^{-3} IBA (87%). Regenerated, rooted plants were acclimatized to *ex vitro* conditions, planted in pots, and placed in a greenhouse.

Keywords: micropropagation, shoot multiplication, explant, organogenesis, barbed skullcap, PGR

INTRODUCTION

Scutellaria L. is a genus that includes over 350 species distributed worldwide [Wang et al. 2012]. The most famous species of this genus include *Scutellaria baicalensis* Georgi and *Scutellaria lateriflora* L. This genus also includes the barbed skullcap (*Scutellaria barbata* D. Don), which is valuable for its adaptogenic and healing properties. *Scutellaria barbata* naturally occurs mainly in northern China, Korea, and Japan. This perennial plant in its natural habitats can grow up to 50 cm. The leaves are lanceolate or triangular in shape and are about 3 cm long. The flower is 1 cm long, the color is purple-blue, slightly hairy [Wang et al. 2020]. The name *Scutellaria* comes from the Latin word ”scutella” meaning a cup, or a shield, this is the shape of the products of the calyx cover of *Scutellaria* flowers. It blooms from May to July. It occurs in wet meadows, near ponds and streams [Wang et al. 2012]. In recent years, studies have been carried out on the chemical composition of *S. barbata*, which have proven the presence of numerous compounds that are

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used in the treatment of human diseases and ailments. *S. barbata* contains alkaloids, steroids, flavonoids, diterpenoids, volatile oils, polysaccharides, and aromatic components. The great importance has been attributed to neo-clerodane diterpenoids from *S. barbata* due to their anti-inflammatory, antiviral, and antitumor effects [Feng et al. 2021, Li et al. 2023]. Single chemical compounds or extracts from *S. barbata* have anticancer activity against gynecological cancer cells, ovarian cancer, breast cancer, prostate cancer, liver cancer, lung cancer, skin and blood cancer [Perez et al. 2010, Wang et al. 2012, Brearley et al. 2014, Sun et al. 2024]. Flavonoids like scutellarin, carthamidin, apigenin, and luteolin are mainly responsible for the anticancer properties of the plant [Chen et al. 2012, Gao et al. 2019, Lema-Rumińska et al. 2023]. According to numerous studies, *S. barbata* also has strong cardiovascular, antibacterial, antiviral, anti-inflammatory, and antioxidant activity, can alleviate memory deficits and neuronal damage, and possesses insecticidal activity [Chen et al. 2020].

Due to the great interest in herbal medicines, the demand for herbal raw materials is growing. Intensive exploitation of herbs in their natural environment, together with human impact on nature, may lead to a reduction in the population of these valuable species. Tissue and cell culture enable the preservation of biological diversity and may be helpful in the rational management of natural resources. By properly selecting micropropagation methods, we can obtain plants with preserved genetic integrity with the mother plant, which ensures high quality of the medicinal raw material, also allows for controlled breeding conditions and obtaining a larger number of healthy plants. Moreover, thanks to this technique, it is also possible to eliminate diseases and pathogens that appear in traditional cultivation methods, which contributes to increasing the safety and quality of plant raw materials [Pant 2014, Brearley et al. 2014].

As demonstrated in the study by Pasternak and Steinmacher [2024] for the optimization of *in vitro* culture, the balance of nutrients and the presence of competent cells that can differentiate into stem cells, and the regulation of endogenous synthesis of hormones such as auxins and their distribution in the plant are also important. Auxin is a hormone responsible for the processes of shoot and root morphogenesis *in vitro*. It can be transported polarly, create gradients, and determine cell functions involved in the formation of all plant organs, including primary and lateral roots [Roychoudhry and Kepinski 2022]. Auxins and cytokinins are the main regulators of plant growth and development. Acting at low concentrations, which exclude their nutritional effect, they influence plant growth and development. In addition, cytokinin and auxin regulate the synthesis of each other, often act as an antagonistic hormone pair, showing a mutual feedback mechanism, which is important for many developmental processes in plants [Kurepa and Smalle 2022]. The main effect of cytokinin in *in vitro* tissue culture is the induction of shoots. It should be noted that cytokinins induce axillary as well as adventitious shoot formation from meristematic explants. The physiological function of cytokinins is, among others, the stimulation of cell division. In addition, they activate RNA synthesis and stimulate protein synthesis and affect enzyme activity. Application of cytokinins effectively limits the length of shoots while increasing their number, limits the surface area of leaves, and stimulates the formation of meristematic centers [Mishra et al. 2019, Hnatuszko-Konka et al. 2021, Pasternak and Steinmacher 2024, Figas et al. 2025].

Among the factors that have the greatest influence on the processes of growth and multiplication in *in vitro* cultures is the type and concentration of the carbon source applied to the medium. This factor influences the number of plants obtained in the process of micropropagation [Brearley et al. 2014]. In *in vitro* culture conditions, carbohydrates are a source of carbon that is used as energy, and regulate the osmotic potential for various physiological processes requiring energy [Yaseen et al. 2013, Naidu Mahadev et al. 2014, Van den Ende 2014]. Carbohydrates can protect against stress factors, and they participate in the regulation of defense reactions of plants exposed to stress. They have an osmoprotective effect and participate in signal transmission [Ciereszko 2018].

Previous studies on *S. barbata* conducted by Lema-Rumińska et al. [2023] included seven new genotypes with significant differences in morphology and metabolite content. However, these studies did not examine the effect of growth regulators on micropropagation and rooting rates. Therefore, our study complements earlier studies and focuses on the L7 line, which is the most efficient in terms of the valuable metabolite (scutellarin) content.

In the described experiment, the influence of different plant growth regulators (PGRs) and carbon source was investigated to determine the most efficient and effective micropropagation protocol for the new L7 line of *S. barbata,* which will help in rapid reproduction of desired lines of plant with valuable medicinal properties and increase the herbal biomass production for pharmaceutical and medical industries.

MATERIAL AND METHODS

The research was conducted at the Department of Biotechnology of the Bydgoszcz University of Science and Technology (Poland). The plant material (ten homogeneous plants from the L7 line) came from previous studies published by Lema-Rumińska et al. [2023]. The explants used for the research were single-node shoot fragments (explants) of *S. barbata* isolated from mother plants growing in greenhouse conditions.

Preliminary test

A preliminary test was performed to determine the most effective and consistent sterilization procedure for the plant material used in the *in vitro* culture of *S. barbata*. In the first stage of disinfection, the explants were rinsed in running water with detergent, then they were placed in 70% C_2H_5OH (Chempur, Piekary Śląskie, Poland) for 1 minute. In the next stage, the explants were treated with sodium hypochlorite – NaClO (Warchem, Zakręt, Poland) solutions at concentrations of 2.0%, 3.0%, and 5.0% with the addition of Tween 20 – 300 µL/100 mL (Sigma-Aldrich, Burlington, MA, USA) for 12 minutes. The process of disinfection of shoot explants was completed by rinsing the disinfected shoot fragments three times in sterile double-distilled water. The control was explants sterilized with only 70% C_2H_5OH (EtOH). Sterile explants were inoculated into MS medium [Murashige and Skoog 1962] without PGR. The composition of the medium used was: 4.4 $g \cdot dm^{-3}$ MS basal medium (Sigma-Aldrich, Burlington, MA, USA), 30 $g \cdot dm^{-3}$ sucrose (Chempur, Piekary Śląskie, Poland), 7 $g \cdot dm^{-3}$ agar (Vitro LAB-AGAR, BioMaxima, Lublin, Poland). The pH of the medium was set at 5.8. In each of the sterilization variants 10 single-node shoot fragments were inoculated onto the medium. *In vitro* cultures were grown at a temperature of 24 ±1 °C, light intensity of 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (fluorescent lamps L36W/77, OSRAM, Munich, Germany) and photoperiod of 16 h light/8 h dark in test tubes, each containing 20 mL of solidified MS medium and one explant. The effectiveness of the disinfection process was determined 14 days after inoculation of the explants onto the culture medium.

Induction of axillary bud development

Stimulation of the development of axillary buds on nodal explants derived from mother plants, sterilized with NaClO at the concentration selected in the preliminary experiment, was carried out on the MS medium with 3 $mg \cdot dm^{-3}$ 6-benzylaminopurine (BAP), 1 $mg \cdot dm^{-3}$ BAP + 0.5 $mg \cdot dm^{-3}$ 1-naphthaleneacetic acid (NAA), or 0.5 $mg \cdot dm^{-3}$ BAP + 1.0 $mg \cdot dm^{-3}$ NAA. The control medium was MS without growth regulators; 10 explants were placed on each medium variant, and the culture was carried out for 12 weeks. This stage of the culture was carried out in test tubes, each containing 20 mL of solidified MS medium and one explant (Table 1).

Shoot multiplication

Shoots obtained by stimulating the development of axillary buds were divided into nodal fragments and transferred in the amount of 30 in each variant to propagation medium containing BAP, gibberellic acid (GA_3) and kinetin (KIN) in the amount of 1 $mg \cdot dm^{-3}$ and 1 $mg \cdot dm^{-3}$ BAP, KIN, thidiazuron (TDZ), zeatin (ZEA) supplemented with 0.1 $mg \cdot dm^{-3}$ NAA (Table 2) and 0.09 M sucrose or glucose as a carbon source. The explants were placed into Erlenmeyer flasks containing 25 mL of solidified MS medium, with three explants allocated per flask. After 12 weeks of culture the number of shoots obtained in the multiplication process was counted. Observations of the plant's shape and measurement of the length of shoots were also carried out. The morphological traits were assessed by manual measurements with the aid of millimeter-scale graph paper.

Induction of root formation

The shoots were transferred to rooting medium (MS) supplemented with 30 $g \cdot dm^{-3}$ sucrose, with

Table 1. Efficiency of stimulation of the development of axillary buds on the nodal explants of *S. barbata*

PGR (mg·dm^{-3})		Number of explants	Mean number of shoots/explant	Mean length of shoots (cm)
BAP	NAA			
0.0	0.0	10	4.6 ±2.63	1.52 ±0.82
3.0	0.0	10	16.4 ±6.01	0.53 ±0.32
1.0	0.5	10	9.0 ±3.74	2.48 ±1.14
0.5	1.0	10	6.8 ±4.26	1.14 ±0.55
$LSD_{0.05}$		–	2.167	0.295

Results are mean ± SD (standard deviation); LSD – the lowest significant difference (Tukey's confidence half-interval) at $p \leq 0.05$; PGR – plant growth regulators; BAP – 6-benzylaminopurine; NAA – 1-naphthaleneacetic acid

1 or 2 mg·dm^{-3} indole-3-butyric acid (IBA) and indole-3-acetic acid (IAA), 30 shoots were used for each medium variant and cultured in a growth room for 12 weeks in Erlenmeyer flasks containing 25 mL of solidified MS medium, with three shoots per flask.

Acclimatization of rooted shoots

The rooted shoots were adapted to the greenhouse conditions. The plants were placed in containers filled with a substrate consisting of a mixture of horticultural substrate it contains high peat (0–6 mm), supplemented with neutralizing minerals, multicomponent fertilizer, specialized micronutrient fertilizer and Hydrofil (Hartmann, Poznań, Poland), sand, and perlite (Biovita, Tenczynek, Poland) in the proportions 2:1:1. The acclimatization process lasted 14 days and took place in multi-pots under a foil tent to limit transpiration, and then the plants were planted in pots with gardening substrate containing high peat (0–20 mm), supplemented with mineral neutralizers and multicomponent fertilizer (Hartmann, Poznań, Poland) and placed in greenhouse conditions.

Statistical analysis

The obtained analytical results were statistically processed in MS Excel and Statistica 13.3. In the case of results obtained from the micropropagation of *S. barbata*, analysis of variance was performed in a completely random design. At the stage of bud induction and rooting, one-way analysis of variance was used to determine the number of shoots or roots per explant and the length of shoots or roots. Two-way analysis of variance was performed to determine the number of shoots per explant and shoot length during the shoot multiplication phase. Using Tukey's single confidence intervals for the significance level at $p \leq 0.05$, the significance of differences (the lowest significant difference, LSD) was determined.

RESULTS AND DISCUSSION

Preliminary test

Sterilization efficiency of nodal shoot fragments was achieved at 80% using 5% NaClO for 12 min. In the case of a 2% NaClO solution, the disinfection efficiency was lower, it was 40%, slightly better results were achieved when a 3% NaClO solution was used, in this case, the disinfection efficiency was 60%. Of the tested explants, only some of them started growth, the highest number of explants able to start growth and development was recorded in the two highest NaClO concentrations, i.e. 3% and 5%. Sterile explants could not be obtained when only EtOH was used (Fig. 1). The results are consistent with the literature, which indicates that NaClO solutions are used for the elimination of contaminants, although their effect may be due to the concentration, action, and time of treatment [Mganga et al. 2025]. Effective sterilization of explants with active Cl was also achieved in *S. barbata* [Lema-Rumińska et al. 2023], *Leonurus sibiricus* L. [Figas et al 2025]. 10% NaClO has been successfully used to sterilize the stem node and shoot tip of lavender (*Lavandula* sp.) [Kara and Baydar 2012]. NaClO has a disinfecting effect also at lower concentrations. 4% NaClO for the sterilization of nodal segments was used by lavender (*Lavandula angustifolia* Mill.) [Kumari et al. 2024].

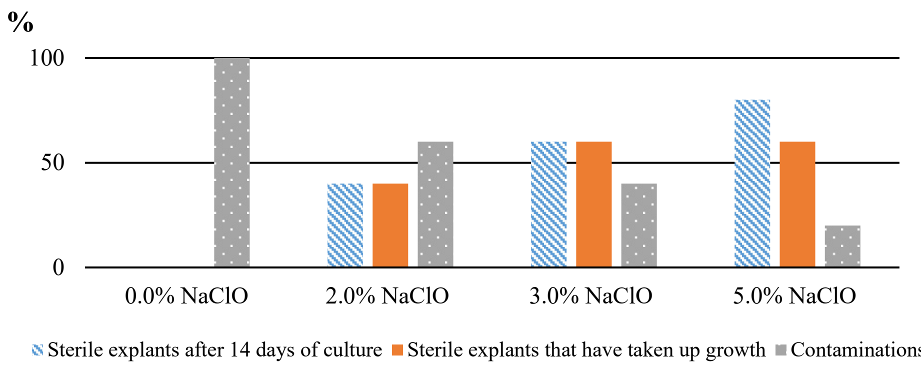

Fig. 1. Effectiveness of the methods used to sterilize *S. barbata* single-node explants

Induction of axillary bud development

The stimulation of the development of axillary buds was performed on MS medium with 3 mg·dm^{-3} BAP, 1 mg·dm^{-3} BAP + 0.5 mg·dm^{-3} NAA, or 0.5 mg·dm^{-3} BAP + 1.0 mg·dm^{-3} NAA. Significant differences were observed in the number and shape of the obtained shoots between the variants of the medium used. The highest effectiveness of inducing this process was found for 3 mg·dm^{-3} BAP. In this variant, it was recorded that one explant yielded on average over 16 shoots, which were characterized by short internodes and a small surface of leaf blades. Lower efficiency of shoot induction was observed on medium with 1 mg·dm^{-3} BAP + 0.5 mg·dm^{-3} NAA or 0.5 mg·dm^{-3} BAP + 1.0 mg·dm^{-3} NAA, where an average of 9 and 6.8 shoots per explant were obtained, respectively. The least useful for induction was the medium without growth regulators (average 4.6 shoots/explant). Measurements and observations were carried out after 12 weeks of culture. The longest shoots were observed on the medium containing 1 mg·dm^{-3} BAP + 0.5 mg·dm^{-3} NAA (2.48 cm) and on the control medium (1.52 cm), whereas the shortest shoots were recorded on the medium with 3 mg·dm^{-3} BAP (0.53 cm), see Table 1. Numerous literature data confirm this regarding the influence of the presence of cytokinins and cytokinins combined with auxins on the efficiency of shoot formation induction.

As other authors have shown, the presence of BAP in the medium is effective in shoot induction in various plant species. In the case of *Phytolacca dodecandra* L'Heit, the authors analyzed the effect of concentration ranging from 2.22 μM BAP to 22.2 μM BAP and achieved 80% shoot induction efficiency on nodal explants and 70% on shoot apical explants at the lowest doses [Daksa et al. 2015]. Good results were also obtained in studies on *Scutellaria alpina* L., where a high average number of axillary shoots was obtained [Grzegorczyk-Karolak 2015].

Analyzing the effect of different BAP concentration on micropropagation of *Scutellaria integrifolia* L., the maximum number of shoots from shoot apices (23 per explant) was obtained on MS medium supplemented with 2.2 μM BAP [Joshee et al. 2007]. In the case of *S. baicalensis* the highest average number of shoots (5.8) was, achieved for the MS + 1.0 mg·dm^{-3} BAP [Dyduch-Siemińska and Gawroński 2024]. Considering the commonly used cytokinins, the application of 5 μM BAP in *in vitro* culture of *S. barbata* and *Scutellaria racemosa* Pers. also resulted in obtaining numerous shoots with nodal explants [Brearley et al. 2014]. The stimulating effect of BAP increases with increasing concentration in Shine Muscat grapevine *in vitro* culture [Kim et al. 2023]. The rate of regeneration process in the presence of different BA doses ranged from 90.4% to 97.8%. The highest efficiency was observed for 2 μM BA, at higher concentration the rate of bud induction decreased. On the other hand, increasing BA concentration increased the number of shoots obtained from nodal explants from 1.05 at a concentration of 1.0 μM BA up to 1.38 at a concentration of 8 μM BA, however, the highest concentration (16 µM BA) turned out to be less useful in this case because the number of shoots per nodal explant was 1.29 [Kim et al. 2023].

Shoot multiplication

Shoot multiplication occurred most intensively on medium with sucrose under the influence of 1 mg·dm^{-3} KIN, where approximately 10 shoots per explant were obtained on average. Other results were presented by Grzegorczyk-Karolak et al. [2015] in the *in vitro* culture of *S. alpina* where the most effective cytokinin was BAP at a concentration of 2 µM, with the highest average number of axillary shoots (25.0 per explant). Kinetin supported the overall increase in shoot length, but did not have such a pronounced effect on the number of shoots as BAP [Grzegorczyk-Karolak 2015]. The combination of two cytokines, BAP and TDZ, effectively induced shoot organogenesis in *in vitro* cultures of *Scutellaria araxensis* Grossh. Optimal effects were obtained using BAP (0.5–2 mg·dm^{-3}) with TDZ (0.1–1.5 mg·dm^{-3}), which were more effective than the combinations of BAP with auxins (NAA and IBA) [Gharari et al. 2022].

In our research, the highest multiplication efficiency was obtained for KIN. BAP also has a positive effect on shoot proliferation, although its action was less effective compared to KIN. The use of BAP at a concentration of 1 mg·dm^{-3} contributed to the improvement of shoot growth, but this effect was less pronounced than in the case of KIN. The longest shoots of *S. barbata* were obtained on the medium with 0.09 M sucrose and 1 mg·dm^{-3} GA_3, where the average shoot length was 10 cm. GA_3 has also been successfully used for elongation *in vitro* cultures of

Table 2. Effect of different PGRs and carbohydrates on shoot number and shoot length of *S. barbata*

Carbohydrate	MS medium and PGR (mg·dm^{-3})	Mean number of shoot/explant	Mean length of shoots (cm)
0.09 M sucrose	1.0 BAP	6.73 ±3.90	4.60 ±3.01
	1.0 GA_3	1.70 ±0.96	10.00 ±2.36
	1.0 KIN	9.80 ±3.68	5.00 ±2.77
	1.0 BAP +0.1NAA	6.10 ±3.93	4.20 ±3.21
	1.0 KIN +0.1NAA	6.50 ±3.86	6.00 ±3.40
	1.0 TDZ +0.1NAA	4.70 ±2.84	4.30 ±2.52
	1.0 ZEA +0.1NAA	5.00 ±3.38	4.20 ±2.89
Mean		5.78 ±2.12	5.47 ±1.81
0.09 M glucose	1.0 BAP	4.90 ±1.90	4.00 ±2.02
	1.0 GA_3	1.30 ±0.71	6.20 ±1.48
	1.0 KIN	6.00 ±2.78	4.20 ±1.22
	1.0 BAP +0.1NAA	4.90 ±2.56	3.60 ±2.05
	1.0 KIN +0.1NAA	4.70 ±2.02	4.70 ±2.10
	1.0 TDZ +0.1NAA	4.30 ±2.07	4.10 ±2.08
	1.0 ZEA +0.1NAA	4.20 ±2.01	3.80 ±2.45
Mean		4.33 ±1.26	4.37 ±0.76
$LSD_{0.05}$ for PGR (I)		0.462	0.456
$LSD_{0.05}$ for sugars (II)		1.278	1.291
Interaction I/II		0.435	0.460
Interaction II/I		1.746	1.848

Results are mean ± SD (standard deviation); LSD – the lowest significant difference (Tukey's confidence half-interval) at $p \leq 0.05$; MS – Murashige and Skoog medium; PGR – plant growth regulator; BAP – 6-benzylaminopurine; NAA – 1-naphthaleneacetic acid; GA_3 – gibberellic acid; KIN – kinetin; TDZ – thidiazuron; ZEA – zeatin

Fig. 2. Shoot multiplication of *S. barbata* on Murashige and Skoog medium [1962] with carbon source: sucrose (A), glucose (B), the rooted plants of *S. barbata* on MS medium with 2 mg·dm^{-3} IBA (C) and 2 mg·dm^{-3} IAA (D), acclimatized plants of *S. barbata* in greenhouse conditions (E), (bar = 1 cm)

S. araxensis [Gharari et al. 2022], *Dietes bicolor* Stend. [Silva 2020], and *Dalbergia latifolia* Roxb. [Boga et al. 2012]. GA_3 affects stem elongation when plants are exposed to different temperatures between day and night [Andini et al. 2020]. This growth phytohormone promotes cell expansion and, even at low concentration, enhances the activity of various cytokinins, thereby facilitating *in vitro* shoot and root elongation, multiple shoot induction, and cell differentiation [Ahmad et al. 2021]. The physiological function of gibberellins is cell elongation by changing the elasticity of the cell wall. They plays an important role in seed germination, stem elongation, leaf expansion and reproductive development [Shani et al. 2013, Teszlák et al. 2013]. In our study, the number of shoots obtained and their length on medium with sucrose were higher than with glucose (Table 2, Fig. 2A, B). In contrast to studies of Brearley et al. [2014], which prove that the addition of fructose or glucose to the medium results in a higher average shoot in *S. barbata* than the addition of sucrose. However, for *S. racemosa*, the results for sucrose after 21 days of culture were higher together with maltose compared to other carbon sources. It follows that the selection of the appropriate type of carbohydrate depends on the plant species and the type of explant. Sucrose is a commonly used carbon source in *in vitro* cultures. In most plant species, sucrose is the main form of assimilated carbon produced during photosynthesis. Long-distance sucrose distribution from the green source tissues to energy-demanding sink tissues is mediated by phloem [Naidu Mahadev et al. 2014, Bavnhøj et al. 2023]. The increase in seedling growth rate and size is related to the volumetric increase in the cell, which is influenced by the osmotic pressure regulated by sucrose [Sumaryono et al. 2012]. Sucrose was used in the effective propagation of *Mentha piperita* L. [Sujana and Naidu 2011], *Stevia rebaudiana* Bertoni [Preethi et al. 2011], *Solanum nigrum* L. [Sridhar and Naidu 2011], and *Solanum viarum* Dunal [Naidu Mahadev et al. 2014]. The effect of sucrose is difficult to determine because autoclaving contributes to the hydrolysis of sucrose into glucose and fructose and a large amount of it is decomposed during seedling growth [Rahman et al. 2010]. As a result of hydrolysis during *in vitro* culture, the concentration of sucrose also decreases due to the action of invertase, the source of which is explants. By examining the composition of the medium during culture, it is possible to determine the presence of both sucrose and glucose and fructose [Naidu Mahadev et al. 2014]. The evaluation of the effect of different carbohydrates on the regeneration process was carried out by Akyüz [2025]. Among the carbohydrates tested for hybrid chestnut cultivar Marsol (*C. sativa* × *C. crenata*) sucrose and then glucose added to the medium resulted in the best regeneration. A much weaker effect was noted for the other analyzed sugar – maltose. Such a diverse effect of carbohydrates used in *in vitro* cultures results from the different ability of plants to metabolize these compounds. Sucrose in *in vitro* plant cultures is usually applied at a concentration of 2–3%. In banana (*Musa banana* L.) cultures, shoot induction was more effective on sucrose medium, while shoot multiplication efficiency was highest in the presence of both 3% sucrose and 3% glucose

[Madhulatha et al. 2006]. As described by Tarinejad and Amiri [2019] in micropropagation of grapevine (*Vitis vinifera* L.), 3% sucrose proved to be the best carbon source, enabling the highest cutting height and the maximum number of shoots obtained from one explant. It was found, that 3% sucrose is also the most useful carbohydrate for effective micropropagation of *Nauclea diderrichii* (De Wild.) Merr. [Pitekelabou et al. 2015] and kohlrabi (*Brassica oleracea* var. *gongylodes* L.) [Ćosić 2020].

Rooting and acclimatization

Auxins such as IAA and IBA were used in the rooting process of *S. barbata* plants. The main factors enabling root formation in *in vitro* cultures are intensive auxin biosynthesis in developing shoots and efficient auxin transport through vessels from the site of synthesis [Pasternak and Steinmacher 2024]. An important role in the process of shoot and root formation is played by the concentration of auxins in tissues and organs, and their reactivity [Koike et al. 2020, Roychoudhry and Kepinski 2022]. In our experiment, the elongated shoots were transferred to MS medium with 1 or 2 mg·dm^{-3} IBA and IAA. The best rooting response was observed on a rooting medium supplemented with 2.0 mg·dm^{-3} IBA (Fig. 2C), with the highest number of rooted plants – 26 and the highest average number of roots – 8 (Table 3). In the plant, endogenous hormones play a fundamental role in morphogenesis. Exogenous growth regulators are applied to ensure balance with endogenous hormones, thereby influencing physiological responses as stimulators of cell division and elongation [Nurhanis et al. 2019]. Exogenous IBA proved to be most useful for stimulating the rhizogenesis process. Similar results were obtained by authors studying *in vitro* plants of the *Scutellaria* genus, namely *S. integrifolia* [Joshee et al. 2007], *S. barbata*, *S. racemosa* [Brearley et al. 2014], *S. havanensis* [Irvin et al. 2021], *S. araxensis* [Gharari et al. 2022], and other species such as *Centella asiatica* (L.) [Panathula et al. 2014]. The use of auxins in different concentrations clearly influenced the number of rooted shoots and root morphology, which is confirmed by the results of Joshee et al. [2007]. They found out that a lower concentration of IBA (4.9 M) was more beneficial for the rooting of *S. integrifolia* plants than 9.8 M IBA, because the higher concentration also caused callusing and swelling in addition to rooting. In our study, the use of IAA in the rhizogenesis process in *S. barbata* did not have a significant effect on the number of roots, but 2 mg·dm^{-3} IAA led to the development of the longest roots – 9.0 cm (Table 3, Fig. 2D). As a main auxin, IAA is a key factor in cell elongation, which explains the observed results. Exogenously supplied IAA affects protein synthesis in plant tissues, changes in the permeability of cell walls occur, and cell division and elongation are intensified, which may result in increased root length [Nurhanis et al. 2019].

After 12 weeks, the rooted plants were transferred to the multi-pots under a foil tent. In the final phase of acclimatization, the plants were placed in individual pots and placed in a greenhouse (Fig. 2E). The accli-

Table 3. Rooting efficiency of shoots on different medium variants after 12 weeks of *S. barbata* culture

MS medium and PGR (mg·dm^{-3})	Number of rooted plants	Mean root length (cm)	Mean number of roots/explant
1.0 IBA	18	5.0	6.0
1.0 IAA	20	8.0	3.0
2.0 IBA	26	5.5	8.0
2.0 IAA	22	9.0	6.0
$LSD_{0.05}$	–	1.533	1.252

Results are mean ± SD (standard deviation); LSD – the lowest significant difference (Tukey's confidence half-interval) at $p \leq 0.05$; MS – Murashige and Skoog medium; PGR – plant growth regulator; IBA – indole-3-butyric acid; IAA – indole-3-acetic acid matization efficiency of *S. barbata* plants in the greenhouse was 60%.

CONCLUSIONS

An efficient plant regeneration system for the new L7 line of *S. barbata* (with the high scutellarin content) using nodal explants was developed. The conducted preliminary studies have shown the high effectiveness of 5% NaClO for the sterilization of nodal explants (80%). Among the growth regulators used, the highest effectiveness in the process of shoot development induction was demonstrated for 3 mg·dm^{-3} BAP. Shoot multiplication occurred most intensively on the medium with the combination of 0.09 M sucrose and 1 mg·dm^{-3} KIN, however, the highest degree of shoot elongation was observed after the application of 1 mg·dm^{-3} GA_3. Root formation was most intensive when the medium was supplemented with 2 mg·dm^{-3} IBA, although the longest roots were noted in the presence of 2 mg·dm^{-3} IAA. According to the presented results, the described micropropagation procedure for the L7 line of *S. barbata* may help in the rapid propagation of desired lines of plant with valuable medicinal properties and can be successfully used in commercial micropropagation.

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REFERENCES

Akyüz, B. (2025). Effect of different carbon sources and concentrations on in vitro propagation of chestnut. Plant Cell Tiss. Organ Cult., 160, 25. https://doi.org/10.1007/s11240-024-02960-w

Ahmad, A., Ahmad, N., Anis, M., Alatar, A.A., Abdel-Salam, E.M., Qahtan, A.A., Faisal, M. (2021). Gibberellic acid and thidiazuron promote micropropagation of an endangered woody tree (*Pterocarpus marsupium* Roxb.) using *in vitro* seedlings. Plant Cell Tiss. Organ Cult., 144, 449–462. https://doi.org/10.1007/s11240-020-01969-1

Andini, R., Luthfia, N., Rahmawati, M., Bakri, S., Akhir, J., M.I. Sulaiman, M.I. (2020). *In-vitro* propagation: application of gibberellic acid (GA_3) in enhancing germination in 'Jernang' or 'Dragon Blood' (*Daemonorops* sp.). The 1st International Conference on Agriculture and Bioindustry 2019 IOP Conf. Ser.: Earth Environ. Sci., 425, 012067. https://doi.org/10.1088/1755-1315/425/1/012067

Bavnhøj, L., Driller, J.H., Zuzic, L., Stange, A.D., Schiøtt, B., Pedersen, B.P. (2023). Structure and sucrose binding mechanism of the plant SUC1 sucrose transporter. Nat. Plants, 9, 938–950. https://doi.org/10.1038/s41477-023-01421-0

Boga, A., Ram, B., Reddy, G.R.S. (2012). Effect of benzyl amino purine and gibberellic acid on *in vitro* shoot multiplication and elongation of *Dalbergia latifolia* Roxb. An important multipurpose tree. Res. Article Biotechnol. Bioinf. Bioeng., 2(1), 597–602.

Brearley, T.A., Vaidya, B.N., Joshee, N. (2014). Cytokinin, carbon source, and acclimatization requirements for *in vitro* propagation of *Scutellaria barbata* D. Don and *Scutellaria racemosa* Pers. Am. J. Plant Sci., 5(24), 3662. https://doi.org/10.4236/ajps.2014.524382

Chen, Q., Rahman, K., Wang, S.J., Zhou, S., Zhang, H. (2020). *Scutellaria barbata*: a review of chemical constituents, pharmacological activities and clinical applications. Curr. Pharm. Des., 26(1), 160–175. https://doi.org/10.2174/1381612825666191216124310

Chen, V., Staub, R.E., Baggett, S., Chimmani, R., Tagliaferri, M., Cohen, I., Shtivelman, E. (2012). Identification and analysis of the active phytochemicals from the anti-cancer botanical extract Bezielle. PLOS ONE 7(1), e30107. https://doi.org/10.1371/journal.pone.0030107

Ciereszko, I. (2018). Regulatory roles of sugars in plant growth and development. Acta Soc. Bot. Pol., 87(2), 3583.

Ćosić, T., Savić, J., Raspor, M., Cingel, A., Ghalawnji, N., Vinterhalter, B., Ninković, S. (2020). Effects of different types of sugars and plant growth regulators on kohlrabi seedling growth and development *in vitro*. Arch. Biol. Sci., 72(3), 349–357. https://doi.org/10.2298/ABS200622029C

Daksa, J., Abera, B., Taddese, T. (2015). Micropropagation of *Phytolacca dodecandra* L'Herit (Endod var. E-44). Afr. J. Biotechnol., 14(2), 108–118. https://doi.org/10.5897/AJB2013.12008

Dyduch-Siemińska, M., Gawroński, J. (2024). The influence of cytokinin on the multiplication efficiency and genetic stability of *Scutellaria baicalensis* regenerants in in vitro culture conditions. Appl. Sci., 14(11), 4791. https://doi.org/10.3390/app14114791

Feng, X.-S., Yan, W., Bai, L.-H., Wang, K., Chen, X.-Q. (2021). *neo*-clerodane diterpenoids from the aerial parts of *Scutellaria barbata* with anti-inflammatory activity. Chem. Biodiv., 18(12), e2100693. https://doi.org/10.1002/cbdv.202100693

Figas, A., Tomaszewska-Sowa, M., Gruszka, Z. (2025). Cultivation of Siberian Motherwort plants (*Leonurus* *sibiricus* L.) in in vitro culture. Agronomy, 15(1), 183. https://doi.org/10.3390/agronomy15010183

Gao, J., Yin, W., Corcoran, O. (2019). From *Scutellaria barbata* to BZL101 in cancer patients: phytochemistry, pharmacology, and clinical evidence. Nat. Prod. Commun., 14(10), 1–12. https://doi.org/10.1177/1934578X19880645

Gharari, Z., Bagheri, K., Sharafi A. (2022). High-frequency adventitious shoot organogenesis from *in vitro* stem explants of *Scutellaria araxensis* Grossh. BioTechnologia, 103(2), 143–151. https://doi.org/10.5114/bta.2022.116208

Grzegorczyk-Karolak, I., Kuźma, Ł., Wysokińska, H. (2015). The effect of cytokinins on shoot proliferation, secondary metabolite production and antioxidant potential in shoot cultures of *Scutellaria alpina*. Plant Cell Tiss. Organ Cult., 122(3), 699–708. https://doi.org/10.1007/s11240-015-0804-5

Hnatuszko-Konka, K., Gerszberg, A., Weremczuk-Jeżyna, I., Grzegorczyk-Karolak, I. (2021). Cytokinin signaling and de novo shoot organogenesis. Genes, 12, 265. https://doi.org/10.3390/genes12020265

Irvin, L., Zavala Ortiz, Y., Rivera, K.R., Nanda Vaidya, B., Sherman, S.H., Batista, R.A., Negrón Berríos, J.A., Joshee, N., Arun, A. (2021). Micropropagation of rare *Scutellaria havanensis* Jacq. and Preliminary Studies on Antioxidant Capacity and Anti-Cancer Potential. Molecules, 26(19), 5813. https://doi.org/10.3390/molecules26195813

Joshee, N., Mentreddy, S., Yadav, A.K. (2007). Mycorrhizal fungi and growth and development of micropropagated *Scutellaria integrifolia* plants. Ind. Crops Prod., 25(2), 169–177. https://doi.org/10.1016/j.indcrop.2006.08.009

Kara, N., Baydar, H. (2012). Effects of different explant sources on micropropagation in lavender (*Lavandula* sp.). J. Essent. Oil Bear. Plants, 15(2), 250–255. https://doi.org/10.1080/0972060X.2012.10644043

Kim, S.-H., Zebro, M., Jang, D.-C., Sim, J.-E., Park, H.-K., Kim, K.-Y., Bae, H.-M., Tilahun, S., Park, S.-M. (2023). Optimization of plant growth regulators for *in vitro* mass propagation of a disease-free 'Shine Muscat' grapevine cultivar. Curr. Iss. Mol. Biol., 45(10), 7721–7733. https://doi.org/10.3390/cimb45100487

Koike, I., Watanabe, S., Okazaki, K., Hayashi, K.I., Kasahara, H., Shimomura, K., Umehara, M. (2020). Endogenous auxin determines the pattern of adventitious shoot formation on internodal segments of ipecac. Planta, 251(3), 73. https://doi.org/10.1007/s00425-020-03367-5

Kumari, S., Fatmi, U., Shukla, P. (2024). Sterilization protocol of nodal explants for *in vitro* propagation of lavender (*Lavandula angustifolia).* Plant Arch., 24(2), 1053–1057. https://doi.org/10.51470/PLANTARCHIVES.2024.v24.no.2.148

Kurepa, J., Smalle, J.A. (2022). Auxin/cytokinin antagonistic control of the shoot/root growth ratio and its relevance for adaptation to drought and nutrient deficiency stresses. Int. J. Mol. Sci., 23, 1933. https://doi.org/10.3390/ijms23041933

Lema-Rumińska, J., Sadowska, K., Tymoszuk, A., Andrzejewska, J. (2023). Scutellarin and other metabolites as well as morphological and molecular characterization of the *Scutellaria barbata* lines from *in vitro* and *in vivo* cultivation. Ind. Crops Prod., 195, 116464. https://doi.org/10.1016/j.indcrop.2023.116464

Li, S.T., Xu, D., Jia, J., Zou, W., Liu, JY., Wang, Y., Zhang, K., Zheng, X., Ma, Y.-Y., Zhang, X., Zhao, D.-G. (2023). Structure and anti-inflammatory activity of neo-clerodane diterpenoids from *Scutellaria barbata*. Phytochem., 213, 113771. https://doi.org/10.1016/j.phytochem.2023.113771

Madhulatha, P., Kirubakaran, S.I., Sakthivel, N. (2006). Effects of carbon sources and auxins on *in vitro* propagation of banana. Biol. Plant., 50, 782–784. https://doi.org/10.1007/s10535-006-0131-0

Mganga, S.C., Karanja, B.K., Gesimba, R.M., Abihudi, S.A. (2025). Optimization of surface sterilization protocol for *in vitro* initiation of African star grass (*Hypoxis schimperi*). EJFOOD, 7(4), 44–48. https://doi.org/10.24018/ejfood.2025.7.4.931

Mishra, A.K., Tiwari, K.N., Mishra, P., Tiwari, S.K., Mishra, S.K., Saini, R. (2019). Effect of cytokinin and MS medium composition on efficient shoot proliferation of *Nyctanthes arbor-tristis* L. through cotyledonary node explant and evaluation of genetic fidelity and antioxidant capacity of regenerants. S. Afr. J. Bot., 127, 284–292. https://doi.org/10.1016/j.sajb.2019.09.008

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

Naidu Mahadev, M., Panathula, C., Naidu, C. (2014). Impact of different carbohydrates and their concentrations on in vitro regeneration of *Solanum viarum* (Dunal) – an important anticancer medicinal plant. Am. J. Plant Sci., 5(1), 200–204. http://dx.doi.org/10.4236/ajps.2014.51026

Nurhanis, S.E., Wulandari, R.S., Suryantini, R. (2019). Variations of IAA Concentration to the Growth of Sengon Tissue Culture. IOP Conference Series: Earth and Environmental Science, 394, The 2nd International Conference on Tropical Silviculture: Forest Research and Innovation for Sustainable Development 10–11 September 2019, Bogor, Indonesia.. https://doi.org/10.1088/1755-1315/394/1/012024

Panathula, C.S., Mahadev, M.D., Naidu, C.V. (2014). Effect of different carbohydrates on *in vitro* plant regeneration of *Centella asiatica* (L.) – an important antijaundice medicinal plant. I. J. Med. Aromat. Plants, 4(1), 41–47.

Pant, B. (2014). Application of plant cell and tissue culture for the production of phytochemicals in medicinal plants. In: R. Adhikari, S. Thapa (eds). Infectious Diseases and Nanomedicine II. Adv. Exp. Med. Biol., 808, 25–39. https://doi.org/10.1007/978-81-322-1774-9_3

Pasternak, T.P., Steinmacher, D. (2024). Plant growth regulation in cell and tissue culture *in vitro.* Plants, 13(2), 327. https://doi.org/10.3390/plants13020327

Perez, A.T., Arun, B., Tripathy, D., Tagliaferri, M.A., Shaw, H.S., Kimmick, G.G., Cohen, I., Shtivelman, E., Caygill, K.A., Grady, D., Schactman, M., Shapiro, C.L. (2010). A phase 1B dose escalation trial of *Scutellaria barbata* (BZL101) for patients with metastatic breast cancer. Breast Cancer Res. Treat., 120, 111–118. http://dx.doi.org/10.1007/s10549-009-0678-5

Pitekelabou, R., Aïdam, A.V., Kokou, K. (2015). Influence of various carbohydrates on the in vitro micropropagation of *Nauclea diderrichii* (De Wild &T. Durand) Merrill, an endangered forest species in Togo. Afr. J. Biotechnol., 14(15), 1283–1289. https://doi.org/10.5897/AJB2015.14412

Preethi, D., Sridhar, T.M., Naidu, C.V. (2011). Carbohydrate Concentration Influences *in vitro* Plant Regeneration in *Stevia rebaudiana*. J. Phytol., 3(5), 61–64.

Rahman, M.H., Islam, R., Hossain, M., Islam, M.S. (2010). Role of sucrose, glucose and maltose on conventional potato micropropagation. Int. J. Agric. Technol., 6(4), 733–739.

Roychoudhry, S., Kepinski, S. (2022). Auxin in root development. Cold Spring Harb. Perspect. Biol., 14(4), a039933. https://doi.org/10.1101/cshperspect.a039933

Shani, E., Weinstain, R., Zhang, Y., Castillejo, C., Kaiserli, E., Chory, J., Tsien, R.Y., Estelle, M. (2013). Gibberellins accumulate in the elongating endodermal cells of Arabidopsis root. Proc. Natl. Acad. Sci. U. S. A., 110(12), 4834–4839. https://doi.org/10.1073/pnas.1300436110

Silva, L.A.S., Costa, A., Batista, D., Silva, M., da Silva, M., da Costa Netto, A.P., Rocha, D.I. (2020). Exogenous gibberellin and cytokinin in a novel system for *in vitro* germination and development of African iris (*Dietes bicolor*). Revista Ceres., 67(5). https://doi.org/10.1590/0034-737x202067050008

Sridhar, T.M., Naidu, C.V. (2011). Effect of different carbon sources on *in vitro* shoot regeneration of *Solanum nigrum* (Linn). An important antiulcer medicinal plant. J. Phytol., 3(2), 78–82.

Sujana, P., Naidu, C.V. (2011). Impact of different carbohydrates on high frequency plant regeneration from axillary buds of *Mentha piperita* (L.). An important multipurpose medicinal plant. J. Phytol., 3(5), 14–18.

Sumaryono, Muslihatin, W., Ratnadewi, D. (2012). Effect of carbohydrate source on growth and performance of *in vitro* sago palm (*Metroxylon sagu* Rottb.) plantlets. HAYATI J. Biosci., 19(2), 88–92. https://doi.org/10.4308/hjb.19.2.88

Sun, J., Cao, Y., Liu, Q., Zhou, Z., Xu, Y., Liu, C. (2024). Chemical constituents, anti-tumor mechanisms, and clinical application. A comprehensive review on *Scutellaria barbata*. Molecules, 29, 4134. https://doi.org/10.3390/molecules29174134

Tarinejad, A., Amiri, S. (2019). Influence of plant growth regulators, carbohydrate source and concentration on micropropagation and other physiological traits of grape (*Vitis vinifera* L. cv. Shahroudi) under *in vitro* conditions. J. Plant. Physiol. Breed., 9(1), 75–82. http://dx.doi.org/10.22034/jppb.2019.10378

Teszlák, P., Kocsis, M., Gaál, K., Nikfardjam, M.P. (2013). Regulatory effects of exogenous gibberellic acid (GA_3) on water relations and CO_2 assimilation among grapevine (*Vitis vinifera* L.) cultivars. Sci. Hortic., 159, 41–51. https://doi.org/10.1016/j.scienta.2013.04.037

Van den Ende, W. (2014). Sugars take a central position in plant growth, development and stress responses. A focus on apical dominance. Front. Plant Sci., 5, 313. https://doi.org/10.3389/fpls.2014.00313

Wang, L., Chen, W., Li, M., Zhang, F., Chen, K., Chen, W. (2020). A review of the ethnopharmacology, phytochemistry, pharmacology, and quality control of *Scutellaria barbata* D. Don. J. Ethnopharmacol., 254, 112260. https://doi.org/10.1016/j.jep.2019.112260

Wang, T.S., Wang, S.Q., Xiao, D.L. (2012). A review of phytochemistry and antitumor activity of a valuable medicinal species: *Scutellaria barbata*. J. Med. Plant Res., 6(26), 4259–4275. https://doi.org/10.5897/JMPR012.295

Yaseen, M., Ahmad, T., Sablok, G., Standardi, A., Hafiz, I.A. (2013). Review: role of carbon sources for *in vitro* plant growth and development. Mol. Biol. Rep., 40, 2837–2849. https://doi.org/10.1007/s11033-012-2299-z