Cioloca Mihaela, et.al. International Journal of Engineering Research and Applications
www.ijera.com
ISSN: 2248-9622, Vol. 15, Issue 8, August 2025, pp 20-25

RESEARCH ARTICLE OPEN ACCESS

Micropropagation of sage (*Salvia officinalis* L.) plantlets obtained *in vitro* from seeds and shoots

Cioloca Mihaela*, Tican Andreea*, Popa Monica*
*National Institute of Research and Development for Potato and Sugar Beet, 2 Fundaturii Street, 500470, Braşov, Romania

ABSTRACT
This study aimed to establish suitable *in vitro* cultivation conditions for *Salvia officinalis* L., focusing on medium composition and sterilization parameters. Stem shoots and seeds were used as the initial explants. The surface sterilization protocol for stem shoots involved immersion in a 20% (v/v) commercial bleach solution (sodium hypochlorite, NaOCl) for 5 minutes, followed by 70% ethanol for 1 minute, and three consecutive rinses with sterile distilled water (5 minutes each). Seed sterilization required 10-minutes exposure to 20% (v/v) commercial bleach solution, followed by three 5-minutes rinses with sterile distilled water. Seed germination was successfully achieved on Woody Plant Medium (WPM) supplemented with 30 g/L sucrose, 1 mg/L indole-3-butyric acid (IBA), and 8.8 g/L agar. For shoot micropropagation from both stem-derived and seed-derived explants, the most favorable results were obtained using Murashige and Skoog (MS) medium enriched with 20 g/L sucrose, 0.5 mg/L naphthaleneacetic acid (NAA), 0.02 mg/L kinetin, 1 mg/L gibberellic acid (GA_3), 1 g/L activated charcoal, and 8.8 g/L agar. Cultures were maintained under controlled growth room conditions: a light intensity of 2000 lux, a photoperiod of 16 hours light/8 hours dark, and temperatures of 24 ± 2 °C during the day and 22 ± 2 °C during the night.

Keywords - explants sterilization, *in vitro* cultivation, micropropagation, nutrient medium, *Salvia officinalis*

Date of Submission: 01-08-2025 Date of acceptance: 11-08-2025

I. INTRODUCTION

Salvia officinalis derives its name from the Latin term "*salvare"*, meaning "to heal," reflecting its historical significance as one of the earliest medicinal plants utilized by humankind. Since antiquity, it has been regarded as a panacea, with the physician Galen attributing to it diuretic, tonic, and hemostatic properties. The medicinal use of sage essential oil dates back to the 16th century. Its therapeutic value was highly esteemed in Roman times, as illustrated by the proverb: "Why should a man die, when sage grows in the garden?" [1].

In traditional medicine, *Salvia* species are recognized for their therapeutic potential in the treatment of various human diseases [2]. Sage species are medicinal plants, rich in active compounds with exceptional pharmaceutical potential, and are also valued for their aromatic qualities and ornamental use [3]. Modern research supports sage's oncostatic and anticancer effects, including its ability to slow malignant tumor growth [4], as well as its potent antioxidant properties [5-7]. The leaf extract of *Salvia officinalis* exhibits a broad spectrum of biological activities, including antibacterial, antiviral, anti-inflammatory, and antioxidant properties [8]. Additionally, it has been reported to possess hypoglycemic activity [9].

Plant biotechnology techniques, specifically *"in vitro"* tissue culture methods, enable the production of standardized plant material and bioactive compounds regardless of external environmental conditions [5,8]. Various studies have explored micropropagation of *S. officinalis* using different explants, such as shoot tips [3,10,12], and nodal segments [3,9,11,12].

Regarding the composition of the culture medium used in various studies related to the *in vitro* cultivation of *Salvia officinalis*, for shoot growth and proliferation, the proposed medium formulations are mainly based on Murashige-Skoog medium [13], used without the addition of growth regulators [3,8,11,12], with the addition of cytokinins [11,12] or with the addition of cytokinins and auxins [3,8,9,10,12] in different concentrations.

Concerning the stimulation of seed germination, in different studies, culture media based on Murashige-Skoog (MS) medium with the addition of gibberellic acid in combination with auxin [9] or a cytokinin [8] were tested.

II. MATERIAL AND METHODS

2.1. Initiation of *in vitro* cultures from stem shoots

Actively growing stem shoots, measuring 10–15 cm in length, were collected from mother plants cultivated in a greenhouse at National Institute of Research and Development for Potato and Sugar Beet (NIRDPSB) Brașov. The shoots were first rinsed under running tap water for 15 minutes and then cut into smaller segments. Surface sterilization was carried out by immersing the segments in 20% (v/v) commercial bleach solution (sodium hypochlorite, NaOCl) for 5 minutes, followed by immersion in 70% ethanol for 1 minute. Subsequently, the nodal segments were rinsed three times with sterile distilled water, each rinse lasting 5 minutes. The segments were then dried on sterile paper towels that had been pre-sterilized in an oven at 180 °C for 2 hours. All procedures were conducted under aseptic conditions within a laminar airflow cabinet (Fig. 1).

Figure 1. Sterilization of sage explants (*Salvia officinalis* L.)

Following sterilization, the explants were prepared and inoculated on the culture medium. Preparation involved removing the leaves and part of the petiole (used to protect the buds during sterilization) followed by trimming the explants to the appropriate size. Explant fragments measuring approximately 1.5–2 cm were placed on the culture medium, ensuring correct polarity orientation. The nodal segments contained both axillary and apical buds. Inoculation was conducted under sterile conditions in a laminar airflow cabinet using sterile instruments on a surface disinfected with ethanol.

For initiating *in vitro* cultures, a Murashige-Skoog (MS) medium [13] supplemented with 20 g/L sucrose, 0.5 mg/L NAA (naphthaleneacetic acid), and solidified with 9 g/L agar was used. The pH of the medium was adjusted to 5.7 before autoclaving. The nutrient medium was dispensed into test tubes sealed with aluminum foil. Test tubes were sterilized in an oven at 180 °C for 2 hours, and the culture medium was autoclaved at 120 °C for 20 minutes.

After transferring the explants on medium, the culture vessels were incubated in a growth chamber under artificial lighting conditions provided by neutral-white LED tubes (luminous flux of 2300 lm, 2000 lux light intensity), with a 16-hour light and 8-hour dark photoperiod. The temperature was maintained at 24 ± 2 °C during the day and 22 ± 2 °C during the night.

2.2. Initiation of *in vitro* cultures from seeds

Sage seeds were sourced from donor plants in the *in vivo* medicinal plant collection of NIRDPSB, Brașov. Prior to inoculation, the seeds were subjected to a sterilization procedure that included rinsing under running tap water for 10 minutes, immersion in a 20% (v/v) commercial bleach solution (sodium hypochlorite, NaOCl) for 10 minutes, followed by three rinses with sterile distilled water at 5-minute intervals. After sterilization, seeds were placed in the dark for 20 hours in sterile distilled water with 5 mg/L GA_3 (gibberellic acid), at a temperature of 23 °C.

For *in vitro* germination, seeds were inoculated on Woody Plant Medium (WPM) [14] supplemented with 30 g/L sucrose and 1 mg/L IBA (indole-3-butyric acid), solidified with 8.8 g/L agar. The pH of the medium was adjusted to 5.7 before autoclaving. The nutrient medium was dispensed into test tubes, sealed with aluminum foil. Test tubes were sterilized in an oven at 180 °C for 2 hours, and the culture medium was autoclaved at 120 °C for 20 minutes.

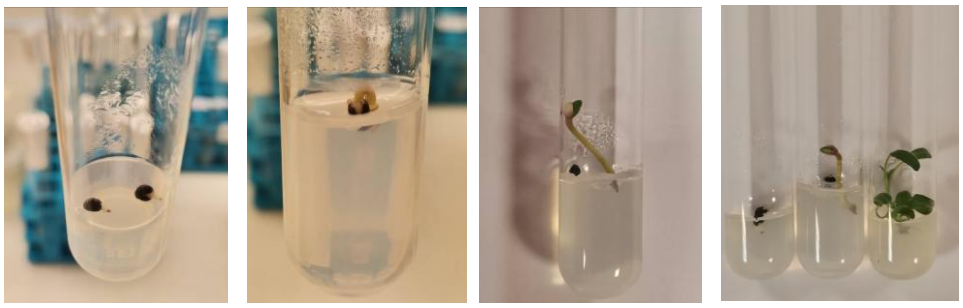

Figure 2. *In vitro* germination of *Salvia officinalis* L. seeds

Once inoculated on the nutrient medium, the seeds were incubated in a growth chamber under controlled conditions: 24 ± 2 °C during the day and 22 ± 2 °C during the night, with a 16-hour light and 8-hour dark photoperiod; neutral-white artificial lighting was provided with LED tubes (same as used for stem shoots). Under these conditions, sage seeds began germinating after 10 days, resulting in the regeneration of microplants (Fig. 2).

2.3. Establishment of shoot culture and microplants regeneration

The *in vitro* cultivation of sage shoots started from nodal explants regenerated from seeds and stem cuttings. They were inoculated on the culture medium, under sterile conditions, using 4 variants of nutrient medium (Table 1).

Table 1. The nutrient medium composition for *in vitro* cultivation of *Salvia officinalis* nodal explants

Components	**Nutrient medium variant**			
	Vo (control)	**V1**	**V2**	**V3**
	Amount (for 1 l medium)			
MS (g)	4.4	4.4	4.4	4.4
Sucrose (g)	20	20	25	30
Agar (g)	8.8	8.8	9	9
Activated charcoal (g)	-	1	1.5	2
NAA (mg)	0.5	0.5	0.5	0.5
Kin (mg)	-	0.02	-	-
BAP (mg)	-	-	1	1
GA_3 (mg)	-	1	0.5	1

MS –Murashige-Skoog medium; NAA – naphthaleneacetic acid; Kin – kinetin; BAP – benzyl aminopurine; GA_3 – gibberellic acid

Activated charcoal, with its fine pore structure, is frequently utilized in tissue culture [15,16] to enhance cell growth and development. It plays a crucial role in micropropagation, promoting rooting [15,16], stem elongation, anther and protoplast culture, [15]. The beneficial effects of activated charcoal on morphogenesis are likely due to its capacity to absorb toxic compounds [15, 17], thereby preventing their buildup in the culture medium.

Following explant inoculation on the culture medium, they were incubated in a growth chamber under controlled conditions: 24 ± 2 °C during the day and 22 ± 2 °C during the night, with a photoperiod of 16 hours light and 8 hours dark, at a light intensity of 2000 lux. The microcutting process was repeated at intervals of approximately 30 days.

About six weeks after inoculation on the four medium variants, the *in vitro* regenerated microplants were evaluated based on stem length, number of leaves per seedling, number of shoots, and main root length.

2.4. *In vitro* rooting of sage microcuttings

After six weeks of *in vitro* cultivation with the four medium variants, the sage explants failed to develop roots, prompting the initiation of a new experiment focused on inducing rhizogenesis.

The microshoots that had regenerated during the multiplication phase were transferred to MS culture medium, where the concentrations of macroelements, microelements, and vitamins were halved (MS½). This medium was supplemented

with 20 g/L sucrose, 20 mg/L yeast extract, 10 mg/L ascorbic acid, and 1 mg/L IBA. Agar (8.8 g/L) was included to solidify the nutrient medium, and the pH was adjusted to 5.8 prior to autoclaving, which was performed for 20 minutes at 120 °C.

III. RESULTS AND DISCUSSION

3.1. Establishment of shoot culture and plant regeneration

The response of *Salvia officinalis* explants varied across the four culture medium variants, depending on the parameter being assessed (Table 2).

Regarding plantlet height, V1 treatment produced the most favorable result, reaching an average of 5.39 cm, which represented a significant increase of 1.94 cm compared to the control group. This enhancement was attributed to the combined effect of 0.5 mg/L NAA, 0.02 mg/L kinetin, and 1 mg/L GA_3, supplemented with 1 g/L activated charcoal, which promoted the growth of sage microshoots.

Analysis of leaf number revealed that V3 variant exhibited the highest average, with 16.11 leaves. This superior performance was attributed to the hormonal treatment comprising 0.5 mg/L NAA, 1 mg/L benzylaminopurine (BAP), and 1 mg/L GA_3, supplemented with 2 g/L activated charcoal and 30 g/L sucrose. Compared to the control group, this represented a significant increase of 8.89 leaves.

V2 variant, containing 0.5 mg/L NAA, 1 mg/L BAP, 0.5 mg/L GA_3, and 1.5 g/L activated charcoal, resulted in the highest average number of shoots, at 3.83, representing a significant increase of 2.83 compared to the control. However, it is noteworthy that although the inclusion of 1 mg/L BAP in the culture medium, in combination with other growth regulators, enhanced both shoot and leaf proliferation, it also led to a reduction in their size and vigor compared to media formulations lacking this cytokinin.

Table 2. The evolution of sage microplants (*Salvia officinalis*) on different nutrient medium variants (after 6 weeks from the cultures initiation)

Culture medium	Plantlet heigh (cm)		
	Average/var.	Diff.	Signif.
Vo (Ct)	3.44	-	-
V1	5.39	1.94	*
V2	4.67	1.22	ns
V3	4.56	1.11	ns
	DL 5% = 1.40 cm; DL 1% = 2.12 cm; DL 0.1% = 3.41 cm		
	Leaf number		
	Average/var.	Diff.	Signif.
Vo (Ct)	7.22	-	-
V1	7.56	0.33	ns
V2	15.50	8.28	ns
V3	16.11	8.89	*
	DL 5% = 8.49; DL 1% = 12.86; DL 0.1% = 20.65		
	Shoot number		
	Average/var.	Diff.	Signif.
Vo (Ct)	1.00	-	-
V1	1.33	0.33	ns
V2	3.83	2.83	**
V3	2.67	1.67	ns
	DL 5% = 1.77; DL 1% = 2.68; DL 0.1% = 4.31		

3.2. *In vitro* rooting of sage microcuttings

The composition of the culture medium (specified at subheading 2.4.) induced the formation of roots, and after an interval of 7 weeks of cultivation under controlled conditions, sage microplants reached an average height of 4.12 cm,

an average number of 9.33 leaves/microplant and formed in an average number of 4 roots/microplant, the average root length being 4.26 cm (Fig. 3).

Figure. 3. The evolution of sage explants (*Salvia officinalis* L.) after 7 weeks from inoculation on the *in vitro* rooting medium

IV. CONCLUSIONS

In recent years, plant tissue culture techniques have gained considerable importance in areas such as plant propagation, disease elimination, genetic improvement, and the production of secondary metabolites. These techniques provide a controlled environment that optimizes growth and multiplication by regulating factors such as nutrient availability, culture medium pH, temperature, and photoperiod.

A suitable protocol for sterilizing the initial material used as a source of explants, as well as appropriate nutrient medium formulations necessary for the *in vitro* cultivation of *Salvia officinalis* microplants have been tested and established. The techniques and controlled conditions employed resulted in the successful *in vitro* production of sage microplants.

These microplants will be used as a source of explants to introduce *Salvia officinalis* into the *in vitro* preserved germplasm collection of the Research Laboratory for Plant Tissue Culture of NIRDPSB Brașov.

ACKNOWLEDGMENTS

This study was supported by the project ADER 4.1.1. (*Research on identifying effective in vitro conservation methods to ensure the preservation of germplasm biodiversity in potato, sweet potato, and medicinal plants*), financed by the Ministry of Agriculture and Rural Development, Romania.

REFERENCES

[1] L.S. Muntean, *Plante medicinale și aromatice cultivate în România* (Editura Dacia, Cluj-Napoca, 1990), p. 178.

[2] A. Marchev, C. Haas, S. Schulz, V. Georgiev, J. Steingroewer, T. Bley, A. Pavlov, Sage *in vitro* cultures: a promising tool for the production of bioactive terpenes and phenolic substances, *Biotechnol Lett.* 2014, *Feb;36(2)*:211-21, doi: 10.1007/s10529-013-1350-z. Epub 2013 Oct 16. PMID: 24129950.

[3] M. Papafotiou, G. Vlachou, A.N. Martini, Investigation of the Effects of the Explant Type and Different Plant Growth Regulators on Micropropagation of Five Mediterranean *Salvia* spp. Native to Greece. *Horticulturae* 2023, *9, 96*, https://doi.org/10.3390/horticulturae9010096.

[4] P. Kubatka, A. Mazurakova, L. Koklesova, T. Kuruc, M. Samec, K. Kajo, K. Kotorova, M. Adamkov, K. Smejkal, E. Svajdlenka, D. Dvorska, D. Brany, E. Baranovicova, V. Sadlonova, J. Mojzis, M. Kello, *Salvia officinalis* L. exerts oncostatic effects in rodent and *in vitro* models of breast carcinoma, *Front Pharmacol* 2024, *February, 23:15:1216199*, doi: 10.3389/fphar.2024.1216199. eCollection.

[5] I. Grzegorczyk, A. Matkowski, H. Wysokińska, Antioxidant activity of extracts from *in vitro* cultures of *Salvia officinalis* L., *Food Chemistry*, *Volume 104, Issue 2*, 2007, 536-541.

[6] P.C. Santos-Gomes, R.M. Seabra, P.B. Andrade, M. Fernandes-Ferreira, Determination of phenolic antioxidant compounds produced by calli and cell suspensions of sage (*Salvia officinalis* L.), *Journal of Plant Physiology*, *Volume 160, Issue 9*, 2003, 1025-1032.

[7] S. Fellah S., P.N. Diouf, M. Pétrissans, D. Perrin, M. Romdhane, M. Abderrabba, Chemical Composition and Antioxidant Properties of *Salvia officinalis* L. Oil from Two Culture Sites in Tunisia, *Journal of Essential Oil Research 18(5):*553-556, 2006, DOI: 10.1080/10412905.2006.9699165.

[8] I. Grzegorczyk, I. Bilichowski, E. Mikiciuk – Olasik, H. Wysokinska, *In vitro* cultures of *Salvia officinalis* L. as a source of antioxidant compounds, *Acta Societatis Botanicorum Poloniae, Vol. 74, No. 1:* 17-21, 2005, DOI: 10.5586/asbp.2005.003.

[9] M. Petrova, M. Nikolova, L. Dimitrova, E. Zayova, Micropropagation and evaluation of flavonoid content and antioxidant activity of *Salvia officinalis* L., *Genetics and Plant Physiology, Volume 5(1)*, 2015, 48–60.

[10] I. Grzegorczyk, H. Wysokinska, Liquid shoot cultures of *Salvia officinalis* L. for micropopagation and production of antioxidant compounds; effect of triacontanol, *Acta Societatis Botanicorum Poloniae, Vol. 77, No. 2:* 99-104, 2008, DOI:10.5586/asbp.2008.013.

[11] A.M. Radomir, R. Stan, I.C. Guță, M.L. Pandelea, D.E. Vizitiu, C.D. Neguț, Effect of gamma irradiation on *Salvia officinalis* L. and *Melissa officinalis* L. *in vitro* plants, *AgroLife Scientific Journal, 12(1)*, 2023, 158–165.

[12] G. Vlachou, A.N. Martini, M. Papafotiou, Effect of plant growth regulators and explant type on *in vitro* shoot multiplication of *Salvia officinalis*. *Acta Hortic. 1327*, 2021, 373-380, DOI: 10.17660/ActaHortic.2021.1327.50.

[13] T. Murashige, F.A. Skoog, A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant*. 1962, *15*, 473–497.

[14] G. Lloyd, B. McCown, Commercially-feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture, *Int. Plant Prop. Soc. Proc.* 30, 1981, 421-427.

[15] T.D. Thomas, The role of activated charcoal in plant tissue culture, *Biotechnology Advances 26*, 2008, 618–631, doi:10.1016/j.biotechadv.2008.08.003.

[16] M. Pan, J. van Staden, The effect of activated charcoal and auxins on root formation by hypocotyl segments of *Daucus carota*, *South African Journal of Botany* 2002, *68:* 349–356.

[17] J.C. Sorgato, J. Schultz Soares, C.R. Damiani, L.M. Ribeiro, Effects of light, agar, activated charcoal, and culture medium on the germination and early development of *Dendrobium* seedlings, *Australian Journal of Crop Science* 2020, *14(04):*557-564.