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Clonal micropropagation *in vitro* of essential oil plants of the family *Lamiaceae* Lindl.

Tetiana Manushkina*

PhD in Agrisciences
Mykolaiv National Agrarian University
54008, 9 Georgiy Gongadze Str., Mykolaiv, Ukraine
https://orcid.org/0000-0001-5843-271X

Tetiana Kachanova

PhD in Agrisciences
Mykolaiv National Agrarian University
54008, 9 Georgiy Gongadze Str., Mykolaiv, Ukraine
https://orcid.org/0000-0003-0032-3996

Mykola Samoilenko

Full Doctor in Agrisciences
Mykolaiv National Agrarian University
54008, 9 Georgiy Gongadze Str., Mykolaiv, Ukraine
https://orcid.org/0000-0002-3292-9717

Olena Petrova

PhD in Agrisciences
Mykolaiv National Agrarian University
54008, 9 Georgiy Gongadze Str., Mykolaiv, Ukraine
https://orcid.org/0000-0001-8612-3981

Abstract. The relevance of research on the development of biotechnology for clonal micropropagation of plants of the family *Lamiaceae* Lindl. is determined by the need for mass production of healthy, pure-grade planting material for the establishment of industrial plantations and the expansion of areas of essential oil crops in Ukraine. The aim of the research was to develop biotechnological methods of clonal micropropagation of essential oil plants of the *Lamiaceae* family – *Lavandula angustifolia* Mill., *Mentha x piperita* L., *Salvia officinalis* L. and *Monarda fistulosa* L. The task of the research was to select optimal conditions for effective cultivation of plants of the *Lamiaceae* family at four stages of clonal micropropagation. The main methods of research: laboratory, field, analytical, mathematical and statistical. The optimal nutrient media for the induction of *in vitro* morphogenesis and the multiplication stage were determined based on the basic medium of Murashige and Skoog: for *L. angustifolia* supplemented with kinetin (1.0 mg/l) and gibberellic acid (1.0 mg/l), for *M. x piperita* – with 6-benzylaminopurine (1.0 mg/l) and gibberellic acid (0.1 mg/l), for *S. officinalis* – with 6-benzylaminopurine (1.0 mg/l) and IOLK (0.5 mg/l), for *M. fistulosa* – 6-benzylaminopurine (1.0 mg/l) and β-indolyl-3-oleic acid (0.1 mg/l). At the stage of multiplication, it is advisable to carry out seven to eight cycles of cultivation. At the stage of rooting of microshoots, the most effective for all studied plant species was determined to be the Murashige and Skuga nutrient medium with a halved concentration of components, supplemented with β-indolyl-3-oleic acid (0.5 mg/l) and β-indolyl-3-acetic acid (0.5 mg/l). The optimal substrate for plant adaptation to *in vivo* conditions is peat: perlite in a volume ratio of 3:1. Incorporation of the developed biotechnology of

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*Corresponding author

clonal micropropagation into the seeding system of essential oil crops of the *Lamiaceae* family will allow to quickly obtain healthy pure-bred planting material and introduce new productive varieties into production

Keywords: *Lavandula angustifolia* Mill.; *Mentha x piperita* L.; *Salvia officinalis* L.; *Monarda fistulosa* L.; reproduction coefficient

INTRODUCTION

Family *Lamiaceae* Lindl. is one of the largest families of flowering plants, which includes about 250 genera and more than 7,000 species distributed throughout the world. It is considered an important source of essential oils and such valuable components as menthol, geraniol, eucalyptol, camphor and thymol (Mesquita *et al.*, 2019). Plants in this family are herbs or shrubs that produce large amounts of essential oil, which allows them to withstand high temperatures in summer (Raja, 2012).

Essential oils in different types of plants accumulate in different organs: flowers, leaves, fruits, seeds, rhizomes, and others. These volatile aromatic substances include a mixture of organic compounds: hydrocarbons, alcohols, monoterpenes, phenols, ethers, aldehydes, ketones, and organic acids (Rozhkov & Ogurcov, 2017; Dehsheikh *et al.*, 2020). The essential oil is obtained by steam distillation and has sedative, diuretic, tonic, antispasmodic and antiseptic properties (Raja, 2012). It is used in the pharmaceutical industry, perfumery, cosmetology and the food industry (Rozhkov & Ogurcov, 2017). Recent scientific studies have proven the antitumor properties of essential oils from plants of the *Lamiaceae* family (Mesquita *et al.*, 2019). The species of this family are promising potential sources of natural antioxidants due to their high content of polyphenols. In addition, increasing scientific and epidemiological evidence links consumption of polyphenol-rich foods to health benefits such as reduced risk of cardiovascular disease through anti-inflammatory effects (Tzima *et al.*, 2018). In Ukraine, narrow-leaved lavender, peppermint, clary sage, clary sage, monarda, hyssop, lemon balm, nepeta, rosemary, savory and others are cultivated as essential oil plants of the *Lamiaceae* family (Rozhkov & Ogurcov, 2017). In modern conditions, the agro-ecological advantages of growing plants of this family are important, such as the ability to grow on unproductive eroded soils, form stable phytocenoses on man-made disturbed lands, and act as phytomeliorants (Dobrovolskyi *et al.*, 2021).

Narrow-leaved lavender *Lavandula angustifolia* Mill. – perennial evergreen semi-shrub containing 1-2.5% of essential oils in inflorescences (Lis-Balchin, 2002; Manushkina, 2019). The main components of lavender essential oil are linalool (10-20%) and linalyl acetate (30-50%) (Küçük *et al.*, 2018; Pokajewicz *et al.*, 2021).

Peppermint *Mentha x piperita* L. is a perennial herb that is a hybrid type of mint that does not occur in the wild. Essential oils are contained in all above-ground organs of plants: leaves (2-4%), inflorescences (4-6%), stems (up to 0.3% of the mass of dry matter). Aerial parts of plants in a withered state or dry leaves are used as raw materials. The main components of peppermint essential oil are menthol (41-92%), menthone (9-25%), pinene, limonene and other substances, as well as phenolic compounds with antioxidant properties (Rozhkov & Ogurcov, 2017).

Medicinal sage *Salvia officinalis* L. is a multi-stemmed subshrub reaching a height of 80 cm. The main biologically active substances are phenolic compounds (flavonoids, tannins, hydroxycinnamic acids) and terpenoids (Jasicka-Misiak *et al.*, 2018). Various medicinal forms created on the basis of a combination of these groups of biologically active substances from plant raw materials are characterized by the high antimicrobial and antioxidant potential of medicines based on sage (Hudz *et al.*, 2020; Schmiderer & Novak, 2020; Francik *et al.*, 2020). The content of phenolic acids and flavonoids depends on the variety of plants and environmental conditions of cultivation (Ćavar Zeljković *et al.*, 2021; Karalija *et al.*, 2022).

Monarda fistulosa L. is a perennial herbaceous and ornamental plant. The essential oil has antiseptic, expectorant and repellent properties (Shanaida & Mashtaler, 2016). It belongs to non-official medicinal plants, it is not included in the State Pharmacopoeia of Ukraine, but it is promising for use as an essential oil plant (Shanaida & Pokryshko, 2015).

In connection with the mentioned economic and ecological advantages of essential oil plants of the *Lamiaceae* family, it is advisable to increase their area in Ukraine, in particular, to grow them as niche crops. The yield and the quality of essential oil depend significantly on the pureness of the planting material used for planting plantations. Since in many species of the *Lamiaceae* family, during seed reproduction, splitting is observed for economically valuable traits, vegetative reproduction is used in seed production. This method allows you to maintain the genetic identity of planting material, but has a low reproduction rate and a high probability of transmission of infectious diseases.

Currently, for effective vegetative reproduction of plants, it is advisable to use the method of clonal micropropagation in *in vitro* culture, which is characterized by a high reproduction ratio, preservation of the genotype, and recovery from pathogens of the planting material. According to the modern classification, two types of clonal micropropagation are distinguished - activation of the development of meristems already present in the

plant and induction of the development of buds or embryoids *de novo* by direct or indirect morphogenesis. For most plants, reproduction *in vitro* culture is carried out according to the first type, since it ensures the preservation of the genotype of the obtained seedlings identical to the original plants. Maintenance of genetic stability of apical meristems is ensured by a number of mechanisms: cells contain a diploid set of chromosomes, are maintained in an embryonically active state, they are organized in the form of discrete zones characterized by high activity of DNA repair systems and negative selection of altered cells. In connection with the above, research on the development of technological measures of clonal micropropagation of essential oil plants of the *Lamiaceae* family for the mass production of pure planting material is relevant.

The task of the research is to choose optimal conditions for effective cultivation of plants of the *Lamiaceae* family at four stages of clonal micropropagation.

The purpose of the research was to develop biotechnological methods of clonal micropropagation of essential oil plants of the *Lamiaceae* family L., *angustifolia* Mill., *M. x piperita L., S. officinalis* L., *M. fistulosa* L. The task of the research is to choose optimal conditions for effective cultivation of plants of the *Lamiaceae* family at four stages of clonal micropropagation.

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MATERIALS AND METHODS

The experimental work was carried out on the basis of the laboratory of clonal micropropagation of farming "Agrolife" (hereinafter referred to as Farm "Agrolife") of the Mykolaiv Region, a branch of the Department of Agriculture, Geodesy and Land Management of the Mykolaiv National Agrarian University. As material for research, lavender plants of the narrow-leaved L. *angustifolia* varieties Sinyeva, Hemus, Imperial Gem, peppermint *M. x piperita* L. varieties Lebedina pisnia, Mama, medicinal sage *S. officinalis* L. variety Shans, monarda tubular *Monarda fistulosa* L. Fortune variety were used.

The main methods of research: general scientific (analysis, synthesis, generalization) and special (laboratory, field). In the course of conducting experimental studies, methods generally accepted in plant biotechnology were used (Kalinin *et al.*, 1984; Kumar & Loh, 2012).

The development of biotechnological measures was carried out in four stages of clonal micropropagation: I stage - isolation of the explant, introduction and initiation of its development *in vitro*; II stage – multiplication; Stage III – rooting of microshoots; Stage IV – adaptation of microplants to *in vivo* conditions.

In stages I and II, Murashige and Skoog (MS) was used as the basic nutrient medium (Murashige & Skoog, 1962). At the III stage, the MS nutrient medium with a halved concentration of components (½ MS) was used as the base. At the first three stages of clonal micropropagation, the composition of hormones in the nutrient medium was changed in various combinations and concentrations to stimulate the necessary path of morphogenesis (Table 1).

Table 1. *Hormones included in nutrient media for clonal micropropagation of plants of the Lamiaceae family*

Full name	Symbolic designation
6-benzyloaminopurine	BAP
6-furfurilaminopurin	kinetin
gibberellic acid	GA
β-indolyl-3-butyric acid	IBA
β-indolyl-3-acetic acid	IAA

Source: *developed by authors*

The acidity of the medium was adjusted to pH 5.5-5.6 using 0.1 n HCl or 0.1 n KOH before autoclaving. The nutrient medium was autoclaved at a temperature of 120°C, a pressure of 0.8 atm. within 20 min.

Donor plants were grown in closed soil conditions. Shoots were separated from the plant, washed in a soap solution, washed in running water and in sterile distilled water. Then they were divided into segments with one pair of buds and staged sterilization was applied according to the scheme: ethanol 70% solution (40 s), sodium hypochlorite 1% solution (5 min). After sterilization, apical or axillary buds were isolated and introduced into *in vitro* culture. Aseptic work was carried out in a laminar box BBP02a. Isolation of explants was carried out under a MBS-9 binocular microscope and cultivated one at a time in chemical test tubes with a volume of nutrient medium of 10 ml.

At the stage of multiplication, microshoots, the development of which was initiated in the first stage, were divided into microcuttings 5-10 mm long with one pair of axillary buds, and additional microshoots were separated and cultivated in 250 ml vessels with a volume of 30 ml of nutrient medium. 6 explants were placed in one culture vessel.

At the stage of rooting, the main shoot of the obtained meristem plants was cut on a 5-10 mm long microcutting with one pair of leaves, and additional microshoots were also separated. Cultivated in vessels with a volume of 250 ml with a volume of nutrient medium of 30 ml for 6 microshoots. To stimulate rhizogenesis, auxins were added to the nutrient medium.

At three stages of *in vitro* clonal micropropagation, explants were cultured in a thermostatically controlled culture room. Cultivation conditions: temperature 25-26°C, illumination 2-3 klk, photoperiod 16 hours, relative humidity 60-70%.

At the stage of adaptation to *in vivo* conditions, micro-plants were grown in cassettes in climatic chambers at a temperature of 20-22°C and constant humidification, with periodic ventilation, the time of which was increased as the adaptation period increased. Substrates for cultivation in cassettes consisted of peat and perlite in different ratios by volume – 1:1; 2:1; 3:1; 4:1; 5:1. Adapted plants were transplanted into pots with a volume of 250 ml and cultivated in climatic chamber conditions for 30-56 days, depending on the type of plants.

The repetition of all experiments is twofold, the sample size is 20 plants.

The multiplication factor was calculated according to formula 1:

$$MF = \frac{Rf \times Nms \times Nls}{100} + \frac{Ffas \times Nas}{100}, \quad (1)$$

where *Rf* – regeneration frequency, %; *Nms* – number of main shoots, pcs.; *Nls* – number of pairs of leaves on the main shoot, pcs.; *Ffas* – frequency of formation of additional shoots, %; Nas – number of additional shoots, pcs.

Mathematical processing of the results was performed in the Microsoft Office Excel 2007 program using descriptive statistics (Rozhkov *et al.*, 2016). The statistical characteristics of quantitative variability were calculated: the arithmetic mean, the error of the arithmetic mean. The reliability of the difference between the mean values was determined by the Student's t-test.

RESULTS AND DISCUSSION

Stage I – isolation of the explant, introduction and initiation of its development *in vitro*. After the use of stepwise sterilization using ethanol and sodium hypochlorite, the infection rate of explants of all types of plants did not exceed 15%, the viability was at least 80%. The regeneration rate reached 90.0-100.0%. However, the hormonal composition of nutrient media significantly influenced the formation of shoots, and therefore, the reproduction coefficient, which is an integral parameter that includes the frequency of regeneration and formation of the main and additional shoots (Fig. 1).

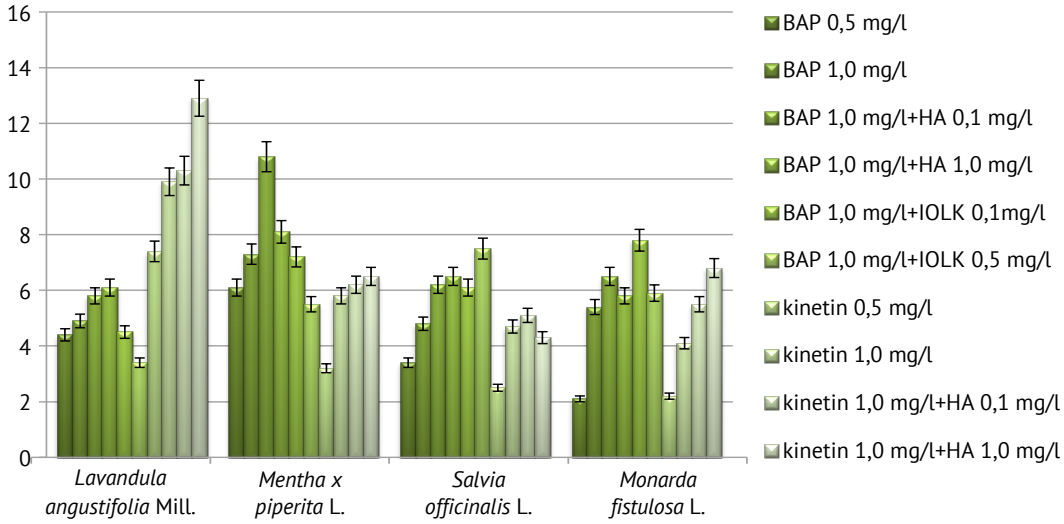

***Figure 1**. The coefficient of reproduction of plants of the Lamiaceae family at the stage of explant isolation, introduction and initiation of its development in vitro depending on the composition of hormones*
***Source**: developed by the authors*

It was determined that nutrient media based on the basic medium of MS are optimal for inducing morphogenesis *in vitro*: for *Lavandula angustifolia* Mill. supplemented with kinetin (1.0 mg/l) and HA (1.0 mg/l), for *Mentha x piperita* L. – BAP (1.0 mg/l) and HA (0. mg/l), for *Salvia officinalis* L. – BAP (1.0 mg/l) and IOLK (0.5 mg/l), for *Monarda fistulosa* L. – BAP (1.0 mg/l) and IOLK (0.1 mg/l).

II stage – multiplication. At the second stage, the multiplication of shoots is achieved in two ways. First, due to the addition of increased concentrations of cytokinins to the nutrient medium to remove apical dominance and stimulate the development of lateral and adventitious buds. Secondly, by carrying out several cycles of subcultivation until obtaining the required number of shoots. By combining these two ways, it is possible to achieve a multiplication factor of up to 105-106 per year (Kalinin *et al.*, 1984). At the multiplication stage, the effectiveness of the hormonal composition of the nutrient media was evaluated by the reproduction coefficient indicator (Fig. 2).

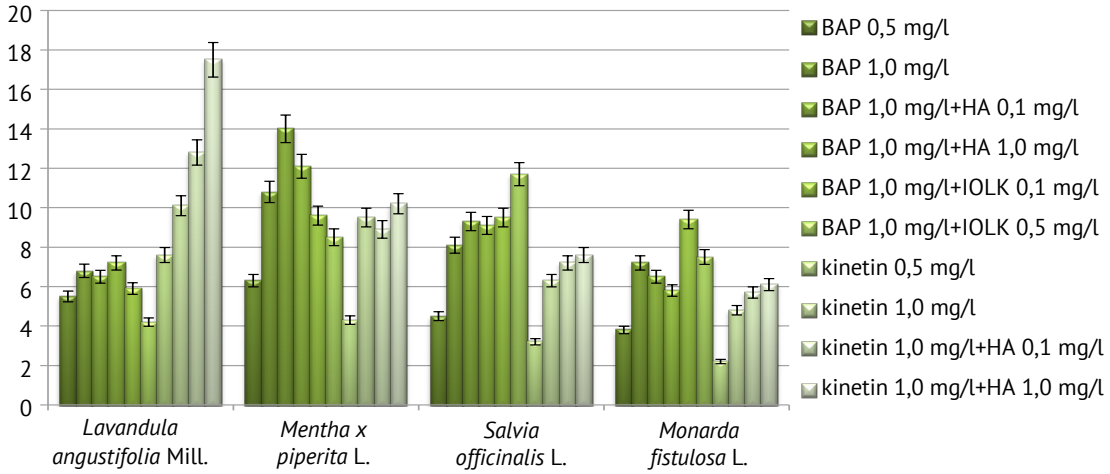

***Figure 2**. The coefficient of reproduction of plants of the Lamiaceae family at the multiplication stage depending on the composition of hormones*
***Source**: developed by the authors*

It was established that for the *in vitro* cultivation of *L. angustifolia* plants at the multiplication stage, the optimal nutrient medium is MS with the addition of kinetin (1.0 mg/l) and HA (1.0 mg/l), which provided the highest multiplication factor – 17.5. A tendency was noted that nutrient mediums with the addition of kinetin stimulated the process of shoot formation more compared to BAP cytokinin, with the use of which vitrified shoots were formed (up to 65%), which cannot be used for further micropropagation. The addition of HA contributed to the increase in the length of the internodes of the shoots, which allows dividing them into separate microcuttings with one pair of buds for further cultivation in the next cycle, and, accordingly, increases the reproduction ratio. The inclusion of auxin IOLK in nutrient media containing BAP caused the formation of callus at the base of the cutting, and with increasing concentration, the frequency and intensity of callusogenesis increased. Callus formation is unacceptable during clonal micropropagation, as indirect hemogenesis and the formation of somaclonal variants are possible.

For the cultivation of explants of *M. x piperita*, *S. officinalis*, and *M. fistulosa*, the addition of MS BAP to the basic nutrient medium was more favorable for stimulating hemogenesis compared to kinetin. On media with BAP, the development of a greater number of additional and adventitious shoots was noted, while their vitrification was not observed as in *L. angustifolia* explants. The addition of HA or IOLK contributed to the optimization of shoot development. Optimum nutrient media for the induction of *in vitro* morphogenesis and the stage of multiplication were determined based on the base medium of MS: for *M. x piperita* – BAP (1.0 mg/l) and IOLK (0.1 mg/l), for *S. officinalis* – BAP (1.0 mg/l) and IOLK (0.5 mg/l), for *M. fistulosa* – BAP (1.0 mg/l) and IOLK (0.1 mg/l).

At the multiplication stage, micropropagation propagation can be carried out in several cultivation cycles to achieve the required number of plants, therefore it is advisable to study the effect of the micropropagation cycle on morphogenesis in *in vitro* culture. This allows to find out the possibility of carrying out subcultivation without changing the morphometric parameters and the reproduction ratio. The dynamics of the reproduction coefficient of plants of the *Lamiaceae* family during ten cycles of cultivation depended on the type of plants (Fig. 3).

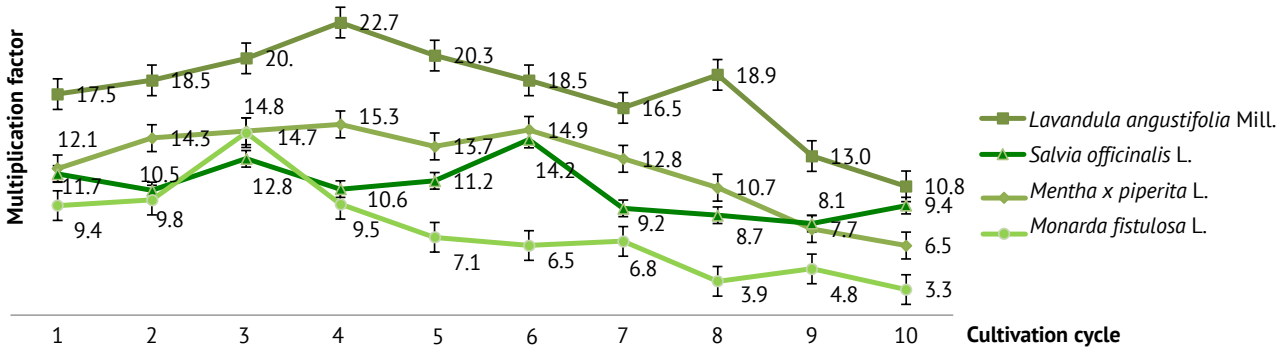

***Figure 3**. The multiplication factor of plants of the Lamiaceae family depending on the cycle of cultivation*
***Source**: developed by the authors*

The highest reproduction ratio was observed in *L. angustifolia* plants, it ranged from 16.5 to 22.7 and remained stably high from the 1st to the 8th cycle of cultivation, and decreased to 10.8-13.0 in the 9th to 10th cycles. A similar trend was observed in *M. x piperita* plants, the reproduction coefficient remained stable during eight cycles of cultivation within the range of 10.7-15.3, and in subsequent cycles it decreased to 8.1-6.5. The multiplication factor of *S. officinalis* was the highest in the 6th cultivation cycle – 14.2, the lowest – 7.7, in the 9th passage, and slightly increased to 9.4 in the 10th passage. The reproduction coefficient of *M. fistulosa* was the lowest among the studied plant species and ranged from 6.5 to 9.4 during 1-7 cycles of cultivation, except for the 3rd cycle, when this parameter was the maximum – 14.7. A significant decrease in the multiplication factor to 4.8-3.3 was observed in 7-10 cycles.

The obtained data make it possible to recommend seven to eight cycles of cultivation during clonal micropropagation of plants of the *Lamiaceae* family, during which they maintain high morphogenetic potentials and provide a stable multiplication factor of 6.5-22.7, depending on the biological characteristics of the species.

Stage III – rooting of microshoots. Stimulation of rhizogenesis in plants is carried out under the influence of auxins, which induce division of shoot parenchyma cells, as a result of which differentiation of root primordia occurs in its basal part. It has been shown that for *in vitro* rooting of microshoots of plants of the *Lamiaceae* family, the nutrient medium of ½ MS supplemented with IOLK (0.5 mg/l) and IOCK (0.5 mg/l) is optimal (Fig. 4). On this nutrient medium, the frequency of rooting was 80.0-97.5%, the highest height shoots were also formed – 56.8-51.5 mm and the largest number of roots – 3.5-4.7 pieces.

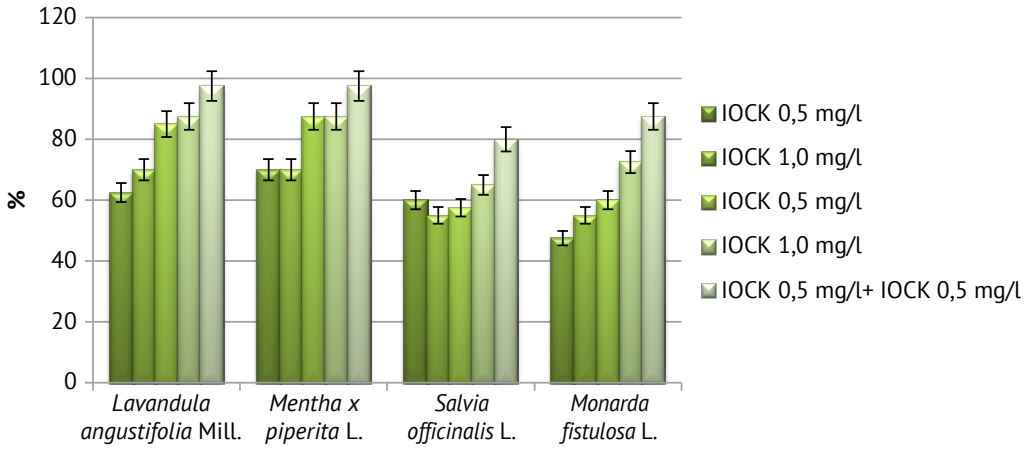

***Figure 4**. The frequency of rooting of microshoots of plants of the Lamiaceae family depending on the composition of auxins*

***Source**: developed by the authors*

It is obvious that IOLK and IOCK auxins in combination provided a synergistic effect. When added individually, they acted less effectively even at a concentration of 1.0 mg/L. The frequency of regeneration was significantly lower compared to this indicator on the medium with a combination of auxins. Also, when the concentration of auxins increased to 1.0 mg/l, inhibition of shoot growth was observed.

Stage IV – adaptation of microplants to *in vivo* conditions. During the transfer of plants from *in vitro* conditions to *in vivo* conditions, cultivation parameters change – temperature, relative humidity, substrate, lighting. Therefore, it is important to develop adaptation measures that contribute to the optimal survival of mericlons.

In plants of the *Lamiaceae* family, after 7-10 days, depending on the species, there were clearly visible signs of the growth of shoots and the formation of the root system. It was determined that 14-20 days are enough for the adaptation of meristem plants, during which 2-3 pairs of leaves were formed. It should be noted that the viability of meristem plants and their growth depended significantly on the composition of the substrate. It was established that the optimal substrate for adaptation is peat: perlite in a volume ratio of 3:1, on which the survival rate of all studied plant species was 82.5-100.0%, while with an increase or decrease in the proportion of perlite in mixture, this indicator decreased.

During the period of adaptation and cultivation in the collection nursery, meristem plants of all studied species had morphological characteristics typical for the varieties. In order to further research the processes of growth and development of plants obtained in *in vitro* culture, a model experiment was established in the collection nursery of the Ukrainian National Academy of Sciences, while the survival rate of plants was 95.0-100.0%.

As a result of the experiments, the processes of morphogenesis in the *in vitro* culture of *L. angustifolia*, *M. x piperita*, *S. officinalis*, and *M. fistulosa* were investigated, and technological measures were developed at the four stages of clonal micropropagation (Fig. 5).

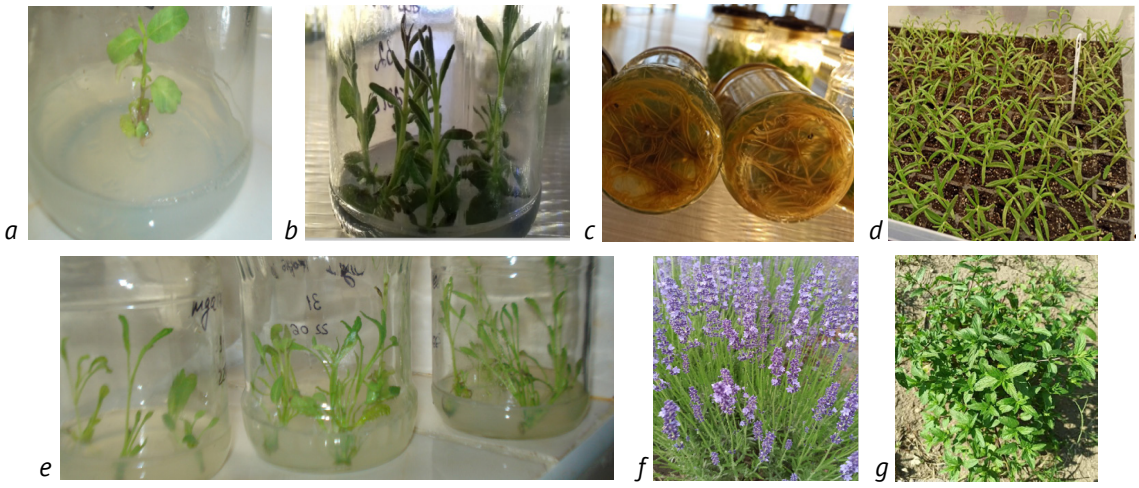

***Figure 5**. Development of plants of the Lamiaceae family at different stages of clonal micropropagation: a – M. fistulosa (I stage); b – L. angustifolia (II stage); c – M. x piperita (III stage); d – L. angustifolia (IV stage); e – S. officinalis (II stage); f – L. angustifolia (collection nursery); g – M. x piperita (collection nursery)*
***Source**: developed by the authors*

The revealed features of cultivation, in particular, the optimal composition of the nutrient medium, the multiplication factor and its dynamics depending on the cultivation cycle, the conditions of rooting and adaptation of microplants differed for *L. angustifolia*, *M. x piperita*, *S. officinalis* and *M. fistulosa*. For the clonal micropropagation of *L. angustifolia*, the most effective at the stages of *in vitro* culture introduction and multiplication was determined to be a nutrient medium based on the MS basic medium, supplemented with kinetin (1.0 mg/l) and HA (1.0 mg/l). D. Leelavathi & N. Kuppan (2013), T. Manushkina (2017) also indicate that the use of kinetin affected the formation of more shoots without morphological changes in *L. angustifolia*. A. Hamza *et al*. (2011), D. Andrys *et al*. (2017) found that plants that produced the highest number of shoots were grown on medium with BAP (0.8-2.0 mg/l). According to A. Kumar *et al*. (2015), the use of MS with the addition of cytokinin BAP (2.0 mg/l) and auxin IOCK (0.5 mg/l) affects the increase in the number of shoots. This study showed that the cultivation of the studied varieties on MS nutrient medium with the addition of BAP (1.0 mg/l) stimulated the development of shoots, but 65% of them were vitrified, excessively hydrated, and the addition of BAP to the nutrient medium together with IOLK caused the formation of callus at the base of the cutting. Both processes are unacceptable in clonal micropropagation. J. Koefender *et al*. (2021) also noted excessive hydration in Lavandula dentata plants grown on MS medium supplemented with BAP (5.0 μM).

For *M. x piperita*, it was determined that in the first two stages of cultivation, the optimal nutrient medium is MS supplemented with BAP (1.0 mg/l) and HA (0.1 mg/l). Such results are consistent with those obtained in the work of A. Islam *et al*. (2017), in which the specified hormones were applied, but in stages. The largest number of shoots was obtained on the medium containing BAP (3.0 mg/l). For further elongation, microshoots were transferred to media with different concentrations of HA. The greatest length of shoots with a frequency of 100% was achieved on the medium containing 1.0 mg/l HA. In the work of T. Talankova-Sereda *et al*. (2016) also determined as optimal the MS medium supplemented with BAP (0.75 mg/l) and HA (0.5 mg/l), but additionally used adenine and IOCK at a concentration of 0.05 mg/l.

Initiation of development and multiplication of shoots in the *in vitro* culture of *S. officinalis* took place most effectively on the nutrient medium of MS with the addition of BAP (1.0 mg/l) and IOLK (0.5 mg/l), and *M. fistulosa* – with the addition of BAP (1.0 mg/l) and IOLK (0.1 mg/l). Similar results were presented by M. Petrova *et al*. (2015), who showed maximum shoot proliferation from seedling segments obtained *in vitro* when cultured in MS medium supplemented with BAP (2.22 μM) and IOLK (0.57 μM). I. Grzegorczyk-Karolak *et al*. (2021) during clonal micropropagation of *Salvia bulleyana* based on leaf segments, the highest regeneration frequency (95%) was obtained on MS medium containing BAP (2.0 mg/l) and naphthylacetic acid (NOC) (0.1 mg/l). However, researchers P. Santos-Gomes & M. Fernandes-Ferreira (2003) showed that the greatest accumulation of essential oils and the greatest increase in shoot biomass was obtained when kinetin (2.0 mg/l) and 2.4 – dichlorophenoxyacetic acid (0.05 mg/l).

At the stage of rooting of microshoots of plants of the *Lamiaceae* family, the highest frequency of rooting (80.0-97.5%) was ensured on nutrient medium ½ MS, supplemented with IOLK (0.5 mg/l) and IOCK (0.5 mg/l). The same auxins in a concentration of 0.5-2.0 mg/l were used in the work A. Islam *et al*. (2017) to root *M. x piperita* microshoots. Among them, the greatest root

proliferation was obtained on the medium containing IOLK (1.5 mg/l). J. Łyczko *et al.* (2020) revealed a high degree of rooting (97-100%) on MS medium with the addition of IOLK (0.5 mg/l). The researchers Andrys *et al.* (2017) show a significant influence of the *L. angustifolia* genotype on the rooting process. For two cultivars, the optimal effect on root length was noted on ¼ MS nutrient medium with the addition of 0.2 mg/l IOLK or (NOC), while for the third cultivar, the best results were obtained on the MS medium with the addition of 0.2 mg/l NOC. In the work Hamza *et al.*, (2011) it was found that the use of ½ MS medium with the addition of NOK (1.0 mg/l) resulted in more roots, but IOLK (2.0 mg/l) had the most positive effect on root length .

The viability of plants obtained by the method of clonal micropropagation in field conditions was 95.0-100.0%. Similar results were obtained in th work of researchers Islam *et al.* (2017).

In general, the direction of morphogenetic processes at individual stages of clonal micropropagation of plants of the *Lamiaceae* family was similar. However, the hormonal regulation of morphogenesis varied, which is obviously due to genotypic differences in the morphogenetic reactions of plants in *in vitro* culture, as well as different explants, growing conditions of donor plants, the season of explantation, and other factors. The works Andrys *et al.* (2017), Islam *et al.* (2017) emphasize that the hormonal conditions for reproduction depend on the species and variety of the source plants. The biotechnological measures of clonal micropropagation of essential oil plants of the *Lamiaceae* family developed in this study are effective and can be recommended for inclusion in the technologies of obtaining planting material.

CONCLUSIONS

Based on scientific research, a biotechnological method of cloning and micropropagation of essential oil plants *Lamiaceae* L. *angustifolia* Mill., *M. x piperita* L., *S. officinalis* L., *M. fistulosa* L. was developed.

The most effective for the stages of introduction into culture *in vitro* and multiplication are nutrient media based on the basic medium of MS, supplemented with hormones: for *L. angustifolia* – kinetin (1.0 mg/l) and HA (1.0 mg/l), for *M x piperita* – BAP (1.0 mg/l) and HA (0.1 mg/l), for *S. officinalis* – BAP (1.0 mg/l) and IOLK (0.5 mg/l), for *M. fistulosa* – BAP (1.0 mg/l) and IOLK (0.1 mg/l).

At the stage of multiplication, plants maintain high morphogenetic potentials in *in vitro* culture and provide a stable multiplication factor of 6.5-22.7 during seven to eight cycles of cultivation, depending on the biological characteristics of the species.

At the rooting stage of microshoots, the nutrient medium ½ MS, supplemented with IOLK (0.5 mg/l) and IOCK (0.5 mg/l), was determined to be optimal, which ensured a rooting frequency of 80.0-97.5%.

The most effective for the adaptation of plants to *in vivo* conditions is a substrate containing peat and perlite in a volume ratio of 3:1, on which the survivability of all studied plant species was 82.5-100.0%.

Prospects for further research are in the development of an integrated system of seed production of essential oil plants with the aim of obtaining standard planting material and studying the characteristics of growth and development, forming the productivity of plants of the *Lamiaceae* family for the development of technological measures for their cultivation in the conditions of the Southern Steppe of Ukraine.

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CONFLICT OF INTEREST

The authors declare that the study was conducted in the absence of any commercial or financial relationships that could be interpreted as a potential conflict of interest.

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Клональне мікророзмноження *in vitro* ефіроолійних рослин родини *Lamiaceae* Lindl.

Тетяна Миколаївна Манушкіна

Кандидат сільськогосподарських наук
Миколаївський національний аграрний університет
54008, вул. Георгія Гонгадзе, 9, м. Миколаїв, Україна
https://orcid.org/0000-0001-5843-271X

Тетяна Володимирівна Качанова

Кандидат сільськогосподарських наук
Миколаївський національний аграрний університет
54008, вул. Георгія Гонгадзе, 9, м. Миколаїв, Україна
https://orcid.org/0000-0003-0032-3996

Микола Олександрович Самойленко

Доктор сільськогосподарських наук
Миколаївський національний аграрний університет
54008, вул. Георгія Гонгадзе, 9, м. Миколаїв, Україна
https://orcid.org/0000-0002-3292-9717

Олена Іванівна Петрова

Кандидат сільськогосподарських наук
Миколаївський національний аграрний університет
54008, вул. Георгія Гонгадзе, 9, м. Миколаїв, Україна
https://orcid.org/0000-0001-8612-3981

Анотація. Актуальність дослідження щодо розробки біотехнології клонального мікророзмноження рослин родини *Lamiaceae* Lindl. зумовлюється необхідністю масового одержання оздоровленого чистосортного садивного матеріалу для закладання промислових плантацій та розширення площ ефіроолійних культур в Україні. Метою досліджень було розробити біотехнологічні заходи клонального мікророзмноження ефіроолійних рослин родини *Lamiaceae* – *Lavandula angustifolia* Mill., *Mentha x piperita* L., *Salvia officinalis* L. і *Monarda fistulosa* L. Завдання дослідження – підібрати оптимальні умови для ефективного культивування рослин родини *Lamiaceae* на чотирьох етапах клонального мікророзмноження. Основні методи дослідження: лабораторний, польовий, аналітичний і математико-статистичний. *Визначено* оптимальні для індукції морфогенезу *in vitro* та етапу мультиплікації живильні середовища на основі базового середовища Мурасиге і Скуга: для *L. angustifolia* доповнене кінетином (1,0 мг/л) і гібереловою кислотою (1,0 мг/л), для *M. x piperita* – 6-бензиламінопуріном (1,0 мг/л) і гібереловою кислотою (0,1 мг/л), для *S. officinalis* – 6-бензиламінопуріном (1,0 мг/л) та ІОлК (0,5 мг/л), для *M. fistulosa* – 6-бензиламінопуріном (1,0 мг/л) та β-індоліл-3-олійною кислотою (0,1 мг/л). На етапі мультиплікації доцільно проводити сім-вісім циклів культивування. На етапі укорінення мікропагонів найбільш ефективним для усіх досліджуваних видів рослин визначено живильне середовище Мурасиге і Скуга зі зменшеною вдвічі концентрацією компонентів, доповнене β-індоліл-3-олійною кислотою (0,5 мг/л) та β-індоліл-3-оцтовою кислотою (0,5 мг/л). Оптимальним для адаптації рослин до умов *in vivo* визначено субстрат торф: перліт у співвідношенні за об'ємом 3:1. *Включення розробленої біотехнології клонального мікророзмноження* до системи насінництва ефіроолійних культур родини *Lamiaceae* дозволить прискорено одержувати оздоровлений чистосортний садивний матеріал та впроваджувати нові продуктивні сорти у виробництво

Ключові слова: *Lavandula angustifolia* Mill.; *Mentha x piperita* L.; *Salvia officinalis* L.; *Monarda fistulosa* L.; коефіцієнт *розмноження*