БІОТЕХНОЛОГІЯ ТА БІОІНЖЕНЕРІЯ

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PHYSIOLOGICAL-BIOTECHNOLOGICAL ASPECTS OF DROUGHT RESISTANCE OF NARROW-LEAVED LAVENDER *(LAVANDULA ANGUSTIFOLIA MILL.)*

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Abstract. *Varieties of narrow-leaved lavender, which have high potential productivity, are zoned on the territory of Ukraine, but due to the variability of the temperature regime and the amount of annual precipitation, it is not fully realized. This causes selectioners to create and introduce into production varieties with high environmental plasticity and resistance to stressors. Today in Ukraine large areas of arable land, especially the steppe zone suffer from insufficient moisture, which leads to 30-50% crop failure, especially in dry years. Therefore, it is especially important to study the physiological and biotechnological aspects of drought resistance of lavender for selection work aimed at creating new, productive varieties adapted to conditions of insufficient humidity. We used modified Murashige and Skoog nutrient media supplemented with different concentrations of cytokinins and osmotically active substances. The content of polyethylene glycol 12000 at a concentration of 7.5% creates sufficiently stressful conditions to obtain drought-resistant lavender lines. At the same time, there is a slowing down of the root formation process, which significantly affects the time required to obtain and adapt stable lines of lavender. Rhizogenesis is much slower, and the root system is less developed, compared with the root system of plants grown on a medium without polyethylene glycol. Alternatively, a nutrient medium with a 5% concentration of PEG can be used. Thus, the percentage of drought-resistant adapted planting material will be higher, although the degree of adaptation to arid conditions will be lower. It is established that the drought factor affects not only the growth of vegetative mass of plants, but also delays their morphogenetic processes.*

Thus, at high concentrations of osmotic agents in the nutrient medium rhizogenesis occurred only in some samples and with a significant delay.

Keywords: *narrow-leaved lavender, in vitro culture, morphogenesis, drought resistance*

Introduction.

Lavender (Lavandula angustifolia Mill.) Is an evergreen essential oil crop that is widely used as a raw material in the pharmacological (López et al., 2017), cosmetic and perfumery (Kunicka-Styczyńska et al., 2009) and food industries (Erland et al. ., 2016), is grown for decorative and recreational purposes (Prusinowska et al., 2014, Min et al., 2016). Industrial cultivation of narrow-leaved lavender and production of lavender oil, widespread in southern Ukraine (Giray et al., 2018, Manushkina et al., 2019), but lavender is not very popular as an agricultural crop and one of the reasons is the lack of affordable and planting material of high quality and varieties adapted to the conditions of Ukraine. A possible way to solve this problem is to develop more intensive breeding methods, in particular, clonal micropropagation *in vitro* (Mitrofanova et al., 2017, Rudnik-Ivashchenko et al., 2019).

Varieties with high potential productivity are zoned on the territory of Ukraine, but due to the variability of the temperature regime and the amount of annual precipitation, it is not fully realized. This causes selectioners to create and introduce into production varieties with high environmental plasticity and resistance to stressors.

Today, large areas of arable land, especially the steppe zone suffer from insufficient moisture, which leads to 30-50% crop failure, especially in dry years. Therefore, it is necessary to optimize the methods of cell selection and microclonal propagation of lavender for selection work aimed at creating new, productive varieties adapted to conditions of insufficient humidity.

The purpose of the work – is to conduct a comparative study of the main physiological and biotechnological parameters of drought-resistant lines of lavender.

Materials and methods of research.

The research was conducted in the Laboratory of Plant Biotechnology of the Department of Ecobiotechnology and Biodiversity of NULES of Ukraine in 2020-2021. Explants 5-7 mm in size isolated from young annual shoots of plants were introduced into the *in vitro* culture.

For morphogenesis and rhizogenesis of narrow-leaved lavender, modified nutrient media Murashige and Skoog (MS) (Murashige et al., 1962) with different concentrations of kinetin MC1 (MS + 0.25 mg / l kinetin) and MSII (MS + 0.5 mg / l kinetin) were used.) Due to the fact that initiation of meristem development and subsequent morphogenesis of lavender is a cytokinin-dependent process (Bona et al., 2012).

To create the *in vitro* stress effect of drought (Szekely-Varga et al., 2020, Lamacque et al., 2019, Marulanda et al., 2019) we used nutrient media supplemented with high molecular weight polyethylene glycol (PEG – 12000) in concentrations of 5% and 7.5%, which is able to mimic water stress without penetrating into cells and act as an osmotic agent (Burnett et al., 2006).

Plants-regenerants were cultivated at a temperature of + 25-26^{0}C, light - 5 thousand lux, 16-hour photoperiod and humidity of 70%.

Statistical processing of the obtained experimental data was carried out using the software package "Analysis of Microsoft Exel spreadsheets", Statistica 6.0

Research results and their discussion.

An important area of application of the method of tissue culture is reproduction in order to obtain genetically identical, valuable genotypes for use in breeding practice. The results of the studies showed that among the two variants of media for morphogenesis (table 1), the best was observed on morphogenic medium MCII, which contained twice the concentration of kinetin. At the same time, the explants differed in more intensive shoot formation compared to the explants planted on the nutrient medium MCI. The coefficient of reproduction was calculated in relation to the number of explants used in the experiment and the average number of shoots per explant. Rhizogenesis of shoots was observed on the 25th day of cultivation among explants planted on morphogenic nutrient medium of variant MCII, whereas on the medium of variant MCI there was only an increase in shoot size.

In the process of cultiuring narrow-leaved lavender regenerants on a medium with PEG 12000, which mimics the effect of drought, measurements of various growth parameters of plants-regenerants were performed to study the changes that occurred in the plant body during the entire cultivation period. These parameters include: the height of the regenerants, the number of shoots per regenerant, the number of internodes on the shoot and the area of the leaf blade, as well as observations of the process of rhizogenesis. Based on the collected and analyzed data, the dynamics of growth and development of lavender under the influence of water stress is shown. The regenerants showed excellent results depending on the concentration of PEG 12000 and the variant of the nutrient medium. 6 separate measurements of growth parameters were performed, which were performed every 10 days of cultivation. The first measurement - on the day of introduction of explants into the culture *in vitro*, the last - on the 50th day.

Regenerants that were cultured at a 7.5% concentration of PEG 12000 had a significantly smaller increase in vegetative mass compared to the control variant and even with plants grown on nutrient medium with a concentration of PEG 12000 in 5.0%. The average increase in vegetative mass in regenerants grown on a medium with twice the concentration of kinetin (medium II) exceeds the increase in regenerants on the first medium (Fig. 1, Fig. 2).

Table 1. **The sequence of individual stages of morphogenesis of lavender in the culture of isolated meristems**

Media	Cultivation time, days			
	Implantation and initial stages of meristem development	The beginning of the main development of the shoot	Induction of numerous shoot formation	Beginning of the development of the root system
MCI	10-11	18-19	25-26	35-36
MCII	10-11	17-18	24-25	35-36

Intensive shoot formation began on day 20-30 of cultivation, regardless of the factors that affected lavender plants. But the activity of shoot formation differed, correlating with the general trend described above.

As can be seen from the presented data, there is a pattern between the number of internodes and the height of plants-regenerants. The higher the plant, the more internodes it will have, but it is also important to consider their length. Thus, control plants, having a significant advantage in the height of regenerants, do not have an equally significant advantage in the number of internodes. This can be explained by the fact that the resources and energy that the plant spends on the elongation of the internodes are spent on counteracting osmotic stress, so the internodes of these plants are on average shorter.

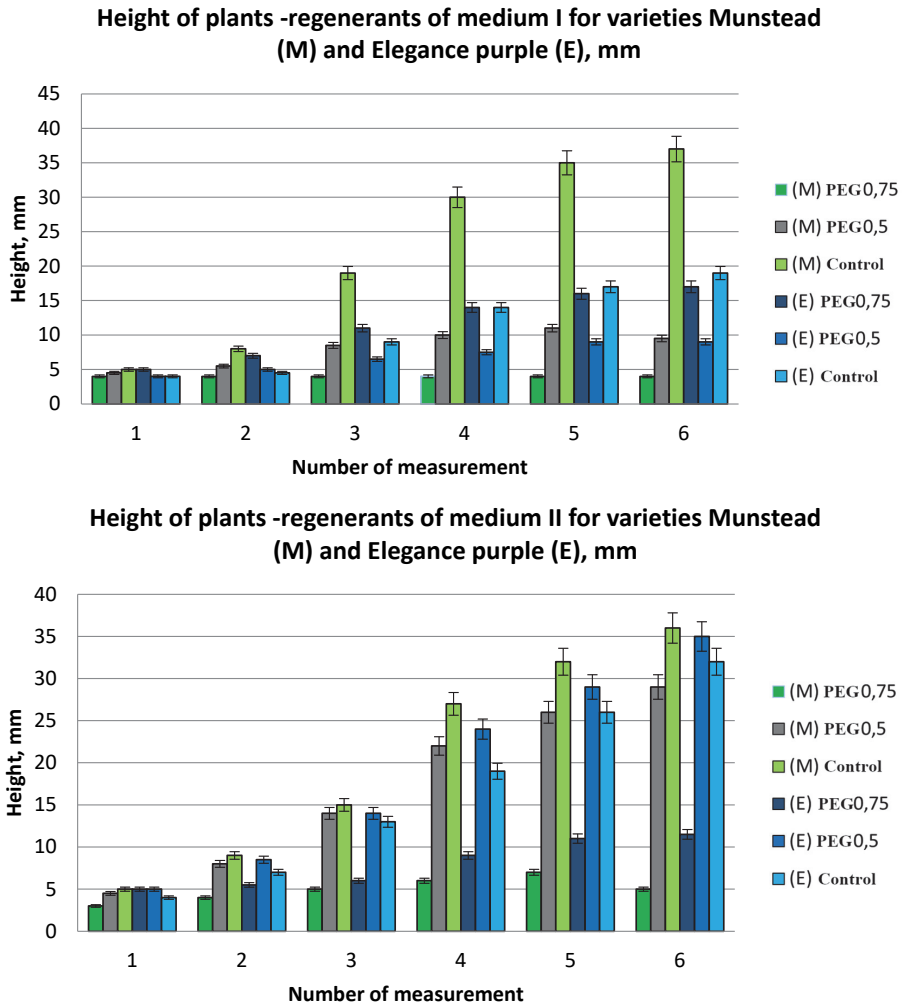

Fig. 1. Graphs showing dependence of height of plants-regenerants, depending on growing conditions

In the study of the leaf surface area of plants-regenerants of lavender narrow-leaved, it was found that the dynamics of change in leaf surface area, although correlated with the general growth trend described above, but the difference between plants-regenerants grown on different nutrient media is not as significant as for other indicators described above.

This can be explained by the fact that increasing the area of the leaf blade in the body is given priority, as it is an organ that has a strong influence on the intensity of photosynthesis, and hence on the viability of the whole plant.

When we studied the effectiveness of the effects of different concentrations of PEG 12000 on the growth and development of culture, the influence of the composition of the nutrient medium, the available growth regulators, it was found that 7.5% concentration of PEG

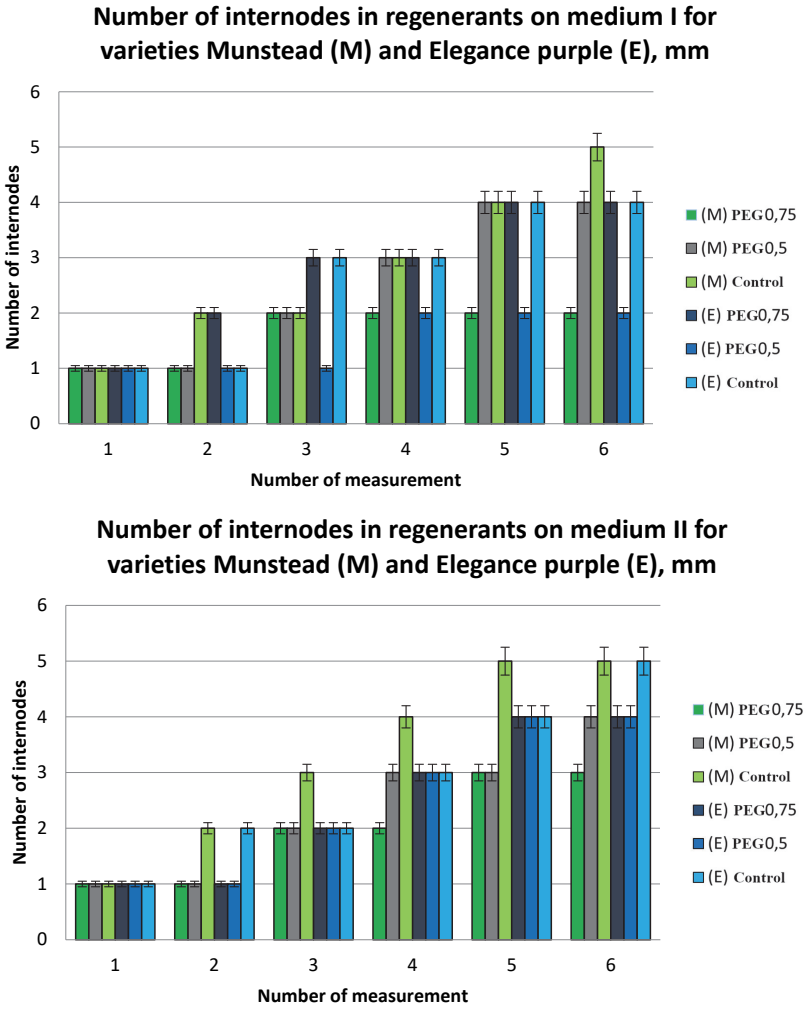

Fig. 2. Graphs of dependence of the number of internodes in regenerants, depending on growing conditions

12000 can be a selective agent, because there are significant differences between the studied samples of narrow-leaved lavender based on reduction of growth of vegetative mass of regenerants, which depending on the concentration of growth hormones available to the plant decreased by 50% or more.

Obtained drought-resistant lines were subsequently used for plant regeneration.

Rhizogenesis *in vitro* is a complex process involving biochemical, histological and physiological changes (Bulavin et al., 2020). Rooting *in vitro* can occur both on the vegetative organs of the plant and on their individual parts. The ratio of auxins and cytokinins is important for rhizogenesis. Shoots or buds are formed at higher concentrations of cytokinins compared to auxins, so one way to induce rhizogenesis involves culturing regenerants on a medium

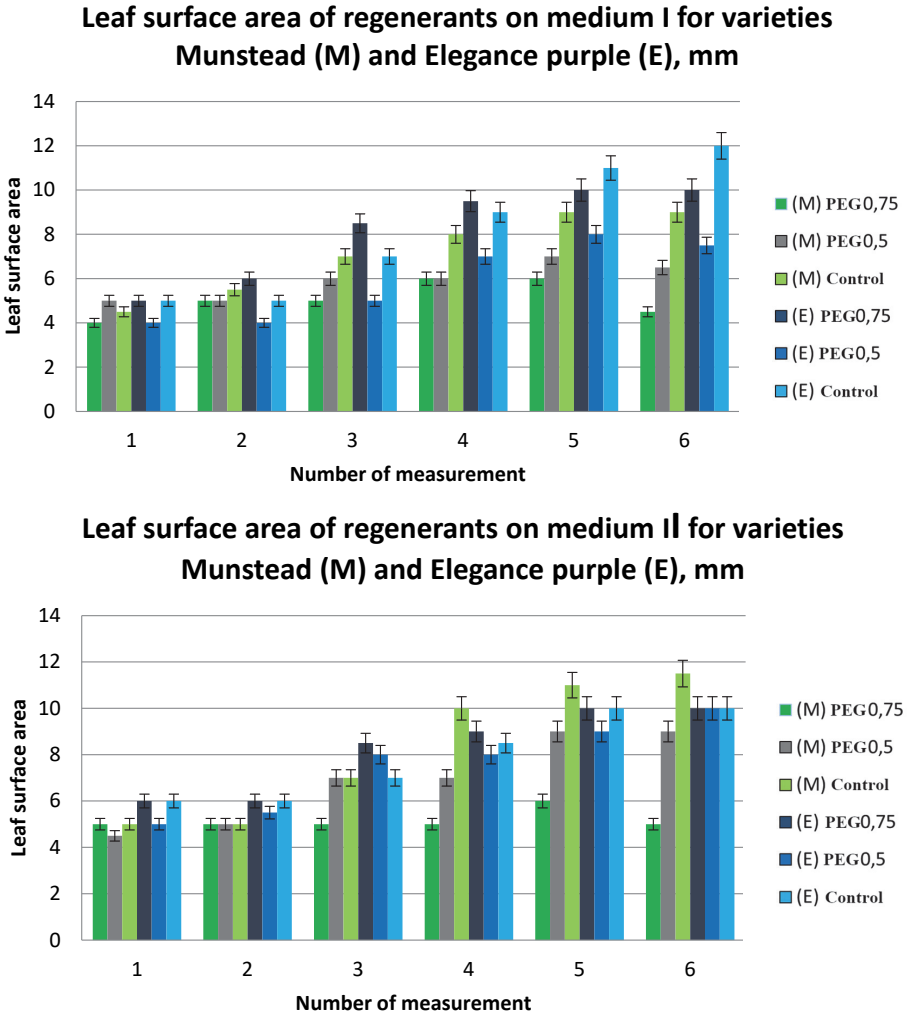

Fig. 3. Graph of the dependence of the leaf surface area depending on growing conditions

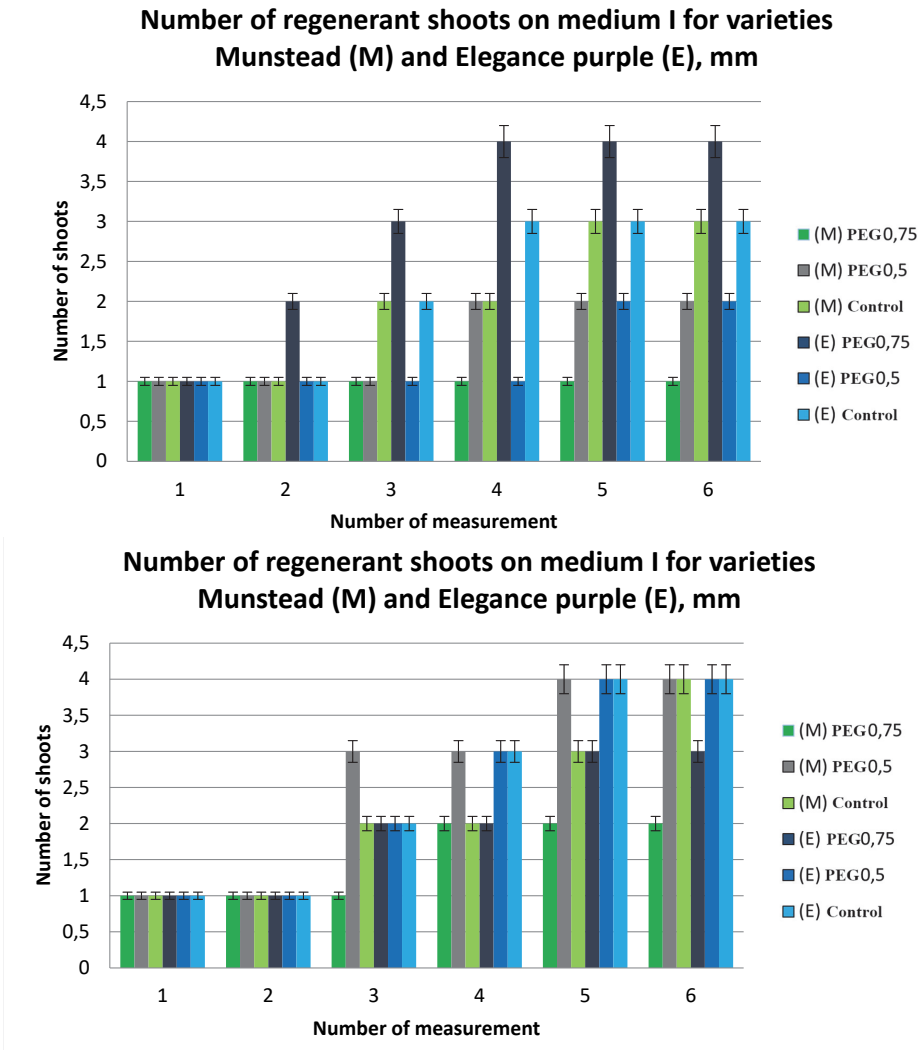

Fig. 4. Graph of the dependence of the number of regenerant shoots depending on growing conditions

Table 2. **Day of the beginning of root system formation of lavender (Lavandula angustifolia Mill.) regenerants**

Variety	Kinetin content, mg / l	Control	The content of PEG 12000	
			5%	7,5%
Munstead	0,25	34,6 days	46,5 days	51,2 days
	0,5	38,5 days	49,7 days	53,1 days
Ellagance Purple	0,25	35,1 days	47,0 days	50,9 days
	0,5	38,7 days	50,4 days	52,6 days

with a higher concentration of auxins than cytokinins (Bulavin et al., 2020). The results of our studies show that osmotically active substances such as PEG 12000 adversely affect the rooting process. The process of rhizogenesis in control samples, which were cultured on medium without osmotically active substances took place for 21 days, while on media with PEG, the root system began to develop with a significant delay (Table 2).

It should be noted that higher concentrations of kinetin in the nutrient medium adversely affect the rooting process.

Conclusions.

A comparative study of the main physiological and biotechnological parameters of drought-resistant lines of narrow-leaved lavender, namely the height of plants-regenerants, leaf surface area, the number of internodes and shoot formation. It was found that the best medium for cultivation and rhizogenesis of plants-regenerants is the environment of MS d by adding kinetin at a concentration of 0.25 mg / l.

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Анотація. *На території України районовано сорти лаванди вузколистої, які мають високу потенційну продуктивність, але через мінливість температурного режиму і кількість річних опадів він реалізується не повністю. Це зумовлює селекціонерів створювати і впроваджувати у виробництво сорти з високою екологічною пластичністю та стійкістю до стресових факторів. На сьогодні на території України великі площі орних земель, особливо зони степу страждають від недостатнього зволоження, що призводить до 30-50% недобору врожаю, особливо в посушливі роки. Тому особливо важливим є вивчення фізіологічно-біотехнологічних аспектів посухостійкості лаванди для селекційної роботи націленої на створення нових, продуктивних сортів пристосованих до умов недостатньої вологості. Нами використано модифіковані живильні середовища Мурасіге-Скуга доповнені різними концентраціями цитокінінів та осмотично активних речовин. Вміст поліетиленгліколю 12000 у концентрації 7,5% створює достатньо стресові умови для отримання посухостійких ліній лаванди. При цьому спостерігається уповільнення процесу корененутворення, що значно впливає на час необхідний для отримання та адаптації стійких ліній лаванди вузколистої. Ризогенез відбувається значно повільніше, а коренева система слабше розвинута, порівняно з кореневою системою рослин вирощених на середовищі без вмісту поліетиленгліколю. Альтернативно можна використовувати живильне середовище з 5% концентрацією ПЕГ. Таким чином відсоток посухостійкого адаптованого посадкового матеріалу буде вищим, хоча ступінь пристосованості до засушливих умов і буде нижчою. Встановлено, що фактор посухи впливає не лише на приріст вегетативної маси рослин, а й затримує їх морфогенетичні процеси. Так, при високих концентраціях осмотичних агентів в живильному середовищі ризогенез відбувався лише в частини зразків і зі значною затримкою.*

Ключові слова: *лаванда вузколиста, культура in vitro, морфогенез, посухостійкість.*