

MULTIPLICATION OF LAVENDER (*L. ANGUSTIFOLIA*) AND LAVANDIN (*LAVANDULA x INTERMEDIA*) IN EXPLANT CULTURE

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Lavender plants are preferably propagated vegetatively. The aim of the work was to propose the composition of nutrient media for *in vitro* multiplication of plants of *Lavandula angustifolia* (var. Krajová and Beta) and *Lavandula x intermedia* (var. Budrovka and Grosso). Explant cultures were initiated from apical or nodal segments. Apical segments were more suitable and regenerated the highest number of shoots in MS medium supplemented with 1 mg/L BAP with or without 0.1 mg/L IAA in all used varieties. Nodal segments are suitable for var. Budrovka, where the number of shoots was not statistically different between the apical or nodal segments and among the media tested. Shoot multiplication of *L. angustifolia* varieties was the most effective in the MS media with 0.5 or 1 mg/L BAP. For variety Grosso, the optimal medium was MS with 1 mg/L BAP, while var. Budrovka regenerated a similar number of shoots in all media supplemented with 0.5 or 1 mg/L BAP with or without 0.1 mg/L IAA. All genotypes rooted with the highest frequency on growth regulator-free medium with a half dose of MS salts. After transplanting into the soil and *ex vitro* acclimatization, plantlets survivability after 10 weeks was 78.9 – 87.7%, except for var. Beta with a lower survivability of 50%. The proposed procedures enable a rapid plant multiplication of varieties Budrovka, Grosso and Krajová. With the Beta variety, it is advisable to continue working on the optimization of nutrient media to verify whether the procedures can be further optimized.

Key words: *Lavandula angustifolia*, *Lavandula x intermedia*, *in vitro* propagation, seedling production, *ex vitro* acclimatization

The genus *Lavandula* belonging to the family *Lamiaceae* comprises 39 lavender species, from which the most cultivated species are *L. angustifolia* (also known as true or English lavender), *L. latifolia* (spike lavender) and *L. x intermedia* (lavandin or Dutch lavender) (Wells *et al.* 2018; Crisan *et al.* 2023). Lavenders are known as a medicinal, aromatic, ornamental and melliferous plant. Plants and products from them are used mainly in medicine for their sedative, anti-inflammatory, antioxidant, and antimicrobial properties, in a wide range of cosmetic products, perfumery, culinary purposes, and insect and pest control (Wells *et al.* 2018;

Crisan *et al.* 2023). Lavender or lavandin plants are especially cultivated and harvested for their essential oils, with main components linalool and linalyl acetate, produced in specialized glandular trichomes located abundantly on the surface of the calyx and on the leaves (Kirimer *et al.* 2017). The demand for natural products including essential oils is globally increasing. Among the hundreds of essential-oil-bearing plants, *L. angustifolia* is one of the most valuable (Crisan *et al.* 2023), with high-quality essential oil, whereas *L. x intermedia*, a sterile hybrid of *L. latifolia* and *L. angustifolia*, is valued for high yield of essential oils (Pokajewicz *et al.* 2022).

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Most of the lavender species can be propagated by seeds, but due to a plant out-crossing, vegetative (clonal) propagation should be preferred to maintain genetic uniformity (Zuzarte *et al.* 2010; Wells *et al.* 2018), especially in bred varieties. Moreover, a hybrid *L. x intermedia*, which lacks fertile seeds can be propagated only vegetatively. In addition to traditional propagation by cuttings, the use of *in vitro* technology can be an alternative, however a more expensive one. It enables the fast propagation of a large number of plants or the production of virus-free plants (Mitrofanova *et al.* 2017) in controlled conditions without the pressure of biotic and abiotic stress factors. Besides, maintained plant tissue cultures can be used as source material in an *in vitro* technology for a continual production of biologically active substances (secondary metabolites) in callus or suspension cultures that can be stimulated by additions of precursors or elicitors or by other changes in cultivation medium composition or cultivation conditions to increase their yield (Espinosa-Leal *et al.* 2018).

Direct organogenesis from pre-existing meristems is an appropriate method of *in vitro* propagation and conservation of vegetatively propagated plants, which minimizes risks of genetic changes during long-term propagation (Gonçalves & Romano 2013). The genetic conformity of *in vitro* cloned plants of lavender multiplied in this way, as well as standard essential oil composition, have been confirmed in previous works (Prasad *et al.* 2015; Kirimer *et al.* 2017). However, some works concerning indirect propagation via callus have also been published (Dronne *et al.* 1999; Leelavathi *et al.* 2020; Al-Tai & Mohamed 2022). Shoot cultures of lavender are usually established from seeds (Kirimer *et al.* 2017; Vinh *et al.* 2017; Miclea & Chifor 2018; Koefender *et al.* 2021) or stem cuttings (Hamza *et al.* 2017; Li *et al.* 2019) from which apical buds or nodal segments are excised. However, only the establishment of culture from cuttings can be considered a strictly vegetative propagation method.

For culture initiation and shoot multiplication of lavenders, cytokinins alone or in combination with auxins are used. There are several papers concerning *in vitro* propagation, especially for *L. angustifolia* (Hamza *et al.* 2011; Kirimer *et al.* 2017; Vinh *et al.* 2017; Miclea & Chifor 2018; Li *et al.* 2019; Leelavathi *et al.* 2020; Al-Tai & Mohammed 2022),

but due to different results obtained, the universal protocol does not exist. Therefore, the micropropagation protocol needs to be optimized not only for each species but also for each variety. Usually, cytokinin BAP (6-Benzylaminopurine) (Kirimer *et al.* 2017; Vinh *et al.* 2017; Li *et al.* 2019; Koefender *et al.* 2021), alternatively kinetin, thidiazuron or zeatin (Hamza *et al.* 2011; Miclea & Chifor 2018) without or with auxins NAA (1-Naphthaleneacetic acid), IAA (Indole-3-acetic acid) or IBA (Indole-3-butyric acid) have been used for shoot induction from initial explants and their multiplication. Plant regulators-free medium (Miclea & Chifor 2018; Koefender *et al.* 2021) or media supplemented with auxins IAA (Vinh *et al.* 2017), NAA (Li *et al.* 2019) or IBA (Hamza *et al.* 2011; Kirimer *et al.* 2017) have been recommended for rooting of shoots. Micropropagation of other lavender species (*L. latifolia*, *L. vera*, *L. pedunculata*, *L. viridis*, *L. spica*, *L. dentata*) have also been described (summarized in Gonçalves and Romano 2013), but there are only several papers concerning micropropagation of *L. x intermedia* by direct or indirect organogenesis (Kara & Baydar 2012; Erland & Mahmoud 2014; Mitrofanova *et al.* 2020).

The aim of our experiments was to establish a protocol for *in vitro* multiplication of *L. angustifolia* (var. Krajová and Beta), *L. x intermedia* (var. Budrovka and Grosso) and to prepare *ex vitro* acclimatized seedlings for the field collection of genetic resources.

MATERIAL AND METHODS

Biological material

In experiments, 4 varieties of lavender or lavandin species were used: *Lavandula angustifolia* Mill. – varieties Beta and Krajová and *Lavandula x intermedia* Emeric ex Loisel. – varieties Grosso and Budrovka. Plant material was provided by the Gene Bank of the Slovak Republic located at the Research Institute of Plant Production in Piešťany.

Var. Beta – Czech lavender bred by free pollination of materials of different origins and individual selections, introduced in 1995. The plants are medium tall with short grey-green leaves and light purple flowers arranged in racemes supported by bracts, condensed into a spike at the top. Its essential oil content is high, and the linalyl acetate content in the essential oil is medium (Čičová 2021).

Var. Krajová – Czech lavender introduced in 1952. It was the only lavender in the list of permitted varieties in Slovakia. The plants form a densely leafy bush with broad greenish-grey leaves and a long, sparse spike inflorescence with light blue-purple flowers (Čičová 2021).

Var. Budrovka – an autochthonous Croatian lavandin originating from the island of Hvar. It is characterized by wider leaves and long flowering stems with darker flowers, and significantly higher oil production compared to true lavender (Jug-Dujaković *et al.* 2022).

Var. Grosso – the most planted and cultivated lavandin in the world, a hybrid with aromatic grey-green foliage with dense spikes of blue-purple flowers. The variety is known for its hardiness and improved oil production, introduced in France in 1972 (Urwin 2014).

Culture initiation

In vitro cultures of lavender were established from plants cultivated in pots in greenhouse conditions. Young shoots, 5–8 cm long were taken in May and washed under tap water for 10 minutes. Then the shoots were sterilised by soaking in 0.15% solution of HgCl₂ with two drops of Tween 20/100 mL for 10 minutes. Sterilised shoots were rinsed 3 times with sterile distilled water.

Shoots were cut into two types of explants: a) apical segments (which consist of an apical bud and one subjacent node) and b) nodal segments, both 4–7 mm long. Explants were inoculated on induction media with the following composition: MS salts and vitamins (Murashige & Skoog 1962), 30 g/L of sucrose, and 10 g/L of agar without growth regulators (control variant MS) or supplemented with 1 mg/L BAP (variant B1) or with 1 mg/L BAP + 0.1 mg/L IAA (variant IB1). Explants were cultivated in test tubes (25 mm in diameter) under a 16/8 h (light/dark) photoperiod with the light intensity of 50 µmol/m²/s provided by cool-white fluorescent lamps and at a temperature of 24°C (day)/20°C (night). Regeneration ability was evaluated after 4 weeks.

Shoot multiplication

Regenerated shoots were cut into nodal segments and transferred to MS medium without growth regulators for 6 weeks to let them grow. For shoot multiplication, one-nodal segments were used for *L. x*

intermedia or apical segments for *L. angustifolia* – due to very short internodia, especially in the case of var. Beta (Figure 1D). Multiplication media were based on MS without growth regulators or supplemented with 0.5 or 1 mg/L BAP (variants B05, B1) and 0.5 or 1 mg/L BAP + 0.1 mg/L IAA (variants IB05, IB1). Explants were cultivated as mentioned above and evaluated after 6 weeks. The number and the height of shoots were measured individually for each explant/shoot but were weighed together in each variant and recalculated to one explant.

In vitro rooting of shoots

In the first experiment for rooting of shoots, MS media with a half dose of MS salts and vitamins (1/2MS) without growth regulators or with 1 mg/L IAA or NAA were compared. According to the results of this experiment, in the second experiment for shoot rooting, the following rooting media were tested: 1/2MS without growth regulators or 1/2MS supplemented with 0.2, 0.5 and 1 mg/L IAA. In both experiments, plantlets (20–30 mm long) were inoculated into the rooting medium and cultivated under conditions mentioned for the initiation phase for 4 weeks, except for var. Beta, which was left on the rooting medium for 6 weeks due to very slow rooting.

Seedling production and acclimatization

For seedling production, shoots were multiplied in medium B1 (*L. x intermedia*) or B0.5 (*L. angustifolia*) and rooted in MS medium without growth regulators in 200 ml baby food jars. Six weeks later, rooted plantlets (22–32 plantlets per variety) were transferred to the garden substrate (KEKKILA Brown OPM 025 W R0332, RKW Finland). Plantlets were protected with a plastic cover to maintain sufficient humidity for 2 weeks and then acclimatized to *ex vitro* conditions by gradually reducing humidity (by partially uncovering the plastic cover) for the next two weeks. Survived plantlets were counted after 4 weeks of cultivation in a growth chamber (16/8 h photoperiod and 22/18°C), then transferred to the greenhouse and counted after another 6 weeks.

Statistical evaluation

Twenty explants (together in two repetitions of experiments) were tested for each variant if not oth-

erwise mentioned. Experimental data were analysed by ANOVA and means were separated by *LSD* (least significant difference) test at $\alpha = 0.05$ using STATGRAPHICS Centurion XVI.II statistical software.

RESULTS

Culture initiation

In the initiation phase, two types of explants and three types of induction media were tested. Nutrient media were based on MS solidified with agar without growth regulators or with cytokinin (1 mg/L BAP) or cytokinin combined with auxin (1 mg/L BAP + 0.1 mg/L IAA). Variety Budrovka of *L. x intermedia* regenerated with 100% effectiveness from both types of explants and on all media tested (Table 1). The number of regenerated shoots was similar on all media used but well developed, and the longest shoots were observed from nodal segments (Figure 1A) on media with growth regulators (BAP or

BAP + IAA; Figure 1B, Table 1). Regeneration on IB1 medium resulted in the formation of a massive callus of yellow to light green colour, up to 20 mm in diameter on the base of nodal segments, or up to 1 cm on the base of the apical segments (Figure 1A). It was very similar for var. Grosso, but in this case, apical segments on growth regulators supplemented media regenerated significantly more shoots than the nodal segments (Table 1). Both varieties of *L. angustifolia* regenerated better from apical segments (Figure 1C, Table 1). Var. Krajová had the significantly highest number of shoots on media supplemented with 1 mg/L BAP + 0.1 mg/L IAA or BAP alone, for var. Beta, the medium with BAP was the most suitable (Figure 1D, Table 1). Brown-coloured calluses up to 5 mm in diameter were formed on the base of the shoot segments in medium with BAP or up to 10 mm in medium with BAP + IAA.

During regeneration, vitrification of shoots often occurred. It was observed through all varieties (15.3% in var. Krajová, 14.58% in var. Beta, 14.25%

T a b l e 1

Induction of shoot regeneration from apical segments (AS) and nodal segments (NS) of four genotypes of *Lavandula*: The average number of shoots per plated explant, the average height of shoots $\pm$ standard deviation (SD), and the frequency of regeneration. Columns with different letters indicate statistically significant differences calculated using the *LSD* test ($\alpha = 0.05$) for each genotype separately

Variant		No. of shoots	Shoot height [mm]	Regeneration [%]	No. of shoots	Shoot height [mm]	Regeneration [%]
Medium Explant		Budrovka			Grosso		
MS	AS	1.86 $\pm$ 0.69 ^a	36.08 $\pm$ 18.83 ^{ab}	100	2.00 $\pm$ 0.89 ^b	24.92 $\pm$ 9.22 ^{ab}	100
B1	AS	2.00 $\pm$ 0.89 ^a	28.08 $\pm$ 12.97 ^b	100	3.33 $\pm$ 0.82 ^a	33.40 $\pm$ 14.03 ^a	100
IB1	AS	2.33 $\pm$ 0.82 ^a	24.07 $\pm$ 18.46 ^{bc}	100	3.00 $\pm$ 0.41 ^a	30.57 $\pm$ 13.03 ^a	100
MS	NS	1.88 $\pm$ 0.35 ^a	15.07 $\pm$ 11.02 ^c	100	2.00 $\pm$ 0.00 ^b	16.25 $\pm$ 8.46 ^b	100
B1	NS	2.00 $\pm$ 0.39 ^a	40.25 $\pm$ 24.06 ^a	100	1.87 $\pm$ 0.35 ^b	25.96 $\pm$ 16.57 ^a	100
IB1	NS	2.00 $\pm$ 0.00 ^a	42.40 $\pm$ 31.14 ^a	100	2.00 $\pm$ 0.71 ^b	25.97 $\pm$ 17.83 ^a	94
		Krajová			Beta		
MS	AS	1.80 $\pm$ 0.84 ^b	19.50 $\pm$ 5.36 ^{ab}	100	1.20 $\pm$ 1.15 ^{cd}	19.50 $\pm$ 5.36 ^b	100
B1	AS	4.00 $\pm$ 1.50 ^a	14.67 $\pm$ 5.84 ^b	100	4.20 $\pm$ 1.30 ^a	26.00 $\pm$ 8.77 ^a	100
IB1	AS	4.00 $\pm$ 0.82 ^a	21.94 $\pm$ 14.16 ^a	100	3.50 $\pm$ 2.07 ^{ab}	20.71 $\pm$ 8.09 ^b	100
MS	NS	0.80 $\pm$ 0.79 ^c	17.00 $\pm$ 3.63 ^{ab}	60	0.80 $\pm$ 0.79 ^c	17.00 $\pm$ 3.63 ^b	60
B1	NS	1.18 $\pm$ 0.98 ^c	25.23 $\pm$ 11.07 ^a	63.6	2.33 $\pm$ 1.32 ^{bc}	17.95 $\pm$ 7.65 ^b	88.9
IB1	NS	0.75 $\pm$ 1.04 ^c	20.17 $\pm$ 4.88 ^{ab}	37.5	1.71 $\pm$ 0.49 ^{cd}	20.17 $\pm$ 9.75 ^b	100

Media: MS; MS + 1 mg/L BAP (B1); MS + 1 mg/L BAP + 0.1 mg/L IAA (IB1). MS – Murashige and Skoog medium; BAP – 6-Benzylaminopurine; IAA – Indole-3-acetic acid; *LSD* – least significant difference.

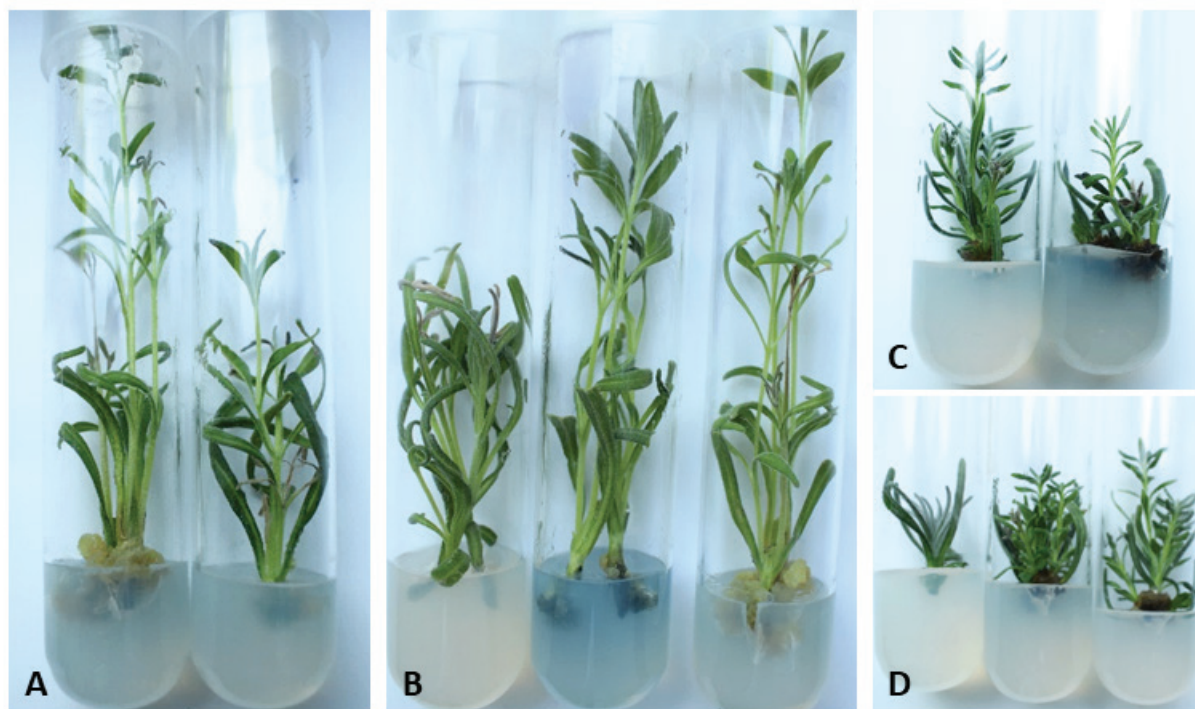


Figure 1. Shoot regeneration of lavender and lavandin: A) nodal segment (left) and apical segment of var. Budrovka in MS medium with BAP + IAA, B) comparison of regeneration of nodal segments of var. Budrovka on media (from left) MS, MS + BAP, and MS + BAP + IAA, C) apical segment (left) and a nodal segment of var. Beta in MS + BAP, D) regeneration of apical segments of var. Beta on media (from left) MS, MS + BAP, and MS + BAP + IAA. MS – Murashige and Skoog medium; BAP – 6-Benzylaminopurine; IAA – Indole-3-acetic acid.

in var. Grosso, and 7.07% in var. Budrovka) and the induction media (at least in MS media – 8.13%) without statistically significant difference. A slightly higher incidence of vitrification was observed in apical segments than in nodal segments (14.38 vs. 11.22%).

Shoot multiplication

For shoot multiplication, media without growth regulators (MS) and with 0.5 or 1 mg/L BAP with or without 0.1 mg/L IAA were tested. Like the induction phase, a higher number of shoots was formed on media supplemented with growth regulators (Table 2). The most suitable medium was MS with 0.5 or 1 mg/L BAP for varieties of *L. angustifolia* and MS + 1 mg/L BAP for var. Grosso, where the highest number of shoots and fresh weight of biomass were observed. In the second variety of *L. x intermedia* Budrovka, there was no difference among media with growth regulators concerning the number of shoots, but the height of shoots and the number of nodal segments produced (Table 2, Figure 2) was the highest in MS medium supplemented with 1 mg/L BAP combined with IAA. The addition of growth regulators en-

abled to increase the shoot multiplication coefficient (calculated as the average number of shoots formed per one explant) by 1.2 times in var. Budrovka, 1.7 times in var. Grosso and to a more significant degree in varieties of *L. angustifolia*, Beta and Krajová, 2.1 times and 2.6 times compared to medium without growth regulators. But when considering the number of nodal segments produced (Figure 2), the multiplication coefficient was more increased by growth regulators compared to MS medium in varieties of *L. x intermedia* (1.78–1.97-times) than *L. angustifolia* (1.2–1.4-times). The shoot height was highest in MS without growth regulators in varieties of *L. angustifolia* (Figure 3), but a different trend was observed in *L. x intermedia*, where the longest shoots were developed on growth regulators supplemented media (Table 2).

In vitro rooting of shoots

In the first experiment for rooting of shoots, MS media with a half dose of MS salts and vitamins without growth regulators or with 1 mg/L IAA or NAA were compared. The highest rooting frequency was observed in medium without growth

regulators (62.5–100% depending on variety), in medium supplemented with IAA 62.5% of shoots rooted (41.7–83.3% depending on variety), and in NAA-supplemented media only 41.1% of shoots rooted (16.7–75% for individual varieties). The

best rooting variety was Budrovka (75–100%), on the other hand, the weakest rooting was recorded in variety Beta (70% in medium without growth regulators, 41.7% in medium with IAA, or 16.7% in medium with NAA).

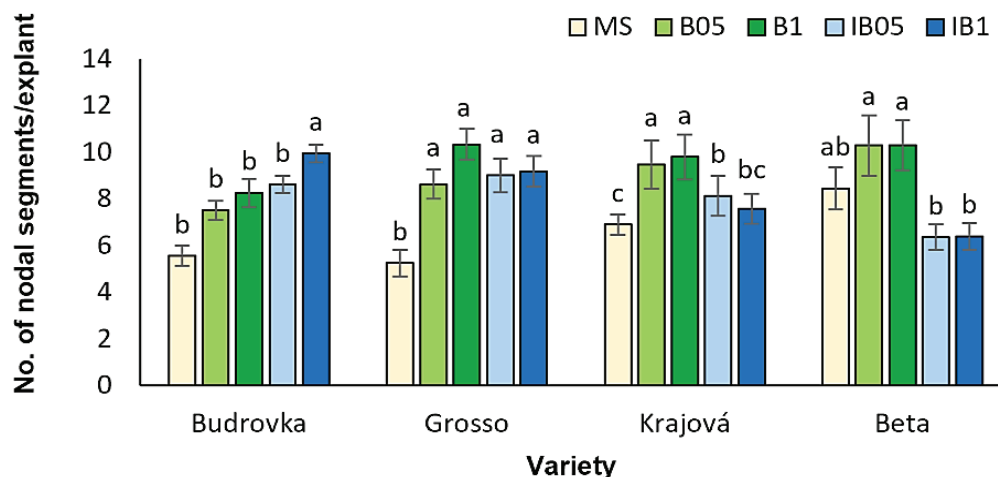


Figure 2. Shoot multiplication of lavender: The average number of nodal segments per regenerated explant $\pm$ standard deviation (SD). Columns with different letters indicate statistically significant differences calculated using the *LSD* test ($\alpha = 0.05$) for each genotype separately. Variants (media): MS, MS + 0.5 or 1 mg/L BAP (B05, B1) and 0.5 or 1 mg/L BAP combined with 0.1 mg/L IAA (IB05, IB1). MS – Murashige and Skoog medium; BAP – 6-Benzylaminopurine; IAA – Indole-3-acetic acid; *LSD* – least significant difference.

T a b l e 2

Multiplication of shoots from nodal segments (*L. x intermedia* – var. Budrovka and Grosso) or apical segments (*L. angustifolia* – var. Krajová and Beta): The average number of shoots per plated explant, the average height of shoots $\pm$ standard deviation (SD), and the biomass fresh weight measured together and recalculated per one explant. Columns with different letters indicate statistically significant differences calculated using the *LSD* test ($\alpha = 0.05$) for each genotype separately.

Medium	No. of shoots	Shoot height [mm]	Biomass FW [mg]	No. of shoots	Shoot height [mm]	Biomass FW [mg]
	Budrovka			Grosso		
MS	2.56±0.70 ^b	38.5±12.6 ^c	111.00	2.63±0.67 ^c	26.0±9.9 ^b	99.89
B05	3.00±0.51 ^a	54.9±13.7 ^b	187.03	2.85±0.46 ^{bc}	33.5±10.4 ^a	140.18
B1	3.08±0.77 ^a	69.4±10.7 ^a	172.63	4.41±2.38 ^a	40.6±12.0 ^a	219.02
IB05	3.00±0.51 ^a	64.8±14.9 ^{ab}	247.15	3.47±1.19 ^{bc}	40.2±12.9 ^a	138.08
IB1	2.92±0.34 ^a	74.8±15.4 ^a	217.44	3.62±1.07 ^{ab}	40.1±10.9 ^a	166.81
	Krajová			Beta		
MS	1.47±0.90 ^c	28.0±8.9 ^a	126.04	1.83±1.15 ^b	27.3±13.1 ^a	103.38
B05	3.65±1.84 ^a	24.6±7.3 ^{ab}	261.35	3.39±2.12 ^a	20.4±6.7 ^b	128.13
B1	3.84±2.03 ^a	22.3±4.9 ^b	225.26	3.88±1.90 ^a	19.7±4.03 ^b	148.98
IB05	3.07±1.75 ^{ab}	18.6±4.4 ^c	137.76	2.06±0.97 ^b	19.82±4.6 ^b	116.40
IB1	2.47±1.22 ^{bc}	22.1±7.4 ^b	167.86	1.83±0.92 ^b	16.83±7.1 ^b	83.76

Variants (media): MS, MS + 0.5 or 1 mg/L BAP (B05, B1) and 0.5 or 1 mg/L BAP combined with 0.1 mg/L IAA (IB05, IB1); FW – fresh weight; MS – Murashige and Skoog medium; BAP – 6-Benzylaminopurine; IAA – Indole-3-acetic acid.

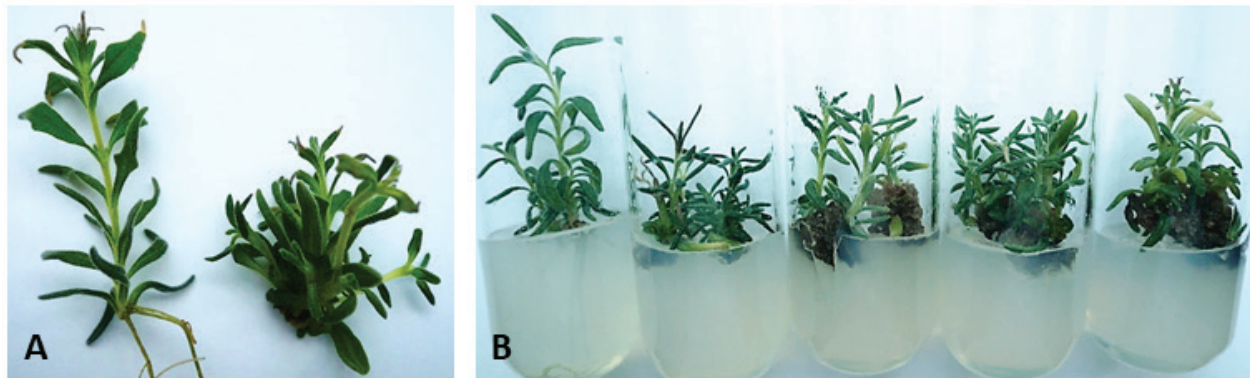


Figure 3. Shoot multiplication of *L. angustifolia*: A) differences in shoot production in var. Krajová in MS medium without growth regulators (left) or supplemented with 1 mg/l BAP, B) shoot multiplication from apical segments of var. Beta on media (from left) MS, MS + 0.5 BAP mg/L BAP, MS + 0.5 mg/L BAP + 0.1 mg/L IAA, MS + 1 mg/L BAP, and MS + 1 mg/L BAP + 0.1 mg/L IAA. MS – Murashige and Skoog medium; BAP – 6-Benzylaminopurine; IAA – Indole-3-acetic acid.

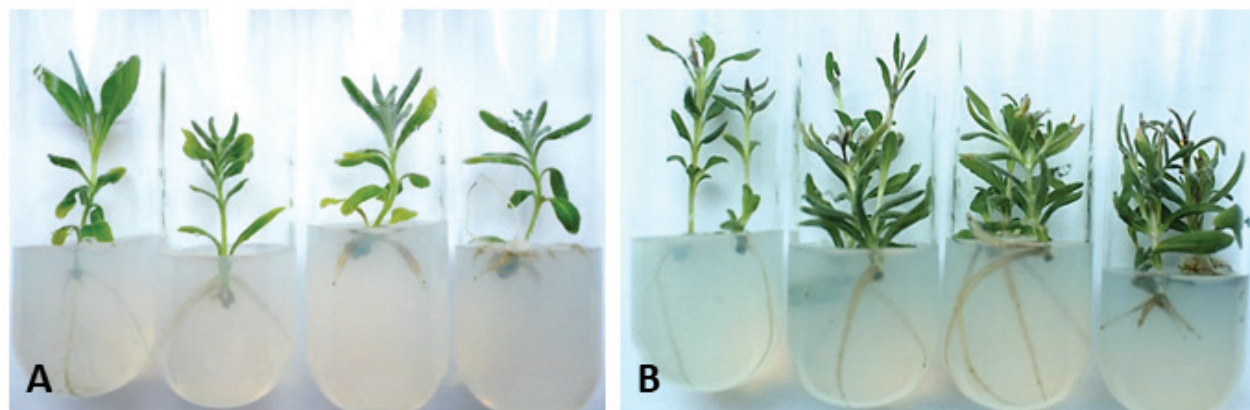


Figure 4. Root formation after 4 weeks of cultivation: A) var. Budrovka and B) var. Krajová in (from left) half-strength MS medium without growth regulators or supplemented with 0.2, 0.5, or 1 mg/l IAA. MS – Murashige and Skoog medium; IAA – Indole-3-acetic acid.

T a b l e 3

Rooting of shoots of used varieties of lavender or lavandin: The rooting frequency, the average number of roots per plated shoot, and the average length of the longest root on shoot $\pm$ standard deviation (SD) in half-strength MS medium (1/2MS) without growth regulators or supplemented with 0.2, 0.5 or 1 mg/L IAA (IAA 02, IAA 05, IAA 1). Columns with different letters indicate statistically significant differences calculated using the *LSD* test ($\alpha = 0.05$) for each genotype separately.

Medium	Rooting [%]	No. of roots	Root length [mm]	Rooting [%]	No. of roots	Root length [mm]
	Budrovka			Grosso		
1/2MS	100	3.8 $\pm$ 0.53 ^a	25.89 $\pm$ 3.63 ^a	95	3.4 $\pm$ 0.45 ^a	30.00 $\pm$ 3.23 ^a
IAA 02	85	1.6 $\pm$ 0.31 ^b	12.11 $\pm$ 3.00 ^b	90	2.0 $\pm$ 0.33 ^b	12.50 $\pm$ 4.29 ^b
IAA 05	80	2.1 $\pm$ 0.84 ^{ab}	9.00 $\pm$ 2.45 ^b	80	2.1 $\pm$ 0.57 ^b	8.75 $\pm$ 2.46 ^b
IAA 1	80	2.2 $\pm$ 0.57 ^{ab}	15.56 $\pm$ 1.98 ^b	75	2.7 $\pm$ 0.40 ^{ab}	11.00 $\pm$ 2.65 ^b
	Krajová			Beta		
1/2MS	90	2.4 $\pm$ 0.40 ^a	34.40 $\pm$ 2.74 ^a	70	1.8 $\pm$ 0.59 ^a	19.57 $\pm$ 4.64 ^a
IAA 02	75	2.2 $\pm$ 0.55 ^a	22.20 $\pm$ 2.97 ^b	30	0.4 $\pm$ 0.22 ^b	8.33 $\pm$ 2.85 ^b
IAA 05	85	2.7 $\pm$ 0.40 ^a	23.11 $\pm$ 3.12 ^b	35	0.5 $\pm$ 0.31 ^b	10.20 $\pm$ 5.23 ^b
IAA 1	80	2.6 $\pm$ 0.56 ^a	16.75 $\pm$ 4.11 ^b	65	2.1 $\pm$ 0.57 ^a	15.71 $\pm$ 4.63 ^b

MS – Murashige and Skoog medium; IAA – Indole-3-acetic acid; *LSD* – least significant difference.



Figure 5. Plants of var. Budrovka rooted in 1/2MS medium (right) and 1/2MS + 1 mg/L IAA (left) and cultivated for 4 weeks in the soil. 1/2MS – half-strength Murashige and Skoog medium; IAA – Indole-3-acetic acid.

According to the results of the first experiment, in the second experiment, different concentrations of IAA were tested: 0, 0.2, 0.5, and 1 mg/L. Rooting was evaluated after 4 weeks, but in var. Beta evaluation was done after 6 weeks because the plants only started to develop roots in the 4th week. Like in the first rooting experiment, the highest frequen-

cy of rooting was observed in the medium without growth regulators (Table 3). The highest number of roots were formed in 1/2MS medium (varieties of *L. x intermedia*) or medium with 1 mg/L IAA (varieties of *L. angustifolia*). The longest roots, determined as the average length of the longest root on the shoot, were formed in growth regulators free medium in all varieties, roots were thin and well-branched, in contrast to roots developed in media with IAA, where thick unbranched roots were formed (Figure 4).

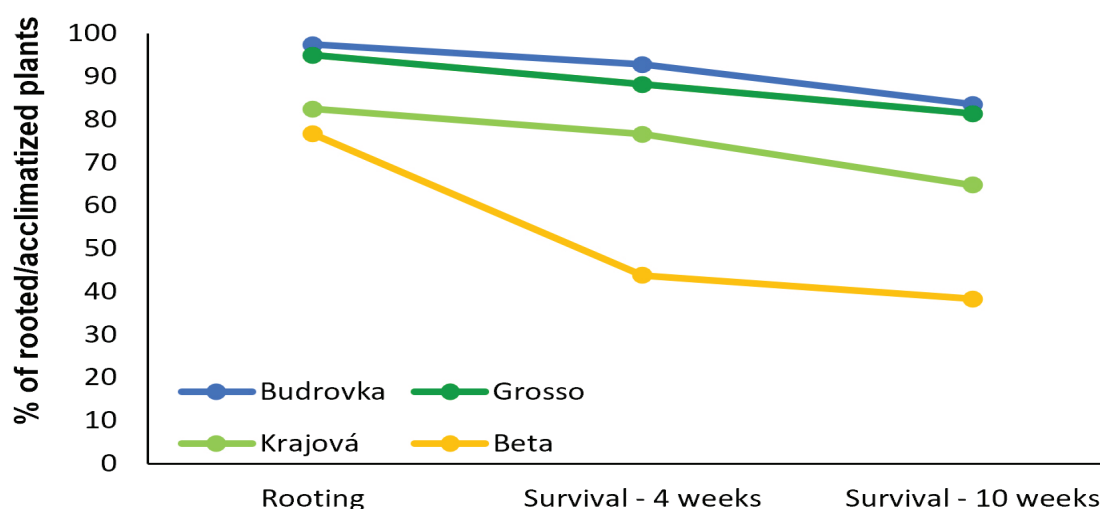
Rooted plantlets of var. Budrovka were transplanted to the soil to compare the ability of plants rooted in medium with 0.5 mg/L IAA and without growth regulator to acclimatise to *ex vitro* conditions. Ninety percent of the plants survived in both cases, but plantlets rooted in medium with IAA stunted the growth of both shoots and roots (Figure 5).

Seedling production and acclimatization

For seedling production, shoots were rooted on the half-strength MS medium that was suitable for rooting all varieties and then the plantlets were



Figure 6. Acclimatization of lavender or lavandin plantlets in soil, varieties (from left): Krajová, Beta, Grosso, Budrovka.



Phase of seedling production

Figure 7. Effectiveness of seedling production from *in vitro* shoots – [%] of rooting and acclimatization of lavender and lavandin plantlets in the soil. All data were calculated as the number of plants rooted or survived/all shoots placed in the rooting medium.

transplanted into the soil (Figure 6). Shoots were rooted with 76.7–95% frequency depending on the variety (Figure 7). The lowest level of survival of plantlets was observed in variety Beta, where 57.1% of plantlets survived after 4 weeks and 50% after 10 weeks. Survival of plantlets was much better for other varieties, 92.9–95.5% after 4 weeks or 78.9 (var. Krajová) – 87.7% after 10 weeks. The effectiveness of seedlings production was calculated as the number of plants that survived acclimatization to *ex vitro* conditions/number of shoots placed in the rooting medium. These results were similar for varieties of *L. x intermedia*, but big differences were shown between varieties of *L. angustifolia* with low effectivity in var. Beta (Figure 7).

DISCUSSION

Regeneration of plants from pre-existing meristems in tissue culture can be a simple way to obtain a large number of genetically uniform and true-to-type plants in controlled conditions within a short time. Although micropropagation has become a widely used propagation method for many plant species in agriculture and horticulture praxis, the capability of plant regeneration, multiplication and

rooting varies among different plant genotypes. In addition to species and genotype, explant type and the composition of nutrient medium, mainly the type and dose of plant regulators, are the most important factors influencing the effectiveness of plant propagation.

To initiate the *in vitro* cultures of lavender and lavandin, two types of stem explants, the nodal segments with lateral buds and apical segments, were used in our experiments. Similarly, Hamza *et al.* (2011) used the same type of initial explants for the propagation of *L. angustifolia* var. Munstead on induction media based on MS and supplemented with BAP, kinetin, or thidiazuron and found out that the nodal segments responded better than the apical segments, with the highest number of shoots in MS medium supplemented with 0.2 mg/L of thidiazuron followed by 0.8 mg/L BAP. They also mentioned problems with explant vitrification (also called hyperhydricity), which was evaluated in the medium with thidiazuron and reached 9.5–45%, according to the dose of MS salts and sugar (saccharose or glucose) in the nutrient medium.

In our experiment, we used four varieties of lavender or lavandin and the effect of BAP or BAP combined with IAA was tested. Because lavenders have shown vitrification tendency, we did not consider

thidiazuron as a suitable cytokinin, as it is known to increase the probability of vitrification and weaken shoot rooting in subsequent subcultures (Lu 1993). The highest number of shoots with the highest length was formed from apical segments in medium with 1 mg/L BAP with or without the addition of 0.1 mg/L IAA, except for the variety Budrovka, where the number of shoots on initial explants was similar in all variants of medium (without statistical differences), but the longest shoots were formed from nodal segments on the medium supplemented with the combination of BAP and IAA. During regeneration, shoot vitrification occurred in all media and varieties, although the incidence of vitrification was half as low in the variety Budrovka. This problem has also been described in other species of lavender, like *L. dentata* (Koefender *et al.* 2021) or *L. pedunculata* (Zuzarte *et al.* 2010). Since vitrification is an irreversible physiological malformation, its occurrence is undesirable because it reduces the efficiency of multiplication. In our experiments, the formation of calluses on the base of shoot segments was observed, especially in varieties of *L. x intermedia* – the larger ones (up to 20 mm in diameter) on var. Budrovka, but they did not interfere with the shoot development, as was also reported in other papers (Mitrofanova *et al.* 2020; Koefender *et al.* 2021).

Li *et al.* (2019) described the most effective shoot induction and multiplication from nodal segments of *L. angustifolia* (without variety specification) on the medium with 1 mg/L BAP combined with 0.3 mg/L IBA. Kara and Baydar (2012) compared the regeneration of shoot tips and stem nodes in the varieties of *L. angustifolia* var. Silver and *L. x intermedia* var. Super A on MS medium with 0.5 mg/L BAP and IBA. They observed the highest shoot length of the shoot tip explant of var. Super A and the highest shoot number on the stem node explant of the “Silver” variety. Mitrofanova *et al.* (2020) described shoot regeneration of four varieties of *L. x intermedia* on media with 0.5–1.0 mg/L BAP or kinetin combined with 0.05–0.1 mg/L NAA and 0.1–0.25 mg/L GA₃. In works where *in vitro* culture of *L. angustifolia* was initiated from seeds, the addition of BAP in doses 0.1–2 mg/L was usually recommended for shoot multiplication (Kirimer *et al.* 2017; Vinh *et al.* 2017).

Considering the published results, we applied BAP at a dose of 0.5 and 1 mg/L with or without the addition of 0.1 mg/L IAA for shoot multiplication in our experiments. Shoot production was similar to the induction phase and the highest number of shoots was produced on media with 0.5 or 1 mg/L BAP. In var. Budrovka, there were not again significant differences in the shoot number among media, but the longest shoots were formed in medium with 1 mg/L BAP + 0.1 mg/L IAA. This difference between Budrovka and other varieties was also clearly visible in Figure 2, which considers the total number of nodal segments produced. Although the figure shows the similarity in the rate of multiplication of all varieties, it does not reflect the reality, because the length of the individual nodal segments was different, the longest being in var. Budrovka (3.84–4.82 mm), medium in varieties Grosso (1.64–3.18 mm) and Krajová (1.06–3.46 mm), and the shortest ones in var. Beta (1.13–2.46). The shortest segments were formed on the media with a higher dose of BAP. This does not allow the shoots to be cut into only single-node segments during multiplication (except for the Budrovka variety). When using two-nodal segments, the speed of multiplication decreases and the real number of nodal explants that can be used for micropropagation is about 1.5 (var. Krajová and Grosso) – 2 times lower (var. Beta) compared to the total number of nodal segments. In the case of var. Beta, the longest shoots with a statistically not different total number of formed nodal segments were observed in a growth regulator-free medium, so also this medium can be used for the multiplication of this variety. From Figure 3 it can also be seen that BAP induces the development of multiple shoots and, on the other hand, in MS medium without growth regulators, fewer but longer and well-developed shoots are produced, which can be cut into nodal segments for multiplication or used for rooting.

Rooting of lavender shoots may be considered a critical step in propagation by stem cuttings (Leelavathi *et al.* 2020; Reed *et al.* 2021) and rooting can be easier using *in vitro* cultures (Gonçalves & Romano 2013). On the other hand, plants propagated *in vitro* under controlled conditions in containers, where the humidity is high, are not ready to survive in the field and must be gradually acclimatized to *ex vitro* conditions. Auxins IAA in doses 0.5 mg/L (Vinh *et*

al. 2017), 1.25–2 mg/L IBA (Hamza *et al.* 2011; Kirimer *et al.* 2017) or 0.4–1 mg/L NAA; (Hamza *et al.* 2011; Kara & Baydar 2012; Li *et al.* 2019), or auxin-free media (Miclea & Chifor 2018; Mitrofanova *et al.* 2020) have been successful in rooting shoots of *L. angustifolia* or *L. x intermedia*. In our first experiment for shoot rooting, we identified that auxin IAA induced rooting with a higher frequency compared to NAA. In the following experiment, doses of IAA 0.2, 0.5 and 1 mg/L were compared for rooting induction with a medium without auxin. A half dose of macro- and micronutrients was used in the rooting media because its low concentration is favoured by lavender plants (Gonçalves & Romano 2013). According to rooting frequency, number and length of roots, medium without auxin, where thin but well-branched roots were formed, was suitable for all genotypes, although the highest number of roots (but not statistically different from MS without auxin) in var. Beta was formed in the medium with 1 mg/L IAA.

Rooting frequency 90–100% and *ex vitro* survivability 79–86% (after 10 weeks in the soil) in varieties of *L. x intermedia* and var. Krajová of *L. angustifolia* can be considered successful. In var. Beta, weaker rooting (70%) and survivability of only 50% was observed. Such poor rooting ability and survivability make a protocol for micropropagation of var. Beta less effective. It is possible that it could be further improved by using other rooting media compositions (e.g., supplemented with auxin), as the quality of roots is crucial for acclimatization, or other plantlets hardening substrate that can significantly improve plantlets survivability, as reported in works of Hamza *et al.* (2011) and Li *et al.* (2019).

From an overall point of view, lavender var. Beta can currently be considered recalcitrant in *in vitro* cultures. On the other side, lavandin var. Budrovka was the easiest to cultivate because it was less susceptible to changes in media composition for individual steps of *in vitro* cultivation and showed fast *ex-vitro* adaptation and plantlets growth.

CONCLUSIONS

Most of the lavender species can be propagated by seeds, but due to a plant out-crossing, vegetative

propagation should be preferred to maintain genetic uniformity. The use of *in vitro* technology can be an alternative way to produce a large number of plants in controlled conditions without the pressure of biotic and abiotic stress factors. In this work, we proposed the composition of media for individual steps of *in vitro* multiplication of *Lavandula angustifolia* (var. Krajová and Beta) and *Lavandula x intermedia* (var. Budrovka and Grosso). For initiation of cultures, apical segments, or nodal segments (var. Budrovka) are suitable. Shoots effectively multiply in media supplemented with BAP or BAP + IAA. For rooting, the addition of growth regulators is not necessary. Concerning shoot propagation, rooting ability and survivability after transplantation into the soil, the suggested media are suitable for the effective multiplication of varieties Budrovka, Grosso, and Krajová. Multiplication of var. Beta may be further optimized mainly from the point of view of rooting and *ex vitro* acclimatization.

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