

Morphological and anatomical features of narrow-leaved lavender plants with prolonged conservation under *in vitro* genebank

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Abstract. Optimization of plants long-term conservation under *in vitro* conditions and identification of their structural and functional features during preservation is the basis for the creation of a genebank of cultured and wild plant species *in vitro*. The aim of our work was to study morphological and anatomical features of plants in lavender cultivar ‘Sineva’ after two years of *in vitro* storage at low positive temperatures. The plant material was evaluated after 24 months of conservation. A significant decrease in the linear parameters of leaves in the preserved plants was noted. Leaf shape altered from narrowly linear to oblong. With prolonged storage, the thickness of the leaf blade slightly increased. The integumentary tissues were thinner, compared with the plants cultured under standard *in vitro* conditions, the cuticle was not clear. The number of stomata decreased. The number of small vascular bundles decreased. The obtained data demonstrate viability of *in vitro* preserved explants, despite significant differences in structure, preservation of chlorophyll-containing tissues, a decrease in transpiration, and an increase of trichomes number. The stability of the vegetative organs functioning was revealed.

1 Introduction

The main task in biological diversity conservation is the comprehensive studies and preservation of genetic resources by replenishing and maintaining collections of living plants, as well as long-term conservation of explants that ensure their viability and stability [1, 2]. Scientific experience indicates that plants, in various culture conditions, can both preserve morphology and anatomy characteristics of a particular genotype, and acquire new features [3-5]. The information available in the publications does not provide a complete picture of the structural and functional changes that occur when plants are conserved in a genebank *in vitro* [1, 6, 7]. At the same time, there is a close correlation between these features and viability of stored explants. Optimization of the conditions for *in vitro* plant long-term conservation and identification of structural and functional features under their storage is the basis for the conservation of valuable genotypes and the creation of the *in vitro* plant genebank [6, 8, 9].

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In the Nikita Botanical Gardens, an *in vitro* collection of ornamental, aromatic and fruit plant cultivars has been created [10]. However, constant replenishment of the collection requires new approaches to *in vitro* preservation of the valuable genotypes, including the analysis of the adaptive capacity in the explants of particular plant species and cultivars under conditions of prolonged conservation.

The object of our work was to identify special morphological and anatomical features in *Lavandula angustifolia* Mill. plants (on the example of the cultivar 'Sineva') after prolonged conservation under the conditions of *in vitro* genebank.

2 Materials and methods

The studies were carried out in the Plant Biotechnology and Virology Laboratory of the Plant Developmental Biology, Biotechnology and Biosafety Department, Federal State Funded Institution of Science "The Labor Red Banner Order Nikita Botanical Gardens - National Scientific Center of the RAS" (NBG-NSC). Methods developed in the Plant Biotechnology and Virology Laboratory were used in the presented work [10].

Meristems 0.5-0.7 mm length were excised from the vegetative buds under a binocular microscope Nikon SM2745T (Japan) and placed on MS culture medium [11]. Viral infection was eliminated by addition 10 mg L⁻¹ virocid ribavirin (Virazole, 1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide, Sigma, USA) to induction medium. For morphogenesis induction MS medium was supplemented with 0.3-1.5 mg L⁻¹ 6-benzylaminopurine (BAP, Sigma, USA), 0.3-1.5 mg L⁻¹ 6-furfurylaminopurine (kinetin, Sigma, USA), 0.025 mg L⁻¹ α-naphthylacetic acid (NAA, Duchefa Biochemie, Holland) in addition to 0.25 mg L⁻¹ gibberellic acid (GA₃, Duchefa Biochemie, Holland) [12]. All culture media were supplemented with 30 g L⁻¹ sucrose and 9 g L⁻¹ agar-agar (Panreac, Spain).

Microshoots cultured *in vitro* for 6 months were used for conservation. Under sterile conditions, 1.0 cm long segments without leaves were separated and placed in the chemical flasks 100-150 ml on ¼ MS medium supplemented with growth inhibitors: 0.2 g L⁻¹ CCC chlorocholinchloride (BASF, Germany) and 60 g L⁻¹ sucrose. Macro- and microelements, vitamins, and 9.0 g L⁻¹ agar-agar were also added in the medium. Retardant-free ¼ MS medium supplemented with 60 g L⁻¹ sucrose was used as the control. The flasks were maintained in refrigerators LIEBHERR FKvsl 4113 (Austria) with a low positive temperature of 6°C, under light intensity 1.25-3.75 μmol m⁻² s⁻¹, and 16-hour photoperiod. The experiments were made three times in ten replications. Temporary histological slides were made according to the standard methods [13]. They were studied with CX41 light microscope (Olympus, Japan) equipped with SC 50 camera (Olympus, Germany) and Cell Sens Imaging Software version 1.17. A comparative analysis of structural changes was carried out with respect to the lavender plants cultured under standard *in vitro* conditions for 6 months. The measurements were made on the sections of 50 leaf blades.

Data statistic analysis was made with common methods using the standard software Microsoft Office Excel (2010).

3 Results and discussions

Our previous studies revealed the main parameters that affect the reduction of the growth kinetics and the preservation of the physiological stability in lavender plants under 24-month conservation: culture medium ¼ MS supplemented with 0.2 mg L⁻¹ CCC and 60.0 g L⁻¹ sucrose, temperature - 6°C. After 24 months of *in vitro* conservation in the genebank, explants of the lavender cultivar 'Sineva' maintained high viability (70-90%) and reduced growth kinetics by two times compared to the control (Fig. 1).

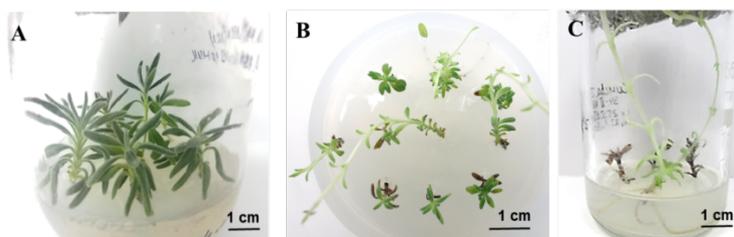


Fig. 1. Plant material of lavender cultivar ‘Sineva’: A – microshoots cultured *in vitro* during 6 months under standard conditions; B (microshoots) and C (spontaneous rooting of microshoots) – after 24 months conservation at *in vitro* genebank

Most explants demonstrated a slight elongation of the microshoots (2.40-2.93 cm), formation of leaves (6-9 ones/shoot) and roots (1-2 ones/shoot, length up to 3.20 cm). Single plants had shoots up to 4.5 cm long with elongated internodes. At the same time, on the cross-sections of microshoots their round or oval shape, clear pith, dermal, vascular and parenchyma tissues were visible (Fig. 2). The dermal tissue of the shoots was represented by a single layer of closed epidermal cells with somewhat thickened outer walls. Some cells formed simple and glandular hairs. Beneath, there were cortex parenchyma cells (chlorenchyma), rarely collenchyma cells were found. The absence of clear mechanical tissues was noted. Endodermis consisted of 1-2 cell layers. Vascular system arranged as a ring, represented by xylem and phloem. The pith consisted of parenchyma cells with thin membranes.

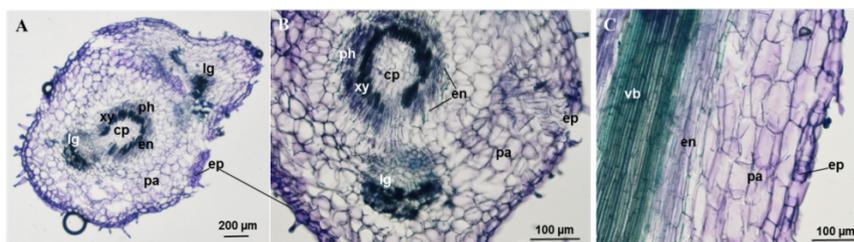


Fig. 2. Slides of *in vitro* deposited shoots of lavender cultivar ‘Sineva’: A, B – cross sections, C – longitudinal sections; Ep – epidermis, pa – parenchyma, en – endodermis, xy – xylem, ph – phloem, lg – leaf gap, cp – central cylinder parenchyma, vb – vascular bundle

Compared to the standard culture conditions *in vitro*, it was noted a trend to decrease the number of collenchyma cells and ones of the central cylinder, as well as layers of the cortical parenchyma; no significant differences in the structure of the microshoots were found. It was revealed a significant decrease (by 1.7-2.5 times compared to the control) in the linear size of leaves in deposited lavender plants. The length of the leaf blade did not exceed 0.7 cm. The shape of the leaves varied from a typical narrow-linear to oblong oval, sometimes – linear (Fig. 1).

Both in standard *in vitro* culture conditions and under prolonged deposition, a dorsiventral type of leaf structure was noted. On the cross sections of the leaf blades, one can noted epidermis with trichomes, vascular bundles, chlorenchyma, phloem, xylem and mechanical tissue (Fig. 3). Under micropropagation and deposition *in vitro* mesophyll included 3-4 cell layers. Under standard conditions, chlorenchyma was differentiated into palisade and spongy tissue (Fig. 3A), and under prolonged conservation in the genebank, it was represented by rounded mesophyll cells without clearly expressed differentiation (Fig. 3B). It should be noted that under the deposition, the leaf blade thickness slightly increased (from 129 µm in the control to 164 µm in the experiment), in some leaves an increase in the

mesophyll intercellular space was noted. Histological analysis demonstrated a decrease in the number of chloroplasts in mesophyll cells and their wall location. These features correspond with a slowdown in the growth processes in the studied explants due to the inhibition of active photoassimilation processes [14]. The number of small vascular bundles in leaves decreased from 6-8 to 2-4, they were rarely accompanied by strands of mechanical tissue. Dermal tissues were thinner than in the case of cultivation under standard *in vitro* conditions. The cuticle was not clear: its average thickness decreased from 3.2 μm to 0.8 μm . Trichomes were less branched, often consisted of 3-4 cells. Under *in vitro* conditions, lavender leaves were of amphistomatic type and stomatal apparatuses were of the anomocytic type. The number of stomata was lower in plants conserved in the genebank *in vitro*: 49-56 stomata/mm² were on the abaxial side, while under standard *in vitro* conditions, they were 142-164 stomata/mm²

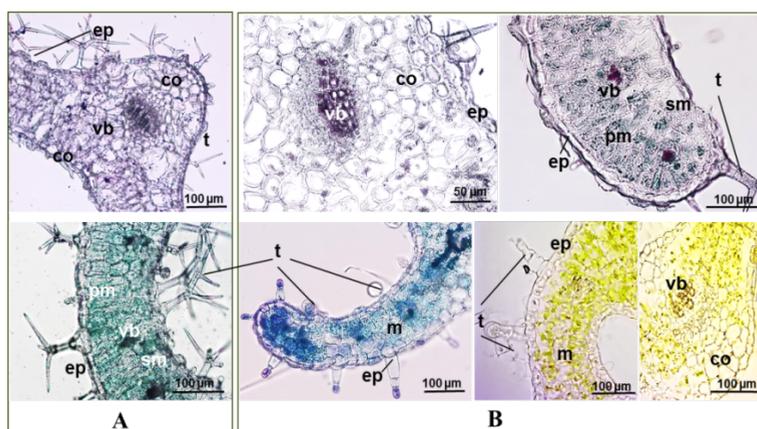


Fig. 3. Slides of the leaf blades from the microshoots of lavender cultivar ‘Sineva’: A – after *in vitro* culture during 6 months under standard conditions; B – after conservation under *in vitro* genebank conditions; ep – epidermis, vb – vascular bundle, t – trichoma, m – mesophyll, sp – spongy mesophyll, pm – palisade mesophyll, co – collenchyma.

4 Conclusions

Thus, on the example of the lavender cultivar ‘Sineva’, it was demonstrated that effective conservation of lavender plants in the genebank *in vitro* is possible with the correct choice of plant growth inhibitors in the culture medium and storage temperature, as well as taking into account both morphometric characteristics of the stored plants and their functional state. Changes in morphology and anatomy of leaves make it possible to suppose the high plasticity of the studied genotype to various *in vitro* culture conditions, and the presence of clear chlorenchyma indicates the ability to restart assimilation after prolonged deposition.

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