ISSN 2959-1864 (Online)
ISSN 2958-0536 (Print)
Volume 4, Number 2
June 2025

Acta Botanica Caucasica

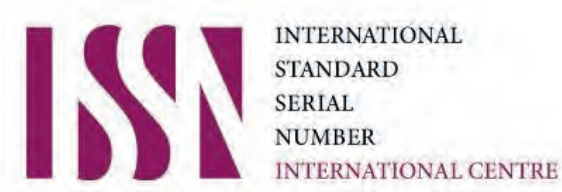
ISSN 2959-1864 (Online)
ISSN 2958-0536 (Print)

MINISTRY OF SCIENCE AND EDUCATION REPUBLIC OF AZERBAIJAN
BAKU STATE UNIVERSITY

Acta Botanica Caucasica

Volume 4, Number 2
June 2025

AZƏRBAYCAN RESPUBLİKASININ PREZİDENTİ YANINDA ALİ ATTESTASİYA KOMİSSİYASI



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COMPARATIVE ECOLOGICAL ANATOMICAL STUDY OF THE STRUCTURAL ADAPTATION OF THE MEDICINALLY IMPORTANT PLANT *SALVIA NEMOROSA* L. UNDER IN SITU AND EX SITU CONDITIONS

Aygun Sardarova |ORCID: 0009-0006-0075-74781

Department of Biology, Azerbaijan State Agricultural University, Ganja, Azerbaijan
*Correspondence: aygun.sardarova4442@gmail.com

Article Info:
DOI: https://doi.org/10.30546/abc.2025.4.2.120
Pages: 81-100
Received: April 30, 2025 | Revised: May 14, 2025 | Accepted: June 4, 2025

Abstract. *The main objective of the research is to conduct a comparative ecological anatomical analysis of the medicinally important species Salvia nemorosa, which belongs to the Lamiaceae family and is distributed in the territory of the Republic of Azerbaijan under both in situ and ex situ conditions. Comparative anatomical analyses were carried out using anatomical, microscopic, histochemical, and biometric methods to determine how anatomical variability may be reflected in the ecotypes of the same species and to explore their environmental adaptation capabilities. Anatomical sections prepared from the studied species were subjected to histological staining using reagents, and permanent slides were then prepared from these samples. Microscopic investigations revealed that in the leaves of S. nemorosa, the functional activity of tectorial and capitate trichomes was identified in the in situ sample, in contrast to the ex situ sample. Granular inclusions were observed in the libriform fibers of the root of the S. nemorosa specimen grown under in situ conditions. A tetrarch type of protoxylem structure was observed in the root of the in situ specimen, while a hexarch type was formed in the ex situ specimen. The identified ecotopic structural adaptations represent an advancement in the field of ecological anatomy and hold significant scientific and practical importance.*

Keywords: *tectorial trichome, capitate trichome, eccentric central cylinder, epistome, granular inclusion structure, parenchymatic excretion*

Introduction

Salvia nemorosa is a plant species belonging to the genus *Salvia* of the family Lamiaceae, naturally distributed in the Republic of Azerbaijan (Fig. 1). It is primarily found on mountain slopes, forest edges, meadows, and similar habitats (Gurbanov, 2024). This perennial species has a quadrangular stem structure and is relatively rigid. The rigidity and shape of the stem are attributed to the collenchyma fibers, which run along the four sides of the stem and can be observed in cross-section, providing mechanical support (Bercu et al., 2012; Aliyeva & Salimov, 2021). As the stem grows vertically, it begins to branch. The branches are arranged straight, with generative organs forming at their ends. The leaves of the plant are lanceolate or ovate-lanceolate, with dentate or undulating margins. They are arranged in an opposite-decussate manner along the stem. The leaf morphology is rugose and covered with dense trichomes (Bagheri et al., 2016a).

The chemical composition of *S. nemorosa* primarily consists of essential oils and terpenoids, phenolic compounds and flavonoids, diterpenoids and triterpenoids, polysaccharides, and other components (Chizzola, 2012; Mahdieh et al., 2018). Among the essential oils

and terpenoids, cineole is aromatic, borneol has antiseptic properties, caryophyllene is antioxidant and antimicrobial, camphene is antibacterial and antifungal, and pinene and linalool exhibit sedative effects. Phenolic compounds such as rosmarinic and caffeic acids have strong antioxidant and antimicrobial properties. Flavonoids such as apigenin, luteolin, and quercetin possess antioxidant properties. Carnose acid has neuroprotective properties, while ursolic acid is cell-protective, antimicrobial, and anticancerous, belonging to the diterpenoid and triterpenoid groups. Mucopolysaccharides act as immunostimulants. These components confirm the phytotherapeutic properties of the plant (Ibadullayeva, 2024).

Considering the potential use of S. nemorosa in folk medicine and modern medicine for therapeutic purposes, its inclusion in ecological-anatomical research is a relevant issue. Furthermore, current studies indicate that investigating species under in situ and ex situ conditions is of great importance for determining their adaptation capabilities and preserving biodiversity (Sərdarova, 2018; Khamraeva et al., 2021; Sun et al., 2022; Sardarova 2024a, 2024b, 2024c). In terms of resistance to climate variability, the study of anatomical and physiological adaptation mechanisms of *S. nemorosa* at the ecotope level is currently a highly relevant research topic discussed on international platforms.

Figure 1. A: *Salvia nemorosa*, Shusha city (in situ); B: *Salvia nemorosa*, Ganja city (ex situ)

Materials and Methods

Collection of Material. *Salvia nemorosa* was collected from the city of Shusha in May 2021 and initially cultivated ex situ in container culture in the Bala Baghman area of Ganja city. After one year, it was transplanted into open soil conditions, where it developed under the region's normal natural conditions without any artificial intervention.

Material Processing. In 2023, samples from both in situ and ex situ plants were collected and placed in fixatives for analytical purposes. Subsequently, anatomical sections were prepared from the fixed material using a microtome (RADICAL, RMT-5, India). During this process, paraffin (BW Blended Waxes, Inc. US) was used as an auxiliary medium both for infiltration and the sectioning process (Erişen et al., 2024). The

thickness of the sections was calibrated using the special micrometric adjustment screw on the microtome, and measurements were taken in microns recorded (Fig. 2). The thickness of the sections obtained using the microtome was determined to be within the range of 7, 8, and 9 µm through micrometric adjustment. After obtaining the microtome sections, histochemical methods were applied, and differential staining was conducted using specific reagents. Special stains used during this process included safranin O (KimyaLab, Turkey), fast green, Sudan III, toluidine blue, phloroglucinol-HCl, and methylene blue (INOVATING SCIENCE, US). Additionally, various chemical reagents such as xylene, carboxylol, and ethylene (Mir Nauki, Russia) were widely used. The staining process, applied for the selective coloring of tissue components, was carried out step by step using the decolorization method (Pradhan Mitra, Loqué, 2014; Moyo et al., 2015; Bozdağ et al., 2016; Da Silva et al., 2020; Engin et al., 2024). The consecutive use of various histological staining techniques allowed for a more precise clarification of the comparative ecological anatomical structure components of S. nemorosa in both ex situ and in situ conditions. For the preparation of permanent slides from the stained sections, Canada balsam (INOVATING SCIENCE, US) was used. A drop of balsam was placed onto the section positioned on a microscope slide (AmScope, USA), and then a cover slip was mounted on top. The prepared slides were placed in a special incubator device with a stable temperature (20–25°C) for complete drying of the Canada balsam, and were kept there until fully dried. Permanent preparations from the plant's transverse sections were then subjected to microscopic analysis.

Figure 2 . Preparation of permanent slides (histology staining stage).

Microscopic Investigations. The research was conducted using the "Carl Zeiss, Axio Imager A2" microscope (ZEISS, Germany), equipped with a LED light source and special objectives (5×, 10×, 20×, 40×, 100×) that minimize aberration (Fig. 3). Additionally, the LCD Digital Microscope NLCD-307B model (Wincom Company Ltd., China) was used (Fig. 4 A, B). During the research, the LCD Digital Microscope NLCD-307B was utilized to monitor the quality of the transverse sections obtained from the plant samples and the effectiveness of the histological staining until the preparation of permanent slides. Final analyses on the prepared slides, photomicrograph acquisition, and the obtaining of statistical measurements were performed using the Carl Zeiss, Axio Imager A2 model. Observations at 100× magnification were carried out using immersion oil (RMY, US). The use of the immersion medium enhanced optical resolution, improving the accuracy and contrast of the microscopic images of the studied samples. Stereoscopic microscopes were also used to perform macroscopic analysis of the samples during the study (Stereo microscope Zeiss Stemi508 (ZEISS, Germany), Stereo YK-SM067B2 (Wincom Company Ltd., China)) (Fig. 4 C, D).

Figure 3. Carl Zeiss, While working with the Axio imager A2 optical microscope model.

Biometric Methods. During the analyses, eyepiece and stage micrometers (MUHVA, China) were used to verify the accuracy of the micrometric indicators obtained automatically through the microscope. First, the eyepiece micrometer was calibrated using the stage micrometer, and then the measurements of the observed objects were calculated based on the scales on the micrometers. Additionally, a digital micrometer (Jiavarry, China) was used to record the macroscopic dimensions of the organs from which the sections were taken. The obtained measurement data were appropriately placed on the photomicrographs (Jambor et al., 2021).

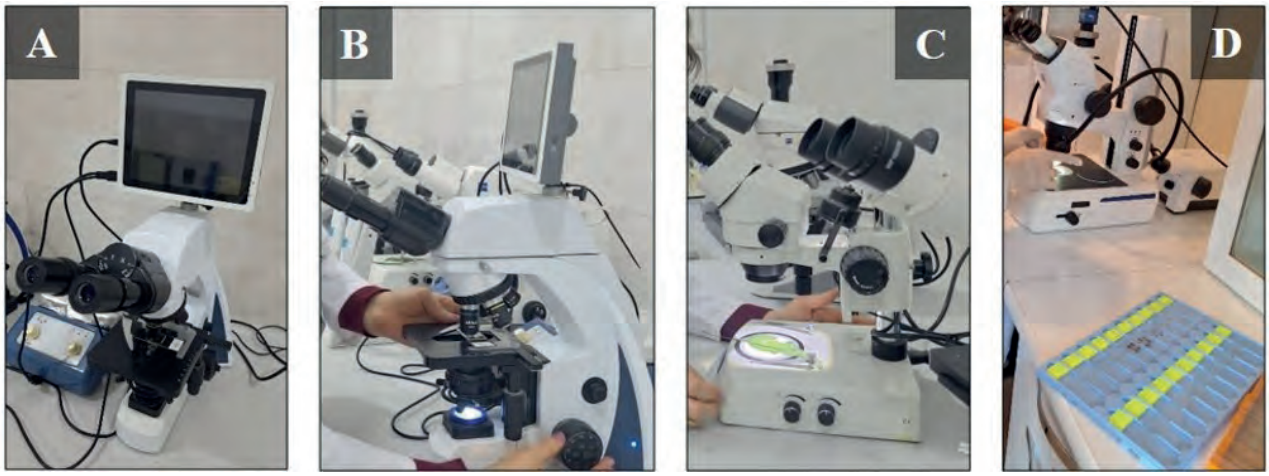

Figure 4. Optical and stereoscopic microscopes used. A, B: LCD Dijital Microskop NLCD-307B; C: Steoroscope Zeiss Stemi508; D: Stereo YK-SM067B2.

Herbarium Preparation. The systematically prepared herbarium specimens from the medicinally valuable *S. nemorosa* plant not only provide a basis for the in-depth analysis of the species' ecology and anatomy but also serve as reference material to assess its potential in pharmacognosy and phytotherapy (Uma & Düzenli, 2012). Herbarium materials prepared from both comparatively studied samples of the *S. nemorosa* species are included in the herbarium collection of the Department of Biology named after Academician Valida Tutayuq at the Azerbaijan State Agricultural University. They are maintained as a systematically organized botanical collection and are open for scientific research, educational, and reference purposes.

Statistical Method. In this study, samples of the *S. nemorosa* species collected from two

regions of the Republic of Azerbaijan with different ecological characteristics were statistically analyzed based on various parameters. From the populations of the same species in both regions, 8–12 individual plants were selected, and samples from the vegetative organs of each plant were taken for the study. For each organ, 10–15 transverse sections were prepared and subjected to anatomical analysis. During the microscopic analyses, various parameters were measured. All measurements were analyzed using the RStudio program (version 2023.09.1, R Foundation for Statistical Computing, Austria). For parameters with confirmed normal distribution, a two-sample ANOVA (analysis of variance) test was applied, while for those without normal distribution, the Mann–Whitney U test was used. All results were presented as mean ± standard deviation (SD), and statistical differences were considered significant at the $P < 0.05$ level.

Results

Flower stem. As observed in the transverse sections of flower stem from both in situ and ex situ conditions of S. nemorosa, they possess four prominent angular structures (Fig. 5 A, B). The central region, composed of pith parenchyma, is predominantly surrounded by well-developed vascular bundles extending toward the corners (Polat, 2004) (Fig. 5 A1, B1). In the in situ specimen, the pith contains large isodiametric parenchyma cells in the central area, while the cells in the perimedullary region appear smaller. In contrast, the transverse section of the ex situ specimen reveals numerous aerenchyma spaces in the center of the pith (Fig. 5 A2, B2). The vascular bundles surrounding the pith are more developed in the in situ specimen. Here, the xylem tissue is thicker, containing more vessel elements and, unlike in the ex situ specimen, also consisting of libriform fibers. In the ex situ specimen, phloem tissue forms continuously along the entire cambial belt. The smaller vascular bundles in the ex situ specimen are separated by medullary rays, with phloem tissue developing only within the bundles (Fig. 5 A4, B4). In the in situ specimen, sclerenchyma tissue has formed outward from the vascular bundles toward the cortex. The epidermis of the flower stem is covered with tectorial and capitate trichomes, which serve a protective function (Viktorov et al., 2017). Microscopic analyses revealed that metabolic processes were more intense in capitate trichomes compared to tectorial trichomes. The in situ sample contained both types of trichomes, with tectorial trichomes being longer than those in the ex situ sample. In the corner regions of the flower stem, collenchyma tissue has formed on the inner side of the epidermis. In the in situ specimen, this tissue consists of a greater number of cells, has a thicker wall, and is more densely packed (Table 1).

Table 1:

Quantitative characteristic of the flower stems of *Salvia nemorosa,* µm

Indicators	*İn situ* (Mean ± SD)	*Ex situ* (Mean + SD)
The height of the epidermis cells	10,64 ± 1,63	19,16 ± 2,72
Radial thickness of the collenchyma tissue	53,24 ± 3,81	51,24 ± 3,17
The thickness of the cortex	51,11 ± 4,08	129,51 ± 8,45
Diameter of the cortical parenchyma cells	17,03 ± 3,29	29,81 ± 2,86
Diameter of the pith parenchyma cells	39,40 ± 4,63	34,07 ± 3,48
Diameter of the xylem vessel	12,77 ± 1,57	12,37 ± 1,14

Note - ANOVA and Mann–Whitney U tests were applied for statistical analysis ($P < 0.05$)

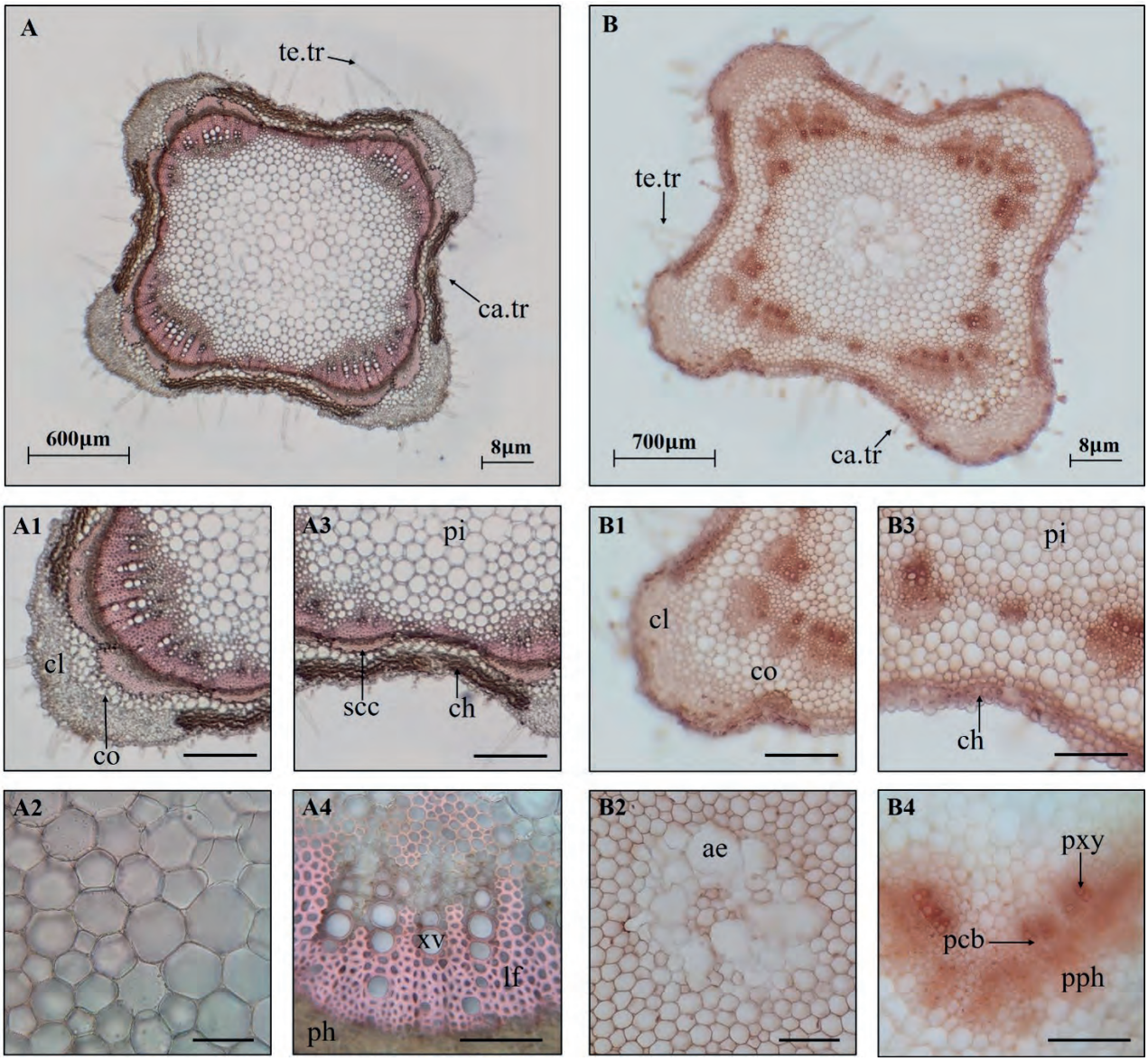

Figure 5. *Salvia nemorosa*. Flower stem. A (in situ), B (ex situ): General view (10x16); A1, B1: Ridges (40x); A2, B2: Pith (40x); A3, B3: Inter-bundle region (40x); A4, B4: Vascular bundle (60x); te.tr-tectorial trichome; ca.tr-capitate trichome; ch-chlorenchyma; cl-collenchyma; co-cortex; pi-pith; scc-sclerenchyma; pxy-protoxylem; pph-proto-phloem; pcb-procambium; xv-xylem vessels; lf-libriform fibres; ph-phloem. (scale bar: A1, B1 – 100µm; A2, B2 - 60µm; A3-4, B3-4 - 100µm)

In the cortical region between the angular corners, chloroplast-containing parenchyma cells are observed in subepidermal areas. In the in situ specimen, these smaller cells form dense layers of four to five rows, accumulating more chloroplasts. In contrast, the ex situ specimen has only two to three layers of such tissue. Toward the pith, the large parenchyma cells of the cortex are positioned beyond the chloroplast-containing cells. This layer consists of one to two cell rows in the in situ specimen, while in the ex situ specimen, it comprises four to five layers (Fig. 5 A3, B3). The cortical parenchyma progressively thickens toward the corners.

Leaf. The leaf of *S. nemorosa* has a dorsoventral structure, with the region traversed by the midrib expanding ventrally. In the ex situ sample, this area is larger in size, and its parenchyma is composed of a greater number of cells. In contrast, in the in situ specimen, the parenchyma cells are smaller, and the tissue complex appears more compact (Fig. 6 A, B) (Table 2).

Table 2:

Quantitative characteristic of the leaves of *Salvia nemorosa*, µm

Indicators	İn situ (Mean ± SD)	*Ex situ* (Mean + SD)
The height of the abaxial epidermis cells	26,85 ± 2,31	25,57 ± 1,03
Diameter of the parenchyma cells	54,98 ± 2,76	62,65 ± 3,32
The height of the central vascular bundle	281,07 ± 5,47	249,84 ± 7,59
Diameter of the xylem vessel	21,86 ± 2,64	20,46 ± 1,91

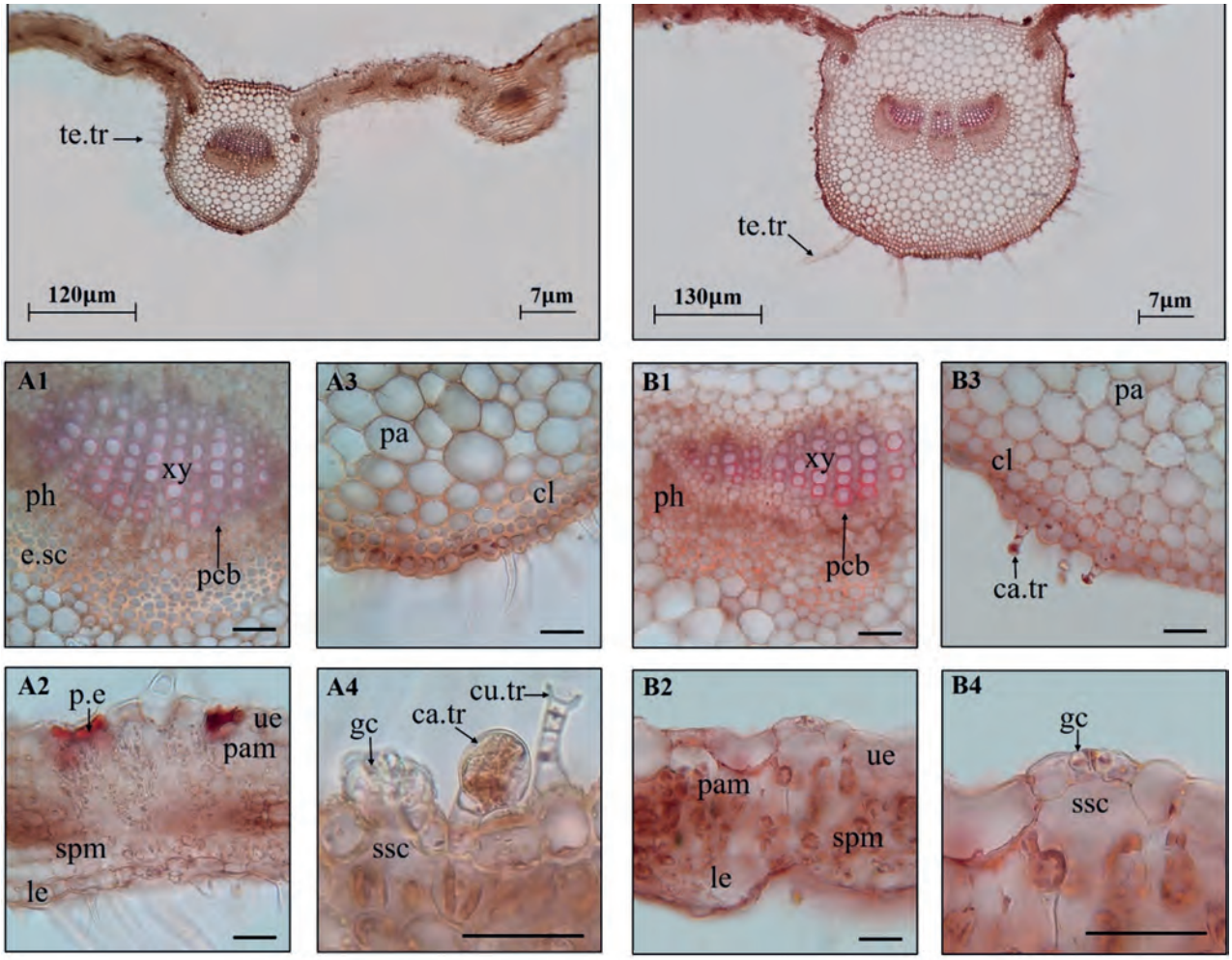

Figure 6. *Salvia nemorosa.* Leaf. A (in situ), B (ex situ): General view (4x16); A1, B1: Central bundle (40x); A2, B2: Mesophyll region (40x); A3, B3: Part of dorsal prominence (40x); A4, B4: Peripheral region (100x). ue-upper epidermis; le-lower epidermis; pa-parenchyma; pam-palisade mesophyll; spm-spongy mesophyll; cl-collenchyma; e.sc-early sclerenchyma; p.e-parenchymatic excretions; xy-xylem; ph-phloem; pcb-procambium; ssc-sub-stomatal cavity; gc-guard cell; ca.tr-capitate trichome; te.tr-tectorial trichome; cu.tr-cup-shaped trichome. (scale bar: A1-3, B1-3 – 50µm; A4, B4 - 40µm)

In the in situ specimen, the main vascular bundle located at the center of the leaf contains a greater number of xylem elements. Thickening of the walls of the xylem vessels is more actively developed in comparison to the ex situ sample. In the ex situ specimen, the central vascular system is divided into three distinct bundles by parenchymal rays. Within the vascular bundles, the procambium undergoes periclinal divisions to produce new phloem and

xylem elements (Rudik & Multyan, 2013; Hardtke, 2023). In the in situ specimen, the primary sclerenchyma, which forms in the ventral region adjacent to the phloem, exhibits normal thickening of cell walls, whereas this feature is less developed in the ex situ specimen (Fig. 6 A1, B1). In the comparative analysis of the leaves of *S. nemorosa*, the visualization of brown-colored parenchymatic excretions localized in the palisade parenchyma of the mesophyll and the adaxial epidermis covering it was conducted using different magnification levels of the objective lenses (Fig. 6 A2, B2). Additionally, dark-colored constitutional substances accumulate in the cells of the abaxial epidermis in the expanded region of the leaf. The lower localization of these substances in the ex situ sample is associated with its presence in a stable ecological environment with minimal stress factors and ensured constant conditions (Sardarova, 2022b). The adaxial and abaxial epidermis of the leaf is covered with multicellular trichomes, including capitate trichomes with a distinct large head, short multicellular trichomes with a cup-shaped small head, and longer tectorial trichomes (Kahraman et al., 2015). The trichome density is higher in the in situ sample, contributing to adaptation to extreme environments (Sardarova, 2011; Mousavi et al., 2014; Kiseleva et al., 2016). In the ex situ sample, the formed tectorial trichomes were observed to be longer compared to those in the in situ sample. Microscopic analyses confirmed the presence of epistomatic structures in the adaxial epidermis of both in situ and ex situ specimens (Kahraman et al., 2009) (Fig. 6 A4, B4). In the in situ specimen, a subepidermal collenchyma layer, composed of two to three cell rows, has formed in the ventrally expanded regions of the leaf. In contrast, this tissue is weakly developed in the ex situ specimen, appearing as a single-cell layer (Fig. 6 A3, B3).**Petiole.** The cr66oss-sectional analysis of the petiole of *S. nemorosa* revealed that the parenchyma tissue is more developed in the ex situ specimen. The vascular system at the center of the petiole is composed of two bundles in the in situ specimen, whereas in the ex situ specimen, it appears as three separate bundles. Additionally, two small-sized vascular bundles are observed at both lateral edges of the petiole (Fig. 7 A, B). In the in situ sample, vascular elements were strongly developed, and thickening of the walls of the xylem vessels proceeded more actively. The xylem, which contains a higher number of elements, exhibits a more organized structure compared to the ex situ specimen. The phloem cells in the vascular bundles of the in situ specimen are more numerous and densely arranged. The cell walls of the phloem fibers also show noticeable thickening. Furthermore, the mechanical cells surrounding the phloem have thicker walls in the in situ specimen than in the ex situ specimen. Some of these mechanical cells have undergone internal wall thickening, leading to the loss of cytoplasm and the formation of dead cells (Fig. 7 A3, B3). Unlike the central bundle, the lateral vascular bundles are larger in the ex situ specimen (Table 3).

Table 3

Quantitative characteristic of the petioles of *Salvia nemorosa,* μm

Indicators	İn situ (Mean ± SD)	*Ex situ* (Mean + SD)
The height of the epidermis cells	20,51 ± 1,03	18,84 ± 1,24
The thickness of the cell outher walls	3,41 ± 0,48	2,56 ± 0,38
Diameter of the parenchyma cells	86,53 ± 5,34	92,14 ± 6,08
The height of the central bundle	298,04 ± 8,26	259,59 ± 7,32
The height of the lateral bundle	132,89 ± 3,81	149,94 ± 4,47
Diameter of the xylem vessel	21,19 ± 2,37	25,18 ± 1,86

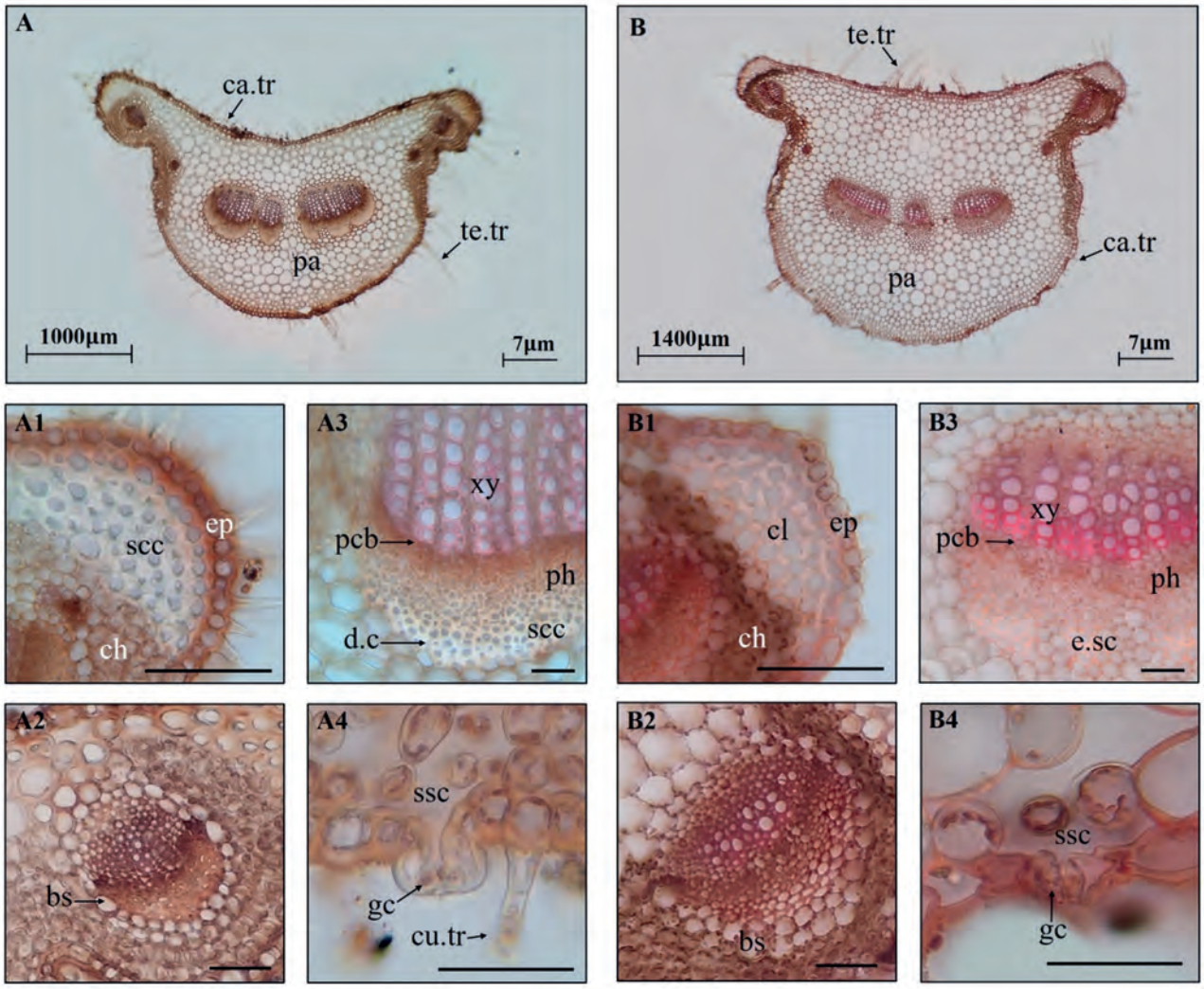

Figure 7. *Salvia nemorosa.* Petiole. A (in situ), B (ex situ): General view (4x16); A1, B1: Petiole wing (40x); A2, B2: Lateral collateral bundle (40x); A3, B3: Central bundle (40x); A4, B4: Stoma structure (100x). ep-epidermis; pa-parenchyma; bs-bundle sheat; ch-chlorenchyma; cl-collenchyma; e.sc-early sclerenchyma; scc-sclerenchyma; xy-xylem; ph-phloem; pcb-procambium; ca.tr-capitate trichome; te.tr-tectorial trichome; cu.tr-cup-shaped trichome; ssc-sub-stomatal cavity; gc-guard cell; d.c-dead cell. (scale bar: A1, B1 – 100µm; A2, B2 - 60µm; A3, B3 - 50µm; A4, B4 -60µm)

In the subepidermal layer of the petiole's outer region, the chloroplast-containing parenchyma is more actively developed. The small vascular bundles in contact with this chlorenchyma are separated from it by sheath cells (Fig. 7 A2, B2). At the ends of the petiole wings, angular collenchyma is present, while lacunar collenchyma develops in the lower subepidermal region (Kılıç M. & Kılıç F., 2022; Bagheri et al., 2016b). In the in situ specimen, collenchyma cells exhibit a higher degree of wall thickening, which is considered an adaptation mechanism to the harsh climatic conditions of the mountainous region where S. nemorosa naturally grows (Fig. 7 A1, B1). The epidermal tissue, which serves as the petiole's protective covering, contains a small number of stomata distributed throughout its surface. In the in situ specimen, the stomata have an epistomatic structure, meaning they are raised above the epidermal surface. In contrast, in the ex situ specimen, the guard cells of the stomata are mostly at the same level as the epidermal cells (Fig. 7 A4, B4). In both in situ and ex situ specimens of *S. nemorosa*, a small accumulation of constitutional substances was observed in the epidermal cells. Microscopic analysis revealed that the surface of the petiole in the in situ sample was covered with densely formed, short trichomes and more sparsely distributed,

elongated, multicellular tectorial trichomes. The majority of the short trichomes consist of multicellular stalks and capitate heads positioned on top, which have a secretory function. Among them, glandular trichomes with a calyx-shaped or cup-shaped small head are also observed (Özkan & Soy, 2007; Kaya, 2024). The remaining portion was composed of small tectorial trichomes. On the surface of the ex situ sample, these trichomes were observed in significantly lower numbers.

Stem. The anatomical analysis of the cross-section of the ex situ *S. nemorosa* stem revealed that each of the four-angled stem corners is well-developed with collenchyma tissue. In the in situ specimen of *S. nemorosa*, the entire tissue complex retains a conservative structure (Fig. 8 A, B). Each of the four corners contains a mechanical tissue composed of six to eight layers of highly sclerified and rigid cells. Using the modern digital Carl Zeiss Axio Imager A2 microscope with a 60x objective lens, it was determined through higher magnification imaging that in the in situ sample, the collenchyma located at the corners of the stem partially differentiated and underwent sclerenchymatization, which is considered an ecotypic indicator reflecting the transition of collenchyma to sclerenchyma in mechanical cells. In the in situ sample, densely arranged, few parenchyma cells were recorded. Conversely, in the ex situ specimen, the cortex is thicker and composed of larger cells (Table 4).

Table 4

Quantitative characteristic of the stems of *Salvia nemorosa*, μm

Indicators	İn situ (Mean ± SD)	*Ex situ* (Mean + SD)
The height of the epidermis cells	12,39 ± 1,46	25,17 ± 1,52
Radial thickness of the collenchyma tissue	74,76 ± 2,35	261,67 ± 4,51
The thickness of the cortex	130,83 ± 6,75	209,34 ± 5,88
Diameter of the cortical parenchyma cells	34,01 ± 2,75	55,98 ± 1,35
Diameter of the pith parenchyma cells	67,62 ± 3,51	73,45 ± 3,25
Diameter of the xylem vessel	18,68 ± 1,74	24,25 ± 1,49

The subepidermal layer, which contains chloroplast-rich parenchyma, is highly developed in the in situ specimen (Fig. 8 A1, B1). In the samples, sclerenchyma tissue was formed toward the cortex from the vascular bundles. In the in situ specimen, the cell walls in this tissue are more thickened, indicating strong sclerification and lignification. The vascular bundles are highly developed, and the xylem vessels have thicker walls compared to the ex situ specimen. Additionally, the phloem elements in the in situ specimen are more abundant, with fiber cell walls thickened and more compactly arranged (Fig. 8 A2, B2). The protective tissue forming the outer layer of the stem consists of large cells in the ex situ specimen, whereas in the in situ specimen, these cells are relatively smaller and contain a higher concentration of biologically active compounds. In both samples, it is observed that the guard cells in the stomata formed in the epidermis are positioned closer to the surface. On the inner side of these small-sized cells, a moderately sized sub-stomatal cavity has formed in the subepidermal layer (Fig. 8 A4, B4). Trichomes, as epidermal derivatives, are denser in the in situ specimen. Most of these trichomes are multicellular, elongated, and pointed tectorial trichomes. Additionally, secretory trichomes are present, including short to medium-length capitate trichomes. These trichomes consist of a glandular head positioned on a stalk composed of two, three, four, or more cells (Eroz Poyraz & Koca, 2007; Esmaeili et al., 2019) (Fig. 8 A3, B3).

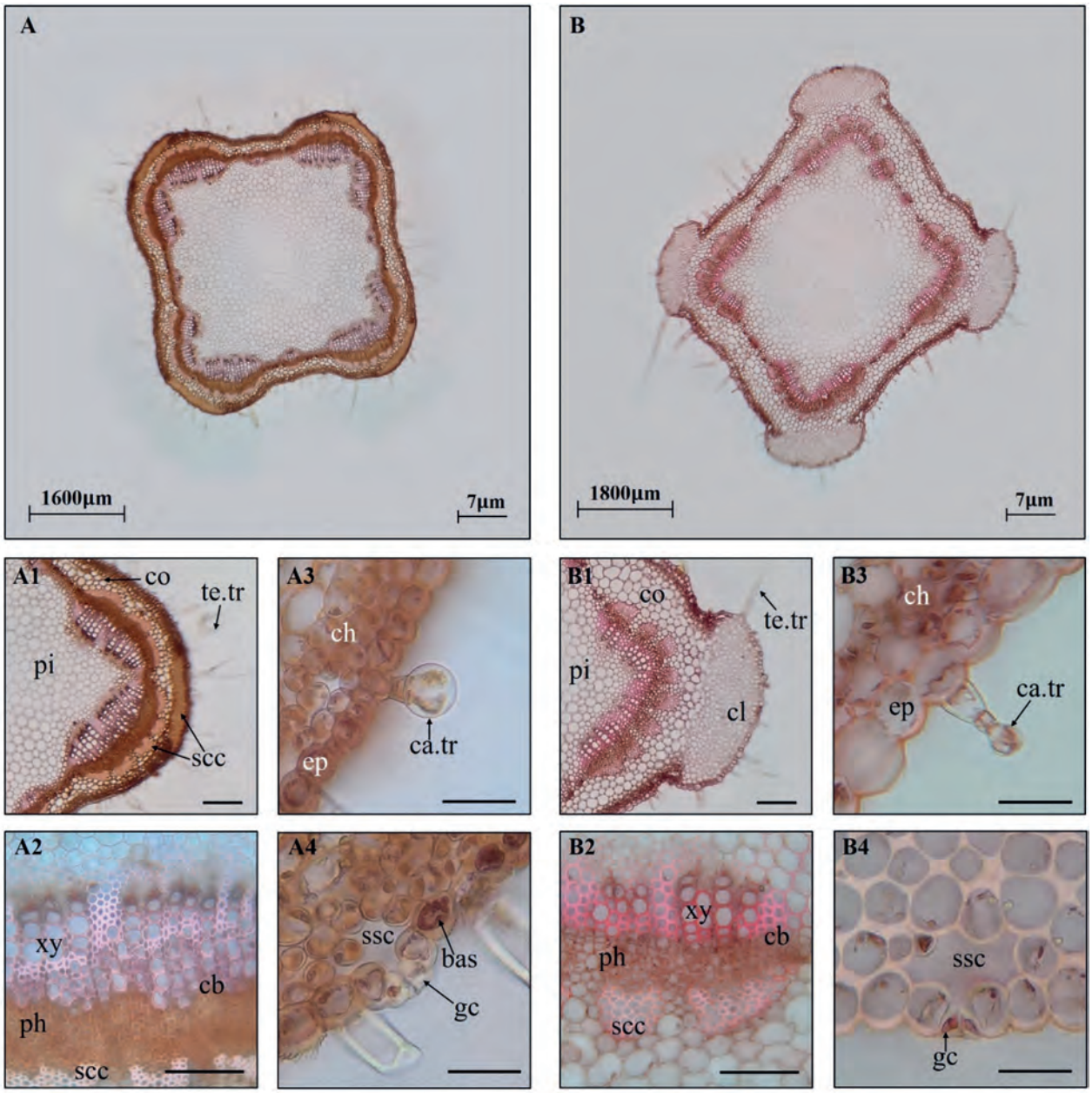

Figure 8. *Salvia nemorosa*. Stem. A (in situ), B (ex situ): General view (10x16); A1, B1: Ridges (40x); A2, B2: Collateral bundles (40x); A3, B3: Capitate trichome (100x); A4, B4: Stoma structure and metobolits in the epidermis cells (100x). ch-chlorenchyma; cl-collenchyma; co-cortex; pi-pith; scc-sclerenchyma; xy-xylem; ph-phloem; ca-cambium; bas-biological active substances; ca.tr-capitate trichome; te.tr-tectorial trichome; ep-epidermis; ssc-sub-stomatal cavity; gc-guard cell. (scale bar: A1, B1 – 200µm; A2, B2 - 100µm; A3-4, B3-4 -

Root. In the ex situ specimen of the *S. nemorosa* root, the central cylinder is large and asymmetrically positioned, denser on one side and oriented towards the cortex. This suggests that, due to environmental factors, the central cylinder in the ex situ sample is not centrally located but instead inclined toward the periphery, exhibiting an uneven expansion towards the cortical tissue (Fig. 9 A, B). In the central part of the root xylem, protoxylem and metaxylem vessels, which are the first-formed elements, can be observed. In the in situ sample, the protoxylem vessels, arranged along radial axes, form four xylem rays, indicating a tetrarch structure in the primary vascular bundle. In contrast, the ex situ sample exhibits six xylem rays, corresponding to a hexarch structure. Additionally, in the ex situ specimen, a region composed

of mechanical cells has developed in the central part, containing several metaxylem vessels (Fig. 9 A2, B2). According to the arrangement of primary elements, the in situ sample has four large medullary rays within the xylem, while the ex situ sample has six. In the in situ specimen, these rays are composed of prosenchymatic parenchymal cells, whereas in the ex situ sample, the cells forming this tissue are relatively prismatic in shape. Microscopic observations of ergastic substances in the parenchyma cells were conducted in both samples (Fig. 9 A5, B5). The sections of xylem tissue, separated by medullary rays, are composed of large xylem vessels and densely arranged small libriform fibers between them (Kahraman et al., 2011; Xu et al., 2023). Additionally, numerous medullary rays, consisting of one or two cell rows, can be observed within these sections (Fig. 9 A1, B1). Anatomical analysis revealed the presence of granular substances within the libriform fibers of the in situ sample, whereas they were found in very small amounts in the second specimen (Fig. 9 A3, B3). In the xylem tissue, particularly in the central elements and some of those near the cortex, an orange-brown substance was observed filling the intracellular spaces. In the in situ sample, a higher sequestration of the mentioned substance was observed. The xylem tissue is surrounded by phloem, within which medullary rays extend into the phloem, dividing it into sections (Fig. 9 A4, B4). In the root of the plant collected from the in situ environment, druse-shaped crystals were observed in the phloem parenchyma as well as in the cortical parenchymal cells located near the periderm. These crystals were less abundant in the ex situ sample. Anatomical investigations determined that sclerenchyma tissue had formed in the phloem region adjacent to the cortex (Özkan & Şenel, 2006). Visual analysis indicated that this tissue consisted of two groups of sclerenchyma fibers. The fiber walls in the phloem-facing region exhibited less thickening, whereas those closer to the cortex underwent intensive thickening, significantly reducing the diameter of the intracellular space. However, it can be clearly stated that the process of sclerification was more prominent in the in situ sample (Fig. 9 A6, B6). The cortex parenchyma cells in the in situ sample are smaller in size and accumulate more ergastic substances (Table 5). In contrast, the cells in the ex situ sample were larger and more elongated (Fig. 9 A7, B7).

Table 5

Quantitative characteristic of the roots of *Salvia nemorosa*, μm

Indicators	İn situ (Mean ± SD)	*Ex situ* (Mean + SD)
The thickness of the periderm	76,81 ± 2,33	68,53 ± 2,16
Diameter of the cortical parenchyma cells	32,62 ± 1,47	43,15 ± 1,78
Diameter of the xylem vessel	66,34 ± 3,15	79,43 ± 3,68

Discussion

In the in situ specimen of *Salvia nemorosa*, the amount of pectin and cellulose increased, leading to thickening and reinforcement of the collenchyma walls, characteristic of classical collenchyma structure. In the in situ specimen of the species, the complete thickening of collenchyma cell walls was considered a transitional stage toward sclerenchyma. This structural adaptation forms in response to the ecological conditions of the area and mechanical stress during plant development (Tutayuk, 1980).

Essential oils (etheric oils) accumulate in the leaves of *S. nemorosa*, primarily composed of diterpenoids and flavonoids (Koshovyi, 2012; Koshovyi et al., 2012). The accumulation of these substances in oil cells and secretory structures is distinctly visible. Microscopic studies revealed the presence of these substances in the epidermal cells of the leaf in the form of diffuse secretion. Additionally, intercellular diffuse secretion and homogeneous secretion were observed in the spongy and palisade parenchyma.

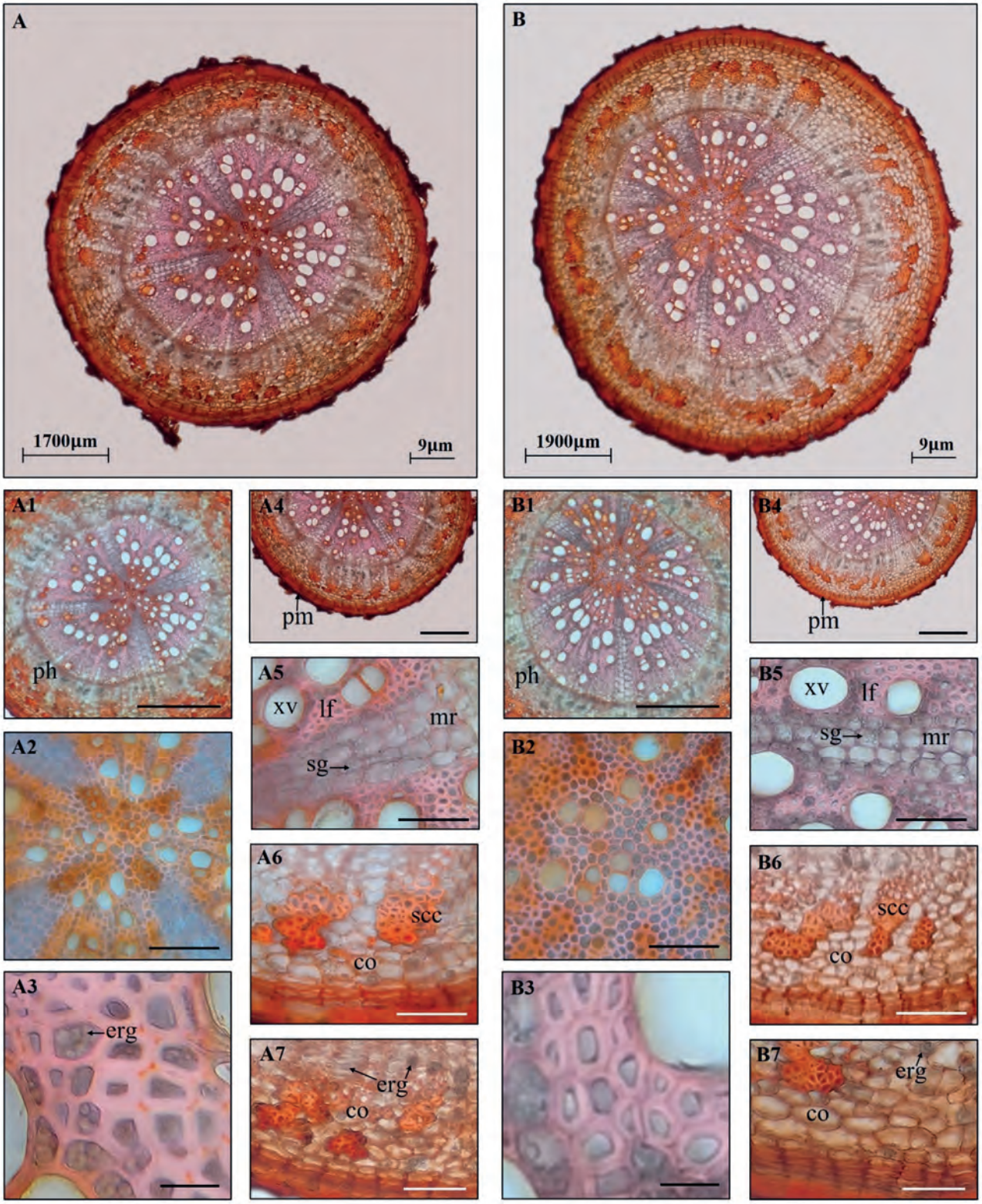

Figure 9. *Salvia nemorosa.* Root. A (in situ), B (ex situ): General view (10x16); A1, B1: Central cylinder (10x); A2, B2: Central part of xylem (40x); A3, B3: Metabolites accumulated in libriform fibers (a larger amount in the in situ sample) (100x); A4, B4: Part of the root (10x); A5, B5: Medullary ray (40x); A6, B6: Sclerenchyma tissue in the cortex (40x); A7, B7: The distribution of metabolites in the root cortex (40x). pm-periderm; co-cortex; scc-sclerenchyma; xv-xylem vessels; lf-libriform fibres; mr-medullary ray; dr-druse; sg-starch granules. ph-phloem; erg-ergastic substances. (scale bar: A1, B1 – 500; A2, B2 - 100µm; A3, B3 - 20µm; A4, B4 - 300µm; A5-7, B5-7 - 100µm)

Bagheri G. et al. (2016a) conducted an anatomical study on the petiole and stem of *S. nemorosa*. For this purpose, specimens were collected from regions with different altitudes in North Khorasan, Iran (Boujnord, Esfidan - 1561 m; Quchan, Oghazkohneh - 1800 m) and fixed in FAA solution. Hand-cut transverse sections were obtained from the samples and stained with green methyl and carmine dyes. The prepared materials were analyzed using a Labomed CZ500 light microscope. The results indicate the formation of two types of trichomes on the plant. Short glandular trichomes were found at a low frequency, and sparsely distributed simple hairs were also observed. In the ex situ specimen we studied, techtorial trichomes were similarly found in low numbers. However, in the in situ specimen, both techtorial trichomes and various types of glandular trichomes were densely distributed on the surface of the petiole (Fig. 10). According to foreign authors, the overall shape of the petiole is crescent-like, with five vascular bundles in the center and two on each side. Anatomical analyses of *S. nemorosa* petioles in both in situ and ex situ specimens revealed three vascular bundles in the central region, while two vascular bundles were present on each side, aligning with previous research findings. In the study by foreign researchers, the collenchyma in the corners of the stem was reported to consist of 6–8 layers of cells. Our microscopic analyses showed that in the in situ specimen, the collenchyma consisted of 5–6 layers of cells, whereas in the ex situ specimen, it comprised 12–13 layers.Bercu and colleagues (2012) investigated the leaf and petiole anatomy of two *Salvia* species (*Salvia nemorosa* and *Salvia nutans*) and their hybrid. Plant samples were collected from the coastal area of the Dobrudja region in Romania. Anatomical analyses revealed that the central vascular tissue in the petioles consisted of several conductive bundles. In *S. nemorosa*, the vascular system comprised two large bundles and one small bundle. In our study, the in situ sample exhibited two very large conductive bundles and one small bundle in the central part. In the ex situ sample, two large bundles and one small bundle were observed. In the study presented by foreign authors, the large petiole bundles consisted of 11 xylem rays, while the small bundle contained 4 xylem rays, with each ray composed of 4-5 xylem vessels. In our anatomical analysis of the in situ petiole of *S. nemorosa*, the large bundles contained 20 xylem rays, each with 8-12 xylem vessels, whereas the small bundle had 6 xylem rays, each formed by 7-9 vessels. In the ex situ sample, the large bundles contained 15 xylem rays, while the small bundles had 6 xylem rays, with each ray composed of 4-6 xylem vessels. Our study included a comparative analysis of the in situ (Shusha) and ex situ (Ganja) conditions of *S. nemorosa* in Azerbaijan, as well as a comparison with in situ samples collected by foreign researchers from the Black Sea coast in the Dobrudja region of Romania. The results indicate that Azerbaijan's mountainous and temperate continental climate positively influences the biomorphological and ecophysiological characteristics of the plant, with Shusha samples exhibiting stronger development. This difference is primarily attributed to the uniqueness of Azerbaijan's climatic and ecogeographical conditions. The country encompasses nine climate types and diverse ecosystems, which enhance plant adaptation potential. Particularly in high-altitude areas like Shusha, the temperate continental climate, high insolation, clean air, and soil minerals contribute to the plant's structural and functional development. Comparative ecological-anatomical analysis shows that in Shusha samples, the development of collenchyma and sclerenchyma tissues in the petiole and leaf, the vascular system, and trichomes is more pronounced, increasing the plant's mechanical resilience. The greater differentiation of the vascular system optimizes water and nutrient circulation. Increased cuticle thickness minimizes water loss. In contrast, *S. nemorosa* samples from the Black Sea coast of Romania demonstrated relatively lower mechanical tissue development and weaker structural reinforcement. This suggests that Azerbaijan's climate and ecosystem conditions are more favorable for the growth of *S. nemorosa*. From an ecological-geographical perspective, Azerbaijan's climatic diversity, multi-climatic adaptation capacity, anatomical resilience

in mountainous environments, photosynthetic optimization in continental climates, and structural reinforcement regulated by ecological stress factors contribute to the formation of the specific structure observed in the in situ *S. nemorosa*. The anatomical structure of the petiole in the *S. nemorosa* specimens collected from the coastal area of the Dobrudja region by Bercu and colleagues closely corresponds only to the ex situ petiole structure of the *S. nemorosa* plants studied in Azerbaijan. As mentioned, the number of xylem rays and xylem vessels in the vascular system of our in situ sample significantly exceeds that of the in situ plant studied by Bercu and colleagues and the ex situ plant examined in our research. During their study, the authors observed capitate glandular and non-glandular trichomes on both the leaf and petiole surfaces, differing in shape and size. They noted that non-glandular trichomes primarily consisted of three cells, though occasionally two or four cells were present. When compared with our findings, this type of covering trichome was also composed of two, three, or four cells, showing consistency. However, the trichomes in our examined plants were more abundant and densely formed on both the leaf and petiole, especially in the in situ samples. In the study by foreign authors, the collenchyma tissue in the abaxial region of the leaf's central vein area was reported to consist of 4–5 layers. Our anatomical analyses confirmed that in the in situ sample, the collenchyma tissue in the subepidermal area consisted of 4–5 layers, whereas in the ex situ sample, it comprised 2–3 layers. The authors observed that the leaf had a dorsiventral structure and was amphistomatic, which was also determined in our study.

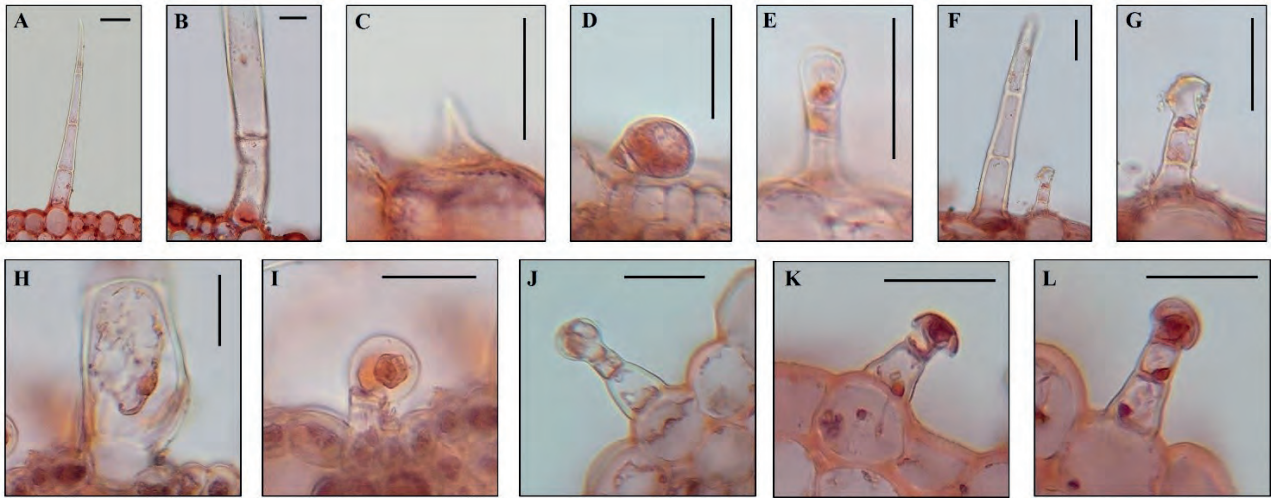

Figure 10. Various types of trichomes in Salvia nemorosa. A, B: Tectorial trichomes on the leaf; C: Conical trichomes on the leaf; D, E: Capitate trichome containing biologically active compounds on the leaf; F, G: Tectorial and capitate trichomes on the petiole; H: Tectorial trichome containing biologically active compounds on the stem; I-L: capitate trichomes containing accumulated substances on the stem; scale bars: 30µm

In the photomicrographic analysis of the ex situ sample of *S. nemorosa*, it was determined that the root had an eccentric central cylinder (Fig. 11). The microscopic image clearly shows that the central cylinder is asymmetrically positioned towards the left and has approached the cortex. The increased development of the sclerenchyma tissue in this direction is associated with enhanced mechanical stability of the root (Pandey, 2012; Prokhorenko et al., 2017). The presence of an eccentric central cylinder in the ex situ sample can be considered as an adaptation to the stable conditions of the introduced environment.

In the in situ sample of S. nemorosa grown under Shusha conditions, the root's xylem development model was found to be tetraarch, while in the ex situ samples grown under Ganja conditions, it was hexarch. This difference is related to the degree of differentiation of the

xylem elements and the dynamics of ontogenetic development, which can be attributed to ecological factors in the growing conditions (such as soil moisture, temperature regime, mineral nutrition, and mechanical stress) (Sardarova, 2022a; Khubieva, 2024). The formation of a hexarch structure in the ex situ samples grown under Ganja conditions can be explained by the longer activity of xylem protoelements and increased hydraulic conductivity. In contrast, the dominance of the tetraarch structure in the in situ samples grown under Shusha conditions is linked to the maintenance of an optimal balance between water transport and mechanical stability, which is suitable for the natural ecosystem. These results suggest that xylem differentiation is regulated by ecological plasticity and adaptation mechanisms.

Figure 11. *Salvia* nemorosa. Central cylinder of the root (10x). A: in situ; B: ex situ; xv-xylem vessels; mr-medullary ray; ph-phloem; scale bar: 500µm.

In the root of *S. nemorosa* grown under in situ conditions, the parenchyma cells are small and relatively elongated, which can be associated with their high intracellular metabolic activity and the increased synthesis of biologically active substances (such as phenols, flavonoids, and terpenoids) for defense purposes. In contrast, under ex situ conditions, the reduction of stress factors results in larger and more elongated parenchyma cells, with a decrease in the amount of biologically active substances. Regarding the variation in the concentration of biologically active substances in both ecotypes, the high concentration of ergastic substances in the root cortex under in situ conditions indicates the plant's adaptation to stress (such as water scarcity, variations in soil conditions, and interactions with microorganisms) (Bertoft, 2017; Lata et al., 2021; Kolupaev & Blume, 2022; Apriyanto et al., 2022; Pramanik et al., 2024). The decrease of these substances under ex situ conditions can be linked to the reduction of stress factors. In the root of the plant grown under in situ conditions, the presence of granular inclusions in the libriform fibers was observed. This may be related to the accumulation of intracellular metabolites or the specific differentiation of structural components.

Sclerenchyma in the cortical regions of the root is more differentiated, which can be regarded as an adaptation for protection against mechanical impacts and ecological factors. Sclerenchyma tissue consists of lignified cells that enhance mechanical stability, and its higher presence in the in situ conditions is likely the result of adaptation to ecological factors such as soil density and water scarcity. The more intensive development of sclerenchyma tissue in the root of *S. nemorosa* grown under in situ conditions can be explained by the influence of

mechanical stress factors in the natural habitat. Under ex situ conditions, however, due to the optimal cultivation conditions, the amount of sclerenchyma decreases, as the plant no longer requires additional mechanical support.

Conclusion

The eccentric differentiation of the central cylinder in the root of the ex situ sample of *Salvia nemorosa* is of theoretical significance in terms of the plant's adaptation to optimal ecological conditions and the regulation of its defense mechanisms. On the side where the central cylinder is oriented, the development of more sclerenchyma tissue in the cortex forms a structure that can contribute to increased mechanical stability and functional adaptation. The formation of tetraarch protoxylem in the in situ sample of S. nemorosa and hexarch protoxylem in the ex situ sample represents the differentiation of vascular system elements, which is an evolution of ecological anatomy and an indicator of the ecotope. Anatomical analyses have revealed that sclerenchyma tissue is intensely developed in the root of S. nemorosa. The increased presence of mechanical tissue serves to enhance the plant's resistance to the ecological pressures of mountainous terrain. Granular inclusions have been found in the root libriform fibers of the *S. nemorosa* sample growing in the in situ conditions, which is a unique finding and represents the plant's ecotypic adaptation structure. In the in situ sample of S. nemorosa, the active biosynthesis of metabolites, the formation of defensive reactions as an ecological plasticity mechanism, and the differentiation of sclerenchyma in plants in terms of resistance to ecological stress are directly related to the unique mountainous climatic factors of the city of Shusha, representing a structural adaptation.

Considering the relevance in the context of climate variability, the comparative ecological anatomical study of structural adaptations such as mechanical tissues, vascular variations, stomatal and trichomal functionality, etc., of *S. nemorosa* species under in situ and ex situ conditions is an important scientific investigation. The results obtained in this study serve as an example in assessing the adaptation potential of flora elements to climate variability in issues such as the sustainability of biodiversity in ecosystems, plant adaptation mechanisms in various ecosystems, and so on. This is a unique fact that will be presented to the contemporary international approach "COP". This work plays an important practical and theoretical role in future climate variability resilience strategies.

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ISSN 2958-0536

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