

"ALEXANDRU IOAN CUZA" UNIVERSITY OF IAȘI
FACULTY OF BIOLOGY
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SUMMARY OF THE DOCTORAL THESIS

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**COMPARATIVE MICROMORPHOLOGICAL,
HISTO-ANATOMICAL, AND
PHYSIOLOGICAL RESEARCH ON SPECIES
OF THE GENUS *ECHIU*M (FAMILY
BORAGINACEAE) IN ROMANIA**

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INTRODUCTION

The family **Boraginaceae** comprises 154 genera according to POWO (2026) and over 2,000 species distributed worldwide (Shinde and Gupte, 2023). In the flora of Romania, 21 genera are present (Guşleac, 1960; Sârbu et al., 2013), comprising herbaceous species, rarely subshrubs, with alternate, simple, exstipulate leaves covered with rough hairs (Ciocârlan, 2009).

Some genera of this family (*Anchusa*, *Borago*, *Cerinth*, *Echium*, *Pulmonaria*, *Symphytum*, etc.) include taxa known for their medicinal uses.

The genus **Echium** includes 68 taxa distributed (native or introduced) across Europe, Western Asia, Asia Minor, North and South Africa, Macaronesia, the Americas, and Australia (POWO, 2026).

In the spontaneous flora of Romania, the genus *Echium* is represented by three biennial or sometimes perennial species: **Echium russicum J. F. Gmel.** (red viper's bugloss / capul şarpelui), **Echium vulgare L.** (viper's bugloss / iarba şarpelui), and **Echium italicum L.** (Italian viper's bugloss / coada vacii) (Grinţescu, 1960; Beldie, 1979; Sârbu et al., 2013). These species possess melliferous (Chwil and Weryszko-Chmielewska, 2007; Weryszko-Chmielewska and Chwil, 2008; Ion et al., 2008), medicinal (Drăgulescu, 1992; Eruygur, 2012; Taravati et al., 2014; Bošković et al., 2017, 2021; Chrzanowska et al., 2024), and ornamental potential (Vidiraşcu and Mititiuc, 2001). Among the three species present in Romania's flora, **Echium russicum J.F. Gmel.** is a species of Community interest, listed in Annex II of the Habitats Directive under code 4047 (Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora). It occurs in approximately 130 Natura 2000 sites, around 45 of which are located within Romania.

In the scientific literature, numerous studies by international researchers focus on phytochemistry and pharmacological activities. A series of studies highlighted that the three species of the genus *Echium* (*Echium italicum* L., *Echium russicum* J. F. Gmel., and *Echium vulgare* L.) contain a wide variety of bioactive compounds

(fatty acids, sterols, terpenoids, flavonoids, tannins, phenolic acids, essential oils, naphthoquinones) and exhibit diverse biological activities (antioxidant, antimicrobial, anti-inflammatory, analgesic, antidiabetic, anxiolytic, cytotoxic) (Özcan, 2008; Nićiforović et al., 2010; Eruygur, 2012; Eruygur et al., 2016; Dresler et al., 2017; Eruygur, 2018; Jin et al., 2020; Bošković et al., 2017, 2021, 2022).

Following a review of the specialized literature, it was found that the studied *Echium* species (*Echium italicum*, *Echium russicum*, *Echium vulgare*) are insufficiently investigated from a histo-anatomical and physiological perspective, both nationally and internationally. This gap highlights the novelty and original character of this doctoral thesis.

Thus, the present study sets forth the following **aim and objectives**: **The aim of the study** is to contribute new micromorphological, histo-anatomical, biochemical, and physiological data to supplement the scientific information published in the literature regarding the biology of *Echium* L. species present in the flora of Romania.

The objectives of the present thesis are:

- **O.1.** Identifying the distribution of the three wild species of the genus *Echium* present in the flora of Romania.
- **O.2.** Analyzing the micromorphological characteristics of the surface of vegetative and reproductive organs of plants belonging to the studied *Echium* L. species.
- **O.3.** Analyzing the histo-anatomical features of the vegetative organs of plants belonging to the studied *Echium* L. species.
- **O.4.** Determining selected biochemical and physiological parameters in plants belonging to the studied *Echium* L. species.
- **O.5.** Studying the nutlet germination process and seedling growth of the investigated *Echium* L. species under experimental conditions.
- **O.6.** Investigating the allelopathic potential of aqueous plant extracts obtained from the aerial parts of wild-

harvested *Echium* species on selected test dicotyledonous plant species.

PART I – THEORETICAL CONSIDERATIONS

CHAPTER I: SPECIES CHARACTERIZATION

I.1. General Overview of the Genus Echium L.

The genus *Echium* L. belongs to the family Boraginaceae. The name derives from the Greek word *echion* ("snake/viper"), referring to the corolla's resemblance to an open snake's mouth (Grințescu, 1960). It comprises herbaceous or suffrutescent plants (biennial, rarely annual), covered with stiff and rough hairs; the flowers are zygomorphic (5-merous), with a bilabiate corolla of varied colors (blue, dark red, yellow, or white) and 5 stamens. The fruit is an erect, rugose, ovoid-trigonous nutlet (Grințescu, 1960; Gibbs, 1972).

The genus includes 68 taxa (POWO, 2026) distributed across Europe, Western Asia, Asia Minor, Africa (North and South), and Macaronesia (Romeiras et al., 2007; POWO, 2026) (Fig. 1.1.1.). A total of 44 endemic taxa are known — 26 of which are in the Canary Islands, along with others in Morocco (5), Madeira (3), Cape Verde (3), Corsica, Sardinia, Spain, Algeria, Iran, Turkey, and Syria (Mozaffarian, 1994; Bacchetta et al., 2000; Romeiras et al., 2007; Carvalho et al., 2010; Delage, 2018).

The species *Echium plantagineum* and *Echium vulgare* are considered invasive on two continents, in Africa and Australia (Zhu et al., 2017).

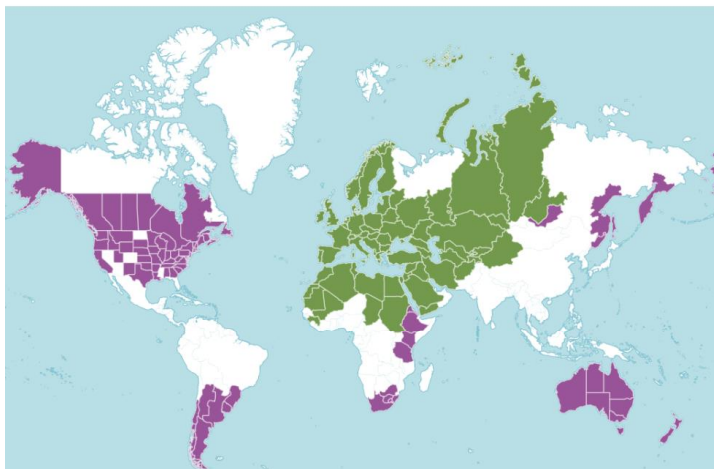


Fig. 1.1.1. Global distribution of *Echium* L. taxa. Green: native species, Purple: introduced species (POWO map, 10/01/2026)

1.2. General Information on Echium L. Species in the Flora of Romania

The genus *Echium* is represented by 3 species in the flora of Romania (Sârbu et al., 2013).

1.2.1. *Echium italicum* L. (*E. altissimum* Jacq.)¹

Echium italicum L. (syn. *E. altissimum* Jacq.) (Fig. 1.2.1.), commonly known under the popular name *coada vacii* (Italian viper's bugloss) (Tomescu, 2016). It is a biennial hemitherophyte, hispid-

¹ A portion of these data has been published as abstracts:

Sava, A. R., Stratu, A., Zamfirache, M. M., **2021.** Aspects regarding the ecology and importance of *Echium vulgare* L. and *Echium italicum* L. species. *Journal of Experimental & Molecular Biology*, 22(2);

Sava, A. R., Zamfirache, M. M., Stratu, A., **2022.** *Echium italicum* L. (family Boraginaceae): biological characteristics and potential uses. *Journal of Experimental & Molecular Biology*, 23(2).

pubescent, with a greyish appearance and a thick (6–15 mm), dark red taproot; the stem is erect, rigid, 40–100 (150) cm tall, covered with long, prickly setaceous hairs (white or yellow) arising from small tubercles, as well as short, soft hairs. Basal leaves are lanceolate (10–15 x 10–20 mm), while lower cauline (stem) leaves are linear-lanceolate, attenuated into a petiole. The inflorescence is an elongated pyramid composed of scorpioid cymes (8–9 cm); flowers are short-pedicellate, yellow, pink, or bluish-white. Corolla is infundibuliform (funnel-shaped) (10–12 mm). It possesses 4–5 stamens (3 being much longer than the corolla), pale pink anthers, and a short, pubescent style with a bifid stigma. The flowering period is June–August. The fruit is a trigonous, yellowish, tuberculate/verrucose, slightly glossy nutlet, 2.5–3 mm in size (Grințescu, 1960; Gibbs, 1972). It is a heliophilous, subthermophilous, xero-mesophilous, neutrophilous, and euryntrophilous species (Sârbu et al., 2013).



Fig. 1.2.1. *Echium italicicum* L.: – localization: Agigea, Constanța, România (original)

1.2.2. *Echium russicum* J.F.Gmel. (*E. maculatum* L., *E. rubrum* Jacq., non Forssk., *Pontechium maculatum* Böhle & Hilger)²

The species *Echium russicum* J.F. Gmelin is known in the specialized literature under several botanical synonyms, such as *Echium rubrum* Jacq., non Forssk. (Gibbs, 1972), *Pontechium maculatum* Böhle & Hilger (Böhle and Hilger, 2000), and *Echium maculatum* L. (Sârbu et al., 2013).

The genus *Pontechium* was officially described and validly published in 2000 by Böhle and Hilger (Böhle & Hilger, 2000), being segregated from *Echium* within the family Boraginaceae based on morphological and molecular evidence. Prior to this publication, the taxon was traditionally treated as *Echium maculatum* L. (Linnaeus, 1759). Following its introduction, the genus *Pontechium* was not immediately or universally adopted (Böhle & Hilger, 2000); although cited by specialists as a distinct taxonomic lineage, several regional floras continued to include it in the genus *Echium*.

Echium russicum J. F. Gmelin (Fig. 1.2.2.), commonly known under the popular name *capul șarpelui* (red viper's bugloss) (Sârbu et al., 2013). It is a biennial therophyte–hemitherophyte species, hispid-pubescent, with a greyish appearance (Grințescu, 1960). Taproot present; fusiform and branched rhizome (Beldie and Chiriță, 1967; Sava et al., 2019). The stem is erect, rigid, 30–90 cm tall, cylindrical, covered with white, rigid, setiform hairs arising from tuberculate bases, along with short and soft hairs. Basal leaves are lanceolate (10–15 cm long, 10–20 mm wide); lower cauline (stem) leaves are linear-lanceolate, attenuated into a short petiole (7–10 x 5–10 cm), whereas upper cauline leaves become progressively smaller. Inflorescence is cylindrical, 25–30 cm long, composed of short, dense cymes. Flowers are short-pedicellate, dark red (rarely white). Corolla is weakly

² A portion of these data was presented at Sesiunea științifică anuală a studenților naturaliști, Ediția a IV-a, Iași, 31 octombrie 2020, în lucrarea cu titlul: Contribuții la cunoașterea distribuției și stării de conservare a speciei *Echium russicum* J. F. Gmelin. în situri natura 2000 din România, Autori: **Alexandra Roxana Sava**, Irina Irimia, Maria-Magdalena Zamfirache

infundibuliform (12–17 mm), with nearly equal lobes and a tube at least twice as long as the calyx. It features 5 exerted stamens with reddish, glabrous filaments, as well as a long, exerted style with a subcapitate stigma. The flowering period is June–July. The fruit is a trigonous, markedly tuberculate/verrucose nutlet, 2–3 mm in size, with a flat base and an acutish apex (Grințescu, 1960; Gibbs, 1972).

Echium russicum has been declared a species of Community interest within the Natura 2000 network (code 4067). It is also included in Annexes II and IV of the Habitats Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora (revised in 2007), as well as in Annex I of Resolution 6 (1998) of the Bern Convention (revised in 2011) (EUNIS, 17/03/2021).

According to the European Nature Information System (EUNIS), the species *E. maculatum* has been inventoried in 132 sites of Community importance across Europe, of which 45 are located in Romania, 45 in Hungary, 14 in Slovakia, 13 in the Czech Republic, 12 in Bulgaria, and 3 in Poland.



Fig. 1.2.2. *Echium russicum* J. F. Gmelin. – localization: ROSCI0221 Sărăturile din Valea Ilenei, Iași, România (original)

Information provided by the Standard Natura 2000 Data Forms in Romania reveals that the vast majority of sites where the species was identified are located in the Transylvanian Plateau, the Moldavian Plateau, Dobrogea, and, to a lesser extent, in Bucovina and the Wallachian Plain (*Câmpia Română*) (Sava et al., 2020).

1.2.3. *Echium vulgare* L.³

Echium vulgare (Fig. 1.2.3.) is a biennial (sometimes perennial) hemitherophyte, hispid and with a greyish appearance, commonly known under the popular Romanian name *limba șarpelui* (viper's bugloss) (Sârbu et al., 2013), as well as other vernacular names such as *capul-viperei*, *viperină*, *ursoaică*, *boranță*, *ochiul-mâței*, or *iarba-năpârcii* (Milică et al., 2012). It develops a black taproot (6–15 mm thick) and an erect, rigid stem of 25–100 cm, covered with short, soft hairs interspersed with long, prickly hairs set on red or violet tubercles. Leaves are linear-lanceolate, with lower leaves arranged in a rosette and attenuated into a petiole (8–10 x 8–15 mm), while cauline (stem) leaves are sessile and uninervate (2–6 x 8 mm). The cylindrical-paniculate inflorescence is composed of 5–15 (20) simple cymes (2–6 cm), arched at maturity. Flowers are short-pedicellate, initially reddish, later turning blue, pink, or white; the infundibuliform and bilabiate corolla (10–22 mm) features a tube shorter than the long, setiform-pubescent calyx. The species has 5 exserted stamens with long, reddish, upward-curved filaments, as well as a thin, pubescent style exceeding the length of the stamens. The fruit is an obliquely ovoid-trigonous, tuberculate nutlet of 3–4 mm, and the flowering period extends from June to September (Grințescu, 1960; Gibbs, 1972).

³ A portion of these data has been published as abstract:

Sava, A. R., Stratu, A., Zamfirache, M. M., 2021. Aspects regarding the ecology and importance of *Echium vulgare* L. and *Echium italicum* L. species. *Journal of Experimental & Molecular Biology*, 22(2);



Fig. 1.2.3. *Echium vulgare* L. – localization: Blocuri Păcurari, Iași, România (original)

1.2.4. Phytosociological Classification

Based on the analysis of specialized literature (Chifu et al., 2014), the phytosociological classification of the three *Echium* species from Romania was established:

- *Echium italicum* occurs in 27 plant associations.
- *Echium russicum* is present in 41 plant associations.
- *Echium vulgare* was identified in 184 plant associations.

1.2.5. Natura 2000 Habitat Types in Which *Echium* L. Species Have Been Recorded

The three *Echium* species from Romania have been identified in habitats of Community interest, some of which are considered priority habitats (marked with *), thereby highlighting their importance for biodiversity conservation. These habitats are included in Annex I of Directive 92/43/EEC.

1.3. Distribution of Echium L. Species in Romania

The analysis of specialized literature contributed to shaping an overview of the national distribution of these species.

1.3.1. Echium italicum L.

It was recorded in: Jud. Arad, Jud. Bihor, Jud. Caraș-Severin, Jud. Călărași, Jud. Constanța, Jud. Dolj, Jud. Hunedoara, Jud. Iași, Jud. Mehedinți, Jud. Satu Mare, Jud. Sălaj, Jud. Suceava, Jud. Tulcea și în Dobrogea.

1.3.2. Echium russicum J.F.Gmel.

It was recorded in: Jud. Alba, Jud. Arad, Jud. Bacău, Jud. Botoșani, Jud. Brașov, Jud. Buzău, Jud. Caraș-Severin, Jud. Călărași, Jud. Cluj, Jud. Dolj, Jud. Galați, Jud. Hunedoara, Jud. Iași, Jud. Mehedinți, Jud. Mureș, Jud. Neamț, Jud. Prahova, Jud. Sălaj, Jud. Suceava, Jud. Tulcea, Jud. Vaslui.

1.3.3. Echium vulgare L.

It was recorded in: Jud. Alba, Jud. Arad, Jud. Argeș, Jud. Bacău, Jud. Bihor, Jud. Bistrița-Năsăud, Jud. Botoșani, Jud. Brașov, Jud. Buzău, Jud. Caraș-Severin, Jud. Cluj, Jud. Constanța, Jud. Covasna, Jud. Dolj, Jud. Galați, Jud. Gorj, Jud. Harghita, Jud. Hunedoara, Jud. Iași, Jud. Maramureș, Jud. Mehedinți, Jud. Mureș, Jud. Neamț, Jud. Olt, Jud. Prahova, Jud. Satu Mare, Jud. Sălaj, Jud. Sibiu, Jud. Suceava, Jud. Teleorman, Jud. Timiș, Jud. Tulcea, Jud. Vaslui, Jud. Vâlcea, Jud. Vrancea.

CHAPTER II: CURRENT STATE OF KNOWLEDGE IN THE FIELD

II.1. Brief History of Morpho-Anatomical and Micromorphological Research

The morphology and anatomy of vegetative organs in the family Boraginaceae are well represented in classical literature, yet specific data for the genus **Echium** remain fragmentary and scattered. Fundamental treatises, such as that by Metcalfe and Chalk (1950), describe the general anatomical characters of the family, alongside isolated references to the genus *Echium*.

At the national level, plant histo-anatomical research has a valuable tradition, synthesized in works regarding the development of phytomorphology at the "Alexandru Ioan Cuza" University of Iași (Toma and Toniuc, 2008), which highlight studies on vegetative organs in species belonging to the genera *Borago*, *Cerinthe*, and *Echium* from the flora of Romania (Ifrim and Toniuc, 2006).

At the international level, research on the genus *Echium* has correlated anatomical and morphological aspects with ecological traits and geographical distribution, addressing endemic taxa from the Cape Verde Islands (Romeiras et al., 2008) and Macaronesia (Aldridge, 1981), as well as molecular analyses aimed at clarifying phylogeny (Cohen, 2014). Additionally, numerous qualitative and quantitative comparative studies have analyzed the structure of vegetative organs in related genera (*Anchusa*, *Nonea*, *Coldenia*, *Symphytum*, *Cynoglossum*, *Pulmonaria*, *Echium*) (Akcin and Baki, 2007; Akcin et al., 2010; Akçin et al., 2012; Barykina and Alyonkin, 2016; Barykina et al., 2016; Yeşil, 2017), occasionally extending to fruit and stem structure (Keshavarzi et al., 2013). Particular interest has been paid to early ontogenetic development (Barykina and Alyonkin, 2017, 2018) and the ecological anatomy of wood (Carlquist, 1970; Baas and Schweingruber, 1987; Rao and Kumar, 1989).

Within the genus *Echium*, histo-anatomical research is relatively limited and focused on specific species or organs. For instance, Sava et al. (2019) provided a detailed analysis of the

structure of the rhizome, aerial stem, and leaf in *Echium russicum* within the Sărăturile din Valea Ilenei nature reserve, while Papp et al. (2011) conducted comparative histological studies among *Echium vulgare*, *Pulmonaria officinalis*, and *Symphytum officinale*.

Special attention has been given to structures with high taxonomic value, such as nutlets, whose micromorphology was investigated in *E. italicum* (Özcan, 2013), *E. vulgare* and *E. plantagineum* (Latini et al., 2022), *E. anchusoides* (Bacchetta et al., 2000), and other Boraginaceae (Filimban et al., 2025; Ullah et al., 2025), alongside analyses of the relationship between seed structure and the germination process (Molinelli et al., 2020). Regarding vegetative organs, some studies compared the morphology and histo-anatomy of species from different regions (Abdel Aal and El-Shazly, 1999) or focused on specific traits, such as trichome types and indumentum (Bramwell, 1972), the stomatal apparatus (Dasti et al., 2003), and secretory structures (Chwil and Weryszko-Chmielewska, 2007; Weryszko-Chmielewska and Chwil, 2008).

Overall, integrated studies on the anatomo-functional structure of vegetative organs in *Echium vulgare* and *Echium italicum* remain limited, thus justifying the need for in-depth research that correlates structural variation with physiological functions and ecological adaptations of these species.

II.2. Uses in Traditional Medicine of Echium L. Species.⁴

Plants of the genus *Echium* have a rich history of use in traditional medicine across various regions of the world. For ***Echium italicum***, ethnobotanical studies conducted in Turkey show that the root and aerial parts were applied externally (poultices or ointments) for wound healing, bruises, abscesses, and the relief of rheumatic pain

⁴ A portion of these data has been published as abstracts:

Sava, A. R., Stratu, A., Zamfirache, M. M., **2021**. Aspects regarding the ecology and importance of *Echium vulgare* L. and *Echium italicum* L. species. *Journal of Experimental & Molecular Biology*, 22(2);

Sava, A. R., Zamfirache, M. M., Stratu, A., **2022**. *Echium italicum* L. (family Boraginaceae): biological characteristics and potential uses. *Journal of Experimental & Molecular Biology*, 23(2).

(Altundag and Ozturk, 2011; Fujita et al., 1995; Gürbüz et al., 2021; Han and Bulut, 2015; Özgökçe and Özçelik, 2004; Tabata et al., 1994). In traditional Iranian medicine, the flowers and aerial parts are used to treat wounds, sore throats, and colds, as well as for their carminative, soporific, nervous system relaxing effects (Ghasemi et al., 2013; Sahranavard et al., 2009), and antidiabetic properties (Faiezizadeh et al., 2016). In Italy, uses of the aerial parts have been reported (de Natale and Pollio, 2007; Pieroni, 2000), while in Macedonia, the roots form the basis of an anti-inflammatory and wound-healing ointment (Albreht et al., 2009).

Various studies have been carried out to scientifically validate the traditional uses of *Echium* species. Extracts obtained from different parts of the studied plant species have demonstrated diverse biological activities: **antioxidant** (Conforti et al., 2009; Nićiforović et al., 2010; Nogala-Kalucka et al., 2010; Nikolova et al., 2011; Eruygur et al., 2012; Tahmouzi, 2014; Bošković et al., 2017; Alamholo, 2020; Bošković et al., 2021; Uysal et al., 2021; Bošković et al., 2022; Ateşşahin et al., 2023); **analgesic** (Eruygur et al., 2012); **anti-inflammatory** (Conforti et al., 2009); **antibacterial/antimicrobial** (Morteza-Semnani et al., 2009; Karakaş et al., 2012; Bošković et al., 2017; Alamholo, 2020; Bošković et al., 2022; Ateşşahin et al., 2023); **antidepressant** (Moallem et al., 2007); **antilisterial** (Tahmouzi, 2014); **antitumor** (Karakaş et al., 2012); **anxiolytic and hypnotic** (Hosseinzadeh et al., 2012); **cytotoxic** (Conforti et al., 2008; Bošković et al., 2017; Bošković et al., 2021; Mihoğlugil et al., 2023; Ateşşahin et al., 2023, 2025); and **wound healing** (Eruygur et al., 2012, 2016).

II.3. Chemical Composition of Echium L. Species

A wide variety of organic compounds has been identified in different parts of *Echium italicum*, *Echium russicum*, and *Echium vulgare* plants: amino acids, fatty acids, phytosterols, phenolic compounds, volatile oils, naphthoquinones, allantoin, and pyrrolizidine alkaloids (Sava et al., 2021; 2022a).

II.3.1. Chemical Composition of *Echium italicum* L.

In the aerial part of *Echium italicum*, 16 amino acids were identified and quantified by Özcan (2016).

The total oil content in the nutlets was determined using a Tecator Soxhlet System HT and ranged between 19.3% and 23.5% (Özcan, 2013). For the total oil content in seeds, values between 11.94% and 15.70% were obtained (Özcan and Süzerer, 2020).

The fatty acid concentration in the oil composition varied depending on geographical region and altitude (Özcan, 2013; Taravati et al., 2014; Özcan and Süzerer, 2020). Özcan and Süzerer (2020) identified 21 fatty acids in the composition of the seed oil. Polyunsaturated fatty acids predominate in the oil composition of *Echium italicum* (Özcan, 2013). Compared to other *Echium* species (*Echium vulgare*, *Echium plantagineum*, *Echium angustifolium*), the highest amounts of α -linolenic and stearidonic acid were determined in the seeds of *Echium italicum* (Özcan, 2008).

Morteza-Semnani et al. (2009) extracted the essential oil (by hydrodistillation) for the first time from the flowering aerial parts of *Echium italicum* (collected from Iran) and analyzed it by GC-MS (Gas Chromatography-Mass Spectrometry). Twenty-two compounds were identified, representing 17.1% monoterpenes, 3.8% sesquiterpenes, and 69.2% non-terpenoids. The major identified compounds were n-hexadecanol (27.1%) and pulegone (8.8%).

The presence of shikonin and several shikonin derivatives (acetylshikonin, deoxyshikonin, 2-methyl-n-butyrylshikonin, isovalerylshikonin, β -dimethylacrylshikonin) in the root of *Echium italicum* was also reported by Eruygur et al. (2016) and Eruygur (2018) in samples collected from wild populations in Turkey.

Dresler et al. (2017) extracted and quantified shikonin (as total shikonin derivatives) from the root of *Echium italicum* and three other Boraginaceae species (*Echium vulgare* L., *Echium russicum* J.F. Gmel., and *Lithospermum officinale* L.).

II.3.2. Chemical Composition of *Echium russicum*

J.F.Gmel.

For the species *Echium russicum*, data regarding its chemical composition are limited. Fatty acids have been identified in the nutlets, while phenolic compounds, naphthoquinones, and allantoin were identified in the roots and shoots.

The nutlets of *Echium russicum* feature a high content of polyunsaturated fatty acids. The determined fatty acids are: γ -linolenic acid, α -linolenic acid, linoleic acid, oleic acid, palmitic acid, and stearic acid (Guil-Guerrero et al., 2003).

II.3.3. Chemical Composition of *Echium vulgare* L.

Polysaccharides from flowers were extracted by Tahmouzi (2014) from *Echium vulgare* plants harvested in Iran.

The nectar contains sucrose, fructose, and glucose in a ratio of 8.6:1.0:0.7 (Southwick et al., 1981, cited by Klemow et al., 2002).

Three sterols were identified in the leaves and flowers (Conforti et al., 2008).

Barberis et al. (2022) analyzed protein amino acids (via HPLC) and biogenic amines (by high-performance liquid chromatography with a diode-array detector – HPLC-DAD) from the floral nectar of *Echium vulgare* from two natural populations across two different flowering periods.

Total phenolic content and total flavonoid content were determined in various plant parts: root (Eruygur et al., 2012); leaves (Alsanie et al., 2018); aerial part (Nićiforović et al., 2010; Eruygur et al., 2012; Bošković et al., 2022); leaves and flowers (Conforti et al., 2008); and seeds (Nogala-Kalucka et al., 2010).

The pyrrolizidine alkaloids identified in **Echium italicum** and **Echium vulgare** are mainly represented by echimidine, echiumine, intermedine, lycopsamine, and their acetylated and *N*-oxidized derivatives (Skoneczny et al., 2015; Mädge et al., 2020; Stefova et al., 2022; Petreska Stanoeva et al., 2023).

PART II – ORIGINAL RESEARCH

CHAPTER III: BIOLOGICAL MATERIAL AND RESEARCH METHODS

III.1. Plant Material

The plant material that formed the subject of the present research (specimens belonging to the wild species *Echium vulgare* L. and *Echium italicum* L.) was collected from six counties of Romania (*E. vulgare* L. – Table 3.1.1) and from a single county (*Echium italicum* L. – Table 3.3.1), during the years 2021, 2022, and 2023, between June and July. This material was identified using the identification key developed by Sârbu et al. (2013) and verified by Dr. Irina Irimia, biologist and curator of the Herbarium of the Faculty of Biology, "Alexandru Ioan Cuza" University of Iași, where one individual – voucher specimen – was deposited for each sampling location (Table 3.3.1).

Table 3.3.1. Collection sites of the plant material under study

Species	Location		Number of voucher
<i>Echium vulgare</i> L.	Iași	Valea lui David	186547
		Vlădeni	186548
		Pădurea Repedea-Bârnova, Releu	186573
	Bacău	Bacău	186572
	Botoșani	Băiceni	186550
	Neamț	Borca	186549
	Suceava	Suceava	186551
<i>Echium vulgare</i> L. - fasciated	Iași	Valea lui David	207019
<i>Echium italicum</i> L.	Constanța	Agigea	184568

Although in the wild flora of Romania the genus *Echium* is represented by three taxa, plant material was collected from only two species (*Echium vulgare* L. and *Echium italicum* L.), because *Echium russicum* J.F.Gmel is a protected species of community interest (Habitats Directive 92/43/EEC, Annex II). It was not collected from the natural environment, and seeds originating from individuals within or outside natural areas of interest were not used for research.

The plant material used in the research consists of:

- **herbarium material** belonging to the three previously mentioned species, from the collections of four herbaria in Romania;
- **whole specimens** collected from the wild flora belonging to *Echium vulgare* L. and *Echium italicum* L. (fresh, dried, and preserved in 70% alcohol plant material) and seeds of *Echium italicum* L.;
- **nutlets (seeds)** purchased from "Jelitto Perennial Seeds" (Fig. 3.3.4), Germany (*Echium vulgare* L. EA020, *Echium russicum* J.F.Gmel. EA017) and commercially purchased seeds of *Raphanus sativus* var. *sativus* L. ("Cherry Belle" cultivar) and *Sinapis alba* L.

In species of the genus *Echium*, the term *seed* is not used in a strict sense, as species of this genus possess fruits called nutlets, which are dry and indehiscent, with the seed remaining intimately attached to the fruit (Roso et al., 2017).

For the plant material collected from the wild flora, the following analyses were performed:

- **micromorphological analyses** of the surface of vegetative and reproductive organs using a Scanning Electron Microscope (SEM) (*Echium italicum* L.; *Echium vulgare* L.);
- **histo-anatomical analyses** of sections through the vegetative organs of the studied plants (roots, stems, leaves);
- **determination of foliar photosynthetic pigment content;**

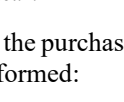
- **determination of foliar water and dry matter content;**
- **germination testing** of nutlets subjected to SEM surface micromorphological analysis (*Echium italicum* L.);
- **preparation of alcoholic extracts** and their biochemical characterization (determination of total polyphenol content, total flavonoid content, and antioxidant activity);
- **preparation of aqueous extracts** and testing of their allelopathic potential.


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- **micromorphological analyses** of the nutlet surface using a Scanning Electron Microscope (SEM) (*Echium vulgare* L. EA020, *Echium russicum* J.F.Gmel. EA017);
- **germination testing** of nutlets (seeds) subjected to SEM surface micromorphological analysis (*Echium vulgare* L. EA020, *Echium russicum* J.F.Gmel. EA017);
- **testing the effect of a biostimulant** on germination and growth processes (*Echium russicum* J.F.Gmel. EA017);
- **testing the effect of heavy metals** on germination (*Echium vulgare* L. EA020, *Echium russicum* J.F.Gmel. EA017);
- **testing the potential allelopathic effect** of aqueous extracts prepared from the aerial parts of *Echium italicum* and *Echium vulgare* on the germination and growth of two dicotyledonous test plant species (*Raphanus sativus* var. *sativus* L. "Cherry Belle" cultivar and *Sinapis alba* L.);
- **determination of total polyphenol content, total flavonoid content, antioxidant activity, and photosynthetic pigment content** in the test plant seedlings thus obtained.

III.2. Research Methods and Techniques

III.2.1 Distribution of Spontaneous *Echium* L. Species in Romania

In collaboration with Dr. Irina Irimia, biologist, data regarding the distribution of the species *Echium italicum* L., *Echium russicum* J.F. Gmel., and *Echium vulgare* L. were analyzed from the Herbarium of the Faculty of Biology at "Alexandru Ioan Cuza" University of Iași, the Herbarium of the "Anastase Fătu" Botanical Garden in Iași, the Herbarium of the "Ion Ionescu de la Brad" University of Life Sciences in Iași, and the Herbarium of the University of Craiova.

III.2.2. Analysis of Environmental Factors in the Sampling Areas

The environmental factors analyzed in the sampling areas of the plant material (represented by specimens of wild *Echium italicum* and *Echium vulgare* subjected to analysis) were: air temperature and relative humidity, atmospheric precipitation, atmospheric cloudiness (cloud cover), and soil type.

III.2.3. Preparation of Aqueous and Alcoholic Extracts

From the dried plant material (aerial parts) of wild *Echium italicum* and *Echium vulgare* specimens, aqueous extracts at concentrations of 1%, 2%, 5%, and 7.5% were prepared according to procedures described in the literature (Moždzeń et al., 2016; Vukajlović et al., 2018).

III.2.4. Micromorphological and Histo-anatomical Determinations

For the micromorphological analysis, the plant material used (nutlets – collected from the wild and purchased; stem fragments, leaf blade, sepals, flowers, and stamens obtained from wild plants) was dried at room temperature, mounted on aluminum stubs covered with double-sided carbon adhesive tape, sputter-coated, and examined under a Scanning Electron Microscope (SEM).

The analyses were carried out using a Tescan Vega II SBH scanning electron microscope at the Electron Microscopy Laboratory of the Faculty of Biology, "Alexandru Ioan Cuza" University of Iași.

The staining technique used for the prepared sections was double staining with iodine green and ruthenium red (Tarnavschi et al., 1974; Șerbănescu et al., 1983).

III.2.5. Determinations of Biochemical and Physiological Indicators

III.2.5.1. Determination of Water and Dry Matter Content

The determination of water and dry matter content was carried out on average samples of fresh leaves and stems of plants collected from the wild.

The water and dry matter content is determined by calculating the percentage of water loss from the fresh biomass of the analyzed sample after maintaining it at a constant temperature of 105 °C until reaching a constant weight (Boldor et al., 1983).

III.2.5.2. Determination of Photosynthetic Pigment Content

A. The photosynthetic pigment content, using 80% acetone as a solvent, was determined from leaves collected from wild individuals during the flowering phenophase as well as the fruiting phenophase. Absorbance was read on a Shimadzu spectrophotometer at wavelengths of 663 nm, 646 nm, and 470 nm against an 80% acetone blank. The obtained values were substituted into the equations for calculating photosynthetic pigment concentration developed by Lichtenthaler and Wellburn (1983). The results are expressed in µg/mL plant extract.

B. The photosynthetic pigments from seedlings experimentally obtained during the germination process were extracted using 96% ethyl alcohol as a solvent. Absorbance was read on a Shimadzu spectrophotometer at wavelengths of 664 nm, 649 nm, and 470 nm against a 96% ethyl alcohol blank. The obtained values were substituted into the equations for calculating photosynthetic pigment concentration developed by Lichtenthaler and Wellburn (1983). The results are expressed in µg/mL plant extract.

The determinations of photosynthetic pigment content in leaves and seedlings were carried out in triplicate.

III.2.5.3. Determination of Total Polyphenol Content

The determination of total polyphenol content was carried out on extracts obtained from dried plant material (aerial parts) of wild *Echium vulgare* L. and *Echium italicum* L., as well as from seedlings of *Raphanus sativus* var. *sativus* L. ("Cherry Belle" cultivar) and *Sinapis alba* L. (Brassicaceae family) grown from seeds subjected to testing with aqueous extracts of the aforementioned *Echium* species.

The total polyphenol content of the plant material was determined using the Folin–Ciocalteu method (Singleton et al., 1999), which is based on the reduction of the Folin–Ciocalteu reagent (a mixture of phosphotungstic acid and phosphomolybdic acid) by the phenolic groups in the studied material. This reduction reaction results in a color change (from yellow to blue), the intensity of which is directly proportional to the polyphenol concentration and is measured using a Shimadzu spectrophotometer at a wavelength of 760 nm.

III.2.5.4. Determination of Total Flavonoid Content

The determination of total flavonoid content was carried out on extracts obtained from dried plant material (aerial parts) of wild *Echium vulgare* L. and *Echium italicum* L., as well as from seedlings of *Raphanus sativus* var. *sativus* L. ("Cherry Belle" cultivar) and *Sinapis alba* L. (Brassicaceae family) grown from seeds subjected to testing with aqueous extracts of the aforementioned *Echium* species.

The total flavonoid content in the plant material was determined using the aluminum chloride (AlCl_3) colorimetric method, according to Jia et al. (1999) and Herald et al. (2012). The method is based on the formation of a complex between the carboxyl or hydroxyl groups of flavonoids and aluminum ions, resulting in a yellow color measured on a Shimadzu spectrophotometer at a wavelength of 510 nm.

III.2.5.5. Determination of Antioxidant Activity

The determination of antioxidant activity was carried out on extracts obtained from dried plant material (aerial parts) of wild *Echium vulgare* L. and *Echium italicum* L., as well as from seedlings of *Raphanus sativus* var. *sativus* L. ("Cherry Belle" cultivar) and *Sinapis alba* L. (Brassicaceae family) grown from seeds subjected to testing with aqueous extracts of the aforementioned *Echium* species.

The determination of antioxidant activity in the plant material was performed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method according to Thaipong et al. (2006) and Herald et al. (2012).

The DPPH radical is purple in color and turns yellow in the presence of antioxidants from the plant material, a color shift attributed to the extract's ability to scavenge free radicals. This color change is measured using a Shimadzu spectrophotometer at a wavelength of 515 nm.

III.2.6. Imbibition, Germination, and Growth Tests under Experimental Conditions

III.2.6.1. Germination Testing of Seeds Subjected to SEM Analysis

To perform the germination test, the seeds (nutlets) initially subjected to SEM analysis were used, their preparation process being described in section III.2.4. For the germination study, a total of 112 nutlets were used (28 for each species). Filter paper moistened with tap water was used as the germination substrate. The germination percentage was evaluated at 7 and 14 days after the start of the experiment. The selection of these evaluation intervals followed the working protocol for crop species (Boldor et al., 1983), adapted for species of the genus *Echium* (Roso et al., 2017). The germination percentage was calculated according to the formula described by Islam and Kato-Noguchi (2014).

III.2.6.2. Testing the Effect of a Biostimulant on Imbibition, Germination, and Growth in *Echium russicum* J.F.Gmel.

As a biostimulatory substance, Atonik solution was used at concentrations of 1‰ and 3‰ applied to the nutlets prior to sowing, the working concentrations being selected according to data from the literature (Djanaguiraman et al., 2005; Kocira et al., 2017).

The experiments were conducted exclusively using *Echium russicum* seeds procured from "Jelitto Perennial Seeds", Germany.

To determine gravimetric imbibition, three replicates were used for each variant (V1, V2, V3), with each replicate containing 12 nutlets; the mass of each sample was determined with an accuracy of 0.0001 g on an analytical balance. The amount of water / Atonik solution absorbed at each time interval was calculated using the formula described by Marler (2019).

For the germination study, nutlets imbibed for 8 hours in distilled water (V1), 1‰ Atonik solution (V2), and 3‰ Atonik solution (V3) were used; non-imbibed seeds were used for the control variant (C). For each variant, three pots were used (1 pot = 12 seeds). The substrate for germination and growth consisted of a commercial universal soil mix.

The universal soil used contained ALONET Sphagnum peat (60%), shredded and sieved leaf mold (15%), compost (10%), washed river sand (10%), softwood sawdust (5%) + perlite. The soil had a loose texture, high water-holding capacity, and a pH ranging between 6 and 7.

The effect of the treatment with 1‰ and 3‰ Atonik solution on the germination of the test species was evaluated by calculating the emergence percentage at 7 and 14 days from the start of the experiment. After 47 days, the seedling survival percentage was determined.

The pots were maintained under laboratory conditions with an ambient temperature of 24 °C–27 °C and an average photoperiod of 15 hours light and 9 hours dark. Soil moisture in the pots was

monitored daily and maintained by watering with tap water. The total duration of the experiment was 61 days.

To evaluate the effect of the treatment with 1‰ and 3‰ Atonik solution on seedling growth, the following morphological indicators were determined: root length, shoot length (hypocotyl + epicotyl), number of leaves per plant, true leaf area, and cotyledonary leaf area. Leaf area was determined using a portable compact AM-100 Area Meter (ADC BioScientific Ltd. Analytical, Hoddesdon, UK), and lengths were measured using a ruler.

*III.2.6.3. Testing the Effect of some Heavy Metals on Imbibition and Germination in **Echium vulgare L.** and **Echium russicum J.F.Gmel.***

To perform the imbibition, germination, and growth tests, nutlets belonging to the species **Echium vulgare** and **Echium russicum** purchased from "Jelitto Perennial Seeds" were used.

The amount of water / solution absorbed at each determination interval was calculated using the formula described by Marler (2019).

For the germination study, the nutlets were immersed in copper sulfate and zinc sulfate solutions (treatment variants) and in distilled water (control variant), respectively, for 4 and 8 hours, respectively. Following the imbibition period, excess water/solution was removed from the seeds using filter paper, and they were subsequently placed to germinate in Petri dishes on filter paper moistened with distilled water.

The Petri dishes were maintained under laboratory conditions with a photoperiod of 12 hours light and 12 hours dark and an average temperature of 21 °C.

The effect of the treatment with CuSO₄ and ZnSO₄ solutions, respectively, on the germination of the test species was evaluated by

calculating four germination indicators according to the formulas described by Islam and Kato-Noguchi (2014).

*III.2.6.4. Testing the Allelopathic Effect of Aqueous Extracts of **Echium vulgare** L. and **Echium italicum** L. on Test Species*

To test the allelopathic potential of aqueous extracts prepared from wild plants belonging to the species *Echium italicum* and *Echium vulgare*, seeds of two test species from the Brassicaceae family were used: *Raphanus sativus* var. *sativus* L. ("Cherry Belle" cultivar) and *Sinapis alba* L.

For the germination study, seeds of the two test species were set up to germinate in Petri dishes moistened with distilled water (control) and with aqueous extracts of **Echium vulgare** (E.v.) and **Echium italicum** (E.i.) at concentrations of 1% (V1), 2% (V2), 5% (V3), and 7.5% (V4), using 20 seeds of each test species per dish, in triplicate.

The Petri dishes were kept in an Aqua Lytic incubator at a constant temperature of 23 °C, with a photoperiod of 12 hours light and 12 hours dark, for 7 days.

The effect of the treatment with aqueous extracts of **Echium vulgare** and **Echium italicum** on seed germination of the test species was evaluated by calculating four germination indicators according to the formulas described by Islam and Kato-Noguchi (2014).

To evaluate the effect of the aqueous extract treatment on seedling growth, the following morphological indicators were determined: root length and hypocotyl length, measured directly using a ruler, and seedling fresh weight, measured using an analytical balance.

III.2.7. Statistical Analysis

The values obtained for all studied parameters are expressed as mean $\pm$ standard error. Statistical differences between variables were evaluated using statistical tests, specifically one-way ANOVA and Tukey's HSD post-hoc test. Statistical analyses were performed using GraphPad Prism 10 software. Graphically presented data represent mean $\pm$ standard deviation. Statistical significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. Values without asterisks are not statistically significant ($p \geq 0.05$).

CHAPTER IV: RESULTS AND DISCUSSION

IV.1. Distribution of Spontaneous *Echium* L. Species in Romania⁵

The data obtained from the specimens in the consulted herbaria were centralized by species and county.

This analysis revealed that the oldest specimen for *Echium italicum* L. was recorded in June 1904 (voucher 3678) from Vârciorova, Caraș-Severin County; for *Echium russicum* J.F.Gmel. in May 1898 from Cîrlig and Mârzești, Iași County (vouchers 3681–3684); and for *Echium vulgare* L. in June 1896 from Bârnova, Iași County (voucher 3687), preserved in the Herbarium collection of the Faculty of Biology, "Alexandru Ioan Cuza" University of Iași.

⁵ Part of these data have been presented at **Trends in Biology: from molecules to complex systems 2026**, 15-18 octombrie 2026, în lucrarea cu titlul: Distribution of *Echium* species in Romania based on herbarium data, Autori: Alexandra Roxana Sava, Irina Irimia, Maria-Magdalena Zamfirache

According to the specimens from the consulted herbaria, *Echium italicum* L. is found in Romania in the following counties: Caraș-Severin, Constanța, Dolj, Galați, Giurgiu, Ilfov, and Vaslui (Fig. 4.1.1).

According to the consulted herbarium specimens, *Echium russicum* J.F.Gmel. is found in Romania in the following counties: Alba, Bacău, Bistrița-Năsăud, Botoșani, Brașov, Buzău, Caraș-Severin, Cluj, Covasna, Dolj, Galați, Iași, Ilfov, Olt, Suceava, Vaslui, and Vrancea (Fig. 4.1.2).

According to the consulted herbarium specimens, *Echium vulgare* L. occurs in Romania in the following counties: Bacău, Botoșani, Brăila, Buzău, Caraș-Severin, Cluj, Constanța, Galați, Harghita, Iași, Mureș, Neamț, Olt, Satu Mare, Suceava, Vaslui, and Vrancea (Fig. 4.1.3).

The analysis of herbarium data and its centralization at the county level is important for integrating dispersed records into organized spatial datasets. As noted in the literature (Heberling et al., 2019), this action aids in creating species distribution maps, supports ecological and climatic studies, and provides applicable information for biodiversity conservation.

IV.2. Analysis of Environmental Factors

IV.2.1. Analysis of Climatic Factors

Climatic factors play an important role in plant life, acting upon them through intensity, rate of change, and duration of exposure (Burzo, 2014).

In the research forming the subject of this thesis, the following climatic factors were investigated: air temperature, relative humidity, atmospheric precipitation amount and duration, and cloud cover (nebulosity).

In the collection areas of the studied **Echium** species, a higher amount of atmospheric precipitation was recorded during 2021

compared to 2022 and 2023; relative humidity was higher in 2021, the year 2022 was characterized by the lowest cloud cover, and 2023 was the warmest year.

According to the recorded data, in the area of Iași city (Iași County), higher precipitation was recorded in 2021 compared to 2022 and 2023; mean, minimum, and maximum air temperatures over the three years gradually increased from 2021 to 2023, atmospheric precipitation decreased during 2022, and cloud cover recorded its lowest value in 2022 compared to 2021 and 2023.

In the area of Agigea commune (Constanța County), low precipitation and a predominance of sunny over cloudy days were recorded during 2022, whereas in 2021 higher relative air humidity was observed compared to the other two calendar years.

In the area of Bacău city (Bacău County), the highest amount of atmospheric precipitation and a predominance of cloudy days were recorded throughout 2021.

In the area of Suceava city (Suceava County), a higher amount of atmospheric precipitation was recorded in 2021 compared to 2022 and 2023.

In the area of Băiceni locality (Botoșani County), higher precipitation was recorded in 2021 compared to 2022 and 2023.

In the area of Borca commune (Neamț County), mean air temperatures in 2021 were lower than in 2022 and 2023, whereas atmospheric precipitation, relative humidity, and cloud cover showed higher values during 2021.

In the area of Vlădeni commune (Iași County), higher atmospheric precipitation was recorded in 2021 compared to 2022 and 2023 (Figs. 4.2.19–4.2.21).

The study areas displayed the same climatic trend observed at the national level, characterized by an increase in mean, minimum, and maximum atmospheric temperatures over the 2021–2023 period.

Air temperature, together with other climatic factors, influences all vital plant processes: germination, growth and development, photosynthesis, plant biomass accumulation, respiration, and transpiration (Acatrinei, 1991; Chifu et al., 1998; Toma and Jităreanu, 2007; Enache, 2009).

Temperature and atmospheric precipitation are considered main factors controlling the onset of ontogenetic cycle stages, such as

growth, flowering, fruiting, and senescence (Enache, 2009; Zhang et al., 2023).

IV.2.2. Soil Type Analysis

The soil type for each collection site of the studied plant material (*Echium* species) was identified based on the following documents: *Soil Map of Romania* at a scale of 1:200,000 (ICPA) (Fig. 4.2.1), *Pedological Map of the Socialist Republic of Romania*, scale 1:500,000 (Florea et al., 1971), and literature data (Posea et al., 1982).

The soil type names from the consulted sources for each locality were correlated according to the Romanian Soil Taxonomy System (SRTS), using the taxonomic equivalences presented by Florea and Munteanu (2012).

The soils in the sampling areas are represented by Chernozems (Kastanozems, Chernozems, Phaeozems), characteristic of the steppe and forest-steppe zones – Valea lui David, Agigea, Vlădeni, Suceava; Luvisols (Preluvosols) – Băiceni, Vicovul de Sus, Repedea-Bârnova; Cambisols (Eutricambosols) – Borca; and Protisols (Alluviosols) – Bacău.

The description of the physico-chemical and ecological characteristics of the soils was performed according to Chiriță et al. (1967), Secu and Rusu (2007), and Paulette (2008).

Echium italicum and *Echium vulgare* are heliophilous species (Chifu et al., 2006; Sârbu et al., 2013), eurythermal (*Echium vulgare*) or subthermophilous (*Echium italicum*) (Sârbu et al., 2013). They prefer sunny areas and dry soils. They possess the capacity to adapt to diverse habitats, including heavily anthropized areas (*Echium vulgare*), such as pastures, dry grasslands, wastelands, roadsides, etc. (Grințescu, 1960; Sârbu et al., 2013). Being biennial (sometimes perennial) species, the plants develop a robust root system, enabling them to survive in nutrient-poor soils and withstand seasonal thermal and hydrological variations.

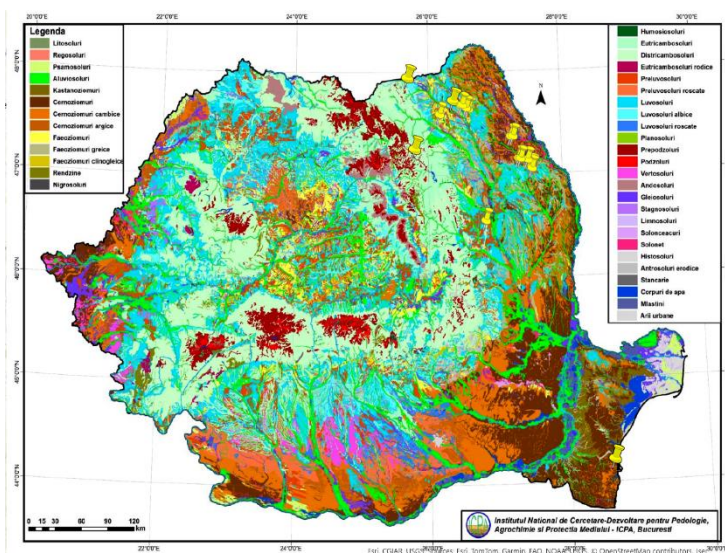


Fig 4.2.1. Soil map of Romania with collection sites (map adapted after "Figure 3. Soil map of Romania at 1:200,000 scale" – ICPA)

IV. 3. Micromorphological and Histo-Anatomical Features of Vegetative and Reproductive Organs in Selected *Echium* L. Species

IV. 3.1. Micromorphology of Aerial Organ Surfaces in *Echium* L. Species.

Micromorphological studies conducted using scanning electron microscopy (SEM) complement classical anatomy, bringing to light fine descriptive details that provide both taxonomic diagnostic features and ecological implications for the genus *Echium*. Since the presence of an abundant indumentum constitutes a characteristic and stable trait of the Boraginaceae family, research focused on the peripheral protective complex of the stem and leaf (upper and lower epidermis, analyzed at the base and apex of the leaf blade).

Qualitative examination aimed at assessing the shape of epidermal cells, the degree of sinuosity of anticlinal walls, trichome typology, the development of basal epidermal rosettes, and cuticular ornamentation. In *Echium* species, the dominant protective trichomes are unicellular, filiform, or subulate (with a broadened base and pointed apex), persistent from early ontogenetic stages. These can be directly inserted (sessile) or supported by radially arranged epidermal cells (basal rosettes covered by cuticle), which provide them with strong mechanical anchoring. In addition to protective trichomes, less numerous, structurally reduced glandular (secretory) trichomes are present (composed of one or several cells, with a terminal secretory cell), located mainly along the margin and veins of the leaf blade (Barykina and Alyonkin, 2016).

The base of protective trichomes in *Echium* species is heavily mineralized with calcium carbonate and silicon dioxide; silicon is predominantly deposited at the apex or impregnates the cell wall, whereas calcium carbonate is found throughout the rest of the trichome structure. Besides conferring mechanical rigidity that makes the plants rough to the touch, these mineral deposits represent a mechanism for immobilizing excess cellular calcium (common in calcicolous Boraginaceae species). Furthermore, the pattern and arrangement of cystolith deposits constitute a phylogenetic trait with diagnostic value at the species level.

IV.3.1.1. Micromorphology of the Surfaces of Vegetative Organs and Selected Floral Parts in Echium vulgare L. – Repedea-Bârnova

This subchapter presents the micromorphological characteristics of the peripheral protective complex of aerial vegetative organs (leaf, stem – sampled at different levels) and floral parts (sepals, petals, stamens). Features of epidermal cells, protective trichomes and their associated microstructures, as well as stomata were analyzed in **Echium vulgare** individuals collected from

populations in Repedea-Bârnova, Iași County, under the climatic conditions of 2021 on July 15.

The Leaf

Regarding the micromorphology of the upper epidermis (Fig. 4.3.1) of the leaf blade (basal, median, and apical levels), the analyses revealed the following:

- The surfaces are dominated by long, rigid, setiform protective trichomes, characteristic of the species; these trichomes exhibit well-developed multicellular bases (basal rosettes), and their surface is covered by a distinctly rough cuticle.
- Among the long trichomes, small-sized trichomes frequently occur, observed in deeper planes of the leaf surface; many of these exhibit thinner walls and a collapsed appearance.
- Stomata are present on both epidermises, confirming the amphistomatic nature of the leaf; they are positioned at the level of the epidermis or slightly prominent above it; stomatal pores (ostioles) are frequently observed in a closed or semi-open state.
- The presence of numerous exogenous particles, unevenly distributed, is noted, covering the epidermis and sometimes hindering detailed observation of other basal microstructures.
- Cuticular relief displays localized variations.

Overall, the upper epidermis shows a relatively uniform appearance along the entire length of the leaf blade, with differences between the basal, median, and apical regions being expressed mainly through trichome density, the frequency of exogenous deposits, and the degree of prominence of various specific epidermal micromorphological details.

The lower surface of the leaf blade (Fig. 4.3.2), examined under SEM, is characterized by:

- an abundant trichome cover, consisting of long and short, predominantly setiform protective trichomes, frequently provided with multicellular bases arranged in a rosette; the surface of the trichomes exhibits a rough relief, whereas the cuticle at the epidermal level is relatively smooth or slightly wrinkled.
- numerous stomata, frequently visible between trichomes, with many displaying open ostioles (stomatal pores).
- variable accumulations of airborne particles (pollen grains and possibly fungal spores) retained by the dense leaf surface cover.
- overall, along the leaf blade, moderate variations in trichome density and distribution are observed, while the general organization of the epidermis remains relatively constant, confirming the conservative nature of the micromorphology of the peripheral protective complex in *Echium vulgare*.

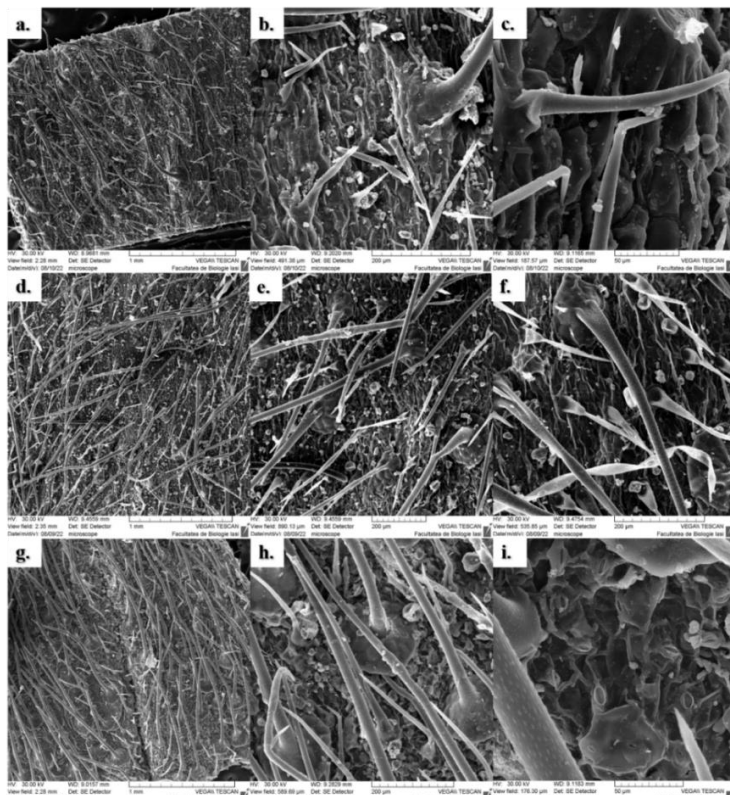


Fig. 4.3.1. Micromorphology of the upper epidermis of the leaf blade in *Echium vulgare* L. collected from Repedea-Bârnova – Iași: at basal level (a, b, c), median level (d, e, f), and apical level (g, h, i); a, d, g – overview images: dominated by long trichomes with a basal rosette and fewer short protective trichomes; b, e, h – simple insertion of short protective trichomes and the basal rosette of long trichomes; c, f, i – details of trichomes and epicuticular relief of epidermal cells.

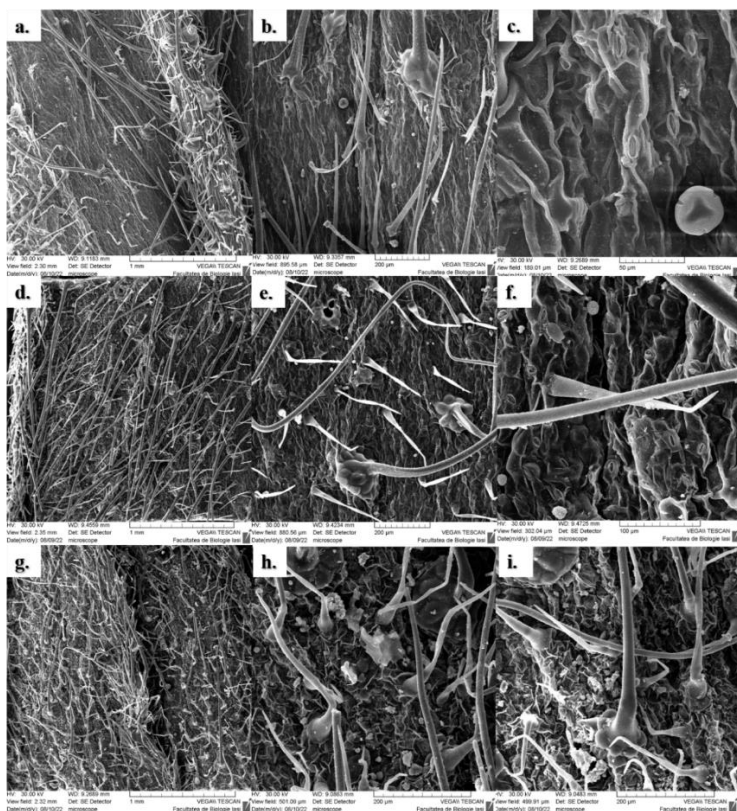


Fig. 4.3.2. Micromorphology of the lower epidermis of the leaf blade in *Echium vulgare* L. collected from Repedea-Bârnova – Iași: at basal level (a, b, c), median level (d, e, f), and apical level (g, h, i); a, d, g – overview images: dominated by long trichomes with a basal rosette and fewer short protective trichomes; b, e, h – simple insertion of short protective trichomes and the basal rosette of long trichomes; c, f, i – details of trichomes and epicuticular relief of epidermal cells.

The Stem

The stem surface is characterized by an abundant cover of protective trichomes, variable in size, unevenly distributed, and without a defined orientation at the epidermal level (Fig. 4.3.3).

Detailed observations reveal the presence of two main categories of protective trichomes:

- long and robust trichomes, inserted on well-developed multicellular bases / basal rosettes;
- shorter and thinner trichomes, much more numerous, distributed among the large-sized trichomes;
- the surface of both trichome categories exhibits roughness (mineral deposits), similar to those observed on the leaf blade;
- here and there, short trichomes appear bent or partially collapsed, a feature that may be explained by the fact that, being younger, they were affected by the material preparation procedure for SEM examination.

Epidermal cells are elongated, with relatively well-defined outlines, and are covered by a predominantly smooth cuticle; exogenous particles of variable shapes and sizes (fine particles, fragments of organic matter, other airborne formations, possibly pollen grains, spores, or dust particles) are frequently present on the examined surfaces, retained by the dense trichome cover of this organ.

Consequently, the stem exhibits a well-developed trichome cover, predominantly composed of setiform protective trichomes with basal rosettes, characteristic of the genus **Echium**. The high trichome density and their capacity to retain exogenous particles contribute to the heterogeneous surface appearance and may play a protective role against environmental factors and herbivore attack.

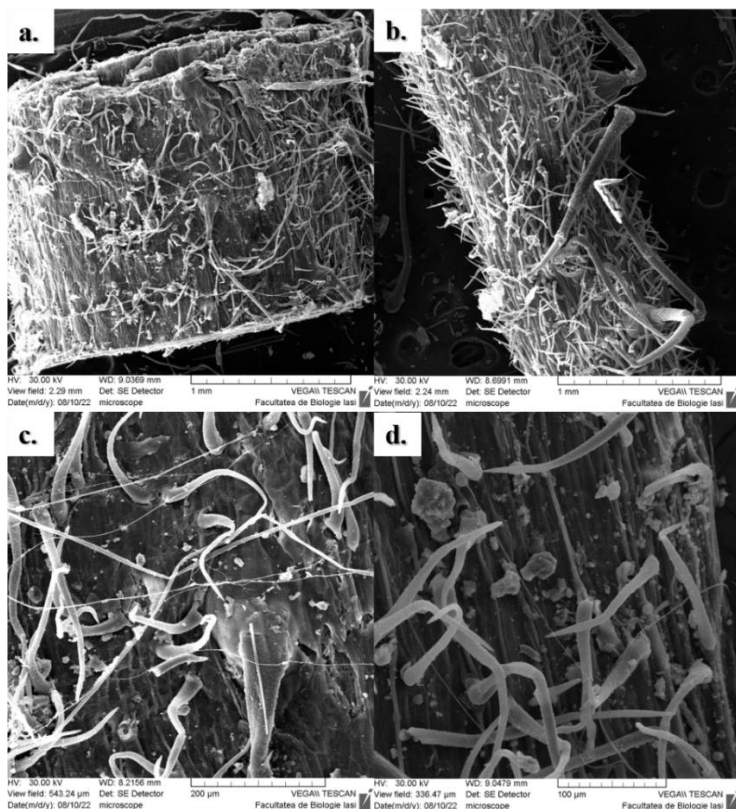


Fig. 4.3.3. Micromorphology of the stem epidermis in *Echium vulgare* L.: a, b – overview images showing the dominance of shorter trichomes; c – detail of a long trichome with a basal rosette and numerous short trichomes; d – details of short trichomes and elongated epidermal cells.

Sepals

SEM analysis of the sepals reveals the following (Fig. 4.3.4):

- a very abundant trichome cover / very high density (predominantly short, rigid, unicellular, subulate protective trichomes, generally oriented towards the apex of the sepals), as well as long trichomes with a well-developed multicellular base formed by cells arranged in a rosette; the trichome

surface is covered by a cuticle with mineral deposits showing a rough or finely granular appearance;

- an epidermis composed of cells with irregular outlines and distinctly sinuous anticlinal walls; the outer surface of the epidermal cells is covered by a heavily wrinkled cuticle, creating a pronounced microrelief;
- relatively numerous stomata in areas with fewer protective trichomes, distributed among epidermal cells.

In conclusion, sepals exhibit the highest trichome density observed so far among the analyzed organs. The association between numerous protective trichomes, wrinkled cuticular microrelief, and the presence of stomata confers a high structural complexity to the sepal surface, superior to that identified in vegetative organs. These characteristics confirm the protective role of sepals and align with the common trend of **Echium** species to develop a dense trichome cover on external floral elements.

SEM analysis of the petals highlights differences between their outer and inner surfaces:

- the outer surface, especially at the base, is covered by protective trichomes, relatively evenly distributed, contributing to their pubescent appearance;
- the inner surface exhibits a considerably smaller number of trichomes, with the surface being dominated by specialized epidermal cells.

Detailed observations highlight:

- epidermal cells with irregular outlines and a complex microrelief;
- trichomes that are generally thinner than those found on vegetative organs and sepals.

In conclusion, the micromorphology of petals differs from that of vegetative organs. These differences relate to the reduction in trichome density and the presence of specialized, papillose epidermal

cells, which contribute to the complexity of the corolla surface. These characteristics are correlated with the role of petals in interacting with pollinators and complete the picture of micromorphological adaptations identified in the floral parts of **Echium vulgare**.

Stamens

SEM analysis of the stamens highlights specific features of the filaments and anthers, as well as relevant details regarding pollen morphology (Fig. 4.3.6).

Stamen filaments are composed of elongated, longitudinally arranged epidermal cells, giving them a characteristic striated appearance; the surface of the epidermal cells is relatively uniform, without obvious ornamentations comparable to those observed on protective trichomes.

The insertion zone between the filament and the anther is well defined and relatively broad, ensuring morpho-structural continuity between the two stamen components.

Anthers consist of two pollen sacs (thecae), frequently displaying a collapsed appearance, likely resulting from dehiscence and the sample preparation process for SEM examination. The outer surface of the anthers is strongly wrinkled and folded, with depressions and irregularities, giving them a distinctly granular and rough appearance.

Pollen is present on both the surface of the anthers and the stamen filaments. Pollen grains are predominantly spherical in shape, relatively uniform in size, and display fine granular ornamentation. Frequently, pollen grains appear clustered on the anthers within their heavily folded areas after opening.

Overall, the complex microrelief of the anther surface and the filament insertion region may favor the temporary retention of pollen grains after anther dehiscence. In this way, pollen transport to pollinators is made more efficient by retaining it on the floral parts for a longer period.

In conclusion, stamens possess a micromorphology adapted to their function, characterized by filaments with a longitudinally striated

surface, anthers with a wrinkled epicuticular relief, and abundant pollen with a relatively uniform morphology. These features complete the set of micromorphological traits identified in the floral parts of ***Echium vulgare*** and highlight the complexity of microstructures involved in the pollination process.

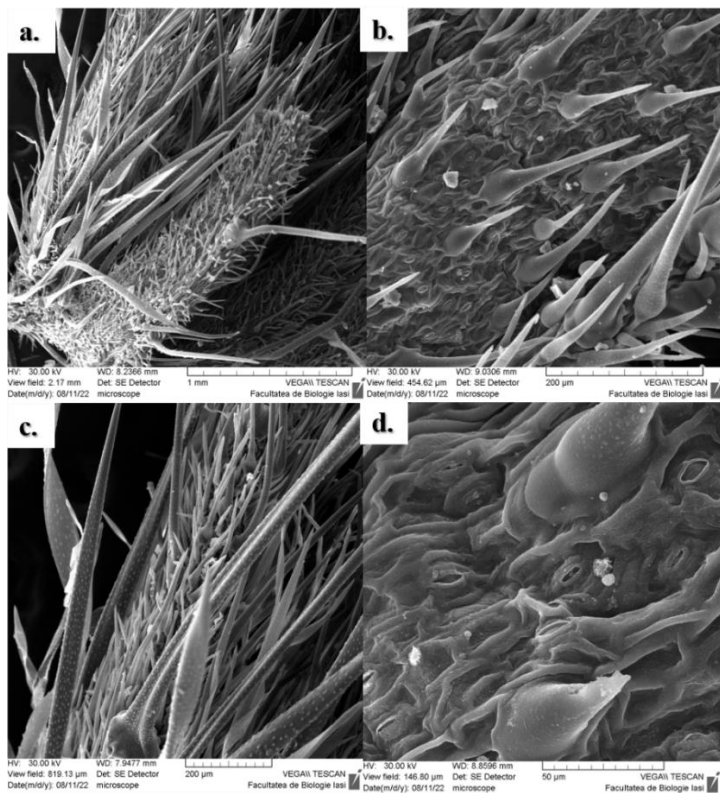


Fig. 4.3.4. Micromorphology of the outer epidermis of sepals in *Echium vulgare* L.: a – overview image showing numerous irregularly arranged trichomes; b – image with short protective trichomes and epicuticular relief of epidermal cells; c – detail of a long trichome with parietal ornamentations; d – detailed image showing short protective trichomes and epicuticular relief of epidermal cells. **Petal**

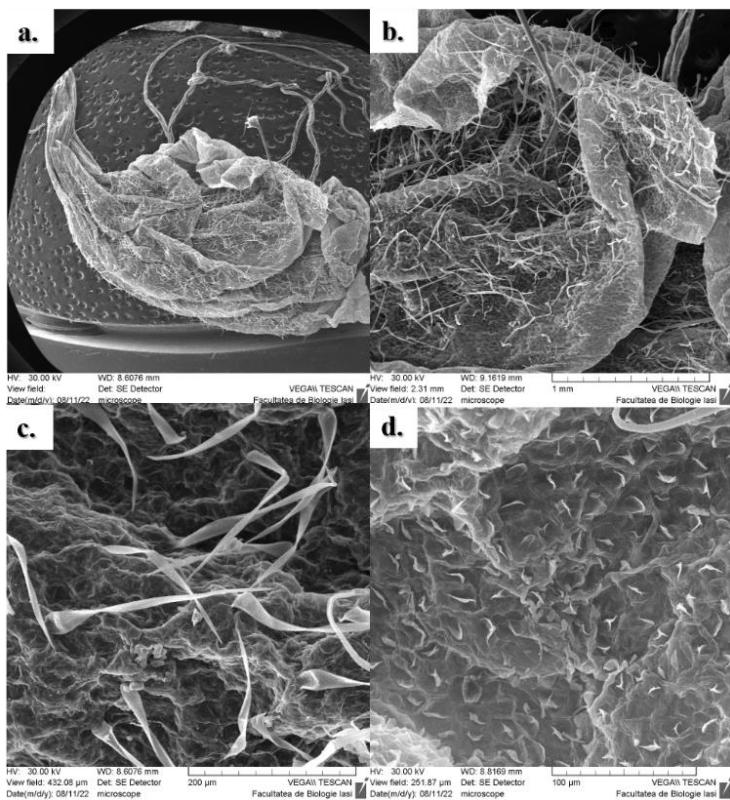


Fig. 4.3.5. Micromorphology of the petal epidermis in *Echium vulgare* L.: a – overview image showing numerous trichomes on the outer surface; b – image showing the appearance of the outer surface of the petals; c – details showing the outline of epidermal cells on the outer surface and long trichomes; d – details showing the outline of epidermal cells on the inner surface of the petals.

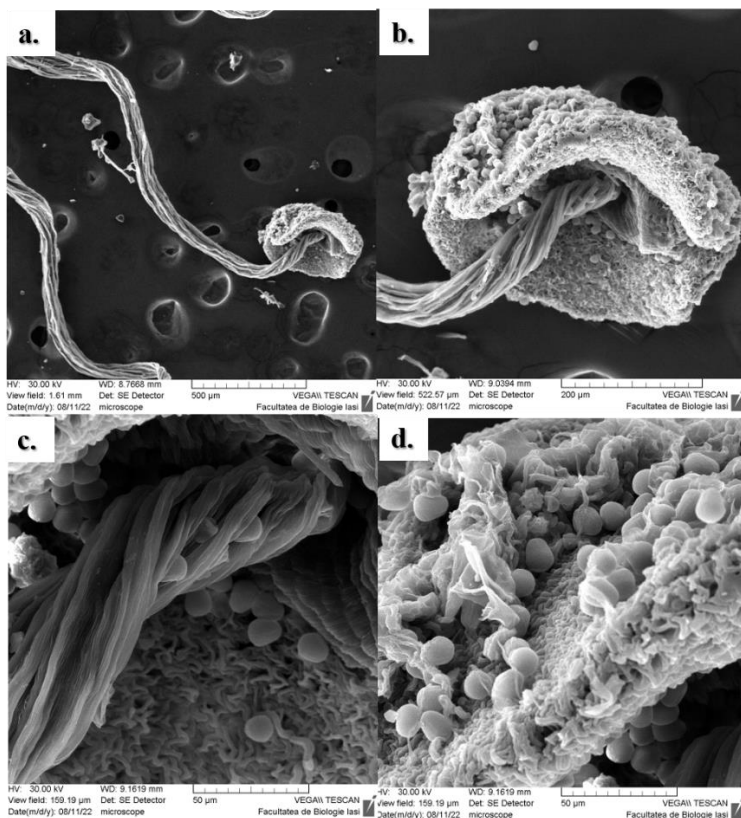


Fig. 4.3.6. Morphology and micromorphology of stamens in *Echium vulgare* L.: a – overview image: filament and anther; b – general view showing the inner and outer surfaces of the anther with pollen grains; c – detail of the insertion zone between the filament and the anther; d – details showing the outline of epidermal cells of the anther and pollen grains.

***IV.3.1.2. Micromorphology of the Surfaces of Vegetative
Organs and Selected Floral Parts in Fasciated Echium vulgare L. –
Valea lui David***

In this subchapter, the micromorphological characteristics of the peripheral protective complex of aerial vegetative organs (leaf, stem – sampled at different levels) and floral parts (sepals, petals, stamens) are presented. Features of epidermal cells, protective trichomes and their associated microstructures, as well as stomata were analyzed in a fasciated *Echium vulgare* individual collected from a population in Valea lui David, Iași County, under the climatic conditions of 2022 on June 30.

The Leaf

The micromorphological characteristics of the lower and upper epidermis, at both the basal and apical levels of the leaf blade, in the fasciated *Echium vulgare* individual are similar to those identified in normal individuals (subchapter IV.3.1.2). Long protective trichomes with a basal rosette and subulate trichomes are more numerous on the lower epidermis (Fig. 4.3.7).

The Stem

The stem surface is similar to that of normal individuals (Fig. 4.3.8).

The sepals, petals, and stamens exhibit micromorphological characteristics similar to those of normal specimens.

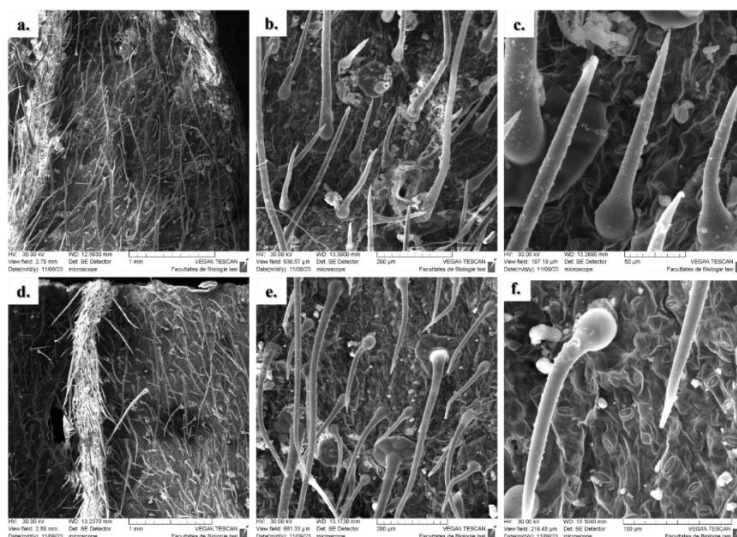


Fig. 4.3.7. Micromorphology of the leaf blade (apical level) in fasciated *Echium vulgare* L. collected from Valea lui David – Iași: upper epidermis: a – overview image: protective trichomes; b – enlarged image: subulate protective trichomes and rare deposits of exogenous material; c – details of subulate protective trichomes with ornamental structures, rare stomata; lower epidermis: d – overview image: protective trichomes; e – subulate protective trichomes and a trichome with a basal rosette; f – detail of a subulate protective trichome with epicuticular ornamental structures; numerous stomata, slightly above epidermal cells; outline of basal epidermal cells.

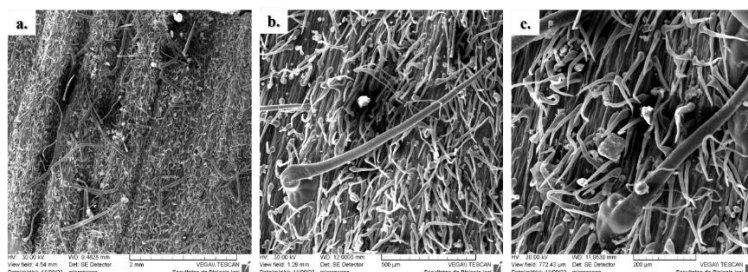


Fig. 4.3.8. Micromorphology of the stem epidermis in fasciated *Echium vulgare* L. collected from Valea lui David – Iași: a – overview showing irregularly arranged trichomes; b – numerous short protective trichomes and a long protective trichome; c – detail: numerous short trichomes and a long protective trichome with a narrow basal rosette.

IV.3.1.3. Micromorphology of the Surfaces of Vegetative Organs and Selected Floral Parts in Echium italicum L. – Agigea

In this subchapter, the micromorphological characteristics of the peripheral protective complex of aerial vegetative organs (leaf, stem – sampled at different levels) and floral parts (sepals, petals, stamens) are presented.

The Leaf

Regarding the micromorphology of the upper epidermis at the apex of the leaf blade (Fig. 4.3.9 a, b, c): On the upper epidermis, the predominant trichomes are simple, unicellular, short protective trichomes and long, unicellular protective trichomes with a less extensive basal cell rosette. The surface of the protective trichomes exhibits finely granular ornamentations. The surface of the epidermal cells is covered with exogenous deposits.

Regarding the micromorphology of the lower epidermis at the apex of the leaf blade (Fig. 4.3.9 d, e, f): On the lower epidermis, numerous simple, unicellular, short protective trichomes are found, along with occasional long, unicellular protective trichomes with a basal cell rosette (1–2 layers). The surface of short protective trichomes presents finely granular ornamentations, whereas long protective trichomes with a basal rosette exhibit larger, tuberculate-type ornamentations. The basal epicuticular relief at the base of the trichomes is smooth; irregularly shaped deposits of exogenous material frequently appear.

Regarding the micromorphology of the upper epidermis at the base of the leaf blade (Fig. 4.3.10 a, b, c): On the upper epidermis, longer and sparser protective trichomes are found compared to the previously analyzed surfaces; among the long trichomes, shorter trichomes also appear, likewise sparser than in other cases. The trichomes are oriented toward the apex of the leaf blade; here and there, crust-like cuticular deposits appear above the epidermal cells; stomata appear positioned at the outer level of the epidermal cells. The cuticular relief has a smooth appearance above the cells forming the basal insertion rosette of the long trichomes; longitudinal folds frequently appear that seem to delimit the basal epidermal cells.

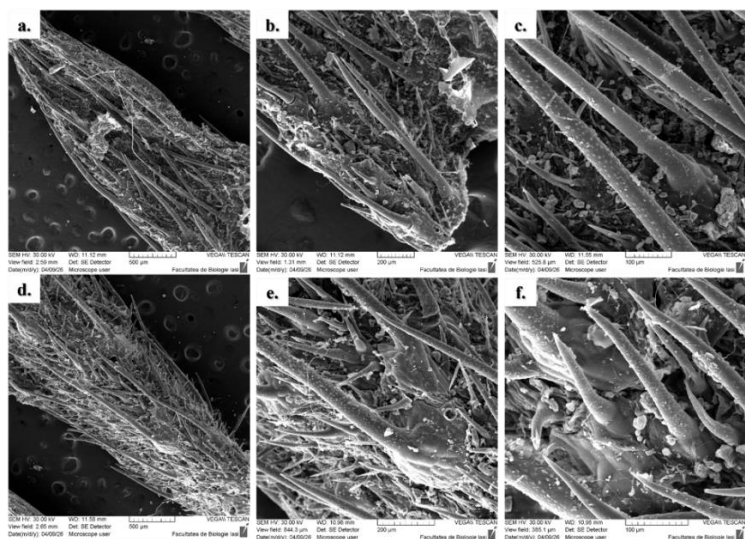


Fig. 4.3.9. Micromorphology of the leaf blade (apical level) in *Echium italicum* L. collected from Agigea – Constanța: upper epidermis: a – overview image: dominated by long protective trichomes oriented toward the apex of the leaf blade; b – simple insertion of protective trichomes along the leaf blade margins; c – surface of long trichomes; short trichomes and deposits of exogenous material; lower epidermis: d – overview image: dominance of long protective trichomes oriented toward the apex of the leaf blade; short trichomes in the background; e – details of long trichomes highlighting the basal rosette; short trichomes in the background; f – details of short trichomes with a rough surface; insertions of long and short trichomes; deposits of exogenous material.

Regarding the micromorphology of the lower epidermis at the base of the leaf blade (Fig. 4.3.10 d, e, f): On the lower epidermis, numerous long, unicellular protective trichomes with a basal cell rosette (1–2 layers) and fewer simple, short trichomes are present. The surface of short protective trichomes exhibits small, finely granular ornamentations, whereas long protective trichomes with a basal rosette display larger, tuberculate-type ornamentations. The basal epicuticular relief has an embossed (goffered) appearance and is covered in places by irregularly shaped deposits of exogenous material. Here and there, stomata with open ostioles are observed.

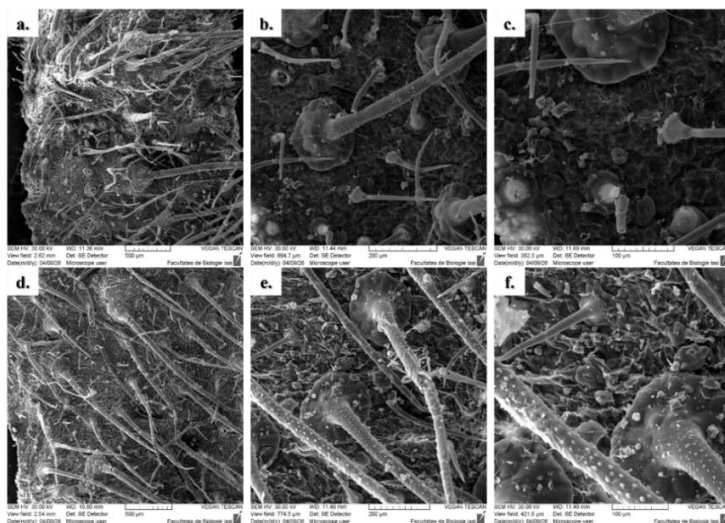


Fig. 4.3.10. Micromorphology of the leaf blade (basal level) in *Echium italicum* L. collected from Agigea – Constanța: upper epidermis: a – overview image; dominated by short protective trichomes and fewer long trichomes with a basal rosette; b – simple insertion of short protective trichomes and the basal rosette of long trichomes; c – details of trichomes and epicuticular relief of epidermal cells; lower epidermis: d – overview image with numerous long trichomes; e – details of long trichomes with parietal ornamentations; highlighting of the basal rosette; few short trichomes in the background; f – details of long trichomes with ornamentations; insertions of long trichomes; deposits of exogenous material; stomata slightly above the outer level of epidermal cells.

The Stem

At the level of the stem epidermis (Fig. 4.3.11 a, b, c), overview images reveal a trichome cover of varying length and thickness, with an irregular arrangement. Numerous short and thin trichomes with finely granular ornamentations are present. Long protective trichomes with a basal rosette exhibit larger, tuberculate-type ornamentations. Here and there, glandular trichomes are also observed.

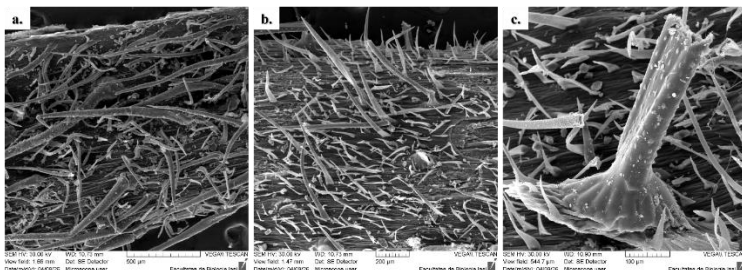


Fig. 4.3.11. Micromorphology of the stem epidermis in *Echium italicum* L. collected from Agiea – Constanța: a – irregularly arranged trichomes of varying length and thickness; b – overview image showing the dominance of shorter trichomes; c – detail of a long trichome with a basal rosette and numerous short trichomes.

The outline of the epidermal cells is elongated. They are covered by a relatively smooth cuticle. Short trichomes, which are more numerous, are often bent. Long trichomes are sparser. Solid deposits of various shapes and sizes (dust, detritus, spores, or pollen) are observed.

Sepals

Sepals (Fig. 4.3.12 a, b, c) exhibit a dense trichome cover, oriented toward their apex. The epidermis consists of cells with irregular outlines, sinuous walls, and a heavily wrinkled cuticular relief. Protective trichomes are rigid, conical-elongated in shape (conical base and acute apex), with a finely granular surface showing a bulbous appearance, while stomata are relatively numerous in areas lacking protective trichomes.

Thick, massive trichomes are also present. The outline of the basal epidermal cells is irregular. Here and there, spherical particles are observed, most likely pollen grains.

Petals

On the outer surface, petals (Fig. 4.3.12 d, e, f) are covered by simple, unicellular trichomes, whereas their inner surface features fewer trichomes. The outline of the epidermal cells is irregular, with some cells likely possessing outer papillae (appearing in the image as dehydrated ridges – Fig. 4.3.12 f).

Trichomes are long, apparently thinner, collapsed, with a granular epicuticular cover; their basal rosette is not visible. The epidermal relief has an irregular appearance, with numerous ridges and depressions. Here and there, solid deposits of inert material are observed.

Stamens

The stamen (Fig. 4.3.14 g, h, i) features an elongated filament with a longitudinally striated surface. The anther consists of two collapsed structures with a granular surface. The filament has a longitudinally striated surface with a fibrillar appearance.

Pollen grains are spherical in shape and relatively uniform in size, with a finely granular surface. Pollen is arranged on a heavily wrinkled tissue surface corresponding to the anther wall after dehiscence.

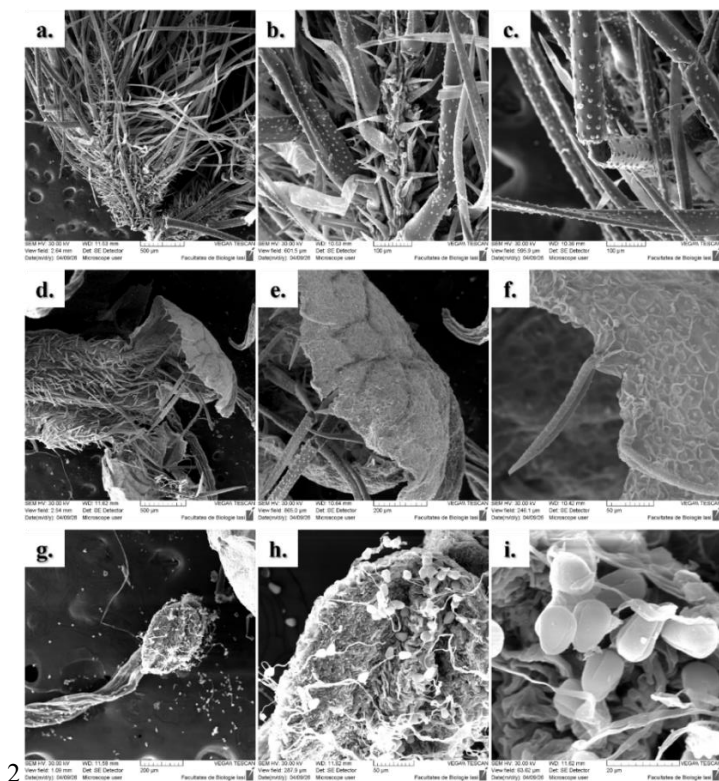


Fig. 4.3.12. Micromorphology of the outer epidermis of sepals in *Echium italicum* L. collected from Agigea – Constanța: a – overview image with numerous irregularly arranged trichomes; b – image showing the dominance of long trichomes with ornamentations, short trichomes in the background; c – details of a long trichome with parietal ornamentations. Micromorphology of the petal epidermis: d – overview image showing numerous trichomes on the outer surface; e – image showing the appearance of the inner surface of the petals; f – details showing the outline of epidermal cells on the inner surface and a long trichome from the outer surface of a petal. Morphology and micromorphology of stamens: g – overview image: filament and anther; h – general view showing the outer surface of the anther with pollen grains; i – details showing the outline of epidermal cells of the anther and pollen grains.

IV. 3.2. Variability of Micromorphological Characteristics of Aerial Vegetative Organs in *Echium vulgare* L. across Different Habitats in North-Eastern Romania

The qualitative micromorphological analysis aimed to identify trichome type and distribution, basal rosette organization, cuticular ornamentation, stomatal position, and the specific features of the anticlinal walls of epidermal cells, being complemented by semi-quantitative scores to evaluate the density of the protective complex.

In parallel, the quantitative analysis involved biometric measurements of protective trichome dimensions, as well as the diameters of insertion bases and epidermal rosettes, allowing the calculation of synthetic indices used to compare the degree of epidermal development across different organs and populations.

By integrating descriptive observations with biometric data and calculated indices, both the stable diagnostic characters of *Echium* species and the micromorphological variability / adaptive plasticity associated with ecological conditions in the analyzed habitats were highlighted.

***IV.3.2.1. Micromorphology of Epidermal Surfaces of Echium vulgare* L. (Sampling Location: Suceava, Suceava)**

The Leaf

Upper epidermis at the apex of the leaf blade (Fig. 4.3.13 a, b, c):
On the upper epidermis, numerous simple, unicellular, short, sessile, subulate-type protective trichomes are found, along with occasional long, unicellular protective trichomes with a basal cell rosette more extensive than on the lower epidermis (composed of cells radially arranged in 2 concentric circles). The surface of short protective trichomes exhibits small, finely granular ornamentations, whereas long protective trichomes with a basal rosette display larger, tuberculate-type ornamentations. Stomata are observed, positioned slightly below the level of the epidermal cells, which have an approximately polygonal outline and moderately sinuous anticlinal

walls. Otherwise, rare deposits of exogenous material can be observed.

Leaf blade – lower epidermis at the apex of the leaf blade (Fig. 4.3.13 d, e, f)

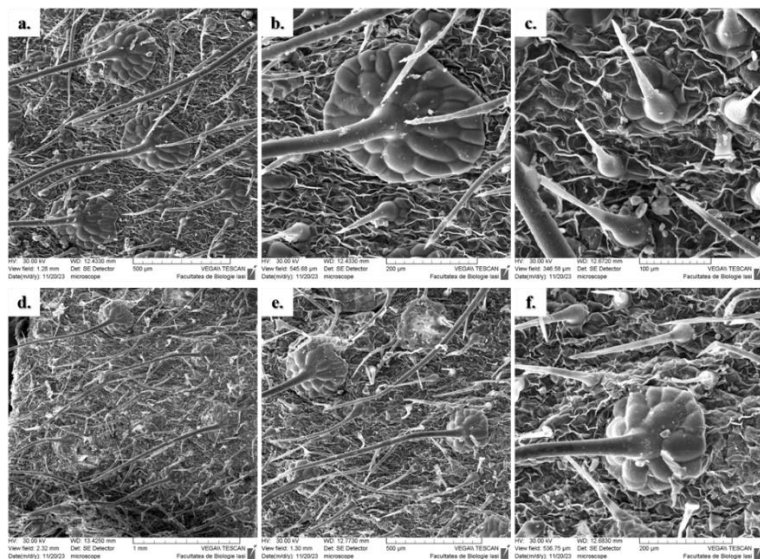


Fig. 4.3.13. Micromorphology of the leaf blade (apical level) in *Echium vulgare* L. collected from Suceava – Suceava: upper epidermis: a – overview image: long and short protective trichomes; b – protective trichome with a well-developed basal rosette covered by a relatively smooth cuticle; c – outline of epidermal cells and short protective trichomes; lower epidermis: d – overview image: protective trichomes of varying lengths and thicknesses; e – detail of long and short trichomes; f – long trichomes with a smaller basal rosette than on the upper epidermis; short subulate trichomes, epidermal epicuticular relief.

Leaf blade – upper epidermis at the base of the leaf blade (Fig.
4.3.14 a, b, c)

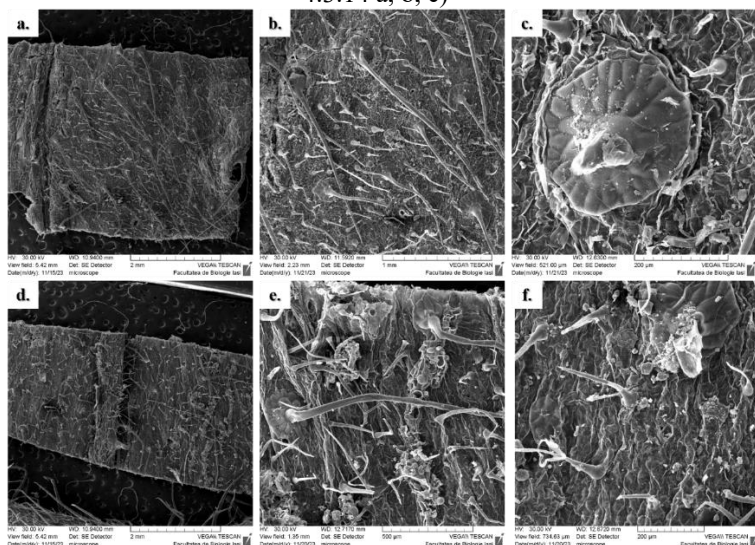


Fig. 4.3.14. Micromorphology of the leaf blade (basal level) in *Echium vulgare* L. collected from Suceava – Suceava: upper epidermis: a – long protective trichomes with a basal rosette and short subulate trichomes; b – long trichomes with a developed basal rosette and short subulate trichomes in the background; deposits of exogenous material; c – detail of a trichome with a basal rosette and an isodiametric, slightly sinuous outline of epidermal cells; lower epidermis: d – overview image: arrangement of trichomes on the vein and leaf blade; e – details of protective trichomes with a reduced basal rosette and short subulate trichomes; f – detail of subulate trichomes.

Leaf blade – lower epidermis at the base of the leaf blade (Fig. 4.3.14 d, e, f): Overview images capture the appearance of trichomes along veins and in their adjacent areas. The trichomes attached to veins are numerous, of varying lengths and thicknesses (though small-sized ones predominate), with a non-uniform orientation. Areas immediately adjacent to veins have a more regular, smooth appearance, with predominantly long trichomes oriented in parallel. Stomata are less easily observable on the upper surface than on the lower surface. Trichome ornamentations are similar on both epidermises.

The Stem

At the level of the stem epidermis (Fig. 4.3.15), overview images reveal a dense cover of trichomes of varying lengths and thicknesses, with an irregular arrangement. Numerous short and thin trichomes with tuberculate to finely granular ornamentations are present, along with long protective trichomes with a basal rosette that exhibit larger, tuberculate-type ornamentations. The outline of epidermal cells is elongated, covered by a relatively smooth cuticle; short trichomes are more numerous, often bent, with walls covered by a rough cuticle; long trichomes are sparser; solid deposits of various shapes and sizes (dust, detritus, spores, or pollen) are observed.

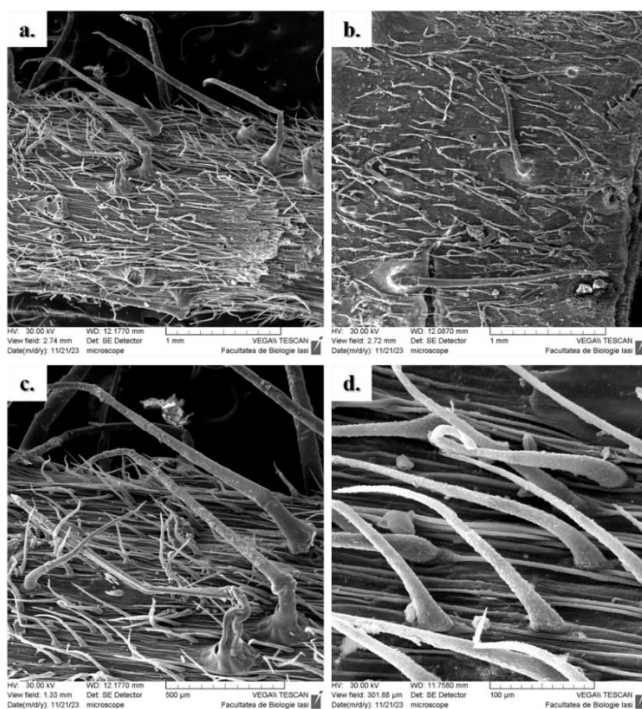


Fig. 4.3.15. Micromorphology of the stem epidermis in *Echium vulgare* L. collected from Suceava – Suceava: a, b – overview of short trichomes with a parallel arrangement and long trichomes with a basal rosette, irregularly arranged; c – numerous short trichomes and long trichomes with a basal rosette; d – details of relatively short, numerous trichomes without a basal rosette; elongated outline of epidermal cells.

Sepals

Sepals (Fig. 4.3.16 a) exhibit a dense trichome cover oriented toward their apex. The epidermis presents sinuous walls with irregularly outlined cells – a heavily wrinkled surface with a pronounced microrelief, rigid protective trichomes of conical-elongated shape (conical base and acute apex) with a finely granular, bulb-like surface; relatively numerous stomata in areas lacking protective trichomes. Thick, massive trichomes and shorter trichomes with a club-shaped base; irregular outline of basal epidermal cells; here and there, spherical particles – possibly pollen grains.

Petals

On the outer surface, petals (Fig. 4.3.16 b, c, d) are covered by simple, unicellular trichomes, whereas the inner surface of the petals features fewer trichomes. The outline of petal epidermal cells is irregular, with some cells likely possessing outer papillae (appearing in the image as dehydrated ridges, Fig. 4.3.16 d).

Stamens

Stamens (Fig. 4.3.16 e, f) feature an elongated filament with a longitudinally striated surface; the anther consists of two collapsed structures with a granular surface. The filament has an elongated structure with a longitudinally striated, fibrillar surface; the filament–anther insertion zone is relatively wide, the surface of the anther is heavily wrinkled, with depressions and folds; pollen is spherical, relatively uniform, present on both the anther and the filament; the microrelief of the anther and of the filament insertion zone contributes to the temporary retention of pollen, facilitating transfer to pollination vectors. Pollen grains are spherical in shape and relatively uniform in size, with a finely granular surface. Pollen is arranged on a heavily wrinkled tissue surface corresponding to the anther wall after dehiscence.

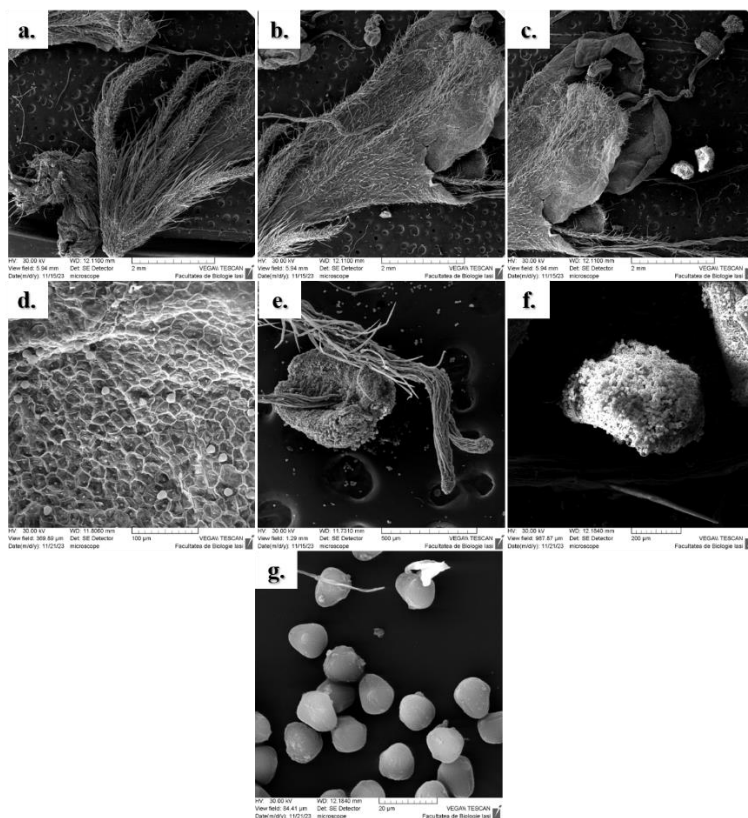


Fig. 4.3.16. Micromorphology of the outer epidermis of sepals in *Echium vulgare* L. collected from Suceava – Suceava: a – overview image of sepals – outer surface with numerous long and short trichomes. Micromorphology of the petal epidermis: b, c – overview image of the outer surface of the petal with trichomes; d – image showing the appearance of the inner surface of the petal. Morphology and micromorphology of stamens: e – overview image of the anther on the dorsal surface; f – overview image of the anther; g – pollen grains.

IV.3.2.2. Micromorphology of Epidermal Surfaces of Echium vulgare L. (Sampling Location: Bacău)

The epidermal surfaces of the leaf blade (upper epidermis and lower epidermis), stem, and selected floral elements (sepals, petals, stamens) were analyzed for *Echium vulgare* collected from Bacău.

IV.3.2.3. Micromorphology of Epidermal Surfaces of Echium vulgare L. (Sampling Location: Borca, Neamț)

The epidermal surfaces of the leaf blade (upper epidermis and lower epidermis), stem, and selected floral elements (sepals, petals, stamens) were analyzed for **Echium vulgare** collected from Borca, Neamț.

IV.3.2.4. Micromorphology of Epidermal Surfaces of Echium vulgare L. (Sampling Location: Băiceni, Botoșani)

The epidermal surfaces of the leaf blade (upper epidermis and lower epidermis), stem, and selected floral elements (sepals, petals, stamens) were analyzed for **Echium vulgare** collected from Băiceni, Botoșani.

IV.3.2.5. Micromorphology of Epidermal Surfaces of Echium vulgare L. (Sampling Location: Valea lui David, Iași)

The epidermal surfaces of the leaf blade (upper epidermis and lower epidermis), stem, and selected floral elements (sepals, petals, stamens) were analyzed for *Echium vulgare* collected from Valea lui David, Iași.

The comparative analysis of *Echium vulgare* populations from different localities highlights a general stability at the micromorphological level, accompanied by predominantly quantitative variations associated with habitat conditions. Thus, specimens from the Borca population are characterized by a high density of long trichomes and well-developed basal rosettes, while

those from Suceava show an intermediate situation (robust trichomes and well-expressed rosettes).

At the organ level, the stem exhibits stability regarding trichome length and base diameter, whereas the leaf displays the highest micromorphological plasticity, showing considerable variations between the base, middle, and apex. Overall, while the histo-anatomical structure (root, stem, leaf) shows high plasticity and large variations in tissue proportions in response to environmental conditions, epidermal micromorphology is much more conservative, maintaining constant trichome typology, the presence of multicellular rosettes, and general epidermal characteristics, with differences between populations mainly affecting trichome density and dimensions.

Quantitative Index-Based Micromorphological Surface Analysis

The indices calculated for the comparative evaluation of trichome cover development and epidermal characteristics across different organs of *Echium vulgare* specimens from different populations are:

- **the robustness index** (the ratio between basal rosette diameter and trichome base diameter);
- **the protection index** (trichome length $\times$ trichome density);
- **the ratio of trichomes with a basal rosette to trichomes without a basal rosette.**

The robustness index exhibited higher values in specimens collected from Suceava on the upper epidermis at the apex of the leaf blade and on the sepals (Fig. 4.3.17 and 4.3.19).

In specimens collected from Borca, the value of the robustness index was higher on the stem, and similar to that from Suceava for trichomes on the upper epidermis at the apex of the leaf blade (Fig. 4.3.18).

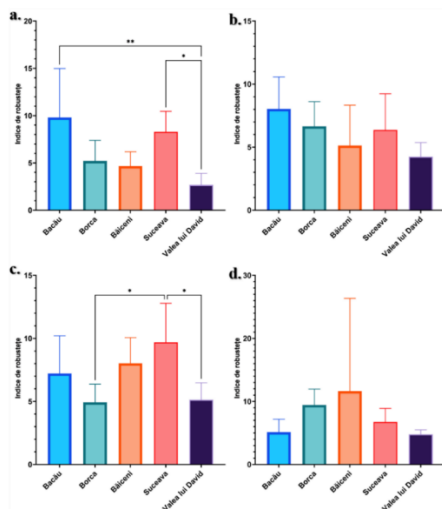


Fig. 4.3.17. Trichome robustness index at the base of the leaf blade: a – upper epidermis; b – lower epidermis; and at the apex of the leaf blade: c – upper epidermis; d – lower epidermis in *Echium vulgare* L. Graphically modeled data represent the mean $\pm$ standard deviation. Statistical significance: * $p < 0.05$. Values without asterisks are statistically non-significant ($p \geq 0.05$).

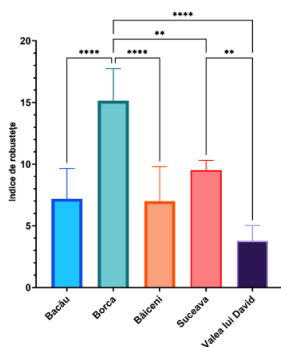


Fig. 4.3.18. Trichome robustness index on the stem of *Echium vulgare* L. Graphically modeled data represent the mean $\pm$ standard deviation. Statistical significance: ** $p < 0.01$; **** $p < 0.0001$.

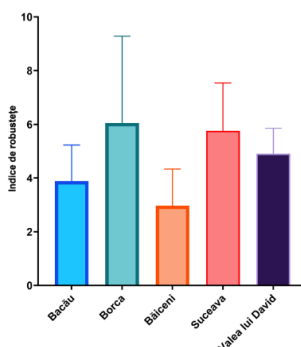


Fig. 4.3.19. Trichome robustness index on sepals of *Echium vulgare* L. Graphically modeled data represent the mean $\pm$ standard deviation. Values without asterisks are statistically non-significant ($p \geq 0.05$).

The protection index of protective trichomes with a basal rosette recorded high values in specimens collected from Valea lui David at the basal level of the leaf blade, on both the upper and lower epidermises, as well as at the blade apex for the upper epidermis. For the lower epidermis at the blade apex, the highest value was recorded in specimens collected from Borca.

For trichomes without a basal rosette, the protection index showed higher values in specimens collected from Valea lui David on both epidermises at the base of the leaf blade; specimens from Borca recorded the highest value for the upper epidermis at the blade apex, while specimens from Bacău showed the highest value for the lower epidermis at the blade apex.

At the stem level, the protection index for both trichomes with a rosette and trichomes without a rosette had significantly higher values in Bacău.

At the sepal level, the protection index recorded a significantly lower value for trichomes without a rosette in Suceava.

At the leaf blade level, the ratio of trichomes with a basal rosette to trichomes without a basal rosette showed higher values in specimens collected from Băiceni at both the base and apex of the blade on both epidermal surfaces.

At the stem level, the ratio between trichomes with a rosette and those without a rosette reached high values in specimens from Borca and Suceava.

At the sepal level, specimens collected from Suceava recorded the highest value for the ratio between trichomes with a rosette and trichomes without a rosette.

From the perspective of organ-specific specialization, a well-defined role is observed for each structure: the leaf exhibits the highest micromorphological diversity and plasticity; the stem features a uniform and dense trichome cover; sepals develop the densest protective trichome layer (including glandular trichomes); petals show reduced pubescence and specialized epidermal cells; and stamens present a microrelief structurally adapted for pollen retention and transfer.

IV. 3.3. Micromorphology of Nutlet Surfaces in *Echium vulgare* L., *Echium russicum* J.F.Gmel., and *Echium italicum* L. with Taxonomic Differentiation Elements ⁶

Botanically, the fruits of *Echium* are termed nutlets and are formed through the schizocarpic fragmentation of the bicarpellary gynoecium into four single-seeded mericarps. Their morphology and micromorphology hold taxonomic relevance within the family Boraginaceae, being frequently used for species differentiation and the interpretation of phylogenetic relationships among them (Selvi and Bigazzi, 2001; Bojnanský and Fargašová, 2007).

Our research indicates that the nutlets of the three analyzed species exhibit the same basic morphology characteristic of the genus *Echium*: trigonal shape, a flat base corresponding to the hilum, a flat or slightly concave ventral surface, and a convex dorsal surface. However, surface dimensions and ornamentations differ, providing a basis for species differentiation as presented below.

The biometric parameter analysis of the nutlets highlights a progression in size from *Echium russicum* to *Echium italicum*, regarding both nutlet dimensions and the development of the ventral keel and lateral ornamentations. Between these two species, *Echium vulgare* occupies an intermediate position depending on the aforementioned parameters.

A comparative analysis reveals gradual differences in ornamentation complexity—from *E. russicum* to *E. vulgare* and *E. italicum*. *E. russicum* nutlets are characterized by a dominance of small ornamentations and the absence of spinous structures, *E. vulgare* nutlets present intermediate traits, while *E. italicum* nutlets develop strong spinous ornamentations. These characters confirm the diagnostic taxonomic value of nutlet micromorphology and support

⁶ Part of these data have been published as an abstract:

Sava, A. R., Zamfirache, M. M., Costică, N., 2023. Research regarding the nutlets micromorphology of species belonging to the genus *Echium* L. (Boraginaceae family) *Journal of Experimental & Molecular Biology*, 24(4): 239-240.

their use in taxonomic and ecological studies of the genus *Echium* (Sava et al., 2023).

Taxonomic Value of Morphological and Micromorphological Nutlet Characters

The obtained results confirm the diagnostic taxonomic value of morphological and micromorphological nutlet characters within the genus *Echium*, in full accordance with specialized literature for the Boraginaceae family (Bojnanský and Fargašová, 2007; Ebadi and Nikzat, 2021; Gillani et al., 2024; Selvi and Bigazzi, 2001).

IV. 3.4. Histo-Anatomical Particularities of Vegetative

Organs in *Echium vulgare* L. and *Echium italicum* L.

In the first part of this subchapter, we present a synthesis of literature data, followed by a description of the histo-anatomical characteristics identified in specimens from our study areas.

In synthetic treatises dedicated to dicotyledon anatomy, such as that by Metcalfe and Chalk (1950), general structural characteristics of vegetative organs in Boraginaceae are presented, along with isolated references regarding the anatomy of certain species within the genus *Echium*.

Carlquist (1970) provides a functional and ecological interpretation of anatomical structures in dicotyledon species, among which we note: xylem vessels are more numerous and narrower in *Echium* species from Mediterranean habitats, thereby reducing the risk of embolism under water deficit conditions.

Sofian (2003) describes the anatomy of vegetative organs in *Echium russicum* J.F. Gmel. (syn. *Echium rubrum* Jacq.), with his data being consistent with those published by Metcalfe and Chalk regarding the general characteristics of the genus, showing specificities related to the differing quantitative development of main tissue types in the investigated taxa.

Research on vegetative organs conducted by Ifrim and Toniuc (2006) on species belonging to the genera *Borago*, *Cerithe*, and *Echium* from the flora of Romania highlighted features of the leaf blade for *Echium vulgare* and *Echium russicum* regarding: epidermal cell outlines; stomatal types—anomocytic (ranunculaceous) in *E. russicum* and anisocytic (cruciferous) in *E. vulgare*, occurring at a higher frequency on the lower epidermis; and protective trichome types—long unicellular protective trichomes with an acute apex and thickened walls supported at the base by a prominent pedestal formed by epidermal cells with straight, thickened walls, and very short, conical unicellular trichomes with a slightly curved apex and a rough surface.

Internationally, studies on the genus *Echium* have focused on both morphological and anatomical aspects, often correlated with the geographical distribution and ecological features of the taxa. Thus, some research has been dedicated to endemic species from regions such as the Cape Verde Islands (Romeiras et al., 2008) or Macaronesia (Aldridge, 1981), while more recent approaches include molecular analyses to elucidate phylogenetic relationships among taxa (Cohen, 2014).

In a landmark study on the anatomy and evolution of Macaronesian *Echium* species, Aldridge (1981) analyzed the anatomical characteristics of several taxa from the Canary Islands, Madeira, and Cape Verde, investigating the relationship between the internal structure of vegetative organs and adaptation to specific insular ecological conditions.

Sava et al. (2019) described the histo-anatomy of vegetative organs (rhizome, stem, leaf blade) in *Echium russicum* and conducted measurements of histological parameters in individuals collected from Sărăturile din Valea Ilenei, Iași County.

IV.3.4.1. Histo-Anatomical Features of Vegetative Organs in Echium vulgare L.

To highlight the structural features of the vegetative organs (root, stem, and leaf), cross-sections were performed on individuals belonging to the species **Echium vulgare**, collected from the Suceava location on July 12, 2022. For each of these organs, sections were

made at three different levels (basal, median, and apical). For the leaf, sections were cut both through the midrib and through the leaf blade (interveinal area).

The following sections descriptively present the histo-anatomical characteristics of each organ, tracking the structural changes that occur along them from base to apex. Overall, the protective, parenchymal, mechanical, and vascular tissues, their spatial organization, and their degree of development were analyzed and described.

This part of our research aims at the structural characterization of the species and the relationship between structure and the functions performed by its various organs. Starting from this baseline description of specific anatomical characters, comparative analyses will subsequently be performed to identify the anatomical variability of ***Echium vulgare*** populations originating from different habitats in northeastern Romania.

The Root

The Primary Root

For the root study, at each of the cross-sections analyzed across the three levels, the following were described: section contour, features of the periderm, cortex, and vascular cylinder (phloem, cambium, xylem, and their ratios), and the pith. Selected images from the performed sections can be observed below, for both the primary root and lateral roots.

Primary Root (Basal Level)

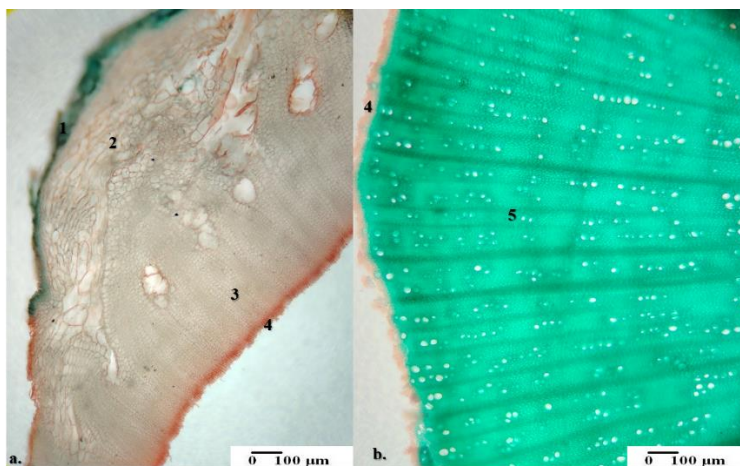


Fig. 4.3.20. Cross-section through the basal level of the primary root of *Echium vulgare* L.: a – Fragment from the outer zone: periderm (1), cortex (2), secondary phloem (3), annular cambium (4); tissue discontinuities (4x10 obj.); b – Fragment from the inner zone of the vascular cylinder: secondary xylem ring (5) delimited by a thin cambial zone (4) – continuing from the one located on the inner side of the secondary phloem in the previous image (4x10 obj.).

From the images above (Fig. 4.3.20), the following characteristics can be observed:

- **The contour of the cross-section is circular**, relatively regular, exhibiting peripheral depressions, which are sometimes more pronounced.

The vascular cylinder is very well developed, occupying most of the section area and reflecting the dominance of secondary vascular tissue development (Fig. 4.3.20, 4.3.21).

In conclusion, the primary root of *Echium vulgare*, analyzed at the basal level, exhibits a typical secondary structure—with the development of both secondary protective tissues and especially vascular tissues, indicating clear activity of secondary meristems, particularly the cambium.

Primary Root (Median Level)

At this level, the root shares the same organizational plan as at the basal level, with the following particularities presented below.

The contour of the cross-section is circular, locally exhibiting exfoliated areas of secondary protective tissues. The section diameter is difficult to estimate precisely from the analyzed sectors, but it appears to be smaller than at the basal level, indicating a primary root in an intermediate to advanced stage of secondary growth. From the exterior to the interior of the sectioned fragment, we distinguish:

The vascular cylinder is well developed, occupying most of the section area and reflecting the predominance of secondary vascular tissues (Fig. 4.3.23).

The cambial zone, located between the secondary xylem and phloem, is difficult to delimit at the cellular level, but clearly identifiable topographically (Fig. 4.3.23 a, c).

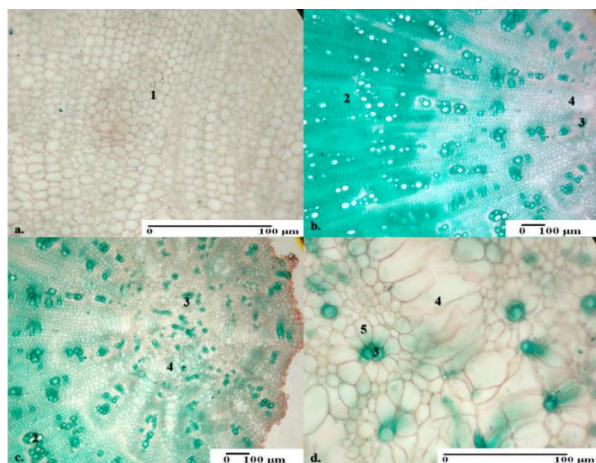


Fig. 4.3.21. Cross-section through the basal level of the primary root: a – Detail from the vascular cylinder: secondary phloem fragment (1) (20x10 obj.); b, c – Details from the vascular cylinder: secondary xylem (2), primary xylem (3), and pith (4) (4x10 obj.); d – Primary xylem vessels separated by radially elongated parenchymal cells (5) (20x10 obj.).

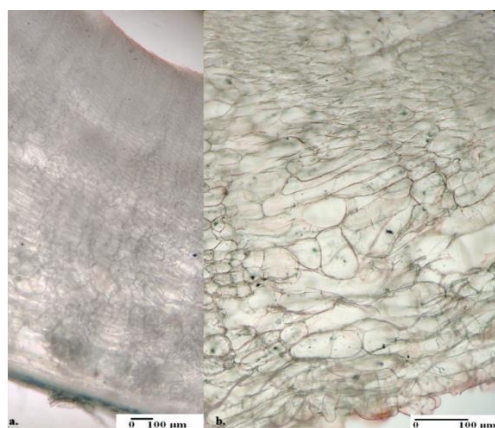


Fig. 4.3.22. Cross-section through the median level of the primary root: a – Fragment from the outer zone of the root of *Echium vulgare*, at the median level: periderm, cortex, secondary phloem, cambial delimitation zone (4x10 obj.); b – Fragment of cortex: parenchymal cells of various shapes and orientations; absence of tissue discontinuities (10x10 obj.).

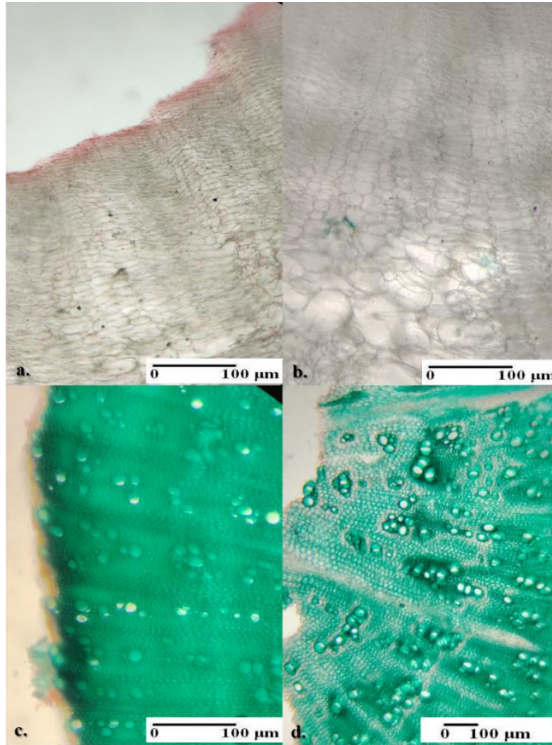


Fig. 4.3.23. Cross-section through the median level of the primary root: a – Fragment from the vascular cylinder: secondary phloem zone, with cambial delimitation (10x10 obj.); b – Details of secondary phloem, delimitation towards the cortex (10x10 obj.); c – Fragment from the vascular cylinder: secondary xylem zone, with cambial delimitation (10x10 obj.); d – Fragment from the vascular cylinder: secondary and primary xylem zone, with medullary delimitation (4x10 obj.).

Primary Root (Apex / Tip)

The apical extremity of the primary root follows the same descriptive pattern as in the previous levels of analysis.

Lateral Roots

Next, the structure of the lateral roots of **Echium vulgare** is analyzed in cross-sections performed at the median level, highlighting the general structural plan and the particularities of the constituent tissues.

The contour of the cross-section is circular, relatively regular, with slight and rare peripheral depressions. The diameter is relatively uniform, suggesting a lateral root in a moderate stage of secondary growth (Fig. 4.3.24 a).

From the exterior to the interior of the section, we distinguish:

- **Periderm**
- **Cortex**
- **Vascular cylinder** (Fig. 4.3.24 a, b), which is relatively well developed, occupying a large portion of the section diameter.

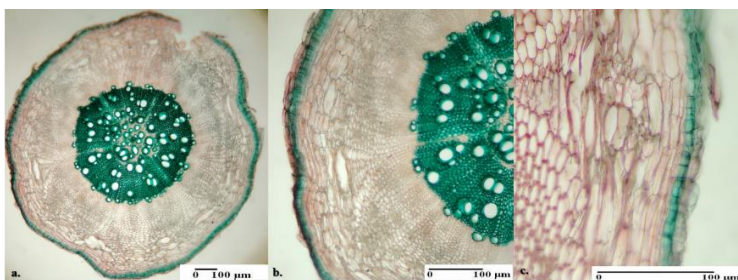


Fig. 4.3.24. Cross-section through the lateral root: a – General overview: periderm, cortex, vascular cylinder; peripheral tissue discontinuities (4x10 obj.); b – Sector of the cross-section showing: fragments of periderm, cortex, vascular cylinder (10x10 obj.); c – Detail of the outer zone: periderm, cortex, outer zone of the phloem ring (20x10 obj.).

The Stem

In the case of the stem, cross-sections were likewise analyzed at its base, middle, and apex, describing: section contour, features of

the epidermis, cortex, and vascular cylinder. The following present selected images from the performed sections along with their descriptions.

Stem (Basal Level) The contour of the cross-section is circular, relatively regular, locally showing uneven surface contours caused by the presence of basal rosettes of long, cystolithic protective trichomes. The section diameter is relatively large, corresponding to a basal stem level with advanced tissue differentiation (Fig. 4.3.25 a). The vascular cylinder (Fig. 4.3.25 a, b) is thick and consists of: a continuous ring of secondary phloem; a xylem zone; vascular cambium.

Stem (Median Level) At this level, the cross-section contour is circular, relatively regular, displaying less frequent tissue breaks or discontinuities. The section diameter is smaller than at the basal level, corresponding to a medium degree of tissue differentiation.

Stem (Apex / Apical Level) The cross-section contour is circular, relatively regular, extremely rarely presenting tissue discontinuities. The section diameter is much smaller than at the median level, corresponding to an incipient to medium degree of tissue differentiation.

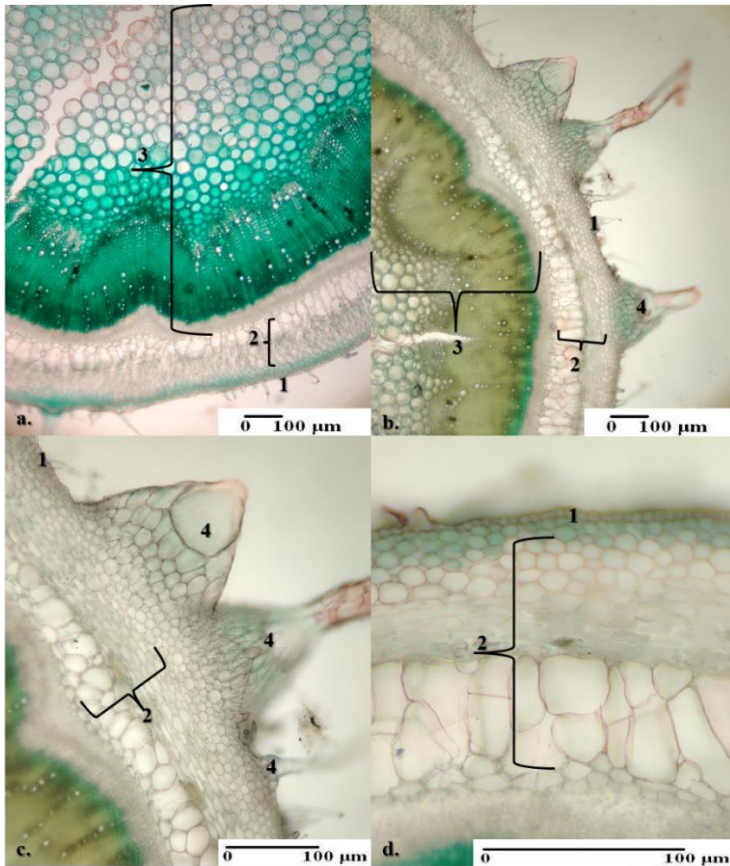


Fig. 4.3.25. Cross-section through the basal level of the stem: a – Sector of the cross-section: epidermis (1), cortex (2), vascular cylinder (3) (4x10 obj.); b – Detail of sector: epidermis (1), cortex (2), vascular cylinder (3) (4x10 obj.); c – Details: epidermis (1) with protective trichomes (4) and cortex (2) (10x10 obj.); d – Details: epidermis (1), cortex (2) (20x10 obj.).

The Leaf (Basal Level)

The leaf structure (basal level) is likewise described at three levels (base, middle, apex), both across the midrib and across the leaf blade, between veins (interveinal area) (Fig. 4.3.26).

At the Midrib (*sau*: At the level of the midrib)



Fig. 4.3.26. Cross-section through the leaf at the basal level across the midrib: a – General overview: adaxial side (1), abaxial side (2), epidermis (3), hypodermis (4), ground parenchyma (5), vascular bundle (6) (4x10 obj.); b – Detail: adaxial side (1), abaxial side (2), epidermis (3), hypodermis (4), ground parenchyma (5), vascular bundle (6) (10x10 obj.).

Analysis of the cross-sections at this level allowed the observation and description of the following structural features (Fig. 4.3.26):

- **The cross-section contour** exhibits bilateral symmetry, with the midrib having a prominent central area and two lateral extensions corresponding to the leaf blade. The adaxial (upper) surface of the midrib is slightly concave, while the abaxial (lower) surface is convex.
- **The epidermis** on both the adaxial and abaxial sides is generally composed of isodiametric cells covered by a visible cuticle.
- **The hypodermis:** subepidermally, smaller cells with slightly thickened cell walls are observed.

- **The ground parenchyma** occupies most of the section area and consists of larger, thin-walled cells adaxially.
- **Vascular bundles:** the central region contains a tangentially elongated collateral vascular bundle, with the xylem oriented towards the adaxial side and the phloem towards the abaxial side.

The Leaf Blade (Interveinal Area)

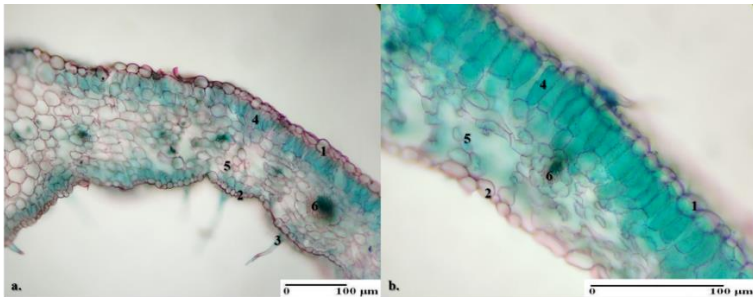


Fig. 4.3.27. Cross-section through the leaf blade at the basal level: a – View through the leaf blade near the midrib, basal level: upper epidermis (on the adaxial side) (1), lower epidermis (on the abaxial side) (2), protective trichomes (3), mesophyll: palisade tissue on the upper side (4); spongy tissue on the lower side (5); lateral (secondary) vascular bundles (6) (10x10 obj.); b – Detailed view: upper epidermis (on the adaxial side) (1), lower epidermis (on the abaxial side) (2), mesophyll: palisade tissue on the upper side (4); spongy tissue (5), lateral vascular bundle (6) (20x10 obj.).

From the images above (Fig. 4.3.27 a, b), the structural characteristics of the leaf blade described below can be identified:

- **The cross-section contour** is relatively uniform and thin, with slight undulations on the abaxial side.
- **The mesophyll** is differentiated into: palisade tissue and spongy tissue.

As a result, the leaf blade exhibits a bifacial (heterofacial) structure with normal dorsiventrality. In certain areas of the blade, larger, rigid protective trichomes supported by a basal rosette of cells are observed, imparting the rough, hispid appearance characteristic of species in the Boraginaceae family.

The Leaf (Median Level)

At the Midrib The cross-section contour maintains its bilateral symmetry, with the midrib forming a prominent central zone towards the abaxial side and two lateral extensions corresponding to the leaf blade. The adaxial (upper) surface of the midrib exhibits, in some sections, a more pronounced concavity than at the basal level, whereas in others it is less marked. Likewise, the abaxial convexity of the midrib varies, being more pronounced in certain sections.

The Leaf Blade (Interveinal Area) The cross-section contour is relatively uniform and thin, displaying slight irregularities caused by the presence of the multicellular pedestal of massive protective trichomes that are cystolithic at the base. At this level as well, the leaf blade exhibits a bifacial (heterofacial) structure with normal dorsiventrality.

The Leaf (Apex / Apical Level)

At the Midrib The cross-section contour maintains its bilateral symmetry, but the midrib is much reduced in development, with the abaxial prominence being less weakly expressed.

The Leaf Blade (Interveinal Area) The cross-section contour is thin, showing prominences and depressions, especially on the lower surface. Consequently, the leaf blade exhibits a predominantly bifacial (heterofacial) structure, with clear differentiation between palisade tissue and spongy tissue.

Results and Discussion

Analysis of cross-sections performed at three levels through the vegetative organs of *Echium vulgare* specimens from the Suceava location confirms that the general structural organization plan is characteristic of each organ and is maintained along its entire length, with variations from one level to another being predominantly quantitative and determined by the dynamics of growth and cellular differentiation.

The primary root exhibits a well-developed secondary structure, defined by the expansion of the periderm and vascular cylinder, where

the secondary phloem forms a continuous ring and the secondary xylem forms a central core.

Lateral roots reflect a less advanced stage of ontogenetic development, having a periderm undergoing differentiation in places, a relatively well-developed cortex, a thinner vascular cylinder, and much less frequent tissue discontinuities.

The stem: Displays a gradual reduction in diameter from base to apex, caused by the thinning of the cortex and a decrease in the degree of development of the vascular cylinder with secondary structure. Towards the apical region, the phloem and xylem rings become thinner, the number and diameter of xylem vessels decrease, and the degree of lignification diminishes. The parenchymal medullary zone (pith) is well developed basally (where it shows tissue discontinuities) and disappears at the apical level, reflecting a clear ontogenetic gradient of maturation (the base being at an advanced stage, while the apex is younger). Unlike in the root (where secondary structure appears simultaneously in both protective and vascular tissues), in the stem, secondary structure is first established in the vascular cylinder. Thus, the protective tissue initially remains primary (epidermis), beginning to exfoliate at the median level as the phellogen generating cork (suber) differentiates.

The leaf: Retains a general bifacial (heterofacial) dorsiventral plan, having palisade tissue on the upper surface and spongy tissue on the lower surface. Towards the apex of the leaf blade, a trend towards an equifacial structure is observed (the appearance of palisade tissue on the abaxial side as well), most likely as an adaptation to the leaf's orientation on the stem relative to light. The midrib, central vascular bundle, ground parenchyma, and mechanical support tissues (hypodermis and bundle sheath) progressively decrease in size from base to apex.

Epidermal appendages: A constant feature on the surface of both organs is the indumentum consisting of simple unicellular protective trichomes (sessile or supported by multicellular epidermal rosettes) and rare secretory trichomes with a club-shaped terminal cell. Stomata were difficult to identify in cross-sections and were analyzed in detail using scanning electron microscopy (SEM).

Consequently, the leaf of ***Echium vulgare*** exhibits structural differences from base to apex, characterized by a progressive reduction in the development of the midrib, mesophyll thickness, and vascular bundle size, without altering the general structural plan. The differences between the analyzed levels are predominantly quantitative and reflect the processes of growth and functional specialization of leaf tissues.

IV.3.4.2. Histo-anatomical characteristics of vegetative organs in fasciated *Echium vulgare* L.⁷

Fasciation represents a morphological deformation characterized by the flattening and widening of plant organs. It most frequently occurs in the stem, a situation in which the shoot apical meristem acquires an unusual band-like or linear meristematic appearance. The name of the phenomenon originates from the Latin *fascis* = bundle or binding.

A fasciated specimen of *Echium vulgare* was identified at Valea lui David, Iași County, under the conditions of 2022. Morphologically, the botanical individual features a strongly transversely flattened stem, with more numerous leaves and deformed inflorescences.

The Root

The root largely retains its normal structure. Areas of periderm differentiation appear, along with a relatively thin cortex exhibiting tissue discontinuities.

The Stem

⁷ A portion of these data was presented at **Trends in Biology: from molecules to complex systems 2026**, 15-18 octombrie 2026, în lucrarea cu titlul: A case of fasciation in *Echium vulgare* L.: histo-anatomical and physiological observations compared to the normal form, Autori: **Alexandra Roxana Sava**, Costică Naela, Stratu Anișoara, Maria-Magdalena Zamfirache

Stem (Basal Level): Exhibits radial symmetry with an epidermis composed of relatively thin-walled cells, yet relatively rich in protective trichomes.

Stem (Median Level): Displays bilateral symmetry with two epidermises composed of cells covered by a distinct cuticle and very numerous protective trichomes.

Stem (Apical Level / Tip): Is at a younger stage of development: it has the same tissue zonation as at the median level, but the constituent tissues are less developed.

The Leaf

At the Midrib: The cross-section contour is modified compared to normal conditions, having a triangular appearance with a visible outgrowth on the abaxial side, in which a well-developed hypodermal collenchyma dominates.

IV.3.4.3. Histo-anatomical characteristics of vegetative organs in *Echium italicum* L.

In order to highlight the structural features of the vegetative organs (root, stem, and leaf), cross-sections were performed on individuals belonging to the species *Echium vulgare*, collected from the Agigea location, Constanța County, on July 14, 2022.

The following presents a descriptive account of the histo-anatomical features of each organ, tracking the changes that occur along their length, from base to apex. Overall, protective, parenchymal, mechanical, and vascular tissues were analyzed and described regarding their spatial organization and degree of development.

The Root

Primary Root For the study of the root, each analyzed cross-section includes descriptions of: section contour, features of the periderm, cortex, and vascular cylinder (phloem, cambium, xylem, and their relative proportions).

The cross-section contour is circular, relatively regular, displaying peripheral depressions that are sometimes more pronounced. The section diameter is considerably large, suggesting a primary root in an advanced stage of secondary growth.

From the exterior to the interior of the sectioned fragments, we distinguish:

- **The periderm** is well developed, resulting from the activity of the phellogen, which generates:
 - relatively thick cork (suber) oriented towards the exterior,
 - a well-represented phelloderm, composed of elongated cells.
- **The vascular cylinder** is very well developed, occupying most of the section area and reflecting the dominance of secondary vascular tissue development.

The Stem

In the case of the stem, cross-sections were analyzed at its base, middle, and apex, describing: section contour, features of the epidermis, cortex, and vascular cylinder. Selected images from the performed sections along with their descriptions are presented below.

Stem (Basal Level) The cross-section contour is circular, without breaks or discontinuities. The epidermis is single-layered, composed of cells generally featuring slightly thickened outer walls covered by a thin cuticle. Thin unicellular protective trichomes are observed fairly frequently. Here and there, stomata situated at the level of the epidermal cells are visible.

The cortex is composed of approximately 14–16 cell layers and is structured into:

- **outer cortex**, consisting of 3–4 layers of small, isodiametric cells;
- **middle cortex**, comprising the next 7 cell layers of larger, isodiametric or tangentially elongated cells;

- **inner cortex**, formed by 3–4 cell layers, generally large, with a predominantly tangential arrangement.

The vascular cylinder is thick and consists of:

- a continuous outer ring of secondary phloem;
- a secondary xylem zone;
- vascular cambium, topographically identifiable but difficult to describe at the cellular level;
- primary phloem, which is very difficult to identify towards the exterior, and primary xylem visible towards the pith, composed of few vessels with small diameters;
- **pith (medulla)**, which is parenchymatous, composed of isodiametric cells.

Stem (Median Level)

The cross-section contour is circular, regular, without tissue breaks or discontinuities. The section diameter is smaller than at the basal level.

Stem (Apex / Apical Level)

The cross-section contour is circular, regular, with local outgrowths caused by the presence of massive protective trichomes with a multicellular basal pedestal formed by epidermal cells. The section diameter is much smaller than at the median level.

The stem of ***Echium italicum*** displays an evident structural gradient from base to apex, characterized by a progressive reduction in organ diameter, thinning of the cortex, and a decrease in the proportion of vascular tissues.

The Leaf (Basal Level)

The leaf structure (basal level) is likewise described at three levels (base, middle, apex), both across the midrib and across the leaf blade, between veins (interveinal area).

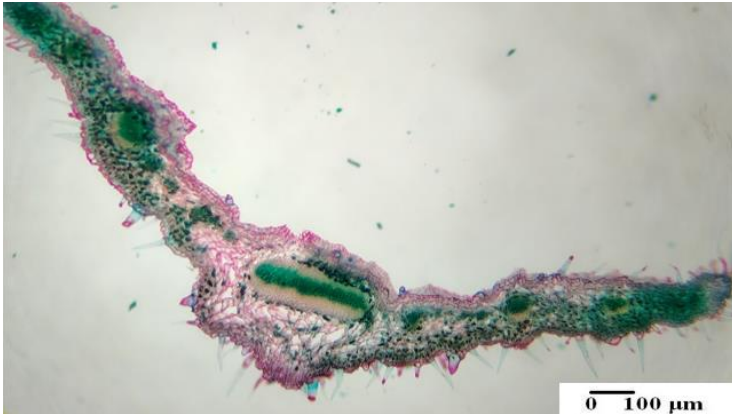


Fig. 4.3.28. Cross-section through the leaf at the basal level across the midrib: general overview; adaxial side, abaxial side, epidermis, hypodermis, ground parenchyma, vascular bundle (4x10 obj.).

At the Midrib (Fig. 4.3.28): The cross-section contour is relatively thin, with frequent local irregularities. It exhibits bilateral symmetry, with a weakly pronounced midrib and two lateral extensions corresponding to the leaf blade. The adaxial surface is flat or slightly convex, while the abaxial surface is convex and weakly prominent.

The Leaf Blade (Interveinal Area) The cross-section contour is thin, showing surface irregularities and folds, especially on the lower surface, opposite the protective trichomes with a multicellular pedestal.

The Leaf (Median Level)

At the Midrib: The cross-section contour displays a wide-open V-shape with bilateral symmetry. The midrib forms a prominent central zone towards the abaxial side and two lateral extensions corresponding to the leaf blade. The adaxial surface of the midrib is almost flat between the two halves of the blade, whereas the abaxial convexity is more pronounced than at the basal level.

The Leaf Blade (Interveinal Area) The cross-section contour is relatively uniform and thin, with rare irregularities caused by the

presence of the multicellular pedestals of massive protective trichomes.

The Leaf (Apex / Apical Level)

At the Midrib: The cross-section contour maintains its bilateral symmetry, but the midrib is somewhat reduced in development, with the abaxial prominence also being visible. The adaxial surface is flat between the two halves of the blade, while the abaxial surface exhibits a visible convexity.

The Leaf Blade (Interveinal Area) The cross-section contour is thin, with rare prominences and depressions. Consequently, the leaf blade exhibits a structure with a trend towards equifaciality, characterized by the development of palisade tissue on both surfaces of the blade, though with a more pronounced development on the adaxial side. Protective trichomes are present on both surfaces of the leaf, contributing to the hispid appearance characteristic of the species.

Comparative Analysis of Histo-Anatomical Characteristics in *Echium vulgare* L. and *Echium italicum* L.

The Root: Displays a secondary structure typical of the genus, but differs from *Echium vulgare* in having a thicker cortex with a tendency towards lignification (highlighted by specific staining with iodine green), as well as secondary and primary xylem richer in storage parenchyma (partially lignified). Ecologically, the strong taproot, early development of the periderm, and well-represented vascularization enable access to water in deep soil horizons, its storage and transport during drought periods, and the accumulation of nutrients required for the biennial cycle. These features confirm the species' preference for drier and sunnier habitats compared to *E. vulgare*.

The Stem: Retains the ontogenetic gradient of thinning from base to apex seen in *E. vulgare*, but develops much more pronounced xeromorphic adaptations: a more abundant and diversified protective indumentum, marked lignification in the outer and inner cortex (periphloemic), xylem, and medullary parenchyma, as well as the presence of large storage cells in the middle zone of the cortex.

Although the general organization plan of the leaf is similar in both species, *Echium italicum* clearly differs from *Echium vulgare* through several structural-xeromorphic adaptations.

IV. 3.5. Histo-anatomical variability of *Echium vulgare* L. in different habitats from North-Eastern Romania

This subchapter includes the research results regarding the comparative structural analysis of vegetative organs (root, stem, leaves), examined at different sectioning levels, from several populations of *Echium vulgare*, under the vegetation conditions of the year 2022 and the specific habitats of: Borca, Neamț; Suceava, Suceava; Bacău, Bacău; Băiceni, Botoșani; and Valea lui David, Iași.

Based on the sections performed, biometric measurements were carried out for the root, stem, midrib, and leaf blade, using ImageJ software.

The aim of this approach was to identify the degree of structural variability in **E. vulgare** individuals originating from different collection sites in North-Eastern Romania and to evaluate the ecological significance of the observed differences. By integrating descriptive histo-anatomical results with biometric and micromorphological data, the objective is to highlight both constant and variable traits depending on habitat conditions. In this manner, a contribution can be made to a better understanding of the species' structural plasticity.

Echium vulgare, which is eurytrophic, is widely distributed from meadows and ruderalized lands to areas with seasonal water deficit. This broad ecological amplitude implies a high adaptive capacity and, implicitly, structural variations among populations originating from different habitats.

IV.3.5.1. *Histo-anatomical variability of the root in Echium vulgare L. in different habitats from North-Eastern Romania*

Sectors of cross-sections performed through the primary root of *Echium vulgare* individuals collected from five different localities indicate a common plan of histo-anatomical organization, characterized by the formation of a well-developed secondary structure. In individuals from all analyzed populations, the following are observed: the periderm, a relatively reduced cortex, and a well-developed vascular cylinder composed of peripherally arranged secondary phloem and a centrally arranged xylem core (hadrome). In all cases, secondary xylem occupies most of the section diameter, giving the root a massive, compact appearance and emphasizing the importance of its conduction and support functions.

Although the general organization plan is similar, differences appear regarding the degree of development of the main tissues. Sectioned individuals from the Suceava population display the largest dimensions of the vascular cylinder and xylem core, while specimens from Valea lui David and Bacău stand out due to the marked development of secondary xylem relative to phloem. The root of individuals from Borca shows a relative reduction of the cortex, leading to high values of the vascular cylinder/cortex ratio, without being accompanied by a marked development of secondary xylem.

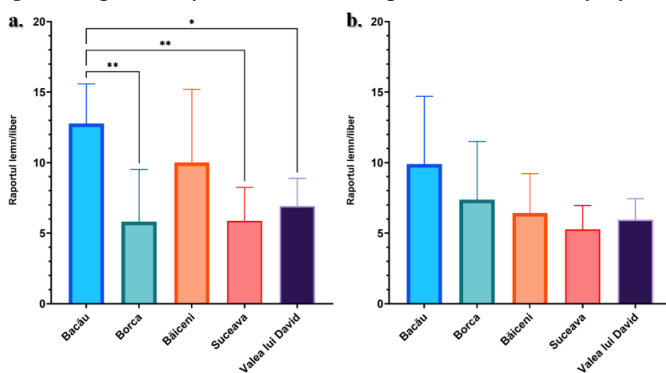


Fig. 4.3.29. Xylem to phloem ratio: a. root base; b. middle of the root in *Echium vulgare* L. (Bacău, Borca, Botoșani, Suceava, and Valea lui David). Graphically plotted data represent the mean $\pm$ standard deviation. Statistical significance: * $p < 0.05$; ** $p < 0.01$. Values without asterisks are statistically non-significant ($p \geq 0.05$).

The differences observed in the development of the main root tissues among individuals from different populations are evident both regarding the development of the vascular cylinder (Fig. 4.3.29) and the proportion of vascular tissues (xylem/phloem) (Fig. 4.3.30), as well as, obviously, organ diameter (Fig. 4.3.31). In individuals from the Bacău population and, to a certain extent, Valea lui David, the root is characterized by high values of the xylem/phloem ratio, indicating marked development of secondary xylem and a possible adaptation to more water-restrictive conditions.

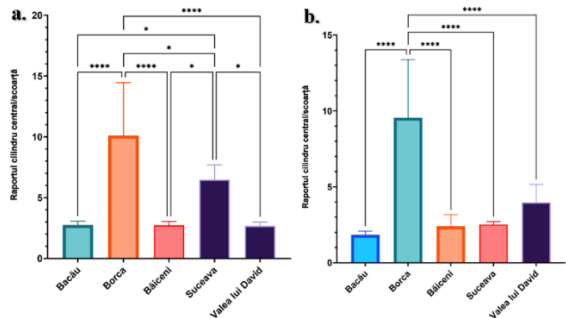


Fig. 4.3.30. Variability of the vascular cylinder to cortex ratio at the basal (a) and median (b) levels of the *Echium vulgare* L. root (Bacău, Borca, Botoșani, Suceava, and Valea lui David). Graphically plotted data represent the mean $\pm$ standard deviation. Statistical significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.0001$. Values without asterisks are statistically non-significant ($p \geq 0.05$).

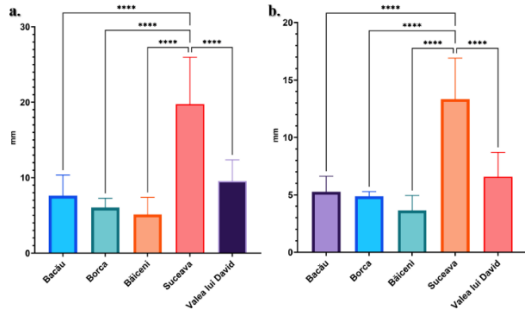


Fig. 4.3.31. Variability of root diameter at the basal (a) and median (b) levels of *Echium vulgare* L. (Bacău, Borca, Botoșani, Suceava, and Valea lui David). Graphically plotted data represent the mean $\pm$ standard deviation. Statistical significance: *** $p < 0.0001$. Values without asterisks are statistically non-significant ($p \geq 0.05$).

The structural variations identified at the root level are evident and are mainly expressed through the development of the vascular cylinder relative to the cortex and through the xylem/phloem ratio. These results indicate the species' structural response to local environmental conditions, demonstrating that the root of **Echium vulgare** exhibits a high degree of anatomical plasticity.

IV.3.5.2. Histo-anatomical variability of the stem in Echium vulgare L. in different habitats from North-Eastern Romania

Analysis of cross-sections performed at the level of the base and median portion of the stem highlights a common structural organization plan, with differences being expressed mainly through the degree of development of the vascular tissues, cortex, and pith (medulla).

Biometric data analysis based on the performed sections reflects the following trends: in the stems of individuals collected from Suceava, all dimensional parameters dominate, in agreement with the root data; in the stems of individuals collected from Bacău, a dominant development of xylem is observed, the same trend being noted in the root as well; the cortex is highly developed in the stems of Suceava individuals and reduced in Bacău; the pith is thin in individuals from Bacău, whereas Suceava exhibits the thickest pith.

Individuals from the analyzed populations differ not only in stem diameter dimensions but also in the degree of development of the secondary structure along the organ. In all cases, an increase in the proportion of xylem towards the base of the stem is observed, but the amplitude of this increase varies among populations from different localities.

IV.3.5.3. Histo-anatomical variability of the leaf in Echium vulgare L. in different habitats from North-Eastern Romania

Comparative analysis of cross-sections performed through leaves originating from **Echium vulgare** populations across five localities (Borca, Suceava, Bacău, Botoșani, and Valea lui David) revealed, first and foremost, the preservation of the general plan of leaf structure. Both at the midrib level and across the interveinal leaf blade, all

specimens from the studied populations display the same histo-anatomical organization, with the observed differences being predominantly quantitative, determined by the degree of development of the constituent tissues.

A constant trait identified is the presence of a gradual leaf development from base to apex.

IV.4. Variations in certain biochemical and physiological indicators in plants belonging to the species *Echium vulgare* L. and *Echium italicum* L. collected from the wild flora

IV.4.1. Foliar photosynthetic pigment content⁸

For the species under study (*Echium vulgare* and *Echium italicum*), the following indicators were analyzed: chlorophyll *a* content, chlorophyll *b* content, carotenoid pigment content, the ratio between chlorophyll *a* and chlorophyll *b* content, and the ratio between total chlorophyll content and total carotenoid pigment content. The obtained results were expressed as mean values $\pm$ standard error, in mg/g fresh plant material.

In the first year (2021), chlorophyll *a* content recorded values ranging from 0.52 mg pigments/g fresh plant material (in samples collected from Suceava) to 0.76 mg pigments/g fresh plant material (in samples collected from Borca and Repedea-Bârnova) (Fig. 4.4.1. a).

Chlorophyll *b* content presented values ranging from 0.21 mg pigments/g fresh plant material (in samples collected from Suceava) to 0.30 mg pigments/g fresh plant material (in samples collected from Bacău) (Fig. 4.4.1. b).

Carotenoid pigments recorded values ranging between 0.23 and 0.30 mg pigments/g fresh plant material. Higher mean values for this

⁸ Part of these data have been published in abstract form:

Sava, A. R., Zamfirache, M. M., Stratu, A., **2022**, Aspects regarding the content of assimilatory pigments in *Echium vulgare* L. species from natural and anthropized habitats (NE region of Romania). *Journal of Experimental & Molecular Biology*, 23(2).

indicator were recorded in samples collected from Valea lui David, Borca, Bacău, and Bârnova-Repedea (Fig. 4.4.1. c).

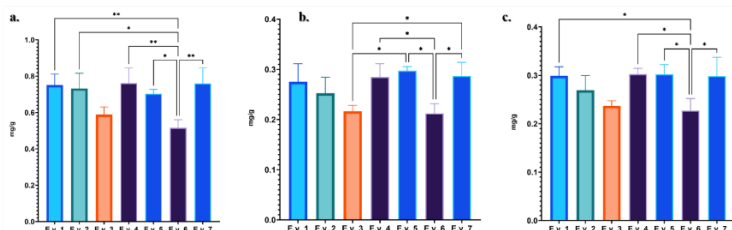


Fig. 4.4.1. Chlorophyll a (a), chlorophyll b (b), and carotenoid pigment (c) content (mg/g fresh plant material) in leaves during the flowering phenophase in plants belonging to the species *Echium vulgare* L. (E.v.) collected from the wild flora in 2021 (Legend: 1 - Valea lui David, Iași; 2 - Băiceni, Botoșani; 3 - Vlădeni, Iași; 4 - Borca, Neamț; 5 - Bacău, Bacău; 6 - Suceava, Suceava; 7 - Repedea-Bârnova, Iași. Graphically plotted data represent the mean $\pm$ standard deviation. Statistical significance: * $p < 0.05$; ** $p < 0.01$. Values without asterisks are statistically non-significant ($p \geq 0.05$)).

In the second year (2022), chlorophyll *a* content recorded values ranging between 0.67 mg pigments/g fresh plant material (in samples collected from Borca) and 0.95 mg pigments/g fresh plant material (in samples collected from Băiceni). Higher mean values for this indicator were recorded in samples collected from Vlădeni and Suceava (Fig. 4.4.2. a).

Chlorophyll *b* content presented values ranging from 0.25 mg pigments/g fresh plant material (in samples collected from Borca) to 0.36 mg pigments/g fresh plant material (in samples collected from Băiceni) (Fig. 4.4.2. b).

Carotenoid pigments recorded values ranging between 0.28 mg pigments/g fresh plant material (in samples collected from Valea lui David) and 0.36 mg pigments/g fresh plant material (in samples collected from Băiceni) (Fig. 4.4.2. c).

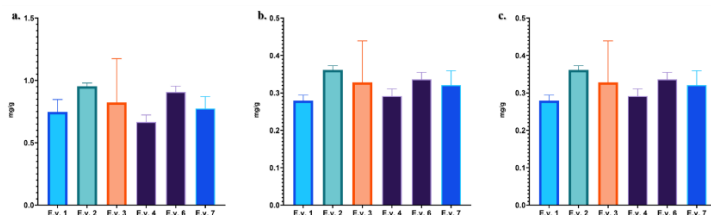


Fig. 4.4.2. Chlorophyll *a* (a), chlorophyll *b* (b), and carotenoid pigment (c) content (mg/g fresh plant material) in leaves during the flowering phenophase in plants belonging to the species *Echium vulgare* L. (E.v.) collected from the wild flora in 2022 (Legend: 1 - Valea lui David, Iași; 2 - Băiceni, Botoșani; 3 - Vlădeni, Iași; 4 - Borca, Neamț; 6 - Suceava, Suceava; 7 - Repedeș-Bârnova, Iași. Graphically plotted data represent the mean $\pm$ standard deviation. Values without asterisks are statistically non-significant ($p \geq 0.05$)).

In the third year, chlorophyll *a* content recorded values ranging between 0.44 mg pigments/g fresh plant material (in samples collected from Vicovu de Sus) and 1.06 mg pigments/g fresh plant material (in samples collected from Borca).

Chlorophyll *b* content presented values ranging from 0.17 mg pigments/g fresh plant material (in samples collected from Vicovu de Sus) to 0.36 mg pigments/g fresh plant material (in samples collected from Borca) (Fig. 4.4.3).

Carotenoid pigments recorded values ranging between 0.22 mg pigments/g fresh plant material (in samples collected from Vicovu de Sus) and 0.41 mg pigments/g fresh plant material (in samples collected from Borca) (Fig. 4.4.3).

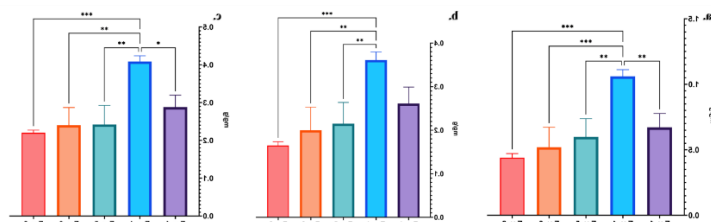


Fig. 4.4.3. Chlorophyll *a* (a), chlorophyll *b* (b), and carotenoid pigment (c) content (mg/g fresh plant material) in leaves during the flowering phenophase in plants belonging to the species *Echium vulgare* L. (E.v.) collected from the wild flora in 2023 (Legend: 1 - Valea lui David, Iași; 4 - Borca, Neamț; 5 - Bacău, Bacău; 6 - Suceava, Suceava; 8 - Vicovu de Sus, Suceava. Graphically plotted data represent the mean $\pm$ standard deviation. Values without asterisks are statistically non-significant ($p \geq 0.05$)).

standard deviation. Statistical significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Values without asterisks are statistically non-significant ($p \geq 0.05$).

In 2021 compared to 2022, slightly lower values for chlorophyll *a*, *b*, and carotenoid pigment contents can be observed in *Echium vulgare* during the flowering phenophase (with a few exceptions) (Fig. 4.4.1, 4.4.2).

The results obtained through our own research contribute to the knowledge regarding the quantitative variation of these foliar biochemical indicators for the species *Echium italicum* and *Echium vulgare*, with the aim of supplementing the specialized literature.

IV.4.1.1. Ratio between chlorophyll a and chlorophyll b content, and ratio between total chlorophyll content and total carotenoid pigment content

In the plants under study in the present research during the flowering phenophase in 2021, the chlorophyll *a* to *b* ratio recorded values lower than the value specified in the literature (3:1). The highest value of this indicator was recorded in *Echium vulgare* collected from the Băiceni locality – 2.90, while the lowest value was in *Echium vulgare* collected from Bacău – 2.37.

In 2022, in the species ***Echium vulgare***, during the flowering phenophase, the ratio between chlorophyll *a* and *b* content was 3.28 for samples collected from Suceava.

In *Echium vulgare* collected from Valea lui David, Băiceni, and Borca, lower values of the discussed ratio were recorded in 2022 compared to 2021 during the flowering phenophase.

For the third year (2023), this indicator recorded values ranging between 2.58 and 2.94.

For the second year (2022), in the case of measurements carried out on samples collected from plants in the fruiting phenophase belonging to the two species, this indicator recorded values ranging between 2.85 and 2.74.

Data analysis regarding the ratio between chlorophyll pigment content and carotenoid pigment content for determinations performed in 2021, 2022, and 2023 (flowering phenophase) revealed variations in values depending on species, sample collection location, and the year in which the determinations were carried out.

For 2021, the maximum value of this indicator was 3.66 in samples collected from location 2 (Băiceni), and the minimum value was 3.21 in samples collected from location 6 (Suceava).

For 2022, values ranging between 3.14 (in samples collected from location 4: Borca) and 4.76 (in samples collected from location 6: Suceava) were obtained, and in 2023, statistically significant differences were recorded among samples collected from all locations.

IV.4.2. Water and dry matter content

IV.4.2.1. Water and dry matter content in the leaf blade

For 2021, in *Echium vulgare* plants, water content recorded the minimum value (75.52%) in samples collected from Bacău and the maximum value (86.36%) in samples collected from Băiceni.

Dry matter content presented values ranging between 13.64% and 24.48%.

For 2022, water content recorded lower values compared to the previous year.

For 2023, in *Echium vulgare* plants, water content recorded values lower than 80% in samples collected from locations 4 (Borca) and 5 (Bacău), and values higher than 80% in samples collected from the other locations.

IV.4.2.2. Water and dry matter content in the stem

For 2022, the lowest water content (68.98%) was recorded in samples collected from location 2 (Băiceni). Samples collected from the other locations displayed a higher water content.

For 2023, water content recorded higher values compared to the previous year.

In the species *Echium italicum*, water content reached values of 72.86% in 2022 and 75.18% in 2023. In the species *Echium vulgare*, dry matter content presented higher values than in *Echium italicum*.

IV.4.3. Total polyphenol content, flavonoid content, and antioxidant activity of the alcoholic extract obtained from the flowering aerial parts of *Echium vulgare* L. and *Echium italicum* L. plants collected from the wild flora

In the present study, the total polyphenol content and total flavonoid content were determined in the ethanolic extract obtained from flowering aerial parts of *Echium vulgare* and *Echium italicum* plants collected from the wild flora. In addition, the antioxidant activity of these extracts was determined using the DPPH method.

Analysis of the obtained results highlighted a higher total polyphenol content in alcoholic extracts obtained from the aerial parts of **Echium vulgare** plants (1.82 mg gallic acid equivalents/g fresh material), this value being 91.57% higher than in *Echium italicum* plants.

Total flavonoid content was higher in alcoholic extracts obtained from the flowering aerial parts of **Echium vulgare** plants (24.21 mg quercetin equivalents/g fresh material) than in extracts from *Echium italicum* plants (by 59.47%).

Analyzing the antioxidant activity, it was found that the ethanolic extract from **Echium vulgare** plants exhibited higher antioxidant

activity than the ethanolic extract prepared from *Echium italicum* plants.

IV.5. Imbibition, germination, and growth tests under experimental conditions

IV.5.1. Germination testing of nutlets (seeds) of *Echium vulgare* L., *Echium italicum* L., and *Echium russicum* J.F. Gmel. after examination of biological material prepared (gold sputtering) for scanning electron microscopy (SEM) observations

In the context of the presented findings, the research constituting the subject of this thesis also includes experiments verifying the germination of nutlets (seeds) belonging to the three *Echium* species under consideration (*Echium italicum*, *Echium russicum*, *Echium vulgare*) prepared by gold sputtering for surface examination via scanning electron microscopy (SEM).

For the species *Echium italicum*, the test variant achieved a germination percentage of 100% at 7 days after the setup of the experiment, remaining unchanged on day 14.

In the case of the species *Echium vulgare*, the test variant had a germination percentage of 71.43% at 7 days after experimental setup, reaching 90.48% at 14 days.

For *Echium russicum*, the germination percentage for the test variant was 57.14% at 7 days after experimental setup, subsequently recording an increase.

Subjecting the seeds of the studied species to gold sputtering positively influenced their germination percentage.

In this context, we consider that future studies monitoring the over-time development of seedlings/plants resulting from the germination of gold-sputtered seeds belonging to the three **Echium** species under focus are necessary—tailored through specific morpho-anatomical,

micromorphological, and biochemical investigations. The results obtained in this manner represent a valuable starting point for future research focused particularly on the seed viability of rare/protected plant species, with a view to testing them via SEM techniques and their subsequent reuse.

IV.5.2. Evaluating the effect of the biostimulant Atonik on seed imbibition, germination, and seedling growth in *Echium russicum* J.F. Gmel.⁹

IV.5.2.1. Seed imbibition

In the one-to-eight-hour interval, the amount of water and Atonik solution absorbed by the seeds through the gravimetric imbibition process showed a distinct pattern depending on the experimental variant: in variant V1, the amount of water absorbed through imbibition increased progressively up to six hours (from 5.40 g% to 13.51 g%), after which it remained constant up to eight hours; in variant V2, the amount of absorbed solution remained constant up to three hours, recorded an increase up to four hours, remained constant up to five hours, and subsequently presented a progressive increase up to eight hours; in variant V3, the amount of absorbed solution remained constant up to two hours, recorded an increase up to five hours, and subsequently remained constant up to eight hours.

The data obtained for the species **E. russicum** show that soaking the seeds in 1‰ Atonik solution had a positive effect on water absorption through imbibition starting from the first hour of monitoring, compared to the control. The higher concentration of the Atonik solution, namely 3‰ (variant V3), resulted in lower values of the amount of water absorbed through the gravimetric imbibition

⁹ This datas were published as a research article: **Sava, A. R.,** Stratu, A., Zamfirache, M.-M., **2026,** Influence of pregermination treatment with Atonik on nutlets imbibition and seedling growth of *Echium russicum* J.F. Gmel. *Studia Univ. VG, SSV, vol. 36,iss.3, 2026*

process compared to those obtained in the control variant (V1), suggesting the occurrence of mild osmotic stress.

IV.5.2.2. Seedling emergence and survival rate following the germination process

In the studied *Echium russicum*, germination/growth is epigeal; the hypocotyl axis exhibits the most pronounced elongation, the epicotyl axis is small in size, and the cotyledons are raised above the soil surface.

From the analysis of the data obtained for the studied plant material (*Echium russicum* individuals obtained through germination from purchased seeds), it was found that 7 days after the initiation of the experiment, the germination percentage presented values ranging between 16.66% and 41.66%, recording higher values in the treatment variants with 1‰ Atonik and 3‰ Atonik solution (by 40% and 50%, respectively) compared to the control variant.

In the variant with seeds imbibed in distilled water (V1), the emergence percentage displayed lower values than in the control.

At 14 days after experiment initiation, the germination percentage ranged between 38.88% and 58.33%.

The seedling survival rate during the interval between day 14 and day 47 was higher in the treatment variants than in the control. Variant V3 stood out with the highest survival percentage value.

The positive effects on the two analyzed indicators (germination percentage and survival percentage) in *Echium russicum* following

seed treatment with 1‰ (V2) and 3‰ (V3) Atonik solution for eight hours are not statistically significant.

In this context, our own results obtained in the presented experiment highlighted that the tested pre-germination treatment with 1‰ and 3‰ Atonik biostimulant stimulated the germination process and increased the seedling survival rate.

IV.5.2.3. Seedling growth parameters

Seed treatment with the biostimulant Atonik (particularly at a 1‰ concentration – variant V2) significantly stimulated the growth and development of all seedling organs compared to the control:

- **Root length:** Recorded higher values in all treatment variants (1‰ Atonik, 3‰ Atonik, and distilled water), with the maximum and statistically significant increase obtained in the variant treated with 1‰ Atonik (V2).
- **Aerial part length (hypocotyl + epicotyl):** Exceeded the control across all variants, with stimulation being progressively amplified from the distilled water variant (V1) to 3‰ Atonik (V3) and reaching its peak at 1‰ Atonik (V2).
- **Total seedling length:** All experimental variants outperformed the control, with the 1‰ Atonik variant generating the largest seedling dimensions, a difference that was statistically significant.
- **Foliar parameters:** Seed imbibition in Atonik solutions favored both the formation of a higher number of leaves per seedling and the expansion of the overall leaf area. Furthermore, the surface area of cotyledonary leaves recorded a considerable expansion in seedlings treated with the biostimulant.

The results of the performed study indicated that 1‰ and 3‰ Atonik solutions applied to seeds prior to sowing positively influence the germination process and the growth of the resulting new individuals (Sava et al., 2026).

IV.5.3. Effects of certain heavy metals on seed imbibition and germination of *Echium vulgare* L. and *Echium russicum* J.F. Gmel. under experimental conditions

IV.5.3.1. Seed imbibition of *Echium russicum* J.F. Gmel. and *Echium vulgare* L. with copper sulfate solutions under experimental conditions

During the monitoring of nutlet imbibition in the two studied *Echium* species in distilled water (C – control) and copper sulfate solutions at concentrations of 20 mg/L (V1), 60 mg/L (V2), and 100 mg/L (V3) (after four and eight hours of imbibition, respectively), variations in values were observed depending on the species, immersion duration, and experimental variant.

Comparative analysis of the results obtained for the two species highlighted that immersing the nutlets in copper sulfate solutions for a period of four and eight hours.

IV.5.3.2 Seed imbibition of *Echium russicum* J.F. Gmel. and *Echium vulgare* L. with zinc sulfate solutions under experimental conditions

The amount of water absorbed through the imbibition process by the nutlets of the two studied *Echium* species during their immersion for four and eight hours, respectively, in distilled water (C – control) and in zinc sulfate solutions at concentrations of 100 mg/L (V1), 200 mg/L (V2), and 300 mg/L (V3) varied depending on the species, immersion duration, and experimental variant.

Overall, the obtained results suggest that copper sulfate and zinc sulfate solutions, respectively, do not affect water absorption through imbibition in *Echium vulgare* and *Echium russicum* nutlets within the defined analysis intervals (four and eight hours, respectively), with the observed changes not being statistically significant ($p > 0.05$).

IV.5.3.3. Seed germination of Echium russicum J.F. Gmel. and Echium vulgare L. in experimentally applied copper sulfate solutions

IV.5.3.3.1. Germination percentage

For both test species, the germination percentage was calculated after a 14-day germination period under experimental conditions.

A. For nutlets subjected to a four-hour treatment duration:

In **Echium russicum**, germination capacity varied depending on the applied treatment. Compared to the control, among the treatment variants, the germination percentage recorded higher values in variant V1 and lower values in variants V2 and V3. In **Echium vulgare** nutlets, germination followed a distinct pattern. In the treatment variants, compared to the control, the germination percentage displayed lower values in variant V1 and higher values in variants V2 and V3.

B. For nutlets subjected to an eight-hour treatment duration:

Analysis of nutlet germination in the two species subjected to experimental treatments highlights an overall superior germinative capacity in **Echium vulgare** compared to **Echium russicum**:

- In **Echium russicum**, the germination percentage reached its minimum level in the control and in variant V1, whereas treatment variants V2 and V3 led to the highest germination values.
- In **Echium vulgare**, the germination rate exceeded the values obtained for **E. russicum** across all experimental variants, with the minimum value observed in the control and the maximum in treatment variants V2 and V3.

In both test species, it was observed that in the treatment variants using copper sulfate solutions containing higher copper concentrations (60 mg/L – V2 and 100 mg/L – V3), the germination percentage recorded higher values than in the control. This finding leads us to consider that the germination process of nutlets from the two species was not negatively affected by the applied treatments.

IV.5.3.3.2. Germination index

The germination index in the two species differs depending on the experimental variant under consideration.

A. For nutlets subjected to a four-hour treatment duration:

- In ***Echium russicum*** nutlets, the germination index recorded the highest value in variant V1 and the lowest value in variant V2.
- In ***Echium vulgare*** nutlets, the germination index recorded the highest value in variant V2 and the lowest value in variant V1.

B. For nutlets subjected to an eight-hour treatment duration:

- In nutlets belonging to the species ***Echium russicum***, the germination index recorded the highest value in variant V2 and the lowest value in variant V1, while in variants V2 and V3 it recorded values 30–32% higher than in the control variant.
- In nutlets belonging to the species ***Echium vulgare***, the germination index recorded the highest value in variant V3 and the lowest value in the control.

IV.5.3.3.3. Mean germination time

A. For nutlets subjected to a four-hour treatment duration:

- In *Echium russicum* nutlets, a decrease in mean germination time of up to 15% compared to the control was observed in variants V1 and V2.

- In *Echium vulgare* nutlets, this parameter recorded values ranging between 9.18 and 10.5, with a reduction of up to 12% compared to the control in variants V2 and V3.

B. For nutlets subjected to an eight-hour treatment duration:

- In *Echium russicum* nutlets, the mean germination time ranged between 6.54 and 7.54, with variants V2 and V3 exhibiting an increase of up to 15% in this germination parameter compared to the control.
- In *Echium vulgare* nutlets, the mean germination time recorded values between 9.52 and 9.93, with variants V1 and V2 showing an increase of up to 4% compared to the control.

IV.5.3.3.4. Germination rate coefficient

A. For nutlets subjected to a four-hour treatment duration:

- In *Echium russicum*, the germination rate coefficient ranged between 10.93 (V3) and 13.36 (V2).
- In *Echium vulgare*, the germination rate coefficient ranged between 9.63 (C1) and 11.05 (V3).

B. For nutlets subjected to an eight-hour treatment duration:

- In *Echium russicum*, the germination rate coefficient ranged between 13.6 (V3) and 15.85 (V1).
- In *Echium vulgare*, the germination rate coefficient ranged between 10.14 (V2) and 10.7 (V3).

From the comparative analysis of the results obtained for the two studied *Echium* species across the copper sulfate treatment variants compared to the control and for both exposure periods, a slight increase in the values of the analyzed germination indicators was observed in most evaluated cases.

*IV.5.3.4. Germination of Echium russicum J.F. Gmel. and Echium vulgare L. seeds in zinc sulfate solutions under experimental conditions*¹⁰

IV.5.3.4.1. Germination percentage

A. For nutlets subjected to a four-hour treatment duration:

- In *Echium russicum* nutlets, the germination percentage presented values ranging between 37.5% and 57.5%.
- In *Echium vulgare* nutlets, the germination percentage recorded values ranging between 55% and 62.5%.

B. For nutlets subjected to an eight-hour treatment duration:

- In *Echium russicum*, the germination percentage presented values ranging between 50% (V1) and 72.5% (C).
- In *Echium vulgare*, the germination percentage recorded values ranging between 47.5% (V1) and 70% (C).

In nutlets belonging to both species, it was observed that in treatment variants using high zinc sulfate concentrations (200 mg/L – V2 and 300 mg/L – V3), the germination percentage recorded a reduction in values compared to the control. However, since this modification was not statistically significant, it can be considered that nutlet germination in the two species was not negatively affected by the applied treatments.

IV.5.3.4.2. Germination index

A. For nutlets subjected to a four-hour treatment duration:

¹⁰This datas were published as a research article: **Sava, A. R.**, Zamfirache, M. M., Stratu, A.,**2023**, The Effect of zinc on germination in species of the genus *Echium* (family Boraginaceae). *Acta Biologica Marisiensis*, 6(1), 46-53.

- In *Echium russicum* nutlets, the germination index reached its highest value in variant V3 (0.99).
- In *Echium vulgare* nutlets, the germination index reached its highest value in variant V1 (0.85).

B. For nutlets subjected to an eight-hour treatment duration:

- In nutlets of *Echium russicum*, the germination index recorded the highest value in the control (1.28) and the lowest in variant V1 (0.8). Compared to the control, the treatment variants showed values lower by 37.5% in V1 and by 21.09% in V2 and V3 (Fig. 4.5.30).
- In *Echium vulgare* nutlets, the germination index recorded the highest value in the control (0.95) and the lowest in variant V1 (0.56). Compared to the control, the treatment variants displayed lower values by 41.05% in V1, 21.0% in V2, and 34.73% in V3 (Fig. 4.5.30).

IV.5.3.4.3. Mean germination time

A. For nutlets subjected to a four-hour treatment duration:

- In *Echium russicum*, the mean germination time ranged between 5.52 and 7.72.
- In *Echium vulgare*, the mean germination time ranged between 8.63 and 10.

B. For nutlets subjected to an eight-hour treatment duration:

- In *Echium russicum*, the mean germination time ranged between 5.9 and 7.37.
- In *Echium vulgare*, the mean germination time ranged between 8.5 and 10.03.

From the data analysis, it can be deduced that the mean germination time is lower in nutlets belonging to the species **Echium russicum**.

IV.5.3.4.4. Germination rate coefficient

A. For nutlets subjected to a four-hour treatment duration:

- In *Echium russicum*, the germination rate coefficient ranged between 13.01 (V1) and 16.77 (C1).
- In *Echium vulgare*, the germination rate coefficient ranged between 10.01 (V2) and 11.7 (V1).

B. For nutlets subjected to an eight-hour treatment duration:

- In *Echium russicum*, the germination rate coefficient ranged between 13.8 (V1) and 17.18 (V2).
- In *Echium vulgare*, the germination rate coefficient ranged between 10.09 (V3) and 11.8 (C).

Analyzing the data, it can be deduced that the germination rate coefficient indicator shows higher values in nutlets belonging to the species *Echium russicum*.

The analysis of the effect of zinc sulfate treatments on germination in **Echium vulgare** and *Echium russicum* highlights a differentiated response depending on exposure duration:

- **4-hour exposure:** Induces a slight stimulation of germination in both species, accompanied, however, by an increase in the time required for germination.
- **8-hour exposure:** Constitutes a chemical stress condition, manifested by delayed germination, a reduction in the germination index, and a prolongation of germination time (a more pronounced effect in *Echium vulgare*).

In the context of the presented findings, we can consider that the results obtained in the present research align with data reported by other authors regarding the effect of different copper and zinc concentrations on the seed germination process of *Echium russicum* J.F. Gmel. and *Echium vulgare* L. tested experimentally.

IV.5.4. Allelopathic potential of aqueous extracts obtained from aerial parts of *Echium italicum* L. and *Echium vulgare* L. plants harvested from wild flora on seed germination and seedling growth of test dicotyledonous plant species

IV.5.4.1. Effects of aqueous extracts obtained from aerial parts of *Echium italicum* L. and *Echium vulgare* L. plants harvested from wild flora on seed germination of test dicotyledonous plant species

According to the obtained results, the analyzed germination indicators exhibited variations in values depending on the **Echium** species used to prepare the extract, the extract concentration, and the target dicotyledonous test species studied.

A reduction in the germination percentage of up to 81% compared to the control was observed. The reduction in germination percentage for both test species was more pronounced when using aqueous extracts at concentrations of 5% and 7.5% obtained from the aerial parts of the two studied Boraginaceae species.

The reduction in germination percentage, germination index, and germination rate coefficient, along with the delay in mean germination time, reveal that aqueous extracts obtained from the aerial parts of plants belonging to *Echium vulgare* and *Echium italicum* species, particularly at concentrations of 5% and 7.5%, have significant effects on the germination of the used test species (*Raphanus sativus* and *Sinapis alba*).

IV.5.4.2. Effects of aqueous extracts obtained from aerial parts of *Echium italicum* L. and *Echium vulgare* L. plants harvested from wild flora on seedling growth of test dicotyledonous plant species

The experiment whose results are presented in this subchapter represents a continuation of the observations and investigations carried out on the previously used test seedlings.

In the case of 7.5% aqueous extracts obtained from *E. vulgare*, no measurements were performed because the seed germination percentage of the test plants (*Raphanus sativus* and *Sinapis alba*) was very low.

Analyzing the root length indicator of newly formed test seedlings, a stimulating effect was observed at low concentrations of aqueous extracts (1% and 2%) obtained from the aerial parts of *E. vulgare* and *E. italicum*, with a statistically significant effect in the case of 1% and 2% *E. vulgare* extracts for both test species (Fig. 4.5.1, Fig. 4.5.2).

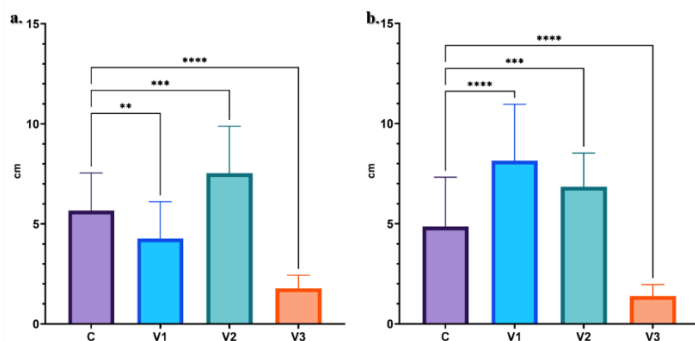


Fig. 4.5.1. Root length of *Raphanus sativus* L. (a.) and *Sinapis alba* L. (b.) test seedlings treated with aqueous extracts obtained from aerial parts of wild-collected *Echium vulgare* L. plants: C – control; V1 – 1%; V2 – 2%; V3 – 5%. Graphically modeled data represent mean $\pm$ standard deviation. Statistical significance: ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. Values without asterisks are not statistically significant ($p \geq 0.05$).

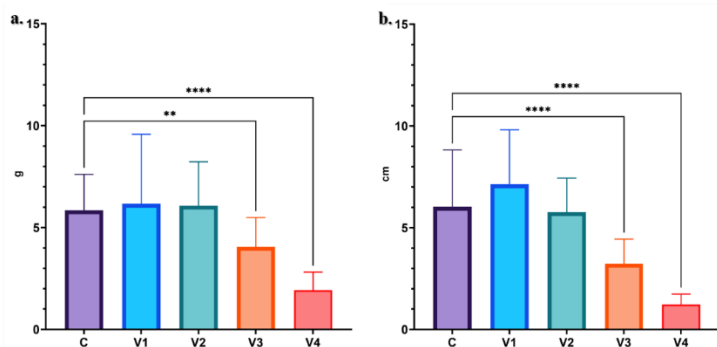


Fig. 4.5.2. Root length of *Raphanus sativus* L. (a.) and *Sinapis alba* L. (b.) test seedlings treated with aqueous extracts obtained from aerial parts of wild-collected *Echium italicum* L. plants: C – control; V1 – 1%; V2 – 2%; V3 – 5%; V4 – 7.5%. Graphically modeled data represent mean $\pm$ standard deviation. Statistical significance: ** $p < 0.01$; **** $p < 0.0001$. Values without asterisks are not statistically significant ($p \geq 0.05$).

Analyzing the hypocotyl length parameter, a stimulating effect was observed in seedlings of both test species when applying 1%, 2%, and 5% aqueous extracts of *E. italicum* and 1% and 2% extracts of *E. vulgare*. A significant retarding effect on hypocotyl elongation was noted only in *Sinapis alba* seedlings upon application of 5% *E. vulgare* and 7.5% *E. italicum* aqueous extracts.

The fresh weight of seedlings in both test species was significantly reduced when using 5% *E. vulgare* aqueous extracts (in the test species *Raphanus sativus*) and 7.5% *E. italicum* extracts (in both test species).

At low concentrations (1%, 2%), extracts obtained from the aerial parts of *Echium italicum* and *Echium vulgare* exerted a stimulating effect on root and hypocotyl elongation in test seedlings; however, at higher aqueous extract concentrations (5%, 7.5%), the observed effect was retarding.

IV.5.4.3. Effects of aqueous extracts obtained from aerial parts of *Echium italicum* L. and *Echium vulgare* L. plants harvested from wild flora on the assimilatory pigment content of seedlings of test dicotyledonous plant species

Further analyzing the evolution of the biochemical composition of test dicotyledonous seedlings (*Raphanus sativus* L. and *Sinapis alba* L.) obtained from seeds treated with aqueous extracts of the aerial parts of the two studied *Echium* species, it is observed that **E. italicum** aqueous extracts across all tested concentrations induced, compared to the control, increasing or decreasing variations in the content of chlorophyll *a*, chlorophyll *b*, and carotenoid pigments in seedlings of both test species (Fig. 4.5.3). The 5% extract concentration stood out through a more pronounced reduction in chlorophyll *a* and carotenoid pigment content in *Sinapis alba*; however, these changes in the assimilatory pigment content of the seedlings were not statistically significant.

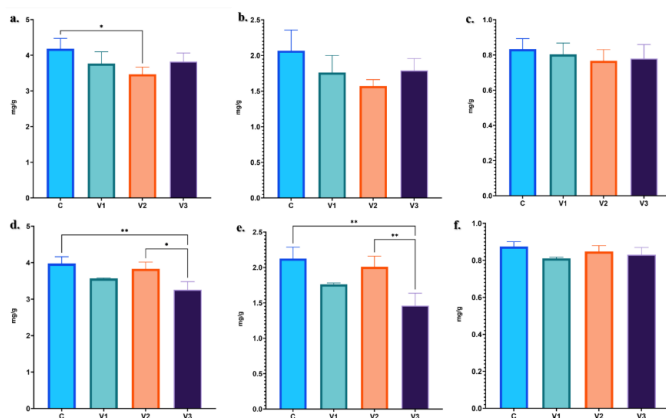


Fig. 4.5.3. Chlorophyll *a* (a., d.), chlorophyll *b* (b., e.), and carotenoid pigment content (c., f.) in test seedlings of *Raphanus sativus* L. (a., b., c.) and *Sinapis alba* L. (d., e., f.) treated with aqueous extracts obtained from aerial parts of wild-collected *Echium vulgare* L. plants: C – control; V1 – 1%; V2 – 2%; V3 – 5%. Graphically modeled data represent mean $\pm$ standard deviation. Statistical significance: * $p < 0.05$; ** $p < 0.01$. Values without asterisks are not statistically significant ($p \geq 0.05$).

Aqueous extracts of **E. vulgare** across all tested concentrations reduced the content of chlorophyll *a*, chlorophyll *b*, and carotenoid pigments in seedlings of both test species.

IV.5.4.4. Effects of aqueous extracts obtained from aerial parts of *Echium italicum* L. and *Echium vulgare* L. plants harvested from wild flora on selected biochemical indicators of seedlings of test dicotyledonous plant species

The analysis of the results obtained for the test seedlings of *Raphanus sativus* L. and *Sinapis alba* L., grown from seeds treated with aqueous extracts prepared from aerial parts of wild-collected *Echium italicum* L. plants, indicated that the analyzed biochemical indicators (total polyphenol content, flavonoid content, and antioxidant activity) exhibited variations in values depending on the species used for extract preparation, extract concentration, and the target test species studied.

Regarding the total polyphenol content in seedlings, an increase in this parameter compared to the control was observed for the treatment variants with 5% aqueous extracts of *E. italicum* and *E. vulgare* in both test species.

The analysis of results concerning the flavonoid content in test seedlings highlighted an increase in this indicator in 75% of the evaluated cases compared to the control.

The antioxidant activity parameter recorded an increase in 58% of the evaluated cases compared to the control. In the case of using 5% aqueous extracts prepared from the aerial parts of plants belonging to both *Echium* species, a situation similar to that described for the total polyphenol content was observed.

The comparative analysis of the results obtained regarding the biochemical indicators of extracts obtained from *Raphanus sativus* L. and *Sinapis alba* L. seedlings grown from seeds subjected to treatments with aqueous extracts prepared from aerial parts of *Echium italicum* and *Echium vulgare* plants highlighted, in both test species,

high values for total polyphenol content, total flavonoids, and antioxidant activity for the 5% concentration extracts.

We also consider that further studies are needed to elucidate the allelopathic potential of *Echium italicum* L. and *Echium vulgare* L. species, including the use of extracts obtained separately from their aerial and/or underground organs, the evaluation of new test species of well-defined economic importance to study the effects of these extracts, as well as the identification of the main chemical compounds in these extracts responsible for the allelopathic phenomenon specific to the two studied **Echium** species.

CONCLUSIONS

This doctoral thesis aimed to complement, through multidisciplinary research—micromorphological, histo-anatomical, biochemical, and physiological—the current information in the specialized literature regarding the biology of *Echium* L. species present in the flora of Romania. In this context, the following key points can be stated:

- **SEM analysis** of the surfaces of vegetative organs and selected floral parts in *Echium vulgare* L. and **Echium italicum** L. highlighted general micromorphological features characteristic of the genus *Echium* L., based on the development of an abundant coat of protective trichomes and relatively uniform peripheral protective complex surfaces. All investigated specimens present a trichome indumentum dominated by long, rigid protective hairs supported by well-developed multicellular basal epidermal rosettes. These structures confer upon the surfaces the hispid appearance characteristic of **Echium** species.
- **Comparative micromorphological analysis** of specimens from populations in the Borca, Suceava, Bacău, Băiceni, and Valea lui David areas revealed a relatively stable micromorphological

pattern, with differences between populations being predominantly quantitative. The results demonstrate that surface micromorphology in the investigated species is characterized by high microstructural conservatism. Trichome types, the presence of basal rosettes, and the general organization of epidermal surfaces remain constant regardless of organ or population. Variability mainly affects trichome density, dimensions, and calculated biometric indices. Based on biometric parameters and indices (robustness index, protection index, and the ratio between trichomes with and without a basal rosette), **Echium vulgare** populations can be differentiated by the degree of development of the trichome coat, without altering the species' micromorphological pattern.

- **Nutlet analysis** confirms the taxonomic value of morphological and micromorphological characters within the genus **Echium** L. The main character is represented by the surface ornamentation type: *E. russicum* J.F. Gmel. exhibits pustulate-tuberculate nutlets, *E. vulgare* L. tuberculate-pustulate nutlets, and *E. italicum* L. tuberculate-echinate nutlets, with well-developed spiny protuberances. Biometric analysis highlights progressive differences from *E. russicum* J.F. Gmel. to *E. italicum* L., with *E. vulgare* L. occupying an intermediate position. Simultaneously, an inverse relationship is observed between the density of small ornamentations and the development of large protuberances.
- **Histo-anatomical analysis** of vegetative organs highlights the existence of a common structural plan among the investigated species, characteristic of the genus *Echium* L., based on the development of a root with a well-represented secondary structure, a stem with moderate secondary growth, and a leaf with a bifacial heterofacial structure. The differences observed in all analyzed taxa between the base, median region, and apex of the organs are predominantly quantitative and reflect the varying degree of ontogenetic tissue development. Secondary structure is more pronounced in basal regions and progressively diminishes toward apical zones.

Echium vulgare L. is characterized by a taproot with a well-developed stele (central cylinder), a stem with moderate secondary structure, and

a typical bifacial leaf, with mesophyll differentiated into palisade and spongy parenchyma. The ensemble of observed anatomical characters suggests the adaptation of the studied plants to open habitats with temporary water deficit periods. The fasciated form of **Echium vulgare** L. retains the same tissue types and general organization, with modifications being primarily expressed through changes in organ geometry and spatial tissue redistribution.

Compared to *E. vulgare* L., *Echium italicum* L. exhibits a more pronounced development of tissues involved in protection, conduction, and storage. The root develops a thicker cortex and more abundant storage parenchyma, the stem shows greater lignification and a denser trichome coat, and the leaf is distinguished by a more compact mesophyll, tending toward an equifacial structure. The anatomical differences between *E. vulgare* L. and *E. italicum* L. suggest an accentuation of xeromorphic characters in *E. italicum* L., in accordance with this species' preference for sunnier and drier habitats.

- **Analysis of biochemical indicators** revealed variability in water content, dry matter, and assimilatory pigments among the investigated species. The values of these parameters were influenced by species-specific traits as well as phenophase, climatic conditions, and habitat characteristics of origin, reflecting the capacity of *Echium* species to respond to environmental variations. Determinations regarding total polyphenol and flavonoid contents, alongside antioxidant activity, highlighted a high biochemical potential of the investigated species, with superior values in *Echium vulgare* L.
- **Germination tests** conducted on nutlets after SEM analysis demonstrated that the preparation and examination process does not affect seed viability. Furthermore, in the case of *Echium russicum* J.F. Gmel., a favorable influence on germination was observed, a result that opens prospects for applying SEM techniques in the study of rare and protected species without compromising biological material.
- **Research on imbibition, germination, and growth** highlighted the stimulating effect of Atonik treatment on seeds of *Echium russicum* J.F. Gmel. The application of the biostimulant favored

both germination and seedling development, confirming previously reported observations regarding the effect of this biostimulant on other species and providing new data on the potential management of the germination process and cultivation of this species of community interest.

- Experiments regarding the **effect of heavy metals** (copper as copper sulfate and zinc as zinc sulfate) showed that, within the tested concentration ranges, imbibition and germination processes were not significantly affected in *Echium vulgare* L. and *Echium russicum* J.F. Gmel. These results suggest a relatively high tolerance of the seeds of these taxa to the concentrations used and complement available information regarding their response to heavy metal-induced stress.
- **Assessment of allelopathic potential** demonstrated the ability of aqueous extracts obtained from flowering aerial parts of *Echium vulgare* L. and *Echium italicum* L. to influence germination, seedling growth, and assimilatory pigment content in test dicotyledonous species. Of the two species, *Echium vulgare* L. exhibited a more pronounced allelopathic effect, while the test species *Sinapis alba* L. proved to be more sensitive to the action of the extracts.
- Overall, the results regarding the biology of the studied species of the genus *Echium* L. highlight complex adaptation and interaction mechanisms with their living environment and complement specialized literature with original data on their morpho-anatomical traits, biochemical composition, germinative capacity, tolerance to stress factors, and allelopathic potential.
- The obtained results highlight and support the fact that the analyzed species of the genus *Echium* L. combine the stability of basic structural traits (conservation of the general structural plan) with the capacity for functional adaptation to the ecological conditions of occupied habitats, thus explaining their ecological success.

- At the same time, the conducted study provides original data regarding population variability and the taxonomic value of nutlets from specimens belonging to the studied *Echium* species, demonstrates the biological characteristics of taxa of the genus *Echium* L. present in the flora of Romania, and opens new directions for investigating their biology at the national level.

DISSEMINATION OF RESULTS

Publications:

1.1. Full-length scientific articles published in journals indexed in international databases (IDB) •

Sava, A. R., Stratu, A., Zamfirache, M.-M., **2026**, Influence of pregermination treatment with Atonik on nutlets imbibition and seedling growth of *Echium russicum* J.F. Gmel. *Studia Univ. V.G. SSV*, vol. 36,iss.3, 2026

Sava, A. R., Zamfirache, M. M., Stratu, A.,**2023**, The Effect of zinc on germination in species of the genus *Echium* (family Boraginaceae). *Acta Biologica Marisiensis*, 6(1), 46-53.

1.2. Papers published as abstracts at conferences •

Sava, A. R., Zamfirache, M. M., Costică, N., **2023**. Research regarding the nutlets micromorphology of species belonging to the genus *Echium* L. (Boraginaceae family) *Journal of Experimental & Molecular Biology*, 24(4): 239-240.

Sava, A. R., Zamfirache, M. M., Stratu, A., **2022**. *Echium italicum* L. (family Boraginaceae): biological characteristics and potential uses. *Journal of Experimental & Molecular Biology*, 23(2):43.

Sava, A. R., Zamfirache, M. M., Stratu, A., **2022**, Aspects regarding the content of assimilatory pigments in *Echium vulgare* L. species

from natural and anthropoized habitats (NE region of Romania). *Journal of Experimental & Molecular Biology*, 23(2):63.

Sava, A. R., Stratu, A., Zamfirache, M. M., **2021**. Aspects regarding the ecology and importance of *Echium vulgare* L. and *Echium italicum* L. species. *Journal of Experimental & Molecular Biology*, 22(2):10.

Conference presentations and scientific events:

National conferences

Tendințe în Biologie: de la molecule la sisteme complexe, Facultatea de Biologie, Iași, 2023 în lucrarea cu titlul: Research regarding the nutlets micromorphology of species belonging to the genus *Echium* L. (Boraginaceae family), autori: **Sava, A. R.**, Zamfirache, M. M., Costică, N.. -Poster

Tendințe în Biologie: de la molecule la sisteme complexe, Facultatea de Biologie, Iași, 2022 în lucrarea cu titlul: *Echium italicum* L. (family Boraginaceae): biological characteristics and potential uses, autori: **Sava, A. R.**, Zamfirache, M. M., Stratu, A. -Poster

Tendințe în Biologie: de la molecule la sisteme complexe, Facultatea de Biologie, Iași, 2022 în lucrarea cu titlul: Aspects regarding the content of assimilatory pigments in *Echium vulgare* L. species from natural and anthropoized habitats (NE region of Romania), autori: **Sava, A. R.**, Zamfirache, M. M., Stratu -Poster

Tendințe în Biologie: de la molecule la sisteme complexe, Facultatea de Biologie, Iași, 2021 în lucrarea cu titlul: Aspects regarding the ecology and importance of *Echium vulgare* L. and *Echium italicum* L. species, autori: **Sava, A. R.**, Stratu, A., Zamfirache, M. M. -Poster

Sesiunea științifică anuală a studenților naturaliști, Ediția a IV-a, Iași, 31 octombrie **2020**, în lucrarea cu titlul: Contribuții la cunoașterea distribuției și stării de conservare a speciei *Echium russicum* J. F.

Gmelin. în situri natura 2000 din România, Autori: **Alexandra Roxana Sava**, Irina Irimia, Maria-Magdalena Zamfirache -Prezentare

International conferences

Trends in Biology: from molecules to complex systems 2026, 15-18 octombrie **2026**, în lucrarea cu titlul: Distribution of *Echium* species in Romania based on herbarium data, Autori: **Alexandra Roxana Sava**, Irina Irimia, Maria-Magdalena Zamfirache – Poster

Trends in Biology: from molecules to complex systems 2026, 15-18 octombrie **2026**, în lucrarea cu titlul: A case of fasciation in *Echium vulgare* L.: histo-anatomical and physiological observations compared to the normal form, Autori: **Alexandra Roxana Sava**, Costică Naela, Stratu Anișoara, Maria-Magdalena Zamfirache - Poster

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