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BAKU STATE UNIVERSITY



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ARTICLE 1

New Distribution Areas of the Genus *Aethionema* R. Br. in the Highland Regions of the Nakhchivan Autonomous Republic

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ABSTRACT

This study reports the newly identified distribution areas of the genus *Aethionema* R. Br. (family Brassicaceae Burnett) in the highland regions of the Nakhchivan Autonomous Republic of Azerbaijan, with particular attention to the Julfa and Ordubad districts. Worldwide, the Brassicaceae comprises more than 3,709 species in 338 genera; in the flora of Azerbaijan the family is represented by 248 species across 74 genera, and in the Nakhchivan Autonomous Republic by 165 species from 67 genera, among which *Aethionema* R. Br. is one of the species-rich genera. During field expeditions and free routes conducted in 2022–2024, covering the period of intensive flowering and fruiting (June–July), the ecological-geographical conditions of the genus were examined and distribution maps were produced. The study areas — the surroundings of the villages of Milakh, Arafsa, Teyvaz, and Bekahmed in the Julfa district and the village of Nurgut in the Ordubad district — lie between 1781 and 2479 m above sea level, and their coordinates were recorded with a Garmin eTrex 30x GPS device. Eight species of *Aethionema* R. Br. were recorded — *A. arabicum*, *A. cardiophyllum*, *A. cordatum*, *A. diastrophis*, *A. edentulum*, *A. fimbriatum*, *A. pulchellum*, and *A. szowitsii* — occurring mainly on dry, rocky, gravelly, and stony slopes of the lower and middle mountain belts. *Aethionema pulchellum* Boiss. & Huet., a multi-stemmed, semi-shrubby plant 15–30 cm high with pink petals, was studied in detail, and the herbaceous-shrub formation in which it participates was analyzed. This formation comprises 28 species; by ecological group, 14 species (50%) are xerophytes, 7 (25%) mesophytes, 4 (14.28%) xeromesophytes, and 3 (10.71%) mesoxerophytes, with *Grammosciadium platycarpum* as the dominant and *Centaurea Behen* as the subdominant species. A table detailing the family, genus, life form, and ecological group of the constituent species, together with a distribution map of the recorded taxa, is provided. The results contribute to the documentation and conservation of Brassicaceae diversity in the arid highlands of Nakhchivan under conditions of global climate change.

Keywords: *Brassicaceae* Burnett, *Aethionema* R. Br., highlands, flora, phytocoenosis, Nakhchivan.

1. INTRODUCTION

In recent years, anthropogenic factors such as drought, mean air temperatures above the annual norm, the irregular use of pastures and meadows, and the expansion of agriculture have been observed to affect the distribution density of many species negatively, as well as their phytocoenology, and some species remain on the brink of extinction. The aim of the wider research programme is to study the impact of the expected global climate change on

biodiversity, including the family *Brassicaceae* Burnett. The work includes identifying species at risk of extinction as a consequence of global climate change and creating a gene pool for biodiversity conservation by collecting seeds of species of the family *Brassicaceae* Burnett, including species of the genus *Aethionema* R. Br. in the Nakhchivan Autonomous Republic. By studying their ecological condition, the research identifies the threats of species extinction and proposes preventive measures for biodiversity

conservation. The genera *Aethionema* R. Br., *Allyssum* L., *Arabis* L., *Draba* L., *Crambe* L., *Iberis* L., and others are described as characteristic components of the natural flora of rocky habitats, in which the proportion of their representatives is high. Most of the studied species (about 85%) were classified as perennial on the basis of their flowering period, and moist highlands are favorable for their growth (Belyaeva, 2021). Rostovtseva et al. (2013) reported studies on a number of seasonal plants, including some members of the cabbage family, in their work “Additions to the Plant Collections of the Penza Botanical Garden,” dividing the species into groups by particular characteristics, among them *Aethionema grandiflorum* Boiss. et Hohen.

A number of authors grouped the species *Aethionema iberideum* (Boiss.) Boiss., *A. lepidioides* Hub.-Mor., *A. eunomioides* (Boiss.) Bornm., *A. heterocarpum* J. Gay, *A. arabicum* (L.) ex D.C., *A. cordatum* (Desf.) Boiss., *A. dumanii* Vural & Adigüzel, *A. subulatum* (Boiss. & Heldr.) Boiss., and *A. turcica* H. Duman & Aytaç by flower color, and provided information on their phenophase, altitudinal distribution, ecological environment, and life form (Aytaç et al., 2020). The Institute for Biodiversity and Ecosystem Research implemented an action plan for the conservation of *Aethionema arabicum* (L.) (Delcheva & Bancheva, 2014). The cabbage family (Cruciferae B. Juss.) has been studied in Europe, European Russia, and the North Caucasus (Chater et al., 1993; Dorofeyev, 2004; Pavlova, 2007). The high genetic diversity of *Aethionema* R. Br. species growing under natural conditions in Turkey may indicate differences in their origin and the presence of new intrageneric taxa and species; the seed-coat macrosculpture of some Turkish *Aethionema* R. Br., their genetic diversity, and their relationships have been revealed by ISSR and RAPD analysis, which proved useful for distinguishing *Aethionema* species (Sunar et al., 2016; Yıldırım & Kılıç, 2016; Pinar et al., 2007). The ecological-geographical distribution, nomenclature, and species composition of the Cruciferae in the central region of the European part of the Russian Federation and some neighboring countries have also been described (Cherepanov, 1995; Dorofeyev, 1998, 2012;

German, 2022). The systematics, phylogeny, and evolution of the Brassicaceae (Cruciferae), together with an information resource on their diversity, have been presented (Al-Shehbaz et al., 2006; Marhold, 2018). In the flora of Iran, the genus *Aethionema* R. Br. is represented by *A. edentulum*, *A. cordatum*, *A. elongatum*, and *A. pulchellum* (Moazzeni et al., 2018). Useful species of the genus *Aethionema* R. Br. occurring in the Shahbuz highlands have also been reported (Nabieva, 2013).

2. MATERIALS AND METHODS

In 2022–2024, expeditions and free routes were carried out to study the current status of species of the family *Brassicaceae* Burnett in arid areas, and distribution maps of species of the genus *Aethionema* R. Br. were produced. The research covered the surroundings of the villages of Milakh, Arafsa, Teyvaz, and Bekahmed in the Julfa district, as well as the village of Nurgut in the Ordubad district of the Nakhchivan Autonomous Republic. During the expeditions and free routes, covering the period of intensive species distribution (June–July), the ecological-geographical conditions of the distribution of the genus *Aethionema* R. Br. were determined. The coordinates of the study areas were recorded with a Garmin eTrex 30x GPS device, as follows: near Milakh, 39°17'26.98" N, 45°45'03.80" E, 1781 m a.s.l.; near Arafsa, 39°19'13.99" N, 45°49'51.04" E, 1883 m a.s.l.; near Teyvaz, 39°14'50.02" N, 45°47'52.02" E, 2038 m a.s.l.; near Bekahmed, 39°15'39.34" N, 45°55'56.22" E, 2479 m a.s.l.; and near Nurgut, 39°12'48.16" N, 45°55'43.41" E, 2441 m a.s.l.

The taxa were identified following Karyagin (1953), Cherepanov (1995), Asgarov (2016), and Talibov et al. (2021), and were clarified according to changes in the international nomenclature from BioLib, ITIS, and EOL. Phytocoenology and plant geography were treated following Gurbanov and Jabbarov (2017).

3. RESULTS AND DISCUSSION

The family *Brassicaceae* Burnett comprises more than 3,709 species belonging to 338 genera worldwide (Al-Shehbaz et al., 2006). In the flora of Azerbaijan, the family is represented by

248 species across 74 genera, while in the Nakhchivan Autonomous Republic 165 species from 67 genera have been recorded (Asgarov, 2016; Talibov et al., 2021). One of the species-rich genera within the Brassicaceae is *Aethionema* R. Br. During the present research, 8 species of the genus *Aethionema* R. Br. were recorded in the highlands of the Nakhchivan Autonomous Republic.

Eukaryota Chatton, 1925

Regnum: Plantae Haeckel, 1866

Divisio: Magnoliophyta Crono., Takht. & W. Zimm., 1966 [Angiospermae Lindl. (P. D. Cantino & M. J. Donoghue)]

Classis: Magnoliopsida Brongn., 1893

Ordo: Brassicales Bromhead

Familia: Brassicaceae Burnett, nom. cons. (= Cruciferae Juss., nom. cons.)

Genus: *Aethionema* R. Br.

1. *Aethionema arabicum* (L.) Lipsky – found in dry, stony, and gravelly areas and debris between the villages of Milakh and Arafsa.

2. *Aethionema cardiophyllum* Boiss. & Heldr. – found on dry, gravelly, and stony slopes near the village of Arafsa.

3. *Aethionema cordatum* (Desf.) Boiss. – distributed on dry, gravelly slopes in the Teyvaz and Bekahmed areas.

4. *Aethionema diastrophis* Bunge – found on

rocky and gravelly slopes between the villages of Arafsa and Bekahmed.

5. *Aethionema edentulum* N. Busch – distributed on dry, stony, and rocky slopes in the Nurgut area.

6. *Aethionema fimbriatum* Boiss. – widely distributed on rocky slopes in the Arafsa, Bekahmed, and Nurgut areas.

7. *Aethionema pulchellum* Boiss. & Huet. – distributed on rocky and gravelly slopes in the Bekahmed, Teyvaz, Arafsa, and Nurgut areas.

8. *Aethionema szowitsii* Boiss. (*A. elongatum* auct.) – widely distributed in the Milakh and Teyvaz areas, mainly on rocky, calcareous slopes.

Aethionema pulchellum Boiss. & Huet., a representative of the genus *Aethionema* R. Br., is a multi-stemmed, semi-shrubby plant 15–30 cm high (Aliyeva, 2022). Its green leaves are linear and elongated. The sepals are sometimes dark, and the petals are pink and 6 mm long. The style is short and columnar. The plant flowers in June and fruits in July. It occurs on dry, rocky, stony slopes in the lower and middle mountain belts. Its attractive leaves and pink flowers are highly valued ornamentally. *Aethionema pulchellum* Boiss. & Huet. was recorded during the expedition at an altitude of 2479 m near the village of Bekahmed.



Figure 1. *Aethionema pulchellum* Boiss. & Huet.



Figure 2. Vegetation around the village of Bekahmed.



Figure 3. Vegetation around the village of Arafsa.

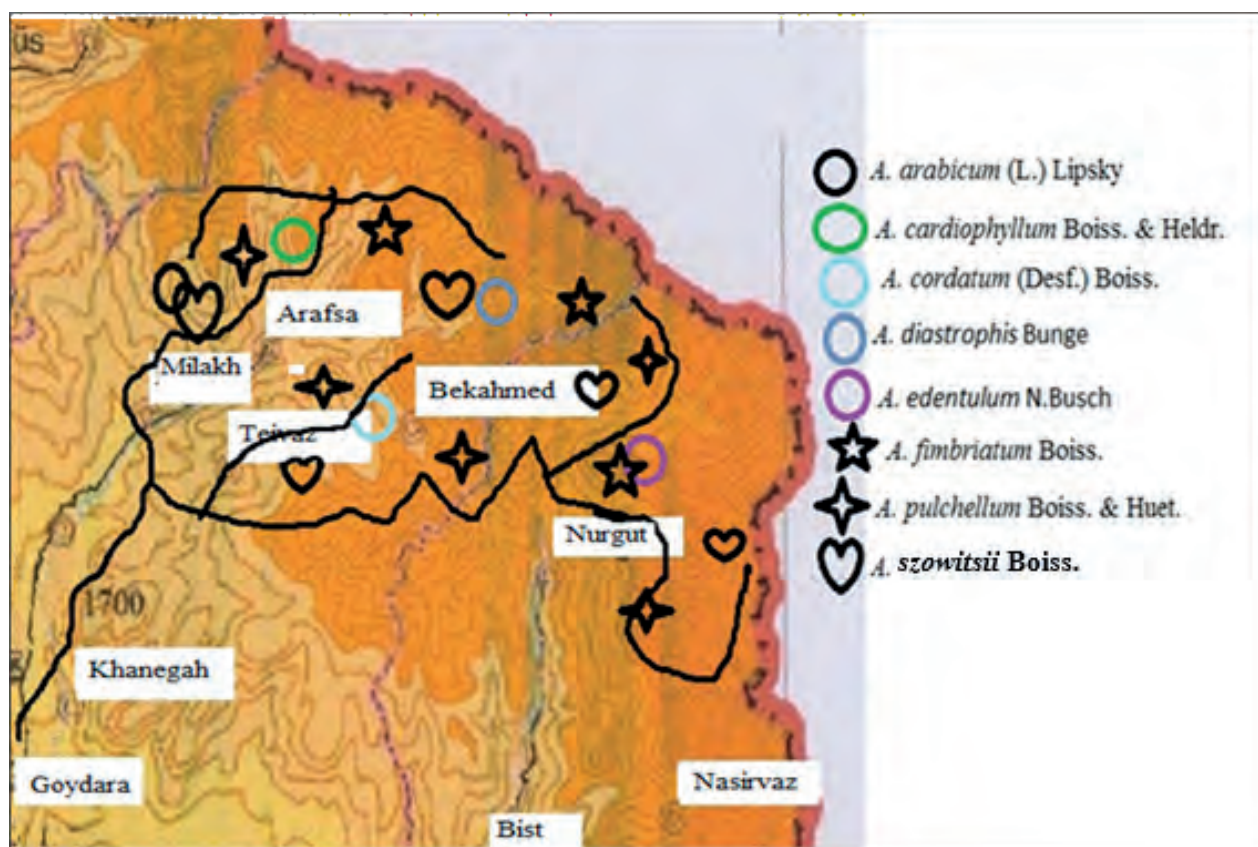


Figure 4. Expedition routes and new distribution zones of species of the genus *Aethionema* R. Br.

The *Aethionema pulchellum* Boiss. & Huet. formation, a diverse herbaceous community, includes species such as *Ranunculus arvensis* L., *Astragalus Kochianus* Sosn., *Crataegus pentagyna* Waldst. & Kit., *Pyrus salicifolia* Pall., *Hordeum bulbosum* L., *Papaver orientale* L., *Papaver fugax* Poir., *Grammosciadium platycarpum* Boiss. & Hausskn., *Hypericum formosissimum* Takht., *Equisetum arvense* L., *Allium atrovioleaceum* Boiss., *Artemisia vulgaris* L., *Trifolium alpestre* L., *Helichrysum callichrysum* DC., *Falcaria vulgaris*

Bernh. [*F. siosides* (Wib.) Aschers.], *Eryngium campestre* L., *Phlomis orientalis* Mill., *Prangos ferulacea* (L.) Lindl. (*P. Biebersteinii* Karjag.), *Euphorbia orientalis* L., *Stachys inflata* Benth., *Iris imbricata* Lindl. (*I. ulfurea* C. Koch), *Rosa canina* L., *Malus orientalis* Uglitzk., *Scorzonera latifolia* DC., *Vicia nissoliana* L., *Juniperus communis* L., *Centaurea Behen* L. [*Microlophus Behen* (L.) Takht.], and *Aethionema pulchellum* Boiss. & Huet. (Table 1).

Table 1. Analysis of the species composition of the genus *Aethionema* R. Br. in herbaceous-shrub formations.

№	Family	№	Genus	Species	Life form	Ecological group
1.	<i>Equisetaceae</i> Michx. ex DC.	1.	<i>Equisetum</i> L.	<i>Equisetum arvense</i> L.	Perennial	Mesophyte
2.	<i>Poaceae</i> Barnhart	2.	<i>Hordeum</i> L.	<i>Hordeum bulbosum</i> L.	Perennial	Xeromesophyte

№	Family	№	Genus	Species	Life form	Ecological group
3.	<i>Rosaceae</i> Juss.	3.	<i>Crataegus</i> L.	<i>Crataegus pentagyna</i> Waldst. & Kit.	Shrub	Mesoxerophyte
		4.	<i>Malus</i> Hill.	<i>Malus orientalis</i> Uglitzk.	Tree	Mesophyte
		5.	<i>Rosa</i> L.	<i>Rosa canina</i> L.	Shrub	Mesophyte
		6.	<i>Pyrus</i> L.	<i>Pyrus salicifolia</i> Pall.	Tree	Xerophyte
4.	<i>Brassicaceae</i> Burnett	7.	<i>Aethionema</i> R. Br.	<i>Aethionema pulchellum</i> Boiss. & Huet.	Semi-shrub	Xerophyte
5.	<i>Fabaceae</i> Lindl.	8.	<i>Astragalus</i> L.	<i>Astragalus kochianus</i> Sosn.	Perennial	Xerophyte
		9.	<i>Trifolium</i> L.	<i>Trifolium alpestre</i> L.	Perennial	Mesophyte
6.	<i>Papaveraceae</i> Juss.	10.	<i>Papaver</i> L.	<i>Papaver orientale</i> L.	Perennial	Xeromesophyte
		11.	<i>Papaver</i> L.	<i>Papaver fugax</i> Poir.	Biennial	Xerophyte
7.	<i>Hypericaceae</i> Juss.	12.	<i>Hypericum</i> L.	<i>Hypericum formosissimum</i> Takht.	Perennial	Xerophyte
8.	<i>Amaryllidaceae</i> J. St.-Hil.	13.	<i>Allium</i> L.	<i>Allium atroviolaceum</i> Boiss.	Perennial	Xerophyte
9.	<i>Asteraceae</i> Bercht. & J. Presl	14.	<i>Artemisia</i> L.	<i>Artemisia vulgaris</i> L.	Perennial	Mesoxerophyte
		15.	<i>Helichrysum</i> Mill.	<i>Helichrysum callichrysum</i> DC.	Perennial	Xerophyte
		16.	<i>Centaurea</i> L.	<i>Centaurea Behen</i> L.	Perennial	Mesophyte
		17.	<i>Scorzonera</i> L.	<i>Scorzonera latifolia</i> DC.	Perennial	Xerophyte
10.	<i>Apiaceae</i> Lindl.	18.	<i>Grammosciadium</i> DC.	<i>Grammosciadium platycarpum</i> Boiss. & Hausskn.	Perennial	Mesoxerophyte
		19.	<i>Falcaria</i> Fabr.	<i>Falcaria vulgaris</i> Bernh.	Biennial (sometimes perennial)	Mesophyte
		20.	<i>Eryngium</i> L.	<i>Eryngium campêtre</i> L.	Perennial	Xerophyte
		21.	<i>Prangos</i> Lindl.	<i>Prangos ferulacea</i> (L.) Lindl.	Perennial	Xeromesophyte

№	Family	№	Genus	Species	Life form	Ecological group
11.	<i>Lamiaceae</i> Martinov	22.	<i>Phlomis</i> L.	<i>Phlomis orientalis</i> Mill.	Perennial	Xerophyte
		23.	<i>Stachys</i> L.	<i>Stachys inflata</i> Benth.	Perennial	Xerophyte
12.	<i>Euphorbiaceae</i> Juss.	24.	<i>Euphorbia</i> L.	<i>Euphorbia orientalis</i> L.	Perennial	Xeromesophyte
13.	<i>Iridaceae</i> Juss.	25.	<i>Iris</i> L.	<i>Iris imbricata</i> Lindl.	Perennial	Xerophyte
24.	<i>Ranunculaceae</i> Juss.	26.	<i>Ranunculus</i> L.	<i>Ranunculus arvensis</i> L.	Annual	Mesophyte
15.	<i>Cupressaceae</i> Rich. ex Bartl.	27.	<i>Juniperus</i> L.	<i>Juniperus communis</i> L.	Shrub	Xerophyte
16.	<i>Fabaceae</i> Lindl. (Faboideae)	28.	<i>Vicia</i> L.	<i>Vicia nissoliana</i> L.	Perennial	Xerophyte

4. CONCLUSION

The elevation belts and coordinates of the study area were determined, and the genus *Aethionema* R. Br. was recorded during the expedition. The range and ecological environment of the species were clarified, and the species composition of the formation to which the plant belongs was studied.

The formation includes 28 species, among which the genus *Aethionema* R. Br. is widespread. In terms of ecological groups, 14 species (50%) are xerophytes, 7 species (25%) mesophytes, 3

species (10.71%) mesoxerophytes, and 4 species (14.28%) xeromesophytes. By life form, the formation comprises 18 perennials (64.28%), 2 biennials (7.14%), 1 annual (3.57%), 2 trees (7.14%), 3 shrubs (10.71%), and 2 semi-shrubs (7.14%). Within the *Rosaceae* Juss., *Apiaceae* Lindl., and *Asteraceae* Bercht., seasonal plants predominate. The dominant species of the phytocenosis is *Grammosciadium platycarpum* Boiss. & Hausskn., while the subdominant is *Centaurea Behen* L.

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ARTICLE 2

The Rare, Endemic and Threatened Vascular Flora of Algerian Wetlands: A Review

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ABSTRACT

This paper presents a review on wetland plants of Algeria, based on a compilation of the data published in the literature and our own field observations during the last 30 years. A total of 361 rare species of vascular plants, distributed in 204 genera and 73 families, were reported for Algerian wetlands, representing about 56% of the whole wetland flora. Out of 51 endemic (s.l.) species (14%), 11 are considered strictly endemic to Algeria. Amongst the rare species of Algerian wetland plants, 58 are threatened (16% of the 361 rare species) and 123 are nationwide protected species (34%). However, 128 rare wetland species (35.5%) are not yet assessed for their threat status, and 238 species (66%) are not yet legally protected in Algeria. In addition, 40 heritage species are the most at risk of extinction, since they have not been seen for at least the past three decades. Targeted field research for these missing species is more than desirable. This is the first comprehensive analysis of the rare flora of Algerian wetlands, for conservation purposes.

Keywords: *conservation status, distribution, endemics, North Africa, rarity, species diversity*

INTRODUCTION

Wetlands, which are among the world's most biodiverse ecosystems, are home to thousands of plant and animal species, including endemic, relict, and rare species (Tedoradze et al., 2023). Moreover, wetlands provide suitable habitats for numerous threatened plant species (Zhang et al., 2014), and their knowledge is essential for wetland conservation programs (Oliveira et al., 2019). Wetlands in Africa are increasingly being recognised as ecosystems of extreme importance to man and biodiversity (Howard & Matindi, 2003), and Algeria's wetlands are of huge importance (Stevenson et al., 1988). Whilst the international importance

of the Algerian wetlands has long been recognised for wintering and breeding waterfowl (Coulthard, 2001), their botanical importance has not received proper recognition (Stevenson et al., 1988), knowing that they contain diverse aquatic plants with many rare and endemic species (Boulaacheb et al., 2011).

Indeed, if the study of Algerian wetlands flora goes back to Gauthier-Lièvre (1931), afterward, they were very poorly studied until the 1990s, when the works by De Bélair and collaborators were published (Benhouhou et al., 2018). Then, during the last three decades until today numerous studies were done. We can mention for instance: De Bélair (1990), Samraoui et al.

(1992), Bensettiti (1992), Géhu et al. (1993, 1994), De Bélair & Samraoui (1994), Bensettiti & Lacoste (1999), Meddour & Laribi (1999), De Bélair (2005), Boulaacheb et al. (2007, 2011), Zouaidia et al. (2008), Belouahem-Abed et al. (2011), Bouldjedri et al. (2011), Laribi (2016), Hamel et al. (2017), Fetnaci et al. (2019), Necer et al. (2019), Miara et al. (2020).

All these works highlight the exceptional richness of the flora of Algerian wetlands and even more reveal the existence of many plant species rare and endemic, some of which are threatened. Despite this renewal, the knowledge on the plant diversity of wetlands in Algeria is far from to be comprehensive. On the other hand, in the North of Algeria, several wetlands have been subject to drying up since 1830, e.g., Ras-sauta marches near Algiers (MATE, 2005; DGF, 2016). This resulted in the partial or even total disappearance of a good number of plant species (see e.g., Faurel, 1959; Mathez et al., 1985; Quézel, 1998). Moreover, their fragile biodiversity remains under threat in the short term from rapidly growing anthropogenic pressures (Belouahem-Abed et al., 2011), and climate change that can significantly alters their nature and function and their floristic diversity (Bouldjedri et al., 2011).

In addition, no study has collated information on the geographic distribution, conservation status, and threats for rare, endemic and threatened wetland plant species in Algeria. If such a study already exists for neighbouring countries, such as Morocco (Hammada et al., 2004) and Tunisia (Ghrabi-Gammar et al., 2009), on the other hand for our country it is still lacking. Thus, we address this information gap by compiling data published in Botanical Works during the last 34 years of research, available to the authors at the present time, and our own field observations in the same time.

The aim of this contribution is accordingly to summarize current knowledge and to provide a

critical review on the taxonomy, diversity, distribution and conservation status of rare wetland plants across the national territory. Common ones were disregarded. Thereafter, we want to answer the following questions: (i) What is the number of rare species recorded for the Algerian wetlands? (ii) What is the number of rare species for each Algerian floristic region and what is the one with the highest number of species? (iii) What are their traits (chorological types, and endemism types)? (iv) Which species are considered threatened or legally protected? and (v) What are the species not observed since several decades (missing species)?

MATERIALS AND METHODS

Terms and definitions

The term “wetland” is used here in accordance with article 1 of Algerian law 11-02 of February 17, 2011, relating to “protected areas in the context of sustainable development”. Its definition is widely inspired by that of the Ramsar Convention (1971), approved by Algeria in 1982. The wetlands comprehend in Algeria different habitat types, of which the typology is based on that in use in the specialised works and international conventions: e.g., lakes, marshes, ponds, etc. Besides, in Maghreb countries, these habitats are recognised under various vernacular names (“oued”, “chott”, “guelta”, “daya”, “sabkha”, etc.) (Figure 1).

In this study, the term ‘wetland plants’ includes species of vascular plants that grow in water or in flooded soils, both obligatory and not obligatory (Zhou et al., 2022).

Data source and literature overview

This study is based upon a nation-wide scientific literature review and databases, which refer to wetland vascular taxa, i.e., species, subspecies and varieties. Hereafter, we will use the term “species” to mention species as well as infraspecific taxa. The “New Flora of Algeria” by Quézel & Santa (1962-1963) and its updated

online version (eflora Maghreb, 2026) were used as a primary data source for this analysis. We supplemented the data with other references, in particular classic Floras covering Algeria (Maire, 1952-1987; Ozenda, 2004). Wetland plant species reported in these Floras were identified with numerous terms (“wetlands”, “water edges”, “lakes”, “marshes”, “ponds”, “swamps”, “dayas”, “wet meadows”, “wet pastures”, etc.), as habitat types. We also used the herbarium of Gérard de Bélair (GDB, 2019), available online (<http://gdebelaire.com/>), and which is the only one to be devoted mostly to wetland flora of North-eastern Algeria. Scientific papers, and Ph.D. theses, published from the 1990s onwards, which contain lists of vascular plants, were compiled, as well as our own field experience over the last three decades. Only research specifically aimed at studying Algerian wetlands were included. We selected the relevant studies (around 45) in which (i) all recorded species were indicated as wetland plants by authors, or (ii) the environment was classified as wetland, such as Samraoui & De Bélair (1997), De Bélair & Samraoui (2000), De Bélair (2005), Kadid et al. (2007), Bouldjedri (2012), Laribi et al. (2016), Megharbi et al. (2016), Allem et al. (2017), Kadid (2019), Kadid & Djaaboub (2019), Bahi et al. (2020), Touarfia et al. (2021), Hammana et al. (2024). We filtered records of species and removed the plants not confined to wetlands. In case of unreliable occurrence in wetlands, the species was not considered. Also, alien or non-native species that were introduced to Algeria, were not considered for this analysis.

It was not in our intention to analyse all known wetland plants in Algeria, so the focus in the present study was on the rare wetland plants. The review is therefore restricted to rare plants, and provide a selective and non-exhaustive list of species. For this purpose, to make the selection of rare species in the global dataset created, we selected a subset and used two

combined criteria for selecting them, i.e., the rarity (rare vs common) and the distribution area (small vs large). Based on these criteria, and with no apparent bias in the selection of species, the data subset was finalised. A third criterion is that Obligate halophytes of continental salt lakes were excluded from the scope of this review, which focuses on freshwater and brackish wetland systems.

Data analysis

From a nomenclatural point of view, the online databases, “Plant of the World Online” (POWO, 2026), “African Plant Database” (APD, 2026) and “Euro+Med PlantBase” (Euro+Med PlantBase, 2026), were followed. In case of discrepancy between these databases, the authors used their own judgement. For example, depending on the taxonomic database used, *Juncus depauperatus* Ten., recognised as a separate species (APD, 2026), is included in *Juncus inflexus* L. subsp. *inflexus* (POWO, 2026), that we followed.

For the attribution of the species to the plant families, we followed the Angiosperm Phylogeny Group IV (APG, 2016), while for pteridophytes (ferns and lycophytes), we used PPG I (2016).

Worldwide distribution of species is essentially based on chorological types according to Pignatti (2005). Chorological types were grouped within the following main categories: Endemic, Mediterranean, Tropical, Northern and Pluriregional. The endemic and subendemic species have been checked according to Dobignard & Chatelain (2010-2013) and POWO (2026). Endemic species are attributed to those species that exclusively occur in Algeria, while subendemic species are species found in Algeria, as well as in the neighbouring countries (Morocco, Tunisia, etc.). We extended this category to the Maghrebian, North-African and Saharan endemics.

Abundance or rarity of the flora is classified

according to Quézel & Santa (1962-1963) into eight levels, ranging from “extremely rare” (RRR) to “extremely common” (CCC). Therefore, in our analysis of the wetland flora, we will retain only the species belonging to four levels of rarity: extremely rare “RRR”, very rare “RR”, rare “R” and quite rare “AR”. However, some species, although listed as common (s.l.), or with indeterminate abundance (R?), are also recorded as they are endemic, threatened, or have a restricted area of occurrence in Algeria, i.e., are present in less than half of the floristic regions (10 or less).

Data on species distribution, in the 20 floristic regions of Algeria (cf. Meddour et al., 2023), were collected from different ancient Floras (Quézel & Santa, 1962-1963; Maire, 1952-1987; Ozenda, 2004). Thus, our knowledge of the precise distribution of the flora is insufficient and outdated, and the areas of distribution of many species are imperfectly known. We completed the distributional data with important recent online resources for herbarium specimens, with their labels, i.e., GBIF (2026), and eflora Maghreb (2026).

Conservation status was provided according to the Red List of Threatened Species of IUCN (2026). All species that have had their extinction risk assessed at various geographic levels - global, continental (Pan-African) or regional (Mediterranean) - are cited in the IUCN global database (www.iucnredlist.org). As well, assessments using the IUCN criteria have been performed for 645 North African aquatic and wetland plants (García et al., 2010; Rhazi & Grillas, 2010).

Finally, the species under legal protection at national level (Executive Decree No. 12-03 of January 4, 2012, setting the “list of protected non-cultivated plant species”, published in the Official Journal of the Algerian Republic No. 03 of 18 January 2012 (OJAR, 2012), are indicated.

RESULTS

Taxonomic diversity

The selected subset of 361 native species of vascular plants encompasses a high proportion of rare species (55.8% of the whole dataset, ca 647 species). The plant list of rare wetland vascular flora includes 305 species, 48 subspecies, 7 varieties and one forma, belonging to 73 families and 204 genera (Supplementary material). The 73 families are shared between 65 Angiosperms and 8 Pteridophytes families. A photographic selection of some representative rare wetland species is depicted in Figure 2.

Rarity levels

Representation (%) of rarity levels within the rare flora of Algerian wetlands is as follow: AR (12.2%), R (28.6%), RR (41.1%) and RRR (2.6%). However, 18 species (5%), although listed as common or quite common, are also selected as they are endemic, threatened, or have a restricted area of occurrence in Algeria, i.e., which are present in 10 or less floristic regions. That is likewise the case of 38 species (10.5%) with indeterminate abundance (R?), but are most likely rare.

Chorological types and endemism

The chorological spectrum of the wetland plants selected is shown on the Table 1. Most of the flora belongs to the typically Mediterranean element (32%, 116 species), followed by the pluriregional element (19.4%, 70). This wetland flora is enriched with plant species of northern origin (17.2%, 62 species), but also of tropical origin (17.2%, 62 species).

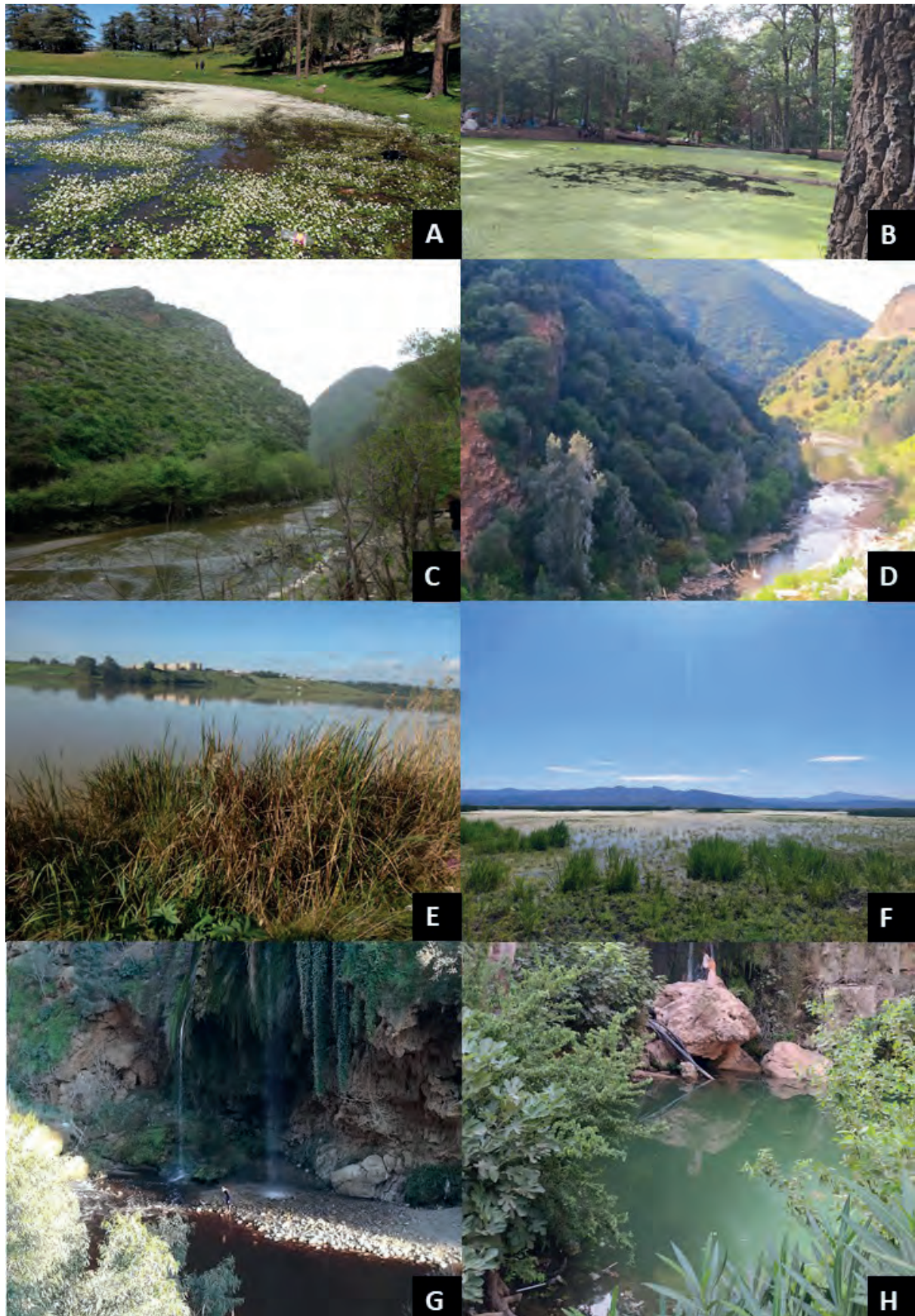


Figure 1. Some views of Algerian wetlands. A. Cedar Lake, Théniet El Had National Park (Tissemsilt), B. Green Lake or Aguelmim Mezzi, Yakouren Forest (Tizi Ouzou), C. Oued Dar El Oued (Jijel), D. Oued Sebaou (Tizi Ouzou); E. Réghaia Lake, Réghaia Nature Reserve (Algiers), F. Oubeira Lake, El Kala National Park (El Tarf), G. Ammal Gorges Waterfall (Boumerdès), H. El Ourit Waterfall (Tlemcen). Photos R. Meddour.



Figure 2. Some rare wetland plant species: A. *Agrostis gigantea*, water source at Tirourda (Djurdjura), August 02, 2012. B. *Cephalaria mauritanica*, water source at Tirourda (Djurdjura), August 02, 2012. C. *Conium maculatum*, Sébaou river (Tizi Ouzou), April 24, 2013. D. *Osmunda regalis*, alder grove, El Tarf, April 25, 2023. E. *Solenopsis bicolor*, alder grove, El Tarf, April 25, 2023. F. *Nymphaea alba*, Tonga Lake (El Kala), April 26, 2023. G. *Marsilea minuta*, Tonga Lake (El Kala), April 26, 2023. H. *Coriaria myrtifolia*, riparian forest, Azzefoun, February 14, 2012. I. *Rumex palustris*, water ditch, Tizi Ouzou, April 15, 2013. J. *Trapa natans*, Oubeira Lake (El Kala), April 26, 2023. K. *Nuphar lutea*, Lake near « Lac Noir », Berrihane, April 26, 2023. Photos R. Meddour.

Table 1. Number and proportion (%) of chorological types within the Algerian wetland flora

Chorological types	Number	%
Endemic		
Algerian Endemic	11	3.0
Algero-Moroccan Endemic	15	4.2
Algero-Tunisian Endemic	11	3.0
Algero-Chadian Endemic	1	0.3
Maghrebian Endemic	7	1.9
North African Endemic	1	0.3
Saharan Endemic	5	1.4
Subtotal	51	14.1
Mediterranean		
Ibero-Maghrebian	6	1.7
Mediterraneo-Montane	5	1.4
Stenomediterranean	29	8.0
Eurimediterranean	45	12.5
Eurimediterranean-Atlantic	17	4.7
Eurimediterranean-Turanian	6	1.7
Subatlantic-Mediterranean	8	2.2
Subtotal	116	32.1
Northern		
European	16	4.4
Europeo-Caucasian	8	2.2
Eurasiatic	29	8.0
Eurosiberian	9	2.5
Subtotal	62	17.2
Pluriregional		
Circumboreal	18	5.0
Cosmopolite	9	2.5
Subcosmopolite	20	5.5
Paleotemperate	23	6.4
Subtotal	70	19.4
Tropical		
Paleosubtropical	8	2.2
Subtropical	26	7.2
Paleotropical	12	3.3
Pantropical	10	2.8
Saharo-Arabian	6	1.7
Subtotal	62	17.2

The endemic species are represented by 51 species (14%), out of which, 11 are strictly endemic to Algeria, i.e., about 3% of all the flora considered, such as *Pulicaria filaginoides* Pomel,

Myosotis speciosa Pomel, *Legousia juliani* (Batt.) Briq., *Ononis megalostachys* Munby, *Rumex algeriensis* Barratte & Murb., and *Agropyropsis lolium* (Balansa ex Coss. & Durieu) A. Camus. The Algero-Tunisian and Moroccan-Algerian subendemics are also numerous (respectively 11 and 15), while only one species is subendemic to Algeria and Chad.

Distribution and numbers of species by floristic regions

On the distribution of the wetland flora, rare species are found within all the floristic regions of the country. However, their distribution is not random and even, i.e., not all areas are equally rich in wetland plant species. Highest numbers of rare species are found in the wetlands of the Numidian floristic region (K3, with 195 species), Algiers littoral (A1, 145 species), Small Kabylia (K2, 115 species), and Great Kabylia (K1, 108 species). Thus, the floristic regions of Numidia (K3) and Algiers littoral (A1) house around 54% and 40% of the rare wetland vascular flora respectively, and their wetlands are of great floristic

diversity. Conversely, we observed a scarcity in the numbers of rare wetland plants for the hyperarid (desertic) floristic regions, namely SS1 (11 species), SM (5), SO (3) and Hd (2) (Figure 3).

On the other hand, about the frequency in which a given species is recorded in all the floristic regions of Algeria, it is clear that some species are restricted to one floristic region, so-called “exclusive species” (148 species, 41%). One can noticed that a great number of these species (102), of special chorological interest, are exclusive to only three floristic regions, - K3 (55 species), A1 (27), and surprisingly SC (20) -, known to host important freshwater wetlands, whereas they are less frequent or absent within the others (e.g., H2, AS2, SM, Hd). Besides the 148 exclusive species, most of them are found in two to 10 floristic regions (210 shared species, 58%), while two common species are present in 10 or more, namely *Juncus bufonius* L., and *Nasturtium officinale* W.T. Aiton. These common plants are though of less conservation concern.

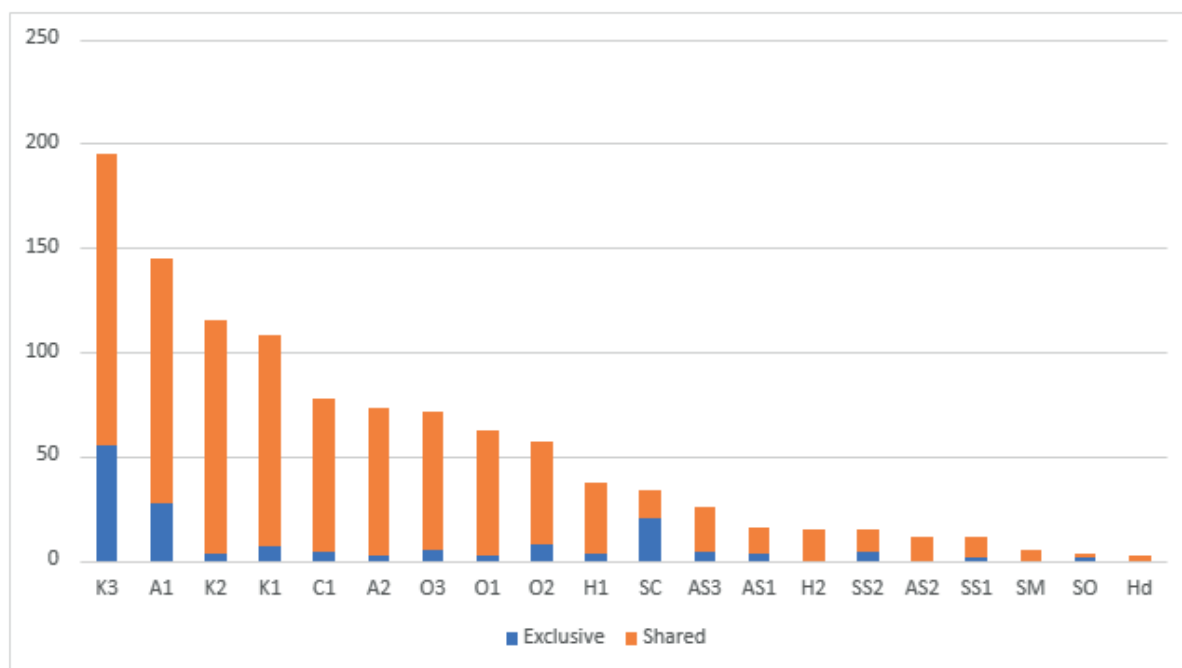


Figure 3. Distribution of Algerian wetland species (exclusive and shared) across floristic regions. Codes for floristic regions: O1: hills of Oran coast; O2: plains of Oran hinterland; O3: mountains of Tlemcen and other mountains of Oran; A1: hills and coast near Algiers; A2: mountains of Algiers Tell; K1: Great Kabylia; K2: Small Kabylia; K3: Numidia; C1: hills of Constantine Tell and mountains of Bibans/Hodna/Belezma axis; H1: Western high plains; H2: Eastern high plains; Hd: Hodna plain (North Saharan enclave); AS1: Western Saharan Atlas; AS2: Central Saharan Atlas; AS3: Aurès and Eastern Saharan Atlas; SS1: NW Sahara; SS2: NE Sahara; SO: Western Sahara; SC: Central Sahara including Ahaggar and Tassili n'Ajjer; SM: Southern Sahara.

Threatened plants on the IUCN Red List

Out of the 361 species of rare wetland plants of Algeria, 194 species were evaluated for their conservation status at the North African level according to the IUCN Criteria. To this figure must be added 39 other species assessed on Mediterranean, Pan-African or global scales. It is therefore for the rare species of the Algerian wetlands, 233 species (64.5% of the total) which are evaluated to date according to the criteria of the IUCN. Amongst them, of the 233 species assessed, 58 are threatened (14 CR, 13 EN or 31 VU) and 50 others are near threatened (NT)

(Table 2). In addition, 109 species are classified as Least Concern (LC), 15 as Data Deficient (DD), and 5 as Not Applicable (NA). Lastly, *Laurembergia repens* P.J. Bergius is considered as Regionally Extinct (RE) in North Africa. Of all species assessed, 26 are endemic or subendemic, including 12 Threatened and 10 Near Threatened species. Nevertheless, 128 rare wetland species (35.5%) are not yet assessed for their threat status at any geographic level according to IUCN.

Table 2. Number and proportion (%) of wetland plant species in each regional Red List Category in Algeria

Table 2. Number and proportion (%) of wetland plant species in each regional Red List Category in Algeria

IUCN Red List Category	Number of all species (%)	Number of endemic/subendemic species (%)
Regionally Extinct (RE)	1 (0.4%)	0 (0%)
Critically Endangered (CR)	14 (6.0%)	6 (23.0%)
Endangered (EN)	13 (5.6%)	1 (3.8%)
Vulnerable (VU)	31 (13.3%)	5 (19.2%)
Near Threatened (NT)	50 (21.5%)	10 (38.5%)
Least Concern (LC)	109 (46.8%)	2 (7.7%)
Data Deficient (DD)	15 (6.4%)	2 (7.7%)
Total number of assessed species	233 (100%)	26 (100%)

National legal protection

In Algeria, a certain conservation status is ensured by designating some species as legally protected plants. Overall, the law protects 123 rare wetland species (34% of the total), which are included in the “List of non-cultivated plant species protected throughout Algeria” since 2012. The remaining 238 species (66%) are not yet legally protected in Algeria. In particular, 52 heritage species, i.e., endemic/subendemic and/or threatened species, are unfortunately in this case.

DISCUSSION

Taxonomic diversity and richness

The Algerian wetland flora, estimated at 361 rare species, belongs to 204 genera and 73 families. More than 50% of aquatic plant species are considered rare to extremely rare at the national level (MATE, 2005). According to our results, rare plant species within the Algerian wetlands,

with 361 species out of 647, represent actually a higher proportion (ca 56%). In comparison, the wetland flora is characterised by a lesser proportion of rare and/or threatened species in Tunisia (29%, according to Ghrabi-Gammar et al., 2009) and Morocco (34%, Hammada et al., 2004). The richness and diversity of rare species in Algerian wetlands has already been pointed out by Faurel (1959). Wetlands accordingly constitute excellent refuges for rare species. The wetlands make a significant contribution to the diversity of the North African flora and constitute a refuge for unusual plant species, the presence of aquatic environments compensating for the aridity of the climate (Rhazi & Grillas, 2010).

Chorology, a pool of species with diverse origins

The Mediterranean element constitutes the backbone of the wetland flora of Algeria (almost a third), as in all the North African region

(Quézel, 1978). Besides, a characteristic of wetlands, crossing different climatic zones (Mediterranean/Steppic/Saharan), is that many species are of wide distribution (or pluriregional) (Zhang et al., 2014). Indeed, widespread species are a significant group in Algerian wetland habitats, where long-distance dispersed species are common due to the presence of active dispersals like migrant birds (Fraga i Arguimbau, 2008). For instance, among the Cyperaceae, many species widely distributed present in Mediterranean Africa, can be linked to transport by migrant birds, such as *Cyperus* spp. (Quézel, 1978).

In North Africa, an appreciable number of rare species, essentially northern (temperate), are linked to wetlands (Quézel, 1998; Ghraïbi-Gammar et al., 2009; Belouahem-Abed et al., 2011). Effectively, the chorological spectrum is characterised by the presence of many plants belonging to the northern origin (17%), especially associated to wetlands, lakes and marshes of the ecoregion of Numidia, which have served as a refuge for European plants (Meddour, 2012). Thus, the presence in wetlands of species of northern origin, pushed back towards the southern latitudes during the last glacial period, constitutes the originality of these wet environments (Quézel, 2000; Rhazi & Grillas, 2010). Such species, which have persisted beyond the last glacial period, exemplify the Tertiary and Pleistocene connexions between Southern Europe and Northern Africa, through Siculo-Tunisian and Ibero-Moroccan bridges (Braun-Blanquet, 1953), and maybe also reveal modern connexions along water-bird migration routes (Daoud-Bouattour et al., 2009). On the other hand, the great diversity of wet habitats and the humid climate have enabled the maintenance and diversification of several species of tropical origin in Algerian wetlands, which are very localised in the form of biogeographical isolates (Grillas et al., 2004), mainly in El Kala region and also in the gueltas of Central Sahara. In fact, several species of tropical origin are reported as exclusive to the El Kala area considered “a subtropical enclave” (De Bélair & Samraoui, 2000). Therefore, in Algeria, the marked interpenetration between the northern and tropical species is confirmed here for the wetlands of the coastal

subsector of Numidia (De Bélair, 2005).

The level of strict endemism in wetland plants is much lower (3%) than that existing in the overall Algerian flora (6.3%) (cf. Dobignard & Chatelain, 2010-2013; Meddour et al., 2023). A low level of strict endemism is predictable in wetland plants because of the connectivity between sites and populations (Rhazi & Grillas, 2010). Nevertheless, the number of endemics and subendemics is higher accounting for 14%. The same proportion is found for the endemic wetland flora of Northern Africa (Rhazi & Grillas, 2010). Finally, this chorological melting pot is explained by the astonishing geomorphological, edaphic, hydrological and bioclimatic diversity of Algerian wetlands.

Distribution in Algerian floristic regions and exclusive species

Previous investigations underlined the exceptional richness of the wetlands of Kabylia, Numidia and Kroumiria (Bouldjedri, 2011), where the most important freshwater wetlands are mainly concentrated (Fetnaci et al., 2019). Indeed, highest numbers of rare species are found in the wetlands of the Kabylia-Numidian floristic region (K1-2-3), but also in Algiers littoral (A1). Therefore, we confirmed that the wetland flora in North-eastern Algeria (Kabylia-Numidian sector) exhibits high species richness, a fact easily explained by the belonging of this area to the “Kabylia-Numidia-Kroumiria” regional hotspot (cf. Véla & Benhouhou, 2007), with the presence in this hotspot of 14 “key biodiversity areas” for plants (Benhouhou et al., 2018). Besides, we highlighted that many species are exclusive to three floristic regions, namely K3 (55 species), A1 (27) and SC (20), where they find their only locations in Algeria. We can mention, for example (see eflora Maghreb, 2026), *Najas pectinata* (Parl.) Magnus, *Serratula tinctoria* L., and *Woodwardia radicans* (L.) Sm., for Numidia region; *Anacamptis laxiflora* (Lam.) R.M.Bateman, *Pridgeon* & M.W.Chase, and *Potamogeton coloratus* Hornem.; for Algiers area; *Hypericum psilophyllum* (Diels) Maire and *Potamogeton hoggarensis* Dandy, for the gueltas of Central Sahara.

We confirm therefore that the Numidian subsector (K3), characterised by the highest

number of rare and exclusive plant species, with many relict species, in wetlands is an important and exceptional centre of plant diversity (De Bélair 2005), within the North African region. More generally, all the wetlands of this regional hotspot, along with those of the Algiers littoral region, and their unique flora, deserve a high conservation priority.

Conservation status and nation-wide legal protection

In the Mediterranean Basin Biodiversity Hotspot and in North African sub-region, among the groups of plants, it is certainly those of the freshwater plants, which are most evaluated by the IUCN (García et al., 2010). Overall, 233 rare wetland species (64.5% of the total) from Algeria are evaluated to date according to the criteria of the IUCN (cf. Rhazi & Grillas, 2010). This number is important to stress, since the IUCN global Red List contains only 345 plant species existing in Algeria, or 7.8% of the total flora of Algeria (Benhouhou et al., 2018). These assessments show above all that 25% of the assessed species are threatened (CR, EN or VU) at national level, and 21.5% others are near threatened (NT), with a real risk of falling into the threatened categories. These figures are alarming when we know that 20% of all species are threatened

with extinction in the Mediterranean region (IUCN, 2026). We already know that one species is regionally extinct (RE), *Laurembergia repens* P.J.Bergius (IUCN, 2026), and *Hydrocotyle vulgaris* L., *Parnassia palustris* L., are likewise extinct in Algeria according to POWO (2026).

However, 128 rare wetland species (35.5%) are not yet assessed at any level by IUCN for their threat status. In particular, we have identified 25 endemic and subendemic species (of the 51 recorded), of conservation interest, that are not yet assessed (Table 3). All these species and subspecies are poorly known in terms of distribution, ecology and population dynamics, making it difficult to assess their conservation status. Anyway, the assessment of the threat status must be applied as a priority on these endemic/subendemic species, sometimes very localised in Algeria, which might already be threatened, e.g., *Centaurea ropalon* Pomel, *Legousia juliani* (Batt.) Briq., etc. (see Faurel, 1959).

Table 3. Heritage wetland species that are yet not evaluated (n.e.) by the IUCN and/or not yet legally protected by national legislation (NP). P: protected by decree. Conservation status is according to IUCN threat categories (North African scale, after Rhazi & Grillas, 2010). The species preceded by an asterisk (*) are endemic or subendemic.

Table 3. Heritage wetland species that are yet not evaluated (n.e.) by the IUCN and/or not yet legally protected by national legislation (NP). P: protected by decree. Conservation status is according to IUCN threat categories (North African scale, after Rhazi & Grillas, 2010). The species preceded by an asterisk (*) are endemic or subendemic.

Heritage species	Conservation Status	National protection
<i>Agrostis tenerrima</i> Trin.	VU	NP
<i>Alopecurus aequalis</i> Sobol.	VU	NP
<i>Althenia orientalis</i> subsp. <i>betpakdalensis</i> (Tzvelev) García-Mur. & Talavera	VU	NP
<i>Carex acutiformis</i> Ehrh.	VU	NP
<i>Carex riparia</i> Curtis	VU	NP
<i>Chrysosplenium dubium</i> J.Gay ex Ser.	VU	NP
(*) <i>Dichoropetalum munbyi</i> (Boiss.) Pimenov & Kljuykov	NT	NP
<i>Frangula alnus</i> Mill. subsp. <i>alnus</i>	VU	NP
<i>Helosciadium inundatum</i> (L.) W.D.J.Koch	VU	NP
(*) <i>Hypericum afrum</i> Lam.	NT	NP

Heritage species	Conservation Status	National protection
<i>Ipomoea sagittata</i> Poir.	EN	NP
(*) <i>Jacobaea gigantea</i> (Desf.) Pelser	NT	NP
<i>Juncus bufonius</i> L.	EN	NP
<i>Juncus sorrentinoi</i> Parl.	EN	NP
<i>Juncus subnodulosus</i> Schrank	VU	NP
<i>Lemna trisulca</i> L.	VU	NP
(*) <i>Lepidium violaceum</i> (Munby) Al-Shehbaz	VU	NP
(*) <i>Limonium durieui</i> (Girard) Kuntze	VU	NP
(*) <i>Linaria fallax</i> Coss. ex Batt.	NT	NP
<i>Marsilea strigosa</i> Willd.	EN	NP
<i>Molinia caerulea</i> (L.) Moench	VU	NP
<i>Nymphoides peltata</i> (S.G.Gmel.) Kuntze	CR	NP
<i>Persicaria amphibia</i> (L.) Delarbre	VU	NP
<i>Pilularia minuta</i> Durieu	CR	NP
<i>Puccinellia convoluta</i> (Hornem.) Fourr.	VU	NP
<i>Puccinellia festuciformis</i> (Host) Parl.	VU	NP
<i>Ranunculus lateriflorus</i> DC.	VU	NP
<i>Ranunculus tripartitus</i> DC.	VU	NP
(*) <i>Serapias lingua</i> subsp. <i>stenopetala</i> (Maire & T.Stephenson) Maire & Weiller	CR	NP
(*) <i>Sisymbrella aspera</i> subsp. <i>munbyana</i> (Boiss. & Reut.) Greuter & Burdet	VU	NP
(*) <i>Sonchus mauritanicus</i> Boiss. & Reut.	NT	NP
<i>Thelypteris interrupta</i> (Willd.) K.Iwats.	EN	NP
<i>Thelypteris palustris</i> Schott	VU	NP
<i>Woodwardia radicans</i> (L.) Sm.	EN	NP
(*) <i>Borago longifolia</i> Poir.	n.e.	NP
(*) <i>Campanula bordesiana</i> Maire	n.e.	NP
(*) <i>Carex distans</i> subsp. <i>oranensis</i> (Trab.) Jahand. & Maire	n.e.	NP
(*) <i>Cephalaria mauritanica</i> Pomel	n.e.	NP
(*) <i>Cerastium atlanticum</i> Durieu	n.e.	NP
(*) <i>Daucus virgatus</i> (Poir.) Maire	n.e.	NP
(*) <i>Erodium garamantum</i> (Maire) Guitt.	n.e.	NP
(*) <i>Heracleum sphondylium</i> subsp. <i>algeriense</i> (Coss. ex Batt. & Trab.) Dobignard	n.e.	NP
(*) <i>Heracleum sphondylium</i> subsp. <i>aurasiacum</i> (Maire) Dobignard	n.e.	NP
(*) <i>Hypericum naudinianum</i> Coss. & Durieu	n.e.	NP
(*) <i>Hypericum psilophyllum</i> (Diels) Maire	n.e.	NP
(*) <i>Isoetes perralderiana</i> Durieu & Letourn. ex Milde	n.e.	NP
(*) <i>Lepidium hirtum</i> subsp. <i>dhayense</i> (Munby) Thell.	n.e.	NP
(*) <i>Lolium mairei</i> (St.-Yves) Banfi, Galasso, Foggi, Kopecký & Ardenghi	n.e.	NP

Heritage species	Conservation Status	National protection
(*) <i>Myosotis speciosa</i> Pomel	n.e.	NP
(*) <i>Pulicaria clausonis</i> Pomel	n.e.	NP
(*) <i>Saharanthus ifniensis</i> (Caball.) M.B.Crespo & Lledó	n.e.	NP
(*) <i>Silene lynesii</i> C.Norman	n.e.	NP
(*) <i>Agropyropsis lolium</i> (Balansa ex Coss. & Durieu) A. Camus	n.e.	P
(*) <i>Centaurea ropalon</i> Pomel	n.e.	P
(*) <i>Legousia juliani</i> (Batt.) Briq.	n.e.	P
(*) <i>Linaria peltieri</i> Batt.	n.e.	P
(*) <i>Ononis megalostachys</i> Munby	n.e.	P
(*) <i>Primula vulgaris</i> subsp. <i>atlantica</i> (Maire & Wilczek) Greuter & Burdet	n.e.	P
(*) <i>Pulicaria vulgaris</i> subsp. <i>pomeliana</i> (Faure & Maire) E. Gamal-Eldin	n.e.	P

On the other hand, if 123 wetland species are protected by national legislation, the remaining 238 species are not yet legally protected in Algeria. Among them, we highlight 52 heritage species (i.e., endemics/subendemics and/or threatened and near threatened plants) that are not so far under law protection (Table 3). We recommend inclusion of all the 27 endemic/subendemic species as well as all the 34 threatened and near threatened ones, in the national list of protected plants. They deserve conservation measures in priority and the status of legally protected species, because they are in danger of extinction due to disturbances to wetlands.

Rare species no longer observed or missing species

Highlighting should be made of many wetland species indicated formerly in ancient Floras covering Algeria, which have not been observed again for a long time in the field, i.e., at least since the past three decades or more. Among the missing species, 40 noteworthy heritage species are the most at risk of extinction (Table 4), of which one species is already declared regionally extinct in North Africa (see above).

Table 4. Noteworthy species not observed for the past 30 years or more. The species preceded by an asterisk (*) are endemic or subendemic. Conservation status at North African scale (after Rhazi & Grillas, 2010). n.e.: not yet evaluated by IUCN (2026).

Table 4. Noteworthy species not observed for the past 30 years or more. The species preceded by an asterisk (*) are endemic or subendemic. Conservation status at North African scale (after Rhazi & Grillas, 2010). n.e.: not yet evaluated by IUCN (2026).

Taxa	Conservation status	Observations
(*) <i>Agropyropsis lolium</i> (Balansa ex Coss. & Durieu) A. Camus	n.e.	Has still not been found again (Mathez et al., 1985), until now
<i>Airopsis tenella</i> (Cav.) Coss. & Dur.	LC	Not seen again in Kabylia (Laribi et al., 2016)
<i>Althenia orientalis</i> subsp. <i>betpakdalensis</i> (Tzvelev) García-Mur. & Talavera	VU	
<i>Anacamptis laxiflora</i> (Lam.) R.M.Bateman, Pridgeon & M.W.Chase,	DD	Has not been recorded since 1976, and has probably gone extinct (IUCN, 2026)

Taxa	Conservation status	Observations
<i>Antinoria agrostidea</i> (DC.) Parl. subsp. <i>agrostidea</i>	LC	Not seen again at Tiaret (Laribi et al., 2016)
<i>Callitriche lusitanica</i> Schotsman	DD	
<i>Carex riparia</i> Curtis	VU	
<i>Catabrosa aquatica</i> (L.) P.Beauv.	VU	
<i>Chrysosplenium dubium</i> J.Gay ex Ser.	VU	Threatened by the destruction of the alder grove in Collo (Faurel, 1959)
<i>Cullen americanum</i> (L.) Rydb.	NT	
<i>Eleocharis caduca</i> (Delile) Schult.	DD	
(*) <i>Heracleum sphondylium</i> subsp. <i>aurasiacum</i> (Maire) Dobignard	n.e.	
<i>Hydrocotyle vulgaris</i> L.	LC	Presumably extinct in Algeria (El Kala) (IUCN, 2026; POWO, 2026)
<i>Juncus sorrentinoi</i> Parl.	EN	
<i>Laurembergia repens</i> P.J.Bergius	RE	Extinct in Algeria (Rhazi & Grillas, 2010; IUCN, 2026)
(*) <i>Legousia juliani</i> (Batt.) Briq.	n.e.	Its location is destroyed (Mathez et al., 1985)
<i>Lemna trisulca</i> L.	VU	In El Kala (Lake Tonga), has not been seen again (IUCN, 2026)
(*) <i>Lepidium violaceum</i> (Munby) Al-Shehbaz	VU	
<i>Lysimachia vulgaris</i> L.	CR	
<i>Marsilea strigosa</i> Willd.	EN	Not been seen again in Oranie (Laribi et al., 2016; Miara et al., 2020); its sites are completely destroyed around Algiers (IUCN, 2026)
<i>Melilotus elegans</i> Salzm. ex Ser.	n.e.	Not been found in El Kala (Hamel et al., 2021)
<i>Mentha cervina</i> L.	CR	
<i>Najas graminea</i> Delile var. <i>graminea</i>	DD	Not been found in Lakes Tonga and Oubeira (DGF, 2016)
(*) <i>Najas marina</i> subsp. <i>arsenariensis</i> (Maire) L.Triest	CR	Not been found in its lone locality, Lac Mellah by Mansouri et al. (2018)
<i>Najas marina</i> subsp. <i>intermedia</i> (Wolfg. ex Gorski) Casper	n.e.	Not been found in Lakes Tonga and Oubeira (DGF, 2016)
<i>Najas minor</i> All.	LC	
<i>Najas pectinata</i> (Parl.) Magnus	VU	
(*) <i>Ononis megalostachys</i> Munby	n.e.	
<i>Parnassia palustris</i> L.	LC	Presumably extinct in Algeria (IUCN, 2026; POWO, 2026)
<i>Potamogeton coloratus</i> Hornem.	NA	Its locations near to Algiers are probably destroyed (IUCN, 2026)

Taxa	Conservation status	Observations
(*) <i>Potamogeton hoggarensis</i> Dandy	DD	Known only in the sterile state, and is perhaps a variety of <i>P. berchtoldii</i> (Ozenda, 2004)
<i>Puccinellia convoluta</i> (Hornem.) Fourr.	VU	Not been found at La Macta (Miara et al., 2020)
<i>Puccinellia festuciformis</i> (Host) Parl.	VU	
(*) <i>Pulicaria clausonis</i> Pomel	n.e.	
(*) <i>Pulicaria filaginoides</i> Pomel	CR	Not been found by Mansouri et al. (2018)
<i>Ranunculus batrachioides</i> Pomel	NT	Not been found in Tiaret (Laribi et al., 2016)
<i>Serratula tinctoria</i> L.	n.e.	Not been observed in El Kala (Hamel et al., 2021)
<i>Spiranthes aestivalis</i> (Poir.) Rich.	n.e.	Not been found again by Martin et al. (2020)
<i>Vallisneria spiralis</i> L.	CR	Has not been seen in Algeria for many years (IUCN, 2026)
<i>Woodwardia radicans</i> (L.) Sm.	EN	Has not been seen again in its lone locality, djebel Edough (Hamel et al., 2017; IUCN, 2026)

This for at least two main reasons: many wetlands have not been investigated, and above all many others have been completely destroyed during the last century. Indeed, the North African ecoregion has experienced the drying up of about fifty lakes, marshes and other wetlands (Alaoui & Himyari, 2006), especially in the permanent aquatic environments of the Mitidja-Algiers region and the Annaba-El Kala region, which are those most disturbed by human activity (Faurel, 1959; Mathez et al., 1985; MATE, 2005), and to a lesser extent in those of the Oranie. It seems essential to us that special attention be given to these missing species, with targeted active research in the field, given the age of the data and the loss of many wetlands.

CONCLUSION

The wetland flora of Algeria is still insufficiently documented. To our knowledge, this work is the first study that focuses on the

diversity of wetland plants overall the territory of Algeria, and our results revealed the distribution patterns, the taxonomic status, the conservation status, the main traits of wetland plants across the wetlands of Algeria. We provided a baseline for future research, and highlighted the need for monitoring of wetland species. Conservation priorities must first be exercised on the endemic plants and/or very localised threatened species and on the hotspot sites. We hope that, in this paper, we contributed significantly to the knowledge of rare wetland species of Algeria, improving the data basis and providing substantial background for planning further conservation strategies.

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ARTICLE 3

Azerbaijani Wild Blackthorn (*Prunus spinosa* L.): Phenological and Morphological CharacteristicsShahmar Hajiyev¹  | <https://orcid.org/0009-0003-5851-6763>Sabina Hajiyeva²  | <https://orcid.org/0009-0006-4481-1915>Vahid Guvendiyev³  | <https://orcid.org/0009-0000-7536-3533>Elchin Hajiyev^{4*}  | <https://orcid.org/0009-0008-6131-7163>¹ Azerbaijan State Agricultural University, Department of General Agriculture, Genetics and Breeding, Ganja, Azerbaijan² Genetic Resources Institute, Department of Molecular Genetics and Genomics, Baku, Azerbaijan³ Genetic Resources Institute, Ecobotany and Systematics Department, Baku, Azerbaijan⁴ Genetic Resources Institute, Department of Immunogenetics, Baku, Azerbaijan

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ABSTRACT

The present research investigates *Prunus spinosa* L., which is widely distributed in Azerbaijan, to evaluate its genetic resources, identify promising genetic material based on phenological and morphological diversity, and enhance scientific knowledge of wild flora biodiversity. A total of 100 distinct genotypes collected in situ from various regions of Azerbaijan were comprehensively analyzed at the Department of General Agriculture, Genetics and Selection of the Azerbaijan State Agrarian University. In phenological studies, the flowering period occurred from 25 March to 20 April, lasting 10–15 days. Leaf formation began in early April (5 April) and was completed by early May and fruit formation from late May to late June. The duration of fruit ripening extended from late July to late September, depending on genotype and environmental conditions. Descriptive statistics, analysis of variance (ANOVA), principal component analysis (PCA), correlation and cluster analysis were applied to assess morphological and pomological traits. Greater variability was observed in vegetative traits than in generative traits, while shape indices of leaves, fruits, and stones remained highly stable. According to the PCA, 91.31% of the total variation was captured by the first three components, with fruit- and stone-related traits making the strongest contribution to intraspecific diversity. The results of ANOVA confirmed the presence of highly significant differences ($p < 0.001$) among genotypes for all traits examined. Cluster analysis separated the genotypes into five major clusters, mainly based on Stem diameter and fruit size, while organ shape showed little variation among clusters. Significant genetic and morphological diversity was confirmed in *Prunus spinosa* L., highlighting its potential as a rich genetic resource for future breeding programs and the development of locally adapted, productive varieties.

Keywords: *Prunus spinosa* L., genetic resources, phenotypic variation, cluster

INTRODUCTION

Genetic resources furnish humans with diverse products, and the global economy, livelihoods, and individual health substantially depend on these resources [Hajiyeva et al., 2024; Guvendiyev et al. 2025a]. The Rosaceae family has 20–40 plum species, several of which intergrade in the wild [Hajiyev et al., 2025a]. Morphological traits can be used to identify genetic differences between and within plant species [(Nikolić et al., 2007]. Some phenotypic variables can be influenced by environmental factors [Tucić et al., 2018]. To prevent genetic

erosion, research, collection, conservation, and sustainable use of wild plant genetic resources are needed, both as selection material and for low-impact fruit production [Shashidhara et al., 2023]. Natural populations of this species are extremely diverse in growth strength, temperature tolerance, ripening time, and resistance to diseases and pests [Petrov et al., 2024]. It grows well on gravelly, sandy, calcareous, and nutrient-poor soils, but it does not do well on dense soils [Dunaevskaya et al., 2021]. Due to these properties, it is often used as a rootstock for commercially important *Prunus* species such

as almonds, apricots, plums, and peaches [Das et al., 2011]. In addition, this species has high potential for carbon sequestration and the accumulation of heavy metals [Petrov et al., 2024]. Morphological assessments of wild blackthorn have been conducted in several studies over recent decades [Vidaković et al., 2024; Vander et al., 2016]. The aim of these studies was to collect important information for the conservation and use of this species.

Azerbaijan's flora is abundant in diverse useful plants, with wild fruit and berry species being of particular interest due to the presence of numerous biologically active compounds in their organs, especially in the fruits [Ibadullayeva and Husneynova, 2021]. *Prunus spinosa* is native to Europe and Western Asia, occurring in countries such as Turkey, Iran, the Caucasus region, and, to a lesser extent, northwest Africa. The species displays a dwarf growth habit and demonstrates resistance to pests, diseases, and abiotic stresses in the field. Its fruit is a 10–12 mm diameter drupe, black with a pale purple-blue waxy coating, ripening in autumn, with thin flesh and a markedly astringent taste when fresh [Kuru Berk et al., 2020]. Azerbaijan, in the South Caucasus region, harbors a flora of remarkable biodiversity, consisting of roughly 4,500 vascular plant species, several of which are endemic or of economic significance [Bayramova 2014]. Particularly important ecological and ethnobotanical roles are played by wild fruit-bearing species. *Prunus spinosa* L., popularly known as blackthorn, is a shrub that is found throughout Azerbaijan, mostly in scrublands, along river valleys, and along forest edges [Ibadullayeva et al., 2023]. Blackthorn fruits are rich in nutrients, vitamins, minerals, dietary fibre, phytochemicals, and particularly antioxidant components. They are also rich in anthocyanins. This species' fruit has both culinary and therapeutic uses. As a result, it has been used for centuries for both nutritional and therapeutic purposes, and it contains substances that are good for human health [Ayla et al., 2017; Karakash et al., 2019].

Because of its anti-inflammatory properties, several plant parts of the species, such as fruits, leaves, and flowers, have been used to treat mucosal sores of the mouth and throat.

Plant extracts from *Prunus spinosa* have long been used as diuretics, to treat gastrointestinal issues, diabetes, and hypertension, as well as to control menstruation [Karakash et al., 2019].

The ecology, floristic distribution, morphological and anatomical characteristics, phytochemical composition, and possible applications of *Prunus spinosa* have been the main areas of study in Azerbaijan [Shiraliyeva et al., 2023; Mailova 2016; Ibadullayeva et al. 2023; Salmanova 2024]. Nevertheless, the majority of current research has been restricted to particular areas, and there is still a dearth of thorough information on the morphological and phenological characteristics of *Prunus spinosa* populations throughout the nation. The current work methodically examines the morphological and phenological traits of *Prunus spinosa* populations spread over Azerbaijan in order to close this information gap.

The main goal of this investigation is to re-search the genetic resources of the widespread *Prunus spinosa* L. species in Azerbaijan, to identify potential genetic resources by evaluating their phenological and morphological diversity and to contribute to scientific understanding of the richness of Azerbaijan's wild flora.

MATERIALS AND METHODS

Plant material

Plant material for this research was collected and evaluated at the Department of General Agriculture, Genetics and Selection of Azerbaijan State Agrarian University, based on 100 in situ-distributed genotypes of *Prunus spinosa* L. gathered from various regions of Azerbaijan. The areas where the evaluated genotypes were collected are shown in Table 1. The fruits were collected at full ripeness, at intervals of 1 to 2 weeks, from July to September. It was not possible to collect all the investigated genotypes simultaneously due to their belonging to different regions and having significant differences in the timing of development and maturation. Phenological observations began from the moment of shoot swelling in pre-determined genotypes. To evaluate the morphological characteristics, 10 fruits were collected from each tree.

Table 1. Populations where *Prunus spinosa* L. genotypes were collected

Code	Population	Code	Population	Code	Population	Code	Population
PS1	Khachmaz	PS26	Ismayilli	PS51	Masalli	PS76	Dashkasan
PS2		PS27		PS52		PS77	
PS3		PS28		PS53		PS78	Goygol
PS4		PS29	Gobustan	PS54	Qakh	PS79	
PS5		PS30		PS55		PS80	
PS6	Quba	PS31		PS56		PS81	
PS7		PS32		PS57		PS82	
PS8		PS33		PS58	Oghuz	PS83	Fuzuli
PS9		PS34	Shamakhi	PS59		PS84	
PS10	Qusar	PS35		PS60		PS85	
PS11		PS36		PS61		PS86	
PS12		PS37		PS62		PS87	
PS13		PS38	Astara	PS63	Sheki	PS88	Khojavend
PS14		PS39		PS64		PS89	
PS15	Shabran	PS40		PS65		PS90	
PS16		PS41	Lerik	PS66		PS91	
PS17		PS42		PS67	Zaqatala	PS92	Shusha
PS18		PS43		PS68		PS93	
PS19	Agsu	PS44	Lankaran	PS69		PS94	
PS20		PS45		PS70		PS95	
PS21		PS46		PS71		PS96	
PS22		PS47		PS72		PS97	Tartar
PS23		PS48		PS73		PS98	
PS24	Ismayilli	PS49		PS74		PS99	
PS25		PS50		PS75		PS100	

Phenological analysis

As part of the research, phenological observations were carried out on the phases of flowering, leaf fall, fruit formation and the ripening of tree fruits. The flowering stage was determined by the number of days from bud opening to petal shedding [Sadigov, 2023]. The period of leaf formation was defined as the period from the day the leaf buds began to open until the day the fully formed leaves appeared. The time of fruit formation was calculated based on the time from the fall of flower petals to the appearance of the first fruits. The fruit maturation period was observed and recorded from fruit emergence to full maturity [Tzoner & Yamaguchi, 1999].

Morphological analysis

A total of 23 morphological characters related to tree, flower, leaf and fruit were assessed in the investigation [Sadigov, 2023]. These assessments were researched in 10 samples for each of the quantitative characteristics and a result was obtained. Quality characteristics were given separately. The quantitative traits evaluated included Stem diameter (TD), plant height (PH), canopy diameter (CD), leaf width (LW), leaf length (LL), leaf shape index (LSI; LL/LW), pedicel length (PL), flower axis length (FAL), sepal length (SL), fruit length (FL), fruit width (FW), fruit shape index (FSI; FL/FW), fruit weight (FWt), pulp weight (PW), stone length (StL), stone width (StW), stone shape index (SSI; StL/StW), stone weight (StWt), and pulp percentage (PP).

Qualitative traits assessed in the study included leaf color (LC), flower color (FC), fruit skin color (FSC), and fruit taste (FT).

Statistical analysis

For statistical analyses, IBM SPSS software (version 25.0) was used to perform correlation analysis of morphological characters. Two-way cluster analysis was conducted in PAST (version 4.11) using Euclidean distances, with clusters constructed by Ward's method based on the distance matrix (Hammer et al., 2001; Ward, 1963). Analysis of variance (ANOVA) and principal component analysis (PCA) were performed in SPSS and included quantitative traits. Varimax rotation with Kaiser normalization was applied to improve component interpretability and standardize factor loadings [Kaiser, 1958, 1960; Jackson, 2005], retaining only components with eigenvalues >1.0 [Abdi & Williams, 2010].

RESULTS AND DISCUSSION

Phenological assessment

Vegetation phenology is considered an

important biological indicator in understanding the behaviour of ecosystems and how it responds to environmental cues [Cosmulescu et al. 2021]. Although all of the genotypes were extremely early flowering and susceptible to spring-frost damage, the phenological traits of the plant previously showed that the blossoming period differed across them [Shamsolshoara et al., 2021]. It should be noted that phenological studies of the genetic resources of wild blackthorn in the world and in our republic are limited. *Prunus spinosa* L. is one of the species of drupe fruit trees with an early growing season. Our research established that although the phenological phases of blackthorn differ somewhat depending on climatic conditions and genotype characteristics, the general sequence of development is stable. This stability may make it important in breeding research. As part of the investigation, phenological observations of wild blackthorn genotypes were carried out and the onset time and duration of each development stage were precisely determined (Table 2).

Table 2. Main phenological phases of *Prunus spinosa* L.

Phenological stage	Start time	End time	Note
Flowering	25 March	20 April	Flowering before leaves appear; duration 10-15 days.
Formation of the leaf	5 April	2 May	It starts immediately after flowering.
Formation of the fruit	28 May	25 June	Fertilization stage, depends a lot on the weather conditions
Fruit ripening	25 July	20 September	Varies depending on genotype and climatic conditions

The investigation revealed that this species enters its flowering stage very early. Flowering began in the last third of March and continued through the second third of April. The flowering phase lasted 10-15 days depending on weather conditions. The flowering process occurred earliest in genotypes common in the Upper Shirvan region and it occurred later in the Karabakh region. It has been noted that after a short winter dormancy, blackthorn blossoms begin to bloom in early spring, usually in March-April [Cosmulescu & Gavrilă, 2020]. According to Salmanova (2024), *Prunus spinosa* L. blooms in

March and bears fruit in August.

Following the completion of flowering, leaf development commenced, and the first fully expanded leaves were observed by the beginning of May. Rapid leaf formation prepares the soil for the next stages of vegetation - photosynthesis and the accumulation of nutrients. The fruit formation phase was observed from the end of May. Fertilization began immediately after the flowers fell and the fruits gradually formed throughout June. At this stage, air temperature and humidity have a serious impact on the quality of fermentation. Fruit ripening times varied

depending on genotype and environmental conditions. Early-ripening genotypes bear fruit from the end of July, while mid- and late-ripening genotypes bear fruit from mid-August to mid-September. Due to differences in maturity between genotypes, it was not possible to collect all the samples at the same time. *Prunus spinosa* fruits are reported to ripen between July and September [Sadigov, 2023]. In conclusion, *Prunus spinosa* can be considered a productive and promising fruit species both in natural ecosystems and in agriculture, with early flowering and medium or high fruit ripening periods.

Prunus spinosa is distributed in situ across various botanical-geographical regions of Azerbaijan, including the Greater Caucasus (northern), Greater Caucasus (western), Greater Caucasus (Quba mountain massif), Lesser Caucasus (northern), Lesser Caucasus (central), Samur–Devechi plain, Caspian lowland, Ganikh–Ayrichay valley, Bozqir plateau, Kura plain, Kura–Araz lowland, Lankaran–Mugan, Lankaran mountainous, Lankaran lowland, and Diabar (Zuvand) regions. It occurs from lowland areas up to the middle mountain belt. The species is mainly found in habitats such as shrublands, forest edges, and river valleys, and belongs to the mesophytic ecological group.

The species *Prunus spinosa* is represented in various ecological groups. It is predominantly mesophilic, meaning it thrives in moderately humid conditions, especially at forest edges and in semi-shaded areas. At the same time, it also acts as a sero-mesophilic (partially adapted to arid climate) plant; this feature allows the species to remain viable even on rocky soils with low moisture content. However, blackthorn is a light-loving species and grows best in open areas and roadsides. The species also has nitrophilous (thriving in nitrogen-rich soils) characteristics, leading to its occurrence in some anthropogenic ruderal areas.

The distribution area of the *Prunus spinosa* species in Azerbaijan is quite wide and covers various physical and geographical regions of the country. The densest distribution is observed in the Lankaran–Astara zone, especially in the foothills of the Talysh Mountains. Here, blackthorn trees grow on the edges of natural forests.

Seminatural forms of blackthorn are often found in the Guba-Khachmaz region, in foothill and forest areas. In the Sheki-Zaqatala and Gakh regions, it is widespread in the shrubforest landscapes of the southern foothills of the Greater Caucasus. In lowland regions such as Karabakh and the Mil plain, blackthorns mainly grow on plains and in irrigated fields. *Prunus spinosa* L. is a vigorously branching shrub or small tree widely distributed in Azerbaijan. The species occurs in forest and shrub vegetation from the lowland to the mid-mountain belts, particularly in thickets, along forest edges, and in river valleys [Ibadullayeva et al., 2023]. Owing to its richness in biologically active substances, the species is also widely used by the local population for medicinal purposes [Shiraliyeva et al., 2023].

Thus, *Prunus spinosa* L. is an important fruit plant of Azerbaijan, which easily adapts to various environmental conditions, is widely distributed in natural landscapes and has genetic diversity. These characteristics make it a promising genetic resource both for biodiversity conservation and for selection and introduction into agriculture.

Morphological assessment

The flora of Azerbaijan is rich in diversity [Qurbanova et al., 2024]. Of the 4,961 species of higher plants common in Azerbaijan, 4,557 species are found in the wild flora of the republic. Since the flora of Azerbaijan was formed in conditions where various elements of the world flora converged in different climatic and soil conditions, the natural plant species common here have complex resistance characteristics. These characteristics can also be successfully used as a valuable resource in selection and hybridization research. The modern vegetation of Azerbaijan was greatly influenced by the flora of Western Asia [Guvendiyev et al., 2025b]. As *Prunus spinosa* L. is a common drupe fruit plant belonging to the Rosaceae family, morphological diversity increases its potential for adaptation and selection in this context. This investigation assessed the main morphological characteristics of the species (Figure 1). It has been established that *Prunus spinosa* has a widespread and multi-branched tree-like form. Descriptive statistics

of pomological and morphological traits used in the investigation of wild blackthorn genotypes are presented in detail in Table 3. Some researchers reported that their studies did not find significant differences for certain variables

(CV = 0.00%), prompting them to exclude these variables from their analyses [Goodarzi et al., 2018; Mostafa et al., 2020; Elwakil et al., 2021; Khadivi et al., 2022].



Figure 1. Photographs of fruits, leaves, and trees from various wild *Prunus spinosa* L. genotypes

Table 3. Descriptive statistics of the studied morphological traits in *Prunus spinosa* L. genotypes

Trait	Abb	Min	Max	Mean	SE	SD	CV%
Trunk diameter (cm)	TD	3.37	15.83	8.07	0.55	5.47	45.27
Plant height (m)	PH	1.40	5.83	3.27	0.11	1.15	33.06
Canopy diameter (m)	CD	1.00	3.70	1.67	0.05	0.54	32.47
Leaf width (cm)	LW	1.20	3.30	2.23	0.06	0.57	25.42
Leaf length (cm)	LL	2.60	5.70	4.11	0.08	0.82	19.91
Leaf shape index	LSI	1.61	2.22	1.89	0.02	0.18	9.57
Pedicle length (mm)	PL	2.87	5.03	3.95	0.07	0.68	17.31
Flower axis length (mm)	FAL	5.40	7.33	6.29	0.06	0.60	9.51
Sepal length (mm)	SL	1.60	3.03	2.20	0.04	0.42	19.26
Fruit length (mm)	FL	13.20	17.20	14.90	0.12	1.23	8.25
Fruit width (mm)	FW	9.90	14.30	11.83	0.14	1.38	11.69
Fruit shape index	FSI	1.20	1.34	1.27	0.00	0.04	3.55
Fruit weight (g)	FWt	1.06	2.14	1.54	0.04	0.35	22.69
Pulp weight (g)	PW	0.60	1.40	0.94	0.03	0.26	27.80
Stone length (mm)	StL	7.87	11.9	9.61	0.08	0.77	12.14
Stone width (mm)	StW	4.90	9.30	6.83	0.05	0.49	13.95
Stone shape index	SSI	1.26	1.61	1.43	0.00	0.04	2.21
Stone weight (g)	StWt	0.15	0.36	0.24	0.01	0.07	27.22
Pulp percentage (%)	PP	52.70	67.50	59.85	0.39	3.87	6.47

Abbreviations: SE-standart error; SD-standard deviation; Abb-abbreviations; CV-coefficient of variation; Max-maximum; Min-minimum.

The stem diameter (TD) ranged from 3.37 to 15.83 cm, with a mean value of 8.07 cm, and the coefficient of variation (CV = 45.27%) was high. This parameter reflects heterogeneity related to plant age, ecological conditions, and genetic diversity. The canopy diameter (CD) also exhibited considerable variability, ranging from 1.00 to 3.70 m, with a mean value of 1.67 m and a coefficient of variation (CV = 32.47%). Gavriela and Cosmulescu (2018) reported Stem diameters ranging between 28.18 and 32.32 mm. The canopy diameter ranged from 113.74 to 151.44 cm, indicating substantial variability among populations. Plant height (PH) ranged from 1.40 to 5.83 m, with a mean value of 3.27 m, and the coefficient of variation was 33.06%. Woldring (2015) described *Prunus spinosa* as a shrub or small tree, typically attaining a height of 3–5 m. According to Gavriela et al. (2018), the coefficients of variation were 26.02–30.87% for stem

diameter, 23.48–32.32% for plant height, and 30.93–37.81% for canopy diameter, indicating notable variability among the measured traits. The leaves of *Prunus spinosa* genotypes varied in color from light green to dark green. The leaf characteristics showed comparatively lower variation. The mean leaf width and length were 2.23 cm and 4.11 cm, respectively, with corresponding coefficients of variation of 25.42% and 19.91%. The leaf shape index (LSI) exhibited low variability (CV = 9.57%), suggesting that leaf shape remained relatively consistent throughout the population. According to Vitkovskii (1978), the length of the leaf blade varied between 3.0 and 7.1 cm, while the width ranged from 1.5 to 4.4 cm. The flower color of *Prunus spinosa* genotypes was white to creamy white. The floral characteristics showed a moderate level of variation. The length of the flower pedicel ranged from 2.87 to 5.03 mm (CV = 17.31%), whereas

the flower axis length (FAL) ranged from 5.40 to 7.33 mm, indicating low variability (CV = 9.51%). Sepal length was characterized by moderate variability (CV = 19.26%). Fruit measurements in the present study revealed average lengths and widths between 11.83 and 14.90 mm, consistent with Herman (1971) and Idžojić (2019), who reported predominantly globose fruits ranging from 10 to 15 mm in size. Krüssmann (1960) documented similar dimensions and further indicated that fruit shape can range from globose to oblate or ovoid, which corresponds with the results obtained in this research. The fruits exhibited a color range from violet-blue to brownish-violet. Genotypes exhibited a range of taste characteristics, including bitter, highly sour, and mildly sour. Compared with vegetative characteristics, fruit traits exhibited greater stability. The observed fruit dimensions varied between 13.2 and 17.2 mm in length and 9.9 and 14.3 mm in width. Coefficients of variation for fruit length and width were 8.25% and 11.69%, respectively, suggesting a high degree of morphological consistency in fruit dimensions. According to Kirca and Mertoglu (2025), there is considerable variability in fruit size among genotypes. The investigation revealed that fruit width showed higher variation compared to fruit length, suggesting greater heterogeneity in fruit width and relatively uniform structure in length. The fruit shape index exhibited very low variability (CV = 3.55%) and confirmed the characteristic stable fruit shape for the species. In contrast, fruit weight and pulp weight (PW) exhibited relatively high variability (CV = 22.69% and 27.80%, respectively), which may be related to environmental factors and the distribution of nutrients. Ozzengin et al. (2023) previously reported a fruit weight of 2.40 g for the wild-grown *Prunus spinosa* genotype, which is comparable to the values obtained in our investigation. Few studies have been conducted on the fruit weight of blackthorn in the existing literature. Smaller fruits exhibit typical traits of wild edible fruits, including those of blackthorn. Kuru Berk et al. (2020) also reported variation in fruit weight among several *Prunus spinosa* genotypes. Kirca and Mertoglu (2025) observed a moderate variation in fruit weight among the

genotypes, reflected by a CV of 11.38%. Fruit size and weight are regarded as key traits because they directly affect consumer preferences [Gorynska-Goldmann et al., 2023]. Ozzengin et al. (2023) reported fruit weight, width, and length of wild-grown *Prunus spinosa* genotypes as 2.40 g, 12.66 mm, and 13.25 mm, respectively, which closely align with the findings of the present research. Idžojić (2019) reported that the length and width of blackthorn stones ranged from 7 to 9 mm and 6 to 8 mm, respectively. In the present investigation, mean stone lengths were slightly lower, ranging from 7.87 to 11.9 mm, while stone widths varied from 4.90 to 9.30 mm. Kernel traits were characterized by low to moderate variability. The coefficients of variation for stone length (StL) and stone width (StW) were 12.14% and 13.95%, respectively. The stone shape index (SSI) exhibited very low variability (CV = 2.21%), indicating high stability of endocarp morphology. In contrast, stone weight (StWt) exhibited relatively high variability (CV = 27.22%). According to Vidaković et al. (2024), fruit and stone traits exhibited pronounced morphological variability among different *Prunus spinosa* populations. The pulp percentage (PP) ranged from 52.70% to 67.50%, with a mean value of 59.85%, and was characterized by a low coefficient of variation (CV = 6.47%). This parameter indicates a high and stable proportion of edible flesh in *Prunus spinosa* fruits, highlighting their nutritional value and potential economic importance. In general, the results demonstrate that vegetative traits in *Prunus spinosa* show higher variability compared to reproductive traits, while shape indices constitute the most stable indicators. This indicates that vegetative organs possess adaptive plasticity, whereas fruit and stone traits serve as reliable indicators for selection and conservation studies.

PCA, or principal component analysis, was used to assess the importance of various characteristics. The appropriateness of PCA for this dataset was validated by the statistical significance of Bartlett's test and the Kaiser-Meyer-Olkin (KMO) test result of 0.890 (Table 4). According to Esadi et al. (2022), a KMO value in the range of 0.7 to 0.8 indicates a robust analysis.

Table 4. KMO and Bartlett’s Test

Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy.		0.890
Bartlett’s Test of Sphericity	Approximate Chi-Square	17645.322
	St.V.	171
	Significance	0.000

Bartlett’s test (Bartlett, 1950) checks whether the correlation matrix is an identity matrix, which would indicate that the variables are unrelated and unsuitable for structure detection. The chi-square value of the Bartlett test was 17645.322, and the degree of freedom (df) was 171. The significance level of the test ($p < 0.001$) indicates high statistical significance [Sarma et al., 2024]. This result confirms that the correlation matrix is not an identity matrix, which means that there are statistically significant correlations between the variables. Thus, the result of Bartlett’s test indicates that the data are suitable for factor analysis and principal components analysis.

The combination of the KMO index and Bartlett’s sphericity test results indicates that the set of variables under investigation is suitable for principal component analysis. A KMO index of 0.890 indicates a sufficient level of fit for the analysis. The high statistical significance of Bartlett’s test confirms the presence of a strong correlation structure between the variables and confirms the reliability of the principal

component analysis results. Therefore, the presented statistical tests provide a sufficient basis for conducting principal component analysis based on the data selected for the study.

The scree plot in Figure 2 shows that there are up to three main components (indicator elements) in the genotypes of *Prunus spinosa* under investigation, each of which successfully explains inter-genotypic variance. However, the explained variance starts to drastically decrease after the third component. Consequently, all subsequent analyses were conducted based on these three selected principal components.

The graph shows that the eigenvalues of the first two components are significantly higher than those of the others: the eigenvalue of the first component is 13.926 and that of the second component is 1.967. This suggests that the majority of the total variation in the dataset (approximately 83.65%) is mainly explained by these two components. Thus, the first two components are of particular importance for understanding the structure of the data.

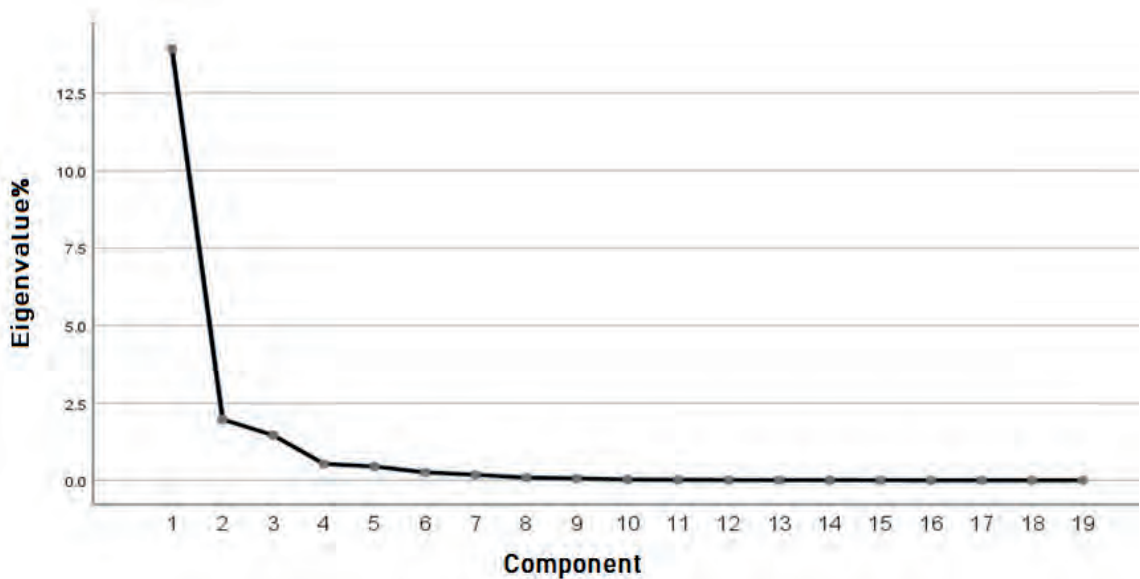


Figure 2. Principal Component Based Scree Plot

The results of the principal component analysis (PCA) conducted on *Prunus spinosa* L. indicated that a large portion of the observed variability among traits is attributed to fruit and other generative organ-related characteristics. The fact that the first three principal components together explained 91.31% of the total variation indicates that the analyzed traits are highly informative in capturing intraspecific differences (Table 5). Similar findings have been reported in several international studies on *Prunus spinosa* [Ilhan, 2023; Papovich et al., 2020; Kirca & Mertoglu, 2025; Vidaković et al., 2024].

The first principal component (PC1) accounted for 73.30% of the total variation and showed

strong positive associations primarily with fruit dimensions, fruit weight, stone size, pulp percentage, and flower morphological traits, confirming that generative traits are the main source of intraspecific morphological variation. According to Vidaković et al. (2024), the second principal component described 30.79% of the overall variability, whereas the first principal component explained 52.05%. Studies conducted in Turkey [Kirca & Mertoglu, 2025] have shown that traits such as fruit length, width, and weight have high loadings on the first principal component and explain the majority of the variation.

Table 5. Principal component analysis (PCA): Eigenvalues and variance explained by the major axes for pomological and morphological traits in the blackthorn genotypes

Trait	Abb	PC1	PC2	PC3
Trunk diameter (cm)	TD	0.081	0.453	0.824
Plant height (m)	PH	0.394	0.595	0.594
Canopy diameter (m)	CD	0.708	0.059	0.059
Leaf width (cm)	LW	0.686	0.622	-0.359
Leaf length (cm)	LL	0.862	0.390	-0.260
Leaf shape index	LSI	-0.067	-0.870	0.451
Pedicle length (mm)	PL	0.990	-0.091	0.008
Flower axis length (mm)	FAL	0.989	-0.088	0.004
Sepal length (mm)	SL	0.980	-0.111	-0.024
Fruit length (mm)	FL	0.982	-0.106	-0.026
Fruit width (mm)	FW	0.988	-0.096	-0.009
Fruit shape index	FSI	-0.957	0.052	-0.036
Fruit weight (g)	FWt	0.989	-0.092	0.003
Pulp weight (g)	PW	0.989	-0.087	0.013
Stone length (mm)	StL	0.988	-0.101	0.004
Stone width (mm)	StW	0.986	-0.095	0.028
Stone shape index	SSI	-0.765	0.098	-0.111
Stone weight (g)	StWt	0.983	-0.098	-0.014
Pulp percentage (%)	PP	0.904	-0.061	0.055
Eigenvalue		13.926	1.967	1.455
Variance (%)		73.296	10.354	7.660
Σ variance (%)		73.296	83.650	91.310

The negative association of fruit shape index (FSI) and stone shape index (SSI) with PC1 indicates that these traits are relatively independent

of size-related characteristics. This finding has also been highlighted in previous studies [Vidaković et al., 2024] and demonstrates that shape

indices, being more stable and less influenced by environmental conditions, play an important role in taxonomic identification and selection programs.

The second principal component (PC2) explained 10.35% of the variation and was associated with plant height and leaf width, indicating that vegetative structure plays a secondary role in morphological variability. The negative loading of the leaf shape index on this component reflects different directions of variation between leaf size and shape. Similar results have been observed in other studies, where vegetative traits were primarily considered as factors reflecting inter-population ecological adaptation [Papovich et al., 2020; Kirca & Mertoglu, 2025].

Although the third principal component (PC3) accounted for a relatively small proportion of the variation, its association with plant height and Stem diameter reflects differences in overall growth vigor and structural development among individuals. This component can be considered an additional source of variation shaped by environmental conditions and local adaptation.

Overall, the results indicate that in *Prunus spinosa* L., morphological variability is primarily driven by fruit and other generative traits, while vegetative traits act as complementary

and secondary factors in this variation. These findings are fully consistent with global investigations and confirm that the PCA method is an effective statistical approach for the morphological characterization of *Prunus spinosa* populations, as well as for assessing the conservation of genetic resources and their selection potential.

Analysis of variance (ANOVA) was employed in the research to evaluate group variability and its significance (Table 6). Whether there are statistically significant differences between features is ascertained with the use of this investigation. The results of one-way analysis of variance revealed highly significant differences ($p < 0.001$) for all studied morphological traits among *Prunus spinosa* L. populations. This confirms the presence of strong phenotypic diversity within the species. Vidaković et al. (2024) reported that ANOVA revealed significant differences ($p < 0.05$) for all measured fruit and stone morphological traits both within and among populations. The highest F-values were observed for vegetative traits, particularly Stem diameter and plant height, indicating that these characteristics play a key role in population differentiation. Leaf size and leaf shape index also exhibited significant variation, confirming the high plasticity of vegetative organs.

Table 6. ANOVA test of the studied morphological traits

Trait	Abb	Sum of sqrs	Mean square	F	p (same)
Trunk diameter (cm)	TD	8883.806	89.735	1099.699	***
Plant height (m)	PH	392.478	3.964	128.576	***
Canopy diameter (m)	CD	87.554	0.884	30.779	***
Leaf width (cm)	LW	94.866	0.958	42.213	***
Leaf length (cm)	LL	199.006	2.010	24.346	***
Leaf shape index	LSI	9.629	0.097	55.284	***
Pedicle length (mm)	PL	138.536	1.399	17.857	***
Flower axis length (mm)	FAL	106.425	1.075	16.247	***
Sepal length (mm)	SL	53.246	0.538	15.742	***
Fruit length (mm)	FL	449.651	4.542	14.437	***
Fruit width (mm)	FW	568.611	5.744	15.034	***
Fruit shape index	FSI	0.598	0.006	13.827	***
Fruit weight (g)	FWt	36.578	0.369	16.615	***
Pulp weight (g)	PW	20.949	0.212	16.742	***

Trait	Abb	Sum of sqrs	Mean square	F	p (same)
Stone length (mm)	StL	178.102	1.799	17.304	***
Stone width (mm)	StW	70.333	0.710	16.458	***
Stone shape index	SSI	0.474	0.005	5.939	***
Stone weight (g)	StWt	1.256	0.013	14.809	***
Pulp percentage (%)	PP	4447.920	44.928	13.886	***

Reproductive traits, including flower, fruit, and stone characteristics, exhibited relatively lower variation compared to vegetative traits, yet the differences were statistically significant. In particular, fruit dimensions and weight, as well as pulp percentage, exhibited considerable variability within populations.

Overall, the ANOVA results indicate that morphological variability in *Prunus spinosa* L. is primarily determined by vegetative traits, while generative traits, despite being more stable, play an important role in population differentiation. These findings scientifically support the ecological adaptability of the species and its significance for selection purposes.

The correlation between the two features was calculated. Correlation analysis can provide valuable information about the most important traits when evaluating genotypes [Norman et al., 2011]. The technique used most often for determining the strength of the linear relationship between two continuous variables is the Pearson correlation coefficient (r), which has a value between -1 and 1 [Pearson, 1900]. By identifying features that have a significant correlation, it is possible to predict in advance the ratio of one feature to another, which can facilitate the selection of suitable genotypes. The results of the correlation analysis are presented in Figure 3.

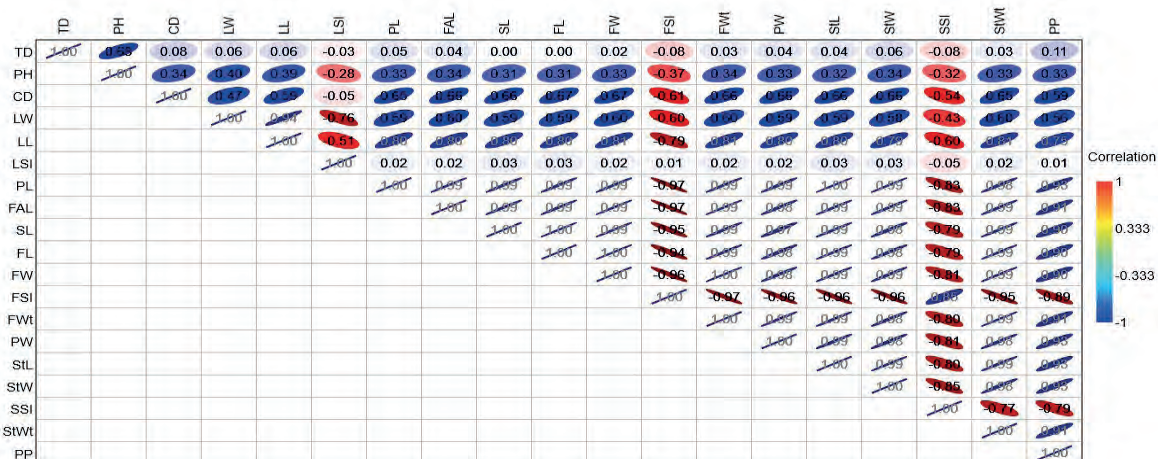


Figure 3. Pearson correlation matrix reflecting the relationships between quantitative traits of blackthorn genotypes (see Table 6 for abbreviations)

The analysis revealed strong positive correlations among some variables. For example, a high positive correlation ($r=0.80$) was observed between leaf width (LW) and leaf length (LL), indicating that as leaf width increases, leaf length also tends to increase. Regarding fruit traits, a

very strong positive correlation ($r=0.94$) was found between fruit length (FL) and fruit width (FW), and a high positive correlation ($r=0.96$) was also observed between fruit width and fruit shape index (FSI). These results indicate that fruit traits are closely interrelated, and that selection

based on one trait may influence the others. Vidaković et al. (2024) reported that fruit and stone morphological traits exhibit significant Pearson correlations at various levels. In our research, some negative correlations were also observed. For example, a negative correlation ($r=-0.37$) was observed between plant height (PH) and flower axis length (FAL), indicating a tendency for flower axis length to decrease as plant height increases. Additionally, a negative correlation ($r=-0.89$) was observed between fruit weight (FWt) and fruit shape index (FSI). Some variables exhibited weak and practically negligible relationships; for instance, the correlation between Stem diameter (TD) and leaf shape index (LSI) was near zero ($r=0.03$). These results indicate that some phenotypic traits act as independent variables and do not exert significant influence on one another.

Overall, the correlation analysis highlights the interactions among plant phenotypes and indicates which traits should be considered together during the selection process.

To assess the relationship between the 100 studied blackthorn genotypes, a two-way cluster analysis was used for 19 traits. The dendrogram was constructed using the Euclidean method in the PAST 4.11 statistical software package (Fig. 4). This analysis facilitates the visual observation of genotype grouping [Mammadova et al., 2024]. One of the most precise ways to express relationships between data is to use the Euclidean distance, which is the linear distance between two points [Hajiyev et al., 2025b; Khadivi et al., 2025].

Ward's approach is used to create clusters based on the distance matrix that was collected. By reducing the variation across groups, Ward's technique guarantees the creation of homogeneous subgroups. This approach is especially recommended for classification analyses where inter-cluster differences must be kept to a minimum [Kaufman & Rousseeuw, 1990; Ward, 1963]. The analysis findings are displayed using a cluster, which also aids in comprehending the connections between quantitative variables and observations (samples). The degree of similarity or distance between the data points is shown by color intensity on the cluster. For instance, separate samples are displayed with more distinct

color tones, whereas comparable samples are depicted with closer color shades [Wilkinson & Friendly, 2009]. Because it swiftly and efficiently visualizes the data's clustering structure, this approach is particularly favored for analyzing big and complicated datasets. As a result, the samples' similarities, differences, and groups may be determined. In this investigation, the similarities and differences between the analyzed samples were visualized using a cluster made using Ward's approach and Euclidean distance coefficients. Quantitative data were used for the analysis procedure, and the findings show which samples are more closely connected to one another and how the data are organized into groups (Figure 4).

In the first part of the dendrogram, quantitative features were initially divided into three main groups (A, B and C) based on an analysis based on the Euclidean distance indicator.

Group A encompasses traits including canopy diameter (CD), sepal length (SL), fruit shape index (FSI), fruit weight (FWt), pulp weight (PW), stone shape index (SSI), stone weight (StWt), and leaf shape index (LSI). These traits reflect the structural and morphological characteristics of both the vegetative and generative organs of the plant, as well as the shape and quantitative parameters of the fruit and stone. In particular, fruit and stone weights are directly associated with yield and quality, while shape indices reflect the morphological stability and genetic determination of the organs.

Group B encompasses traits such as plant height (PH), leaf length (LL), pedicel length (PL), flower axis length (FAL), stone width (StW), and pulp percentage (PP). These traits serve as indicators of the plant's overall growth potential, the developmental status of its reproductive structures, and the internal quality parameters of the fruit. Pulp percentage is one of the most important quality parameters from a consumer perspective, as it determines the proportion of the edible portion of the fruit. In this context, Group B represents traits associated with the plant's height, length, and internal quality attributes.

Group C is characterized by morphological traits including Stem diameter (TD), fruit length

(FL), fruit width (FW), and stone length (StL). These traits represent fundamental macromorphological indicators that reflect the overall vegetative vigor of the plant and the primary dimensional attributes of the fruit and its stone. While Stem diameter is closely associated with the vegetative development of the plant, fruit length and width serve as key indicators determining the fruit's size potential and market value, and stone length provides additional insight into internal fruit morphology. Therefore, Group C primarily characterizes the structural vigor of the plant and the size-related attributes of both the fruit and the stone.

Overall, the clustering observed in the dendrogram indicates a clear differentiation of morphological traits into three distinct groups: structural and morphological characteristics of vegetative and generative organs, along with shape and quantitative attributes of fruit and stone (Group A); growth-related traits and internal quality parameters reflecting plant development and fruit quality (Group B); and fundamental macromorphological traits associated with plant vigor and the primary dimensional attributes of fruit and stone (Group C). This structured classification allows for a more precise assessment of morphological diversity and facilitates the identification of traits of significance for selection among genotypes. Consequently, it provides a solid scientific basis for future breeding programs and conservation strategies.

The genotypes were separated into five major clusters while examining the second section of the cluster. The majority of the 11 accessions that make up cluster 1 are moderately grown plants. Plant height in this cluster varies from 2.1 to 5.8 m, canopy diameter from 1.8 to 2.3 m, and Stem diameter from 5.7 to 15.7 cm. Fruit weight ranges from 1.7 to 2.1 g, while fruit length varies from 15.4 to 17.2 mm. The pulp percentage varies between 63.1 and 64.6%, suggesting that this cluster has intermediate fruit quality characteristics.

There is more morphological variety in cluster 2, which has 25 accessions. Plant height varies from 1.4 to 5.8 m, and Stem diameter ranges from 3.4 to 15.8 cm. Fruits range in length from 13.2 to 16.1 mm and in weight from 1.1 to 1.9 g, making them medium in size. The range of the pulp percentage is 52.7 to 66.5%. These findings corroborate this cluster's significant genetic diversity by showing both lower-quality and comparatively higher-quality genotypes.

With 31 accessions, cluster 3 is regarded as one of the study's most promising groupings. With Stem diameters ranging from 4.3 to 11 cm and plant heights between 1.7 and 5.7 m, vegetative development in this cluster is rather robust. Fruits range in length from 14.2 to 17.1 mm and in weight from 1.4 to 2.1 g, making them quite big. The pulp percentage, which varies from 58.2 to 64.8%, suggests that this cluster has a lot of processing and breeding potential.

The 9 accessions that make up cluster 4 are characterised by comparatively consistent morphological traits. Plant height varies from 1.8 to 4.6 m, and Stem diameter ranges from 6.3 to 13.3 cm. Fruit weight ranges from 1.2 to 2.1 g, while fruit length ranges from 13.2 to 15 mm. The proportion of pulp varies between 52.7 and 61%. The significance of this cluster for selection programs is increased by the existence of genotypes with exceptionally high pulp percentages.

The majority of the 24 accessions in cluster 5 are medium-sized trees. Plant height varies from 1.4 to 5.1 m, and Stem diameter ranges from 3.4 to 14.3 cm. Fruit weight ranges from 1.1 to 1.7 g, while fruit length ranges from 13.2 to 15 mm. This cluster includes genotypes with both moderate and high fruit quality features, as seen by the pulp percentage ranging from 52.8 to 61%.

Blackthorn fruits are known to be easily consumed and spread across greater distances by animals and, to a lesser extent, birds (Vander Mijnsbrugge et al., 2013), particularly in regions lacking significant topographical obstacles. Mohanty et al. (2002) state that *Prunus spinosa*'s long-distance seed dispersion may be effective enough to eliminate phylogeographic genetic patterns and reduce genetic variation among populations.

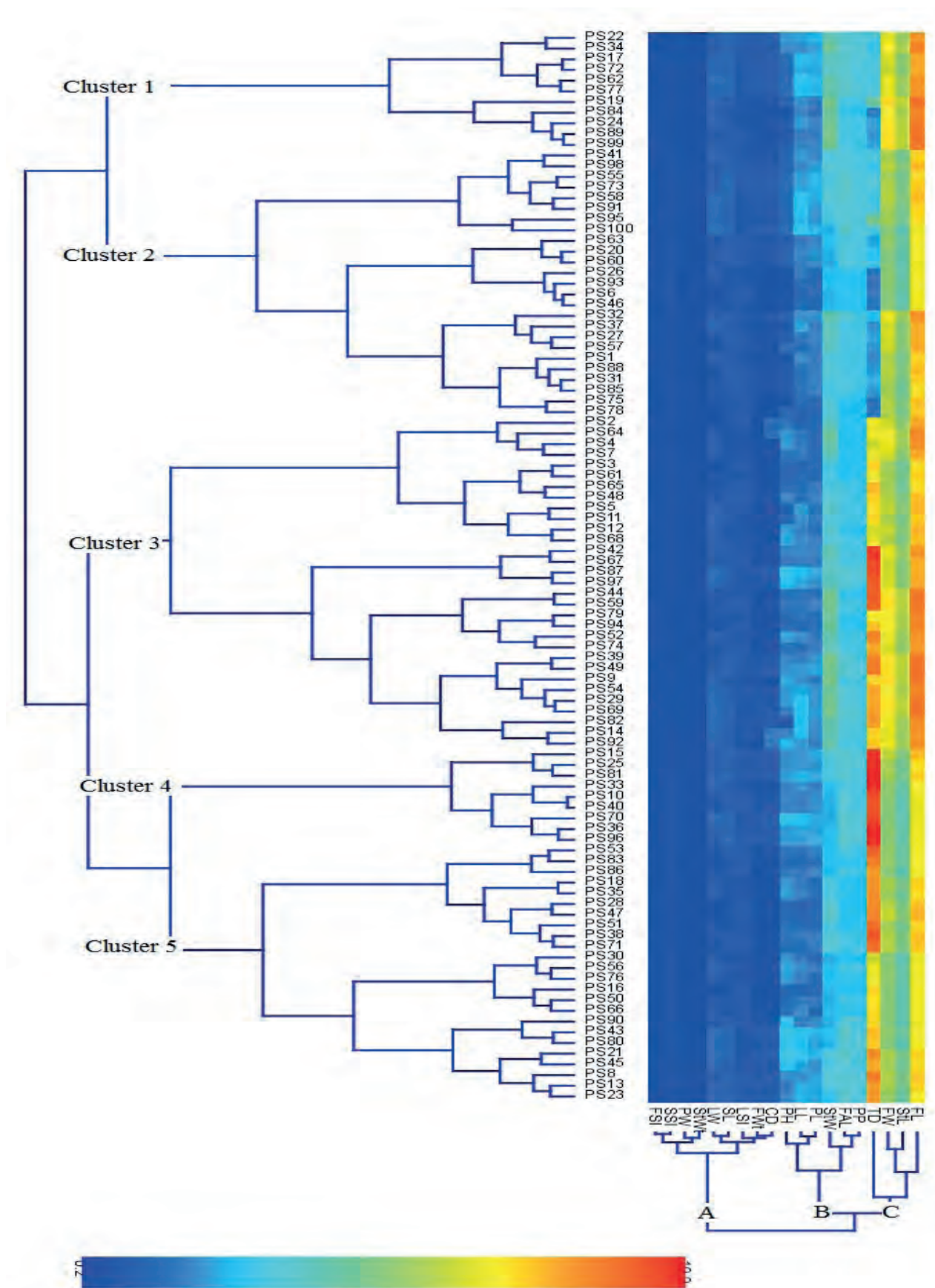


Figure 4. Dendrogram constructed on the basis of morphological characteristics of blackthorn genotypes. (see Table 6 for abbreviations).

Other animal-dispersed species, including *P. avium* L. [Popović & Kerkez, 2016; Rakonjac et al., 2014] and *Sorbus torminalis* (L.) Crantz. [Bednorz, 2007], were also shown to lack spatial structure based on morphological features. Furthermore, no spatial population structure was found in genetic investigations of the population variability of *Prunus sibirica* L. [Wang et al., 2014], *P. africana* [Nzweundji et al., 2020] and *S. torminalis* [Demesure et al., 2000]. In our study as well, as shown in Figure 6, the grouping of genotypes based on morphological traits does not coincide with the regions from which they were collected. According to the cluster analysis, clusters 1, 3 and 4 are the most promising overall, owing to their greater fruit size and higher pulp content — cluster 3, which contains the largest number of accessions (31), stands out as particularly informative for selection. Clusters 1 and 5 include more stable genotypes with intermediate values of the studied traits, while cluster 2 stands out for its significant genetic and morphological variation. These results offer a strong scientific foundation for breeding initiatives and the selection of superior cultivars.

CONCLUSION

The investigation offers a thorough evaluation of the morphological and phenological diversity seen in Azerbaijan's wild *Prunus spinosa* L. populations. The results show that *Prunus spinosa* has a strong ability for adaptation since, despite certain genotype changes, the sequence of phenological developmental phases characteristic of the species stays consistent. Morphological

investigations showed that whereas generative features, such as fruit and stone form indices, are relatively stable and genetically conserved, vegetative traits, such as Stem diameter and plant height, exhibit greater variability and are important in population divergence. Fruit and stone related characteristics are the primary causes of intraspecific variation, according to multivariate statistical studies (PCA, ANOVA, correlation and cluster analysis), which makes them important markers for genotype selection. Fruit size may be somewhat independent of vegetative vigour in some genotypes, according to cluster analysis, which also identified Stem diameter as the main distinguishing characteristic, creating several size-based groups. The function of efficient seed dispersal in preserving genetic connection among populations is supported by the lack of considerable regional organisation. It should be mentioned that there are not many studies on the distribution of Azerbaijani wild blackthorn genotypes inside the Republic's borders, and none on the particular topic this research looks at. Accordingly, the methodological framework adopted in this study offers a significant advancement to the current literature. In conclusion, *Prunus spinosa* L. is a fruit species that shows promise in Azerbaijan due to its steady reproductive features and significant phenotypic variety. The results of this investigation offer a strong scientific foundation for the establishment of breeding programs, the preservation of natural populations, and the use of this species in agricultural practices.

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ARTICLE 4

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ABSTRACT

The morphology and anatomical structure of the fruits of eight representative taxa, one from each of eight genera of the *Oleaceae* family (*Fontanesia*, *Forestiera*, *Forsythia*, *Fraxinus*, *Jasminum*, *Ligustrum*, *Olea*, and *Syringa*), were investigated. For each taxon, the lateral external appearance of the fruit and transverse sections of the ovary and fruit were examined, and the anatomical structure of the exocarp, mesocarp, and endocarp was described; for genera that did not produce fruits, the structure of the pistil was described instead. Mature fruits were collected as the main material, and for several genera fruits at different developmental stages, as well as flowers and flower buds, were also studied. The pericarp anatomy was examined on light-microscope preparations after fixation in 70% ethanol or storage in Strasburger's solution, with transverse sections cut on a microtome and the mechanical elements characterized using phloroglucinol and concentrated hydrochloric acid. The results showed that the exocarp is generally a single layer of thin-walled cells covered by a thick cuticle; that the mesocarp is differentiated into an outer zone of thin-walled parenchyma (about 5–15 layers in some genera and up to roughly 30 layers in *Olea* and *Syringa*) and an inner zone of two to eight or more layers of isodiametric or elongated, heavily thickened sclereids; and that the endocarp is usually a single flattened cell layer. Parenchymatous strands associated with the dehiscence mechanism, vascular bundles at the outer–inner mesocarp boundary, and calcium oxalate crystals were also observed in particular genera. Comparison of material from in situ and ex situ conditions, and of fresh, herbarium, and carpological specimens, showed that the anatomical structure of the mechanical tissues of the outer fruit layer did not differ, indicating that properly preserved dry fruits can be reliably used for carpological study.

Keywords: *Oleaceae*, fruit, anatomy, morphology, carpology.

1. INTRODUCTION

The fruits of *Oleaceae* representatives have long attracted the attention of researchers because of traits that carry significant phylogenetic weight. The subfamily *Oleoideae* is distinguished from the other subfamilies by its single-seeded fleshy fruits. It should also be noted that, in general, the fruits of taxa belonging to the *Oleaceae* family have not been extensively or

comprehensively studied. For example, the inclusion of most species of the genus *Ligustrum* L. in the subfamily *Oleoideae* is based solely on the fact that their fruits are fleshy and dark-colored (Green & Miller, 2009; Ha et al., 2018).

Within the genus *Ligustrum* L., there are taxa with one or several seeds, as well as taxa whose fleshy fruits are completely seedless or indehiscent — a fact that has not been recorded

in most studies of the family (Green & Miller, 2009; Hong-Wa et al., 2023).

These facts indicate that the fruits of the *Oleaceae* family require more detailed investigation, not only to study fruit structure and determine the morphogenetic type, but also to examine pericarp anatomy (Altamura et al., 1987; Turland, 1994; Wu et al., 2021). This is also important for establishing a phylogenetic system of the *Oleaceae* and determining its place within the Angiospermae. Given the wide distribution of *Oleaceae* representatives and their economic importance, the research is particularly relevant, since carpological studies help to resolve various problems of structural botany and also have particular significance for many applied questions (Wu et al., 2021; Iskender, 1993; Iskender et al., 2024).

Previous studies have described the responses of *Pyrus* L. species in the north-eastern Greater Caucasus to various abiotic factors, providing insights into their ecological preferences and their potential for cultivation under changing environmental conditions (Jafarzadeh et al., 2022), and detailed morphological and taxonomic investigations of *Pyrus* L. in the region have clarified species boundaries and intraspecific variation relevant to conservation and ex situ management (Iskender, Jafarzadeh, & Muradova, 2024).

2. MATERIALS AND METHODS

Mature fruits of eight species belonging to eight genera of the *Oleaceae* family were collected as research material, and for many species fruits at different developmental stages were examined. Special studies of fruit development and pericarp histogenesis were carried out for several genera (*Forsythia* — Ha et al., 2018, Wu et al., 2021; *Jasminum* — Green & Miller, 2009, Hong-Wa et al., 2023; *Olea* — Altamura et al., 1987; among others). Flowers and flower buds at different developmental stages were also studied for *Jasminum arborescens*, *J. fruticans*, *J. nudiflorum*, and *J. officinale* (Green & Miller, 2009). Materials were obtained from the Institute of Dendrology of the Ministry of Science and Education of the Republic of Azerbaijan, the Central Botanical Garden, and parks

and gardens in Baku (Iskender, 1993; Iskender et al., 2024).

To study the anatomy of the pericarp, preparations were made for light microscopy. Sections were cut with an MS-2 microtome. Fresh, fleshy fruits were first fixed in 70% ethanol. Mature dry fruits, as well as fruits from carpological and herbarium collections, were stored in Strasburger's solution (distilled water : 96% ethanol : glycerin, 1:1:1) prior to study (Turland, 1994). The duration of storage in Strasburger's solution varied from 10 to 20 days depending on fruit size and the thickness of the sclerenchyma tissue.

For sectioning, the materials were embedded directly in paraffin blocks without preliminary treatment. Transverse sections were prepared from the middle part of the fruit, which is considered the most informative for studying pericarp anatomy (Nesterovich & Deryugina, 1978; Novruzova, 1986; Turland, 1994); in some cases, longitudinal sections were also prepared to clarify structural features. The anatomical structure was studied with a Mikmed-1 light microscope (Turland, 1994).

Fixation of the materials, preparation of solutions, staining of sections, histochemical reactions, and the staining and localization of secondary metabolites were performed by standard methods (Turland, 1994; Wu et al., 2021). Of particular importance were observations of mechanical elements treated with phloroglucinol and concentrated hydrochloric acid, which provided information on cell shape and degree of lignification. Drawings were made with a PA-5 drawing apparatus (Turland, 1994).

3. RESULTS AND DISCUSSION

The morphology and anatomical structure of taxa belonging to the *Oleaceae* family were investigated on the basis of our own research. The genera and species studied are presented in alphabetical order; for genera that do not produce fruits, only the structure of the pistil is described. Comparison of materials from different sources showed that the pericarp structure of the studied species does not change under ex situ conditions relative to in situ conditions (Yang et al., 2008; Wu et al., 2021).

Furthermore, when fruits are properly

preserved in herbaria and carpological collections, no changes occur in the anatomical structure of the dry fruits, and their pericarp can be reliably studied. For example, no differences were noted in the fruit structure or pericarp anatomy of *Fraxinus coriaria* specimens collected from natural habitats and from herbarium material. When herbaria of fleshy fruits are prepared, strong changes are usually observed in the pericarp parenchyma; however, the anatomical structure of the mechanical tissues of the fruit remains stable.

Overall, there are very few studies that comparatively analyze the pericarp structure of *Oleaceae* species; although not central to carpology, they address important aspects of the field (Jafarzadeh et al., 2024; Wallander & Albert, 2000).

***Fontanesia* Labill. Morphology**

The fruits of taxa belonging to the genus

Fontanesia Labill. develop from a superior, bicarpellary, syncarpous pistil. The ovary is bicarpellary, with one anatropous ovule in each locule, and the number of seeds generally corresponds to the number of ovules. *Fontanesia fortunei* Carrière, a representative of this genus, is a shrub that in nature can reach 4–5 m in height, with slender, straight branches. The leaves are lanceolate or ovate, 3–5 cm long and 1–1.5 cm wide, with an elongated apex, entire margins, and a glossy green color. The flowers are whitish, 2–3 mm long, borne on short pedicels in racemose inflorescences. The fruits are ellipsoid, 0.6–0.9 cm long, and reach full development in the first half of July. Mature fruits are considerably flattened laterally, disc-shaped, elliptic, or obovate, with winged ridges, and 6–11 mm in diameter. The central part of the fruit is 3–5 mm thick, dry, smooth, yellowish, and indehiscent. In transverse section, well-defined septa are visible (Figure 1).

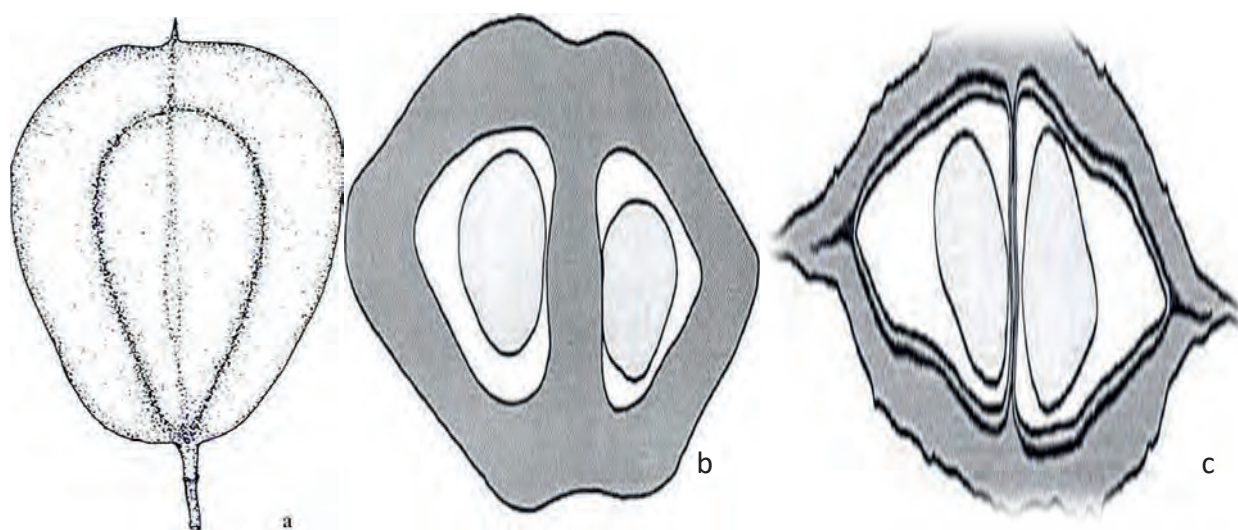


Figure 1. *Fontanesia fortunei*: (a) lateral view of the fruit ($\times 7$); (b) transverse section of the ovary ($\times 25$); (c) transverse section of the fruit ($\times 12$).

Anatomy

The exocarp is composed of thin-walled, single-layered cells that are flattened to varying degrees; numerous stomata are present, and a thick cuticle covers the surface of mature fruits. The mesocarp is divided into two topographic zones, the outer zone consisting of 3–8 layers of parenchyma cells with distinct morphology. Within the parenchyma, numerous thick-walled

small conductive tubes provide mechanical strength to the seed wing. The inner zone of the mesocarp consists of 2–5 layers of elongated, heavily thickened sclereids, arranged rather irregularly and, in the peripheral part of the fruit (wing), mainly oriented along the plane of the wing. The endocarp is represented by a single layer of thin-walled, flattened cells (usually double-layered in *F. phillyreoides*) and is relatively

difficult to observe in mature fruits (Figure 2).

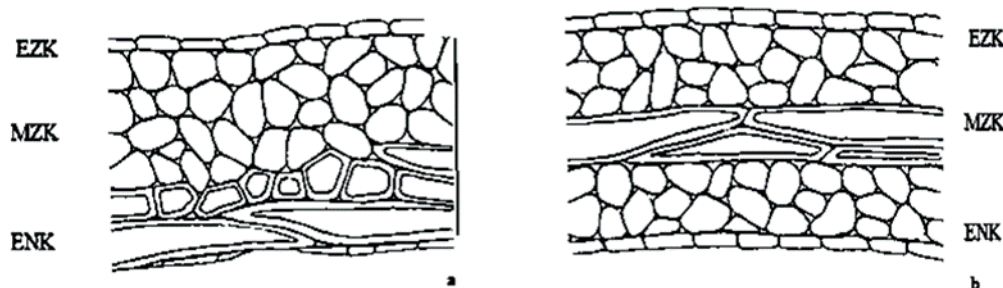


Figure 2. *Fontanesia fortunei*: (a) anatomical structure of the pericarp in the central part of the fruit ($\times 420$); (b) anatomical structure of the pericarp in the peripheral part (wing) of the fruit.

***Forestiera* Poir. Morphology**

The fruits of taxa belonging to this genus develop from a superior, bicarpellary, syncarpous pistil with medial orientation. The ovary is two-loculed, with two anatropous ovules in each locule, of which only one develops. Mature fruits are elliptic or globose, 7–10 mm long and 6–8 mm in diameter. In *Forestiera neomexicana* Gray, the fruits are fleshy, bluish-black, and indehiscent; in transverse section, the septum and traces of degenerated ovules are practically invisible. In nature this species occurs in forests

from New Mexico to southern Colorado and Utah, in desert and semi-desert areas, on dry slopes, and along riverbanks, reaching 1.5–3.5 m in height though generally a small shrub; owing to its favorable branching habit, it can be used as a living hedge. Its green, olive-like leaves turn yellowish in winter, and its light-colored branches remain prominent through winter. The plant produces yellow flowers that bloom before leaf emergence in racemose inflorescences, and the fruits are dark purple. The species is resistant to drought and cold (Figure 3).

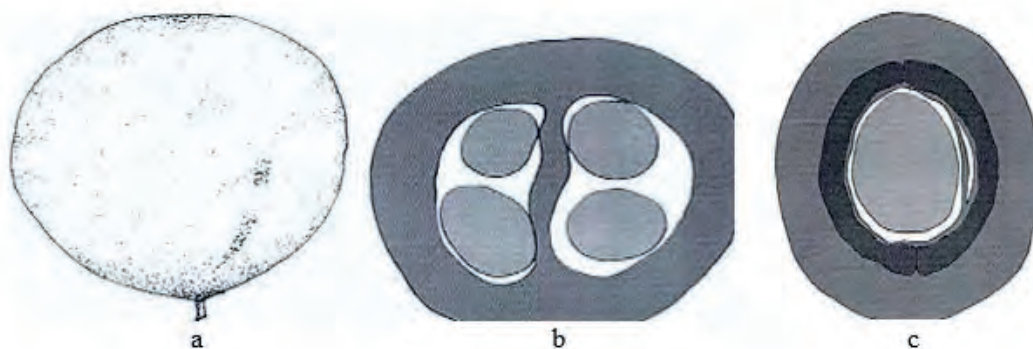


Figure 3. *Forestiera neomexicana* Gray: (a) lateral view of the fruit ($\times 6$); (b) transverse section of the ovary ($\times 20$); (c) transverse section of the fruit ($\times 6$).

Anatomy

The exocarp is formed by a single layer of thin-walled cells that are flattened (sometimes cubical) and covered with a thick cuticle; a few stomata are visible between the exocarp cells, and dark pigments are present within them. The mesocarp is divided into outer and inner zones.

The outer zone is typically composed of 5–15 layers of thin-walled cells, with the parenchyma cells generally radially flattened; in the peripheral parenchyma layers, cells with pigmented protoplasts predominate and give color to the mature fruit. The inner zone contains 2–8 layers of isodiametric sclereids with heavily thickened

cell walls (Figure 4). On the dorsal sides of the carpels, parenchymatous strands formed by very small cells are embedded within the sclerenchyma; these are remnants of the dehiscence mechanism and become difficult to detect once lignification of the inner mesocarp is complete. At the boundary between the outer and inner

mesocarp, a large vascular bundle lacking a mechanical covering is present. The endocarp is composed of a single, highly flattened cell layer that rapidly disintegrates in mature fruits.

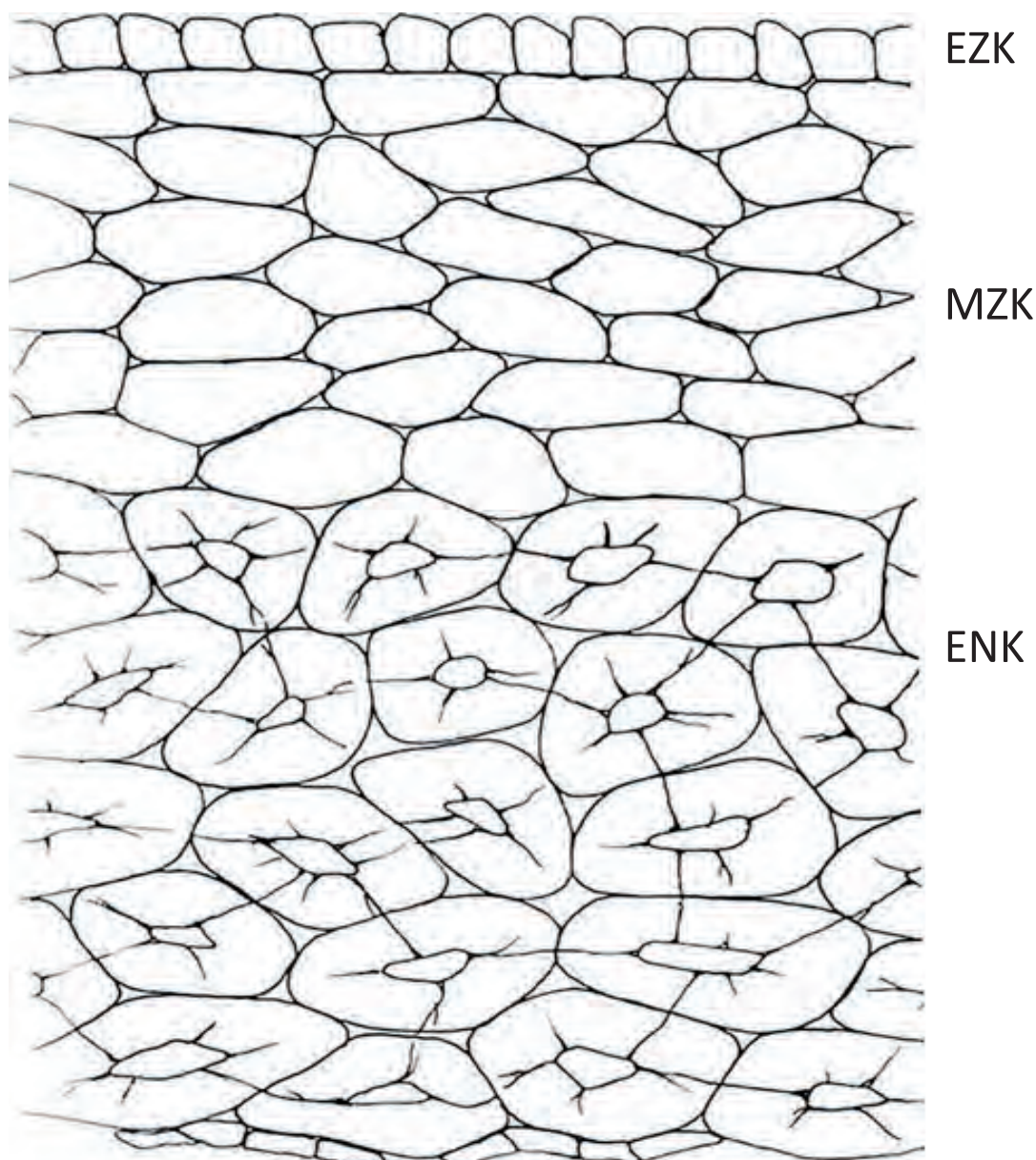


Figure 4. *Forestiera neomexicana* Gray: anatomical structure of the pericarp in the central part of the fruit (×420). EZK, exocarp; MZK, mesocarp; ENK, endocarp.

***Forsythia* Vahl. Morphology**

The fruits of taxa belonging to this genus develop from a superior, bicarpellary, syncarpous pistil with medial orientation. The ovary is two-loculed, containing one anatropous ovule in each locule in *Forsythia europaea*; in

Forsythia suspensa Vahl there are six, and in some cases four, ovules per ovary, each later developing into a winged seed. *Forsythia suspensa* Vahl is a shrub 1–3 m high, with a canopy 2–2.5 m in diameter and quadrangular branches with grayish-brown bark. The leaves are opposite,

with simple petioles, rarely trifoliate; the blades are ovate, with serrate or entire margins, 2–12 cm long. The flowers are bisexual, solitary or grouped in the leaf axils, and bright yellow, with two stamens about the height of the corolla tube. As in some other Oleaceae genera (*Syringa* L., *Olea* L.), the ovary walls secrete sap. The genus is notable for heterostyly, with short- and long-styled flower forms. Mature fruits are ovate, somewhat flattened laterally, elongated,

and curved at the apex, 13–17 mm long and 4–7 mm in diameter; they are dry, rough, and light brown (Figure 5). In transverse section they appear oval or circular-rhomboid, with clearly visible septa. On maturity the fruits dry and split loculicidally into two valves, releasing winged seeds, and may remain on the branches for a long period. Flowering occurs before leaf emergence.

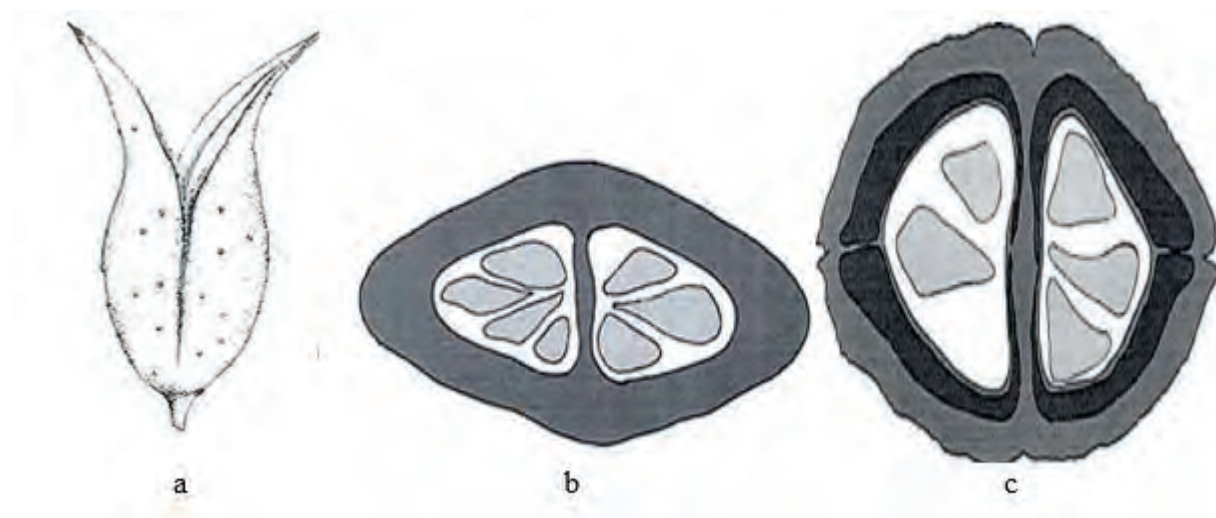


Figure 5. *Forsythia suspensa* Vahl: (a) lateral view of the fruit (×4); (b) transverse section of the ovary (×20); (c) transverse section of the fruit (×7).

Anatomy

The exocarp is mainly composed of a single layer of epidermal cells; during maturation a thick cuticular layer develops on the surface, protecting the fruit. The mesocarp is differentiated into outer and inner zones. In most species the outer mesocarp consists of about 10 layers of parenchyma cells, but in *Forsythia suspensa* it comprises about 20 layers. Within the parenchyma, single or grouped isodiametric sclereids with heavily lignified walls are present, particularly abundant in *Forsythia europaea* and *Forsythia viridissima*. The inner mesocarp typically consists of 4–12 layers of polygonal, tangentially oriented sclerenchyma cells of varying length; isodiametric sclereids are more numerous on the dorsal surfaces of the fruit valves, corresponding to the dehiscence zone. In the dehiscence region, parenchymatous strands of more than ten cell layers — slightly smaller than those in the outer mesocarp — extend along the

dorsal sides of the valves and the central part of the septum, facilitating fruit separation. Several vascular bundles pass along the boundary between the outer and inner mesocarp. The endocarp consists of a single layer of isodiametric, thin-walled cells forming an epidermis.

Fraxinus L. Morphology

The fruits of taxa belonging to this genus develop from a superior, bicarpellary, syncarpous pistil with medial orientation. The ovary is two-loculed, with two anatropous ovules (inverted 180°) in each locule. Mature fruits are strongly flattened laterally, lanceolate or narrowly elliptic, with an acute (occasionally obtuse) apex and downward-bent wings; the fruit is 6–9 mm wide and 15–25 mm long. In *Fraxinus oxycarpa* Willd., fruits can reach 27–30 mm in length. Mature fruits are dry, smooth, yellow, and indehiscent, and typically contain a single seed positioned toward the lower end (Figure

6); in rare cases, transverse sections show degenerated ovules and poorly developed septa. *Fraxinus oxycarpa* Willd. is a medium-sized tree reaching 15–20 m in height, with a crown diameter of 7–10 m; the bark of young trees is smooth and grayish-brown. The buds are pale brown, distinguishing this species from the

closely related *Fraxinus excelsior* L., in which the buds are dark. The leaves of *F. oxycarpa* are pinnately compound, with 2–12 lanceolate, serrate leaflets. Flowering occurs from late April to early May, with yellow-green flowers, and the fruits are dry and winged.

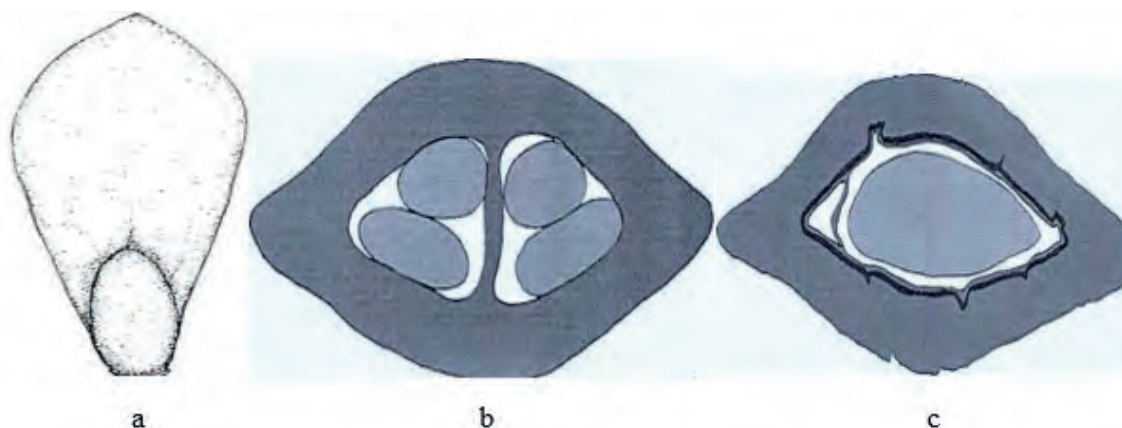


Figure 6. *Fraxinus oxycarpa* Willd.: (a) lateral view of the fruit (×10); (b) transverse section of the ovary (×45); (c) transverse section of the fruit (×15).

Anatomy

In species of this genus, the exocarp is single-layered, composed of thin-walled, generally slightly flattened cells; numerous stomata are present between them, and a cuticular layer covers the surface of mature fruits. In immature fruits, the exocarp bears numerous multicellular peltate trichomes, particularly abundant in glandular-hairy species, serving a protective function; each trichome consists of a stalk cell supporting up to 25 radially arranged thin-walled cells that form the shield-like scutellum. The mesocarp is divided into two zones. The outer mesocarp consists of 3–5 layers of isodiametric parenchyma cells (4 layers in *Fraxinus excelsior* L.; Figure 7), frequently containing calcium oxalate crystals and numerous small vascular bundles with weak mechanical sheaths that support the fruit wings. The inner mesocarp contains 2–4 layers of large, isodiametric sclereids, generally arranged in rows, the outer sclerenchyma layers having thinner walls than the inner ones. The endocarp is composed of a single layer of thin-walled, isodiametric or slightly flattened cells forming the septa. In the peripheral region of the fruit, the mesocarp parenchyma consists of

2–3 cell layers, and the mesocarp sclerenchyma forms a single-layered (occasionally double-layered, as in *Fraxinus americana* L.) connection along which the mechanically reinforced vascular bundles extend; in general, the endocarp does not participate in wing formation.

Jasminum L. Morphology

The fruits of taxa belonging to the genus *Jasminum* L. develop from a superior, bicarpellary, syncarpous pistil with medial orientation; the ovary is positioned above the point of fusion of the floral parts (superior) and is two-loculed, with two anatropous ovules in each locule (Figure 8). Typically, the number of developed seeds corresponds to the number of ovules. Mature fruits are bicarpellary, forming a fused, globular structure; they are dry, smooth, yellow or yellowish-green, fragile, and indehiscent, only rarely opening along the dorsal side, as in *Jasminum nudiflorum* Lindl. and *Jasminum fruticans* L. *Jasminum officinale* L., one of the rare plants of Azerbaijan, is a perennial, deciduous shrub with long, slender, smooth, angular branches; its leaves occur in 2–3 pairs, are lanceolate-elliptic, acuminate, and smooth, with serrate margins, bright green above and light green beneath. The

flowers are white and fragrant, borne on long pedicels in umbels, flowering mainly in April. In most taxa the fruit diameter ranges from 4 to 8

mm, and the fruits are typically enclosed by the adjacent calyx lobes.

EZK

MZK

ENK

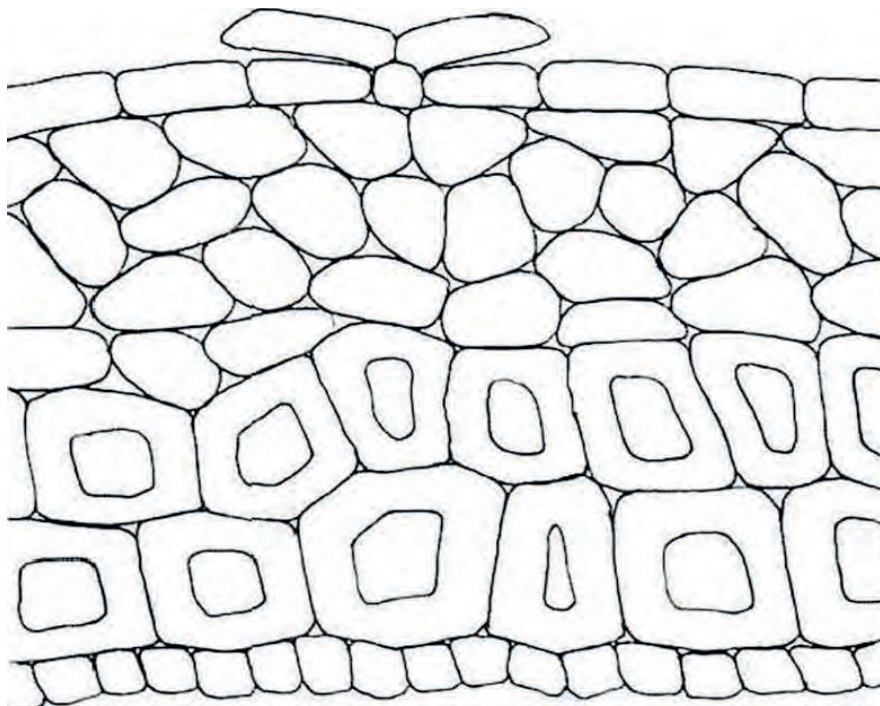


Figure 7. *Fraxinus oxycarpa* Willd.: anatomical structure of the pericarp in the central part of the fruit ($\times 420$). EZK, exocarp; MZK, mesocarp; ENK, endocarp.

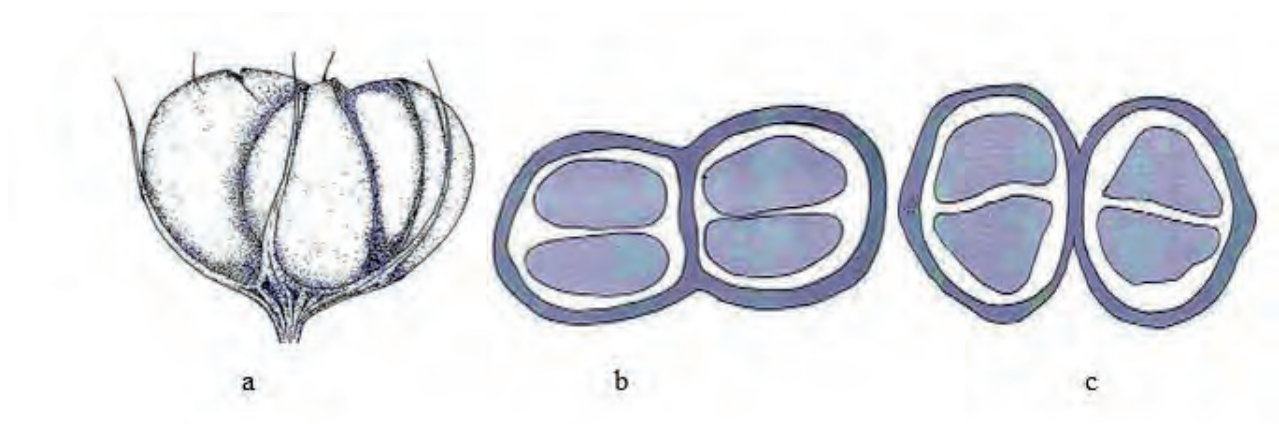


Figure 8. *Jasminum officinale* L.: (a) lateral view of the fruit ($\times 8$); (b) transverse section of the ovary ($\times 20$); (c) transverse section of the fruit ($\times 6$).

Anatomy

The exocarp is small, composed of a single layer of thin-walled cells that are strongly or slightly flattened; for example, in *Jasminum arborescens* Roxb. numerous stomata are present on the lateral surfaces. The cuticle becomes considerably thinner as the fruit matures but remains clearly visible in section. The mesocarp of *Jasminum* taxa is composed of heterocellular

parenchyma with diverse cell types: on the lateral surfaces it generally consists of 4–7 layers of flattened or isodiametric parenchyma (depending on species), the central part of up to 3 layers, and the dorsal surfaces up to 12 layers (Figure 9). In *Jasminum nudiflorum*, isolated cells with thickened, weakly lignified walls occur within the mesocarp of the dorsal surfaces, likely contributing to dehiscence; in some species

such cells are fewer and restricted to the dorsal surfaces, playing a minimal or no role in fruit opening. Within the parenchyma, vascular bundles lacking mechanical sheaths are observed. The endocarp is relatively large and strongly

flattened, composed of a single layer of thin-walled epidermal cells, although in *Jasminum officinale* L. the endocarp of the dorsal portions of the valves is occasionally two-layered.

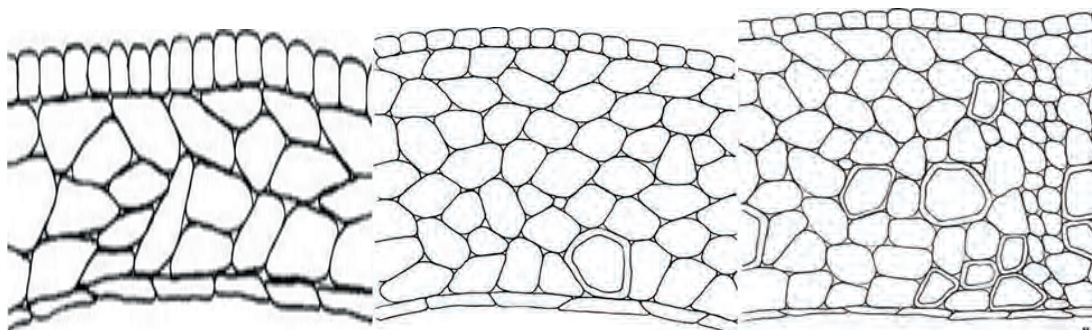


Figure 9. *Jasminum officinale* L.: (a) pericarp in the central part of the fruit ($\times 420$); (b) pericarp in the peripheral part (wing) on the lateral surface ($\times 420$); (c) pericarp in the dehiscence region ($\times 420$).

***Ligustrum* L. Morphology**

The fruits of taxa belonging to the genus *Ligustrum* L. develop from a superior, bicarpellary, syncarpous, medial pistil. The ovary is bicarpellary, with two anatropous ovules in each locule. Mature fruits are primarily ellipsoid, 5–11 mm long in most species (e.g., *Ligustrum sinense* Lour., 7 mm), and globose in some. The fruits of *Ligustrum ibota* Sieb. ex Sieb. et Zucc. are fleshy, black, glossy, and indehiscent. One of the most widely distributed representatives

is *Ligustrum vulgare* L., a deciduous shrub or small tree reaching 4–5 m in height, with opposite, simple, lanceolate, leathery leaves that are dark green above and lighter beneath and have a pointed apex. The flowers are white, fragrant, and arranged in racemose inflorescences, flowering from late June to early July. In transverse sections of mature fruits, one or two seeds may be observed, though typically there is only one; in *Ligustrum vulgare* the fruits may be seedless. The fruit is round and toxic (Figure 10).

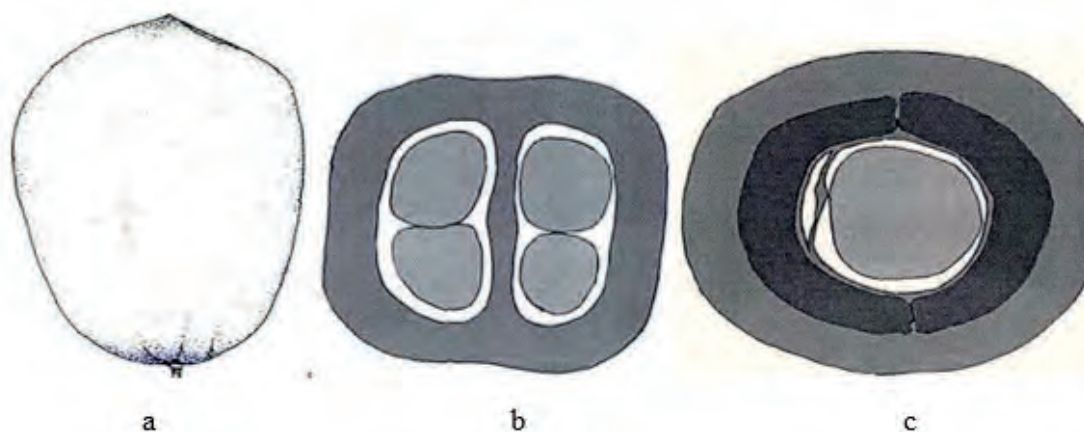


Figure 10. *Ligustrum vulgare* L.: (a) lateral view of the fruit ($\times 5$); (b) transverse section of the ovary ($\times 20$); (c) transverse section of the fruit ($\times 6$).

Anatomy

The exocarp consists of a single layer of papillate or slightly flattened epidermal cells (as in *L. vulgare*); beneath the epidermis, the

hypodermis is composed of cubical cells. The mesocarp is divided into two zones. The outer mesocarp, as in other genera, consists of isodiametric parenchyma cells arranged in

17–19 layers (occasionally 14–15 in *Ligustrum sinense*), within which a set of non-lignified conductive vessels is present. The inner mesocarp is composed of tangentially elongated cells with highly thickened, lignified walls. In most species, within the inner part of the mesophyll, tangentially oriented long sclereids, isodiametric sclereids, and fibrous sclereids occur along the dorsal sides of the fruit valves, and strands of small-celled parenchyma extend along the

dorsal vascular bundles and septa (Figure 11); in transverse section these appear as radial parenchymatous rays piercing the sclerenchyma ring. In *Ligustrum vulgare*, a distinct sclerenchymatous lignification zone is generally not observed; occasionally, groups of isodiametric sclereids occur within the mesocarp parenchyma, likely representing the foundation of the sclerenchymatous region. The endocarp consists of 2–5 layers of thin-walled, flattened cells.

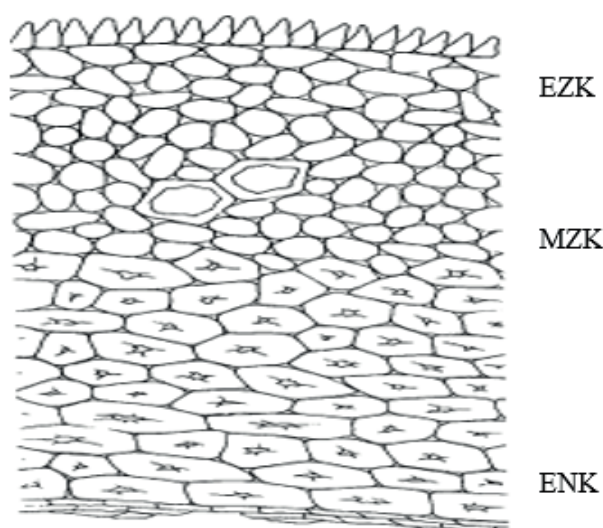


Figure 11. *Ligustrum vulgare* L.: anatomical structure of the pericarp in the central part of the fruit (×420). EZK, exocarp; MZK, mesocarp; ENK, endocarp.

Olea L. Morphology

The fruits of taxa belonging to the genus *Olea* L. develop from a superior, bicarpellary, syncarpous, medially oriented pistil. The ovary is bicarpellary, with one anatropous ovule in each locule, of which only one develops. Mature fruits are ellipsoid with a pointed apex, 15–21

mm long (Figure 12), reaching 25–27 mm in some species; they are fleshy, turn dark when ripe, and are indehiscent. In transverse section of mature fruits, a large smooth stone (endocarp) is observed, along with traces of a septum displaced by the seed and degenerated ovules.

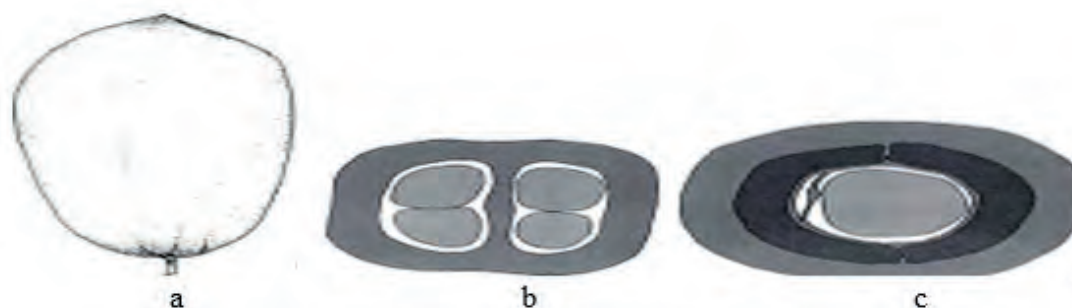
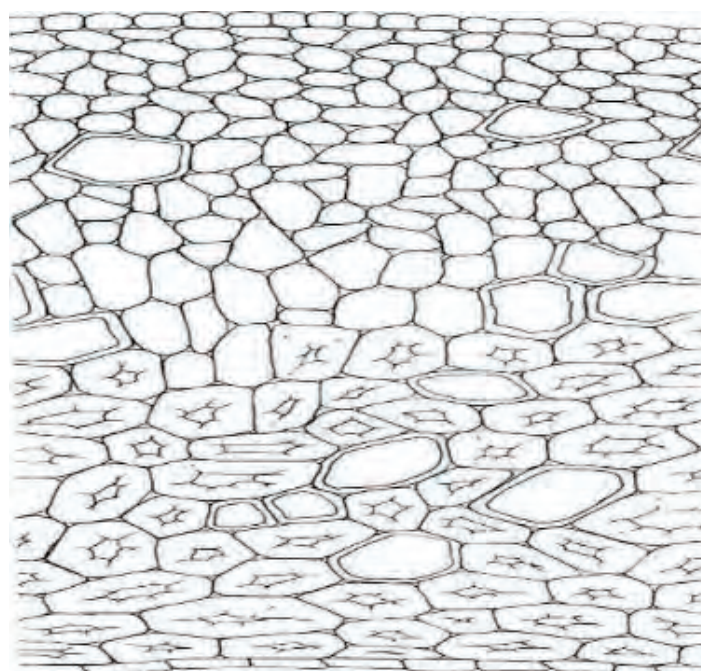


Figure 12. *Olea europaea*: (a) lateral view of the fruit (×3); (b) transverse section of the ovary (×25); (c) transverse section of the fruit (×4).

Anatomy

In taxa of the genus *Olea*, the exocarp consists of a single layer of isodiametric or slightly flattened thin-walled cells (as in *Olea europaea*), covered by a thick cuticle. The mesocarp is divided into two regions. The outer mesocarp is composed of about 30 layers of isodiametric parenchyma cells, within which numerous small isodiametric sclereids with slightly thickened walls are present; the inner mesocarp consists of multiple layers of isodiametric sclereids. Single cells with thickened but non-lignified walls are distributed among the sclereids. Along the dorsal sides of the carpels, parenchymatous connections within the sclerenchyma facilitate dehiscence, representing rudimentary elements of the fruit-opening mechanism; in mature fruits, once lignification is complete, these

connections are compressed by the surrounding sclerenchyma (Figure 13). In nature, the European olive (*Olea europaea*) grows as a tree or shrub 3–5 m high, with gray bark and long, often twisted branches (pendulous in some varieties). The leaves are simple, nearly sessile, leathery, narrowly lanceolate, entire, and gray-green, with a silvery underside, renewed every two to three years. Depending on climate, flowering occurs from late April to the end of June; the flowers are small, fragrant, whitish, and 3–4 mm long, each with two stamens, arranged in racemose inflorescences of 9–35 flowers, and pollination is primarily anemophilous. The fruit is a false single-seeded drupe, maturing 4–5 months after flowering. The species was first cultivated under domestication in Greece.



EZK

MZK

ENK

Figure 13. *Olea europaea*: anatomical structure of the pericarp in the central part of the fruit (×420). EZK, exocarp; MZK, mesocarp; ENK, endocarp.

Syringa L. Morphology

The fruits of taxa belonging to the genus *Syringa* L. develop from a superior, bicarpellary, syncarpous pistil with medial orientation. The ovary is bicarpellary, with two anatropous ovules in each locule, and the number of mature seeds generally corresponds to the number of ovules. Mature fruits are cylindrical, slightly flattened laterally, elongated, and curved at one end, 14–18 mm long and 6–9 mm in diameter; they are dry, protruding, and light brown. In transverse

section, the fruits show oval or circular-rhomboid outlines with clearly visible septa; on maturity they dry and dehisce loculicidally into two valves, releasing winged seeds. One of the widely distributed representatives is *Syringa vulgaris* L., the common lilac, a multi-stemmed, deciduous shrub 1.5–7 m high, with individual stem diameters of 15–20 cm. The bark of older plants is gray or grayish-brown, while young plants have smooth bark. The branches typically terminate in two (occasionally one) buds; the apical buds

are thick, quadrangular, sharp-pointed, greenish or reddish-brown, and 5–10 mm long, the

lateral buds more prominent than those on the main branches (Figure 14).

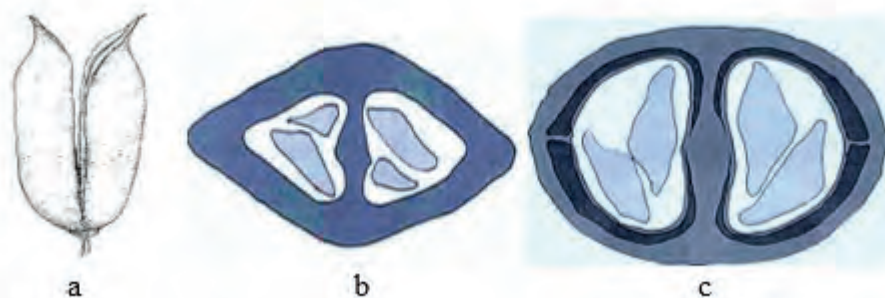


Figure 14. *Syringa vulgaris*: (a) lateral view of the fruit ($\times 8$); (b) transverse section of the ovary ($\times 20$); (c) transverse section of the fruit ($\times 6$).

Anatomy

The exocarp consists of a single layer of cubical (as in *Syringa amurensis*) or slightly flattened thin-walled cells, with a sufficiently thick cuticle on the fruit surface. The mesocarp is divided into two topographic zones. The outer zone is generally composed of parenchyma cells in 15–20 layers (up to 30 layers in *Syringa vulgaris*). Isodiametric sclereids with very thick walls are diffusely distributed, singly or in groups, among the parenchyma cells, and isolated sclereids also occur in the micropylar region. The inner zone consists of polygonal, variably elongated

sclerenchyma cells forming up to 25 layers (up to 30 in *Syringa josikaea*) (Figures 15, 16). On the dorsal sides of the carpels and in the central part of the septum, the thickened sclerenchyma is covered by parenchymatous strands of small isodiametric cells. Numerous vascular bundles run along the boundary between the outer and inner zones of the mesocarp. The endocarp is primarily a single layer of flattened, thin-walled epidermal cells (two epidermal layers in *S. reflexa* and *S. platyphylla*).

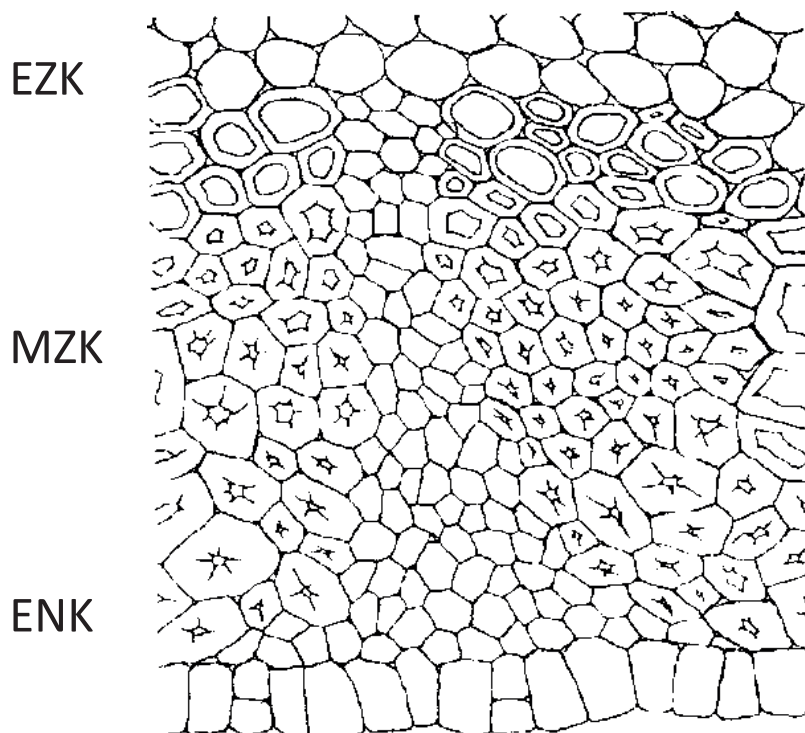


Figure 15. *Syringa vulgaris*: anatomical structure of the pericarp in the central part of the fruit ($\times 420$). MZK, mesocarp.

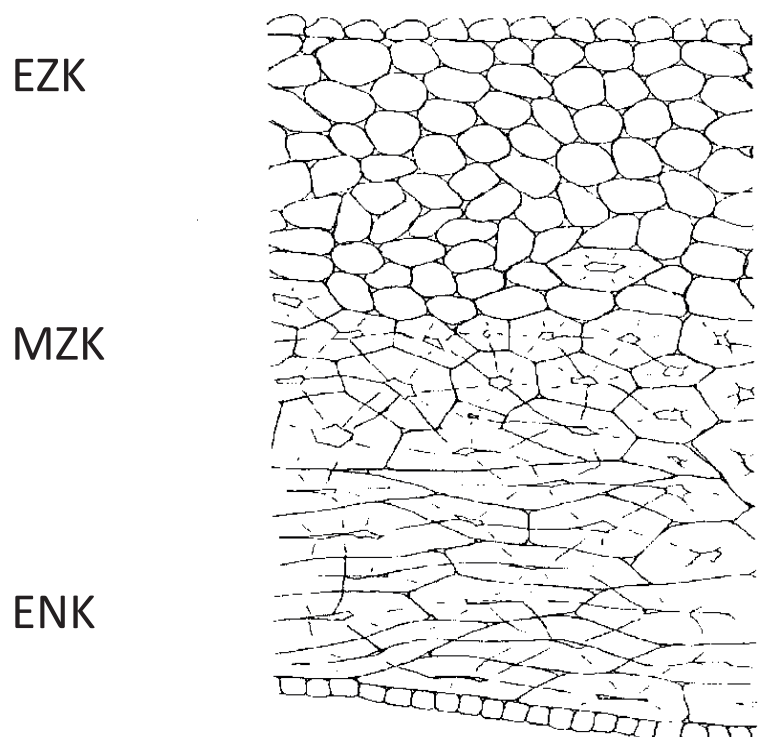


Figure 16. *Syringa vulgaris*: anatomy of the pericarp in the dehiscence region (×420). EZK, exocarp; MZK, mesocarp; ENK, endocarp.

CONCLUSION

This study presents the first comparative carpological analysis of eight genera representing eight taxa of the family Oleaceae — *Fontanesia* Labill., *Forestiera* Poir., *Forsythia* Vahl., *Fraxinus* Tourn. ex L., *Jasminum* L., *Ligustrum* L., *Olea* L., and *Syringa* L. — based on original field and laboratory material collected in situ and ex situ conditions in Azerbaijan. The investigation encompassed both morphological characterization of fruit form, dimension, and dehiscence, and detailed histological analysis of the three principal pericarp zones: exocarp, mesocarp, and endocarp.

Morphological examination revealed considerable diversity in fruit type and form across the studied genera. *Fontanesia* produces dry, laterally flattened, disc-shaped, indehiscent fruits with winged ridges (6–11 mm diameter). *Forestiera* bears fleshy, bluish-black, indehiscent fruits (7–10 mm). *Forsythia* develops dry, dehiscent capsules with winged seeds. *Fraxinus* produces dry, smooth, indehiscent samaras. *Jasminum* fruits are dry, yellowish-green, and predominantly indehiscent, with only rare dorsal dehiscence. *Ligustrum* and *Olea* bear fleshy,

dark-coloured fruits, consistent with their classification as drupes. *Syringa* produces ovate, laterally flattened, indehiscent capsules.

Anatomical analysis of the pericarp demonstrated a fundamentally uniform three-layered organization (exocarp–mesocarp–endocarp) across all studied genera, alongside a range of genus- and species-level structural specializations. The exocarp in all taxa is composed of a single layer of thin-walled epidermal cells covered by a thick cuticle, with stomata present in varying densities. The mesocarp exhibits the greatest histological variation: in dry-fruited taxa it is dominated by sclerenchymatous tissue and vascular bundles, whereas in fleshy-fruited genera (*Olea*, *Ligustrum*) it is primarily parenchymatous, rich in pigmented cells. The endocarp ranges from a single thin-walled epidermal layer in most genera to a double-layered arrangement in certain species of *Fontanesia* and *Syringa*.

A notable finding of this study is that pericarp anatomy is stable under ex situ cultivation conditions and in properly preserved herbarium and carpological specimens: no structural changes were detected relative to in situ material in any of the studied taxa. This result confirms

the reliability of herbarium and carpological collections as sources for comparative anatomical investigations in the Oleaceae.

The data obtained expand the morpho-anatomical knowledge base for the Oleaceae in the Caucasus region and may serve as diagnostic criteria in taxonomic circumscription at both genus and species levels. Collectively, the structural characteristics of the pericarp across the eight

investigated genera reflect the broad ecological and functional diversity of the family, encompassing dry dehiscent and indehiscent fruits, fleshy drupes, and samaras — adaptations that underpin the wide natural distribution and successful introduction of Oleaceae representatives into the dendrological collections of Azerbaijan.

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ARTICLE 5

The Effect of NaCl and Na₂SO₄ on Biometric and Physiological Parameters of the Common Bean Landrace Phaseolus vulgaris ‘Piyada’

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ABSTRACT

The agrochemical properties of a forest soil and the effects of NaCl and Na₂SO₄ salt stress on the local “Piyada” variety of common bean (*Phaseolus vulgaris* L.) were studied. A forest soil sample from the Tovuz–Gazakh belt showed high natural fertility, with a high humus content and adequate supplies of ammonium and nitrate nitrogen, mobile phosphorus, and exchangeable potassium; analysis of its water-soluble salts showed a low salt content and no carbonate salinization, confirming that the soil is non-saline and ecologically stable. Salinization of this soil with NaCl or Na₂SO₄ at increasing concentrations raised the electrical conductivity and shifted the pH from slightly alkaline toward neutral, with NaCl exerting a stronger effect than Na₂SO₄. In the bean plant, increasing salt stress markedly reduced the germination percentage, germination energy, seedling, root and shoot length, and the tolerance index, and lowered the dry biomass of shoots and roots while slightly increasing tissue moisture content, interpreted as an adaptive water-retention response. Salt stress also disrupted the leaf water balance: the relative water content decreased, the water deficit increased, and the leaf area was reduced as an adaptive means of limiting transpiration. In 7-, 10-, and 14-day-old seedlings, the contents of chlorophyll a, chlorophyll b, and carotenoids declined and the chlorophyll a/b ratio rose, indicating damage to the photosynthetic apparatus and a weakening of photosynthesis and of the antioxidant defense system. Across all parameters, NaCl produced a more pronounced negative effect than Na₂SO₄, showing that the type of salt — and the higher toxicity of chloride relative to sulfate ions — plays a significant role in the growth and development of the common bean under salinity.

Keywords: NaCl and Na₂SO₄ salt stress, photosynthetic pigments, common bean (*Phaseolus vulgaris* L.), morphological parameters, biometric parameters, physiological parameters.

INTRODUCTION

Salinity is one of the most significant abiotic stress factors affecting agriculture in the modern era. The accumulation of dissolved salts in the soil, particularly compounds such as NaCl and Na₂SO₄, reduces the osmotic potential of the soil, impairs the ability of plants to absorb water and nutrients, and consequently slows their growth and development (Munns & Tester, 2008; Hasanuzzaman et al., 2018). On a global scale, salinization is widespread and significantly reduces agricultural productivity (FAO, 2015, 2021).

In the Republic of Azerbaijan, soil salinization

is one of the major ecological and agricultural problems. In particular, in the Shirvan, Mil-Mugan, and Absheron regions, a significant portion of the soil has been affected by salinity, which negatively affects the growth and development of agricultural crops. As a result of salinity, the ionic balance in the soil is disrupted, and the predominance of sodium ions weakens the functioning of the root system and limits water uptake (Hanin et al., 2016; Olatunbosun et al., 2023; Jafarzadeh, 2025).

The common bean (*Phaseolus vulgaris* L.) is an important source of food and protein and belongs to a group of plants that are relatively

sensitive to salinity. Under salt stress, the germination percentage and germination energy of bean plants decrease, which is associated with a reduction in the physiological activity of the seeds (Shahbaz & Ashraf, 2020); retardation is also observed during the vegetative growth stage, leading to a decline in productivity (Olatunbosun et al., 2023).

The effects of salinity on the common bean are manifested in various morphological, biometric, and physiological parameters. Specifically, reductions in root and shoot length, as well as decreases in fresh and dry biomass, are observed and are associated with the inhibition of cell division and elongation (Parida & Das, 2005). Moreover, increased water deficit and water loss reflect a disruption of the plant's osmotic balance (Munns & Gilliam, 2015), and a decline in photosynthetic pigments is linked to reduced chlorophyll synthesis and increased oxidative stress (Gill & Tuteja, 2017).

Thus, salinity is a significant stress factor that exerts a complex influence on the morphological, biometric, and physiological parameters of plants. In this regard, studying the response of the common bean to salt stress and investigating its tolerance mechanisms are of great scientific and practical importance for agriculture.

MATERIALS AND METHODS

A soil sample from the Tovuz–Gazakh forest belt was used as the soil culture. The agrochemical properties of the soil (humus; ammonium and nitrate nitrogen; phosphorus; and potassium) and its ionic composition were determined under laboratory conditions. Saline soils at different concentrations (0.2%, 0.4%, 0.6%, 0.81%) were prepared in the laboratory using NaCl and Na₂SO₄, and the electrical conductivity (dS/m) and pH of the treated samples were measured with an EC meter and a pH meter, respectively.

The germination percentage was calculated as $A = (n / m) \times 100\%$, where A is the germination percentage, n the number of germinated seeds, and m the total number of seeds. Dry biomass was determined after drying roots and leaves in a thermostat at 80 °C for 48 h (Tunc-turk, 2019): Dry matter (%) = $m(\text{dry}) / m(\text{fresh}) \times 100\%$, and Moisture (%) = $100 - \text{Dry matter} (\%)$.

The tolerance index was calculated as $TI = (\text{plant height under stress} / \text{plant height in the control}) \times 100\%$.

The relative water content (RWC) of leaves was determined as $RWC = [(M_f - M_d) / (M_t - M_d)] \times 100\%$, where M_f is the fresh mass, M_d the dry mass, and M_t the turgid (water-saturated) mass (Tabbusi, 2005). The water deficit of the leaves was determined following Khramchenkova (2016) as $WD = [(M_t - M_f) / (M_t - M_d)] \times 100\%$.

The photosynthetic pigments — chlorophyll a, chlorophyll b, and carotenoids — were determined spectrophotometrically (Lichtenthaler, 1987). About 0.1 g of fresh plant material was homogenized in a mortar with 5 mL of 96% ethanol, and the absorbance of the extracts was measured at 440.5, 644, and 662 nm with a Genesys 20 spectrophotometer (Thermo Scientific, Madison, WI, USA). Pigment concentrations (mg/L) were calculated using the Holm–Wettstein equations: $Chl\ a = 9.784 \times D_{662} - 0.990 \times D_{644}$; $Chl\ b = 21.426 \times D_{644} - 4.650 \times D_{662}$; $Car = 4.695 \times D_{440.5} - 0.268 \times (Chl\ a + Chl\ b)$. The results were processed statistically (Kerdyatov, 2018); the standard error of the mean and the precision index were calculated, and the precision index was below 3%.

RESULTS AND DISCUSSION

Agrochemical properties of the forest soil

The agrochemical characteristics of the control (forest soil) sample from the Tovuz–Gazakh forest zone are important for assessing soil fertility and nutrient availability. The humus content was 4.5% (Table 1), a high value; humus plays a key role in the formation of soil structure, the improvement of water-physical properties, and the enhancement of nutrient availability (Lal, 2015; Lehmann & Kleber, 2015). The ammonium-nitrogen content was 29.93 mg/kg, indicating a sufficient supply of readily assimilable nitrogen closely associated with soil microbial activity and important during early plant development (Zhang X. et al., 2016). The nitrate-nitrogen content was 43.86 mg/kg, indicating active nitrification; the nitrate form is rapidly absorbed and plays a key role in productivity (Singh, 2021).

Table 1. Degree of nutrient provision of the soil (control, forest soil).

Sample	Humus, %	Ammonium (N-NH ₄), mg/kg	Nitrate (NO ₃), mg/kg	Phosphorus (P ₂ O ₅), mg/kg	Potassium (K ₂ O), mg/kg
Control (forest soil)	4.5±0.2	29.93±1.1	43.86±0.52	43.75±0.51	126.52±0.31

* $p < 0.001$.

The mobile-phosphorus (P₂O₅) content was 43.75 mg/kg, indicating a sufficient phosphorus supply; phosphorus participates in energy metabolism, stimulates root development, and contributes to the formation of generative organs (Shen et al., 2018). The exchangeable-potassium (K₂O) content was 126.52 mg/kg, indicating a high potassium supply; potassium regulates the plant water regime, activates enzymatic processes, and increases resistance to stress (Wang et al., 2019; Raza et al., 2020).

Ionic composition of water-soluble salts

Carbonate ions (CO₃²⁻) were not detected, indicating the absence of carbonate salinization,

and the bicarbonate (HCO₃⁻) content indicated a weakly alkaline character (Table 2). The chloride (Cl⁻) content was low and non-toxic to plants, and although sulfate (SO₄²⁻) ions were present, their levels did not pose a salinization risk (Raza et al., 2020). Among the cations, calcium (Ca²⁺) and magnesium (Mg²⁺) contribute to the formation and stability of soil structure, and the sodium-plus-potassium (Na⁺ + K⁺) content indicated that sodium was not at a hazardous level (Wang et al., 2019). The dry residue was 0.233% and the total salt content 0.204 g/kg, confirming that the soil is non-saline or very weakly saline.

Table 2. Ionic composition of dissolved salts (each cell: % / mg-equivalent).

Sample	CO ₃ ²⁻	HCO ₃ ⁻	Cl ⁻	SO ₄ ²⁻	Ca ²⁺	Mg ²⁺	Na ⁺ +K ⁺	Dry residue, %	Total salts, g/kg
Control	Absent	0.031 / 0.5	0.021 / 0.6	0.093 / 1.94	0.028 / 1.4	0.007 / 0.61	0.024 / 1.03	0.233	0.204

Effect of salt stress on soil electrical conductivity and pH

Increasing soil salinity produced significant changes in the physicochemical properties of the soil (Table 3). The electrical conductivity (EC) in the control was 0.58 dS/m, indicating weak mineralization. NaCl caused a sharp increase in EC (to 1.49, 3.14, and 5.65 dS/m at 0.2%, 0.6%, and 1%, respectively), reflecting a higher concentration of dissolved ions and a rise in salinization (Rengasamy, 2016; Shrivastava & Kumar, 2015). Na₂SO₄ also increased EC (1.30, 2.15,

and 3.53 dS/m), but less than NaCl, which is attributed to the higher mobility and ionic activity of chloride relative to sulfate ions (Isayenkov & Maathuis, 2019). The control pH was slightly alkaline (7.9); NaCl lowered it toward neutrality (7.1–7.0), and Na₂SO₄ produced a smaller shift (7.3–7.2), reflecting the influence of salts on the ionic balance and hydrolysis in the soil solution (Qadir et al., 2018).

Table 3. Effect of salt stress on soil electrical conductivity and pH.

Soil culture	EC (dS/m)	pH
Control	0.58±0.03	7.9±0.31
0.2% NaCl	1.49±0.01	7.1±0.22
0.6% NaCl	3.14±0.02	7.05±0.21
1% NaCl	5.65±0.35	7.0±0.22

Soil culture	EC (dS/m)	pH
0.2% Na ₂ SO ₄	1.3±0.01	7.3±0.23
0.6% Na ₂ SO ₄	2.15±0.01	7.25±0.23
1% Na ₂ SO ₄	3.53±0.15	7.2±0.22

* $p < 0.001$.

Effect of salt stress on germination parameters

In the control, both germination percentage and germination energy were 100%. NaCl caused a sharp decline: germination and germination energy fell to 75% and 60% at 0.2%, to 65% and 40% at 0.4%, and to 40% and 20% at 0.6%, respectively (Figure 1). This reduction reflects the osmotic effect of salt, which impairs water uptake by the seeds, and increased ion toxicity (Shrivastava & Kumar, 2015). Na₂SO₄ produced a weaker effect (germination 90%,

80%, and 60%, and germination energy 80%, 70%, and 50% at 0.2%, 0.4%, and 0.6%, respectively), attributed to the lower toxicity of sulfate relative to chloride ions (Abdiyev et al., 2019; Isayenkov & Maathuis, 2019). The more rapid decline of germination energy than of germination percentage indicates that the early metabolic activity of the seeds is more sensitive to salt stress, owing to the weakening of enzymatic processes and disruption of water balance at the cellular level (Rengasamy, 2016).

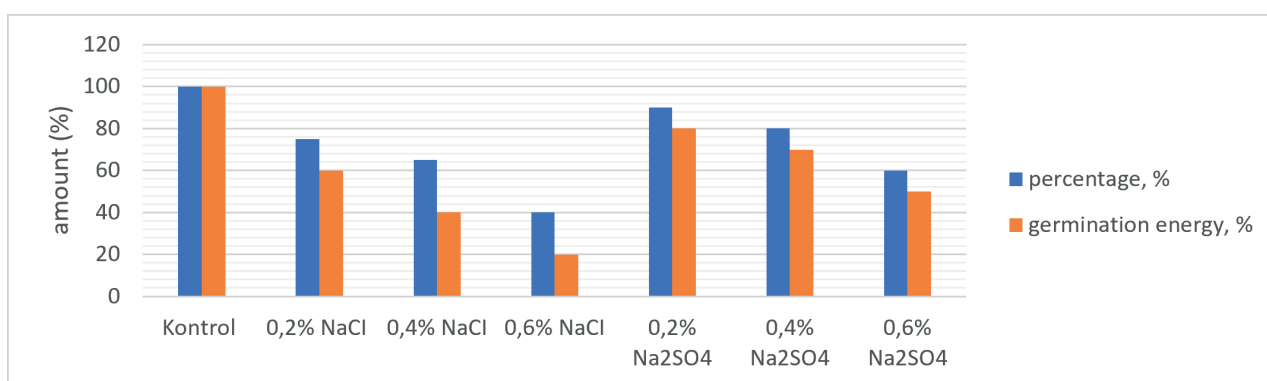


Figure 1. Germination percentage and germination energy of bean seedlings under NaCl and Na₂SO₄ stress.

Effect of salt stress on growth parameters

In the control, the total seedling length was 17.3 cm, root length 8.8 cm, and shoot length 8.5 cm, indicating optimal growth. NaCl sharply reduced all parameters: seedling length fell to 9.3, 7.4, and 3.3 cm at 0.2%, 0.4%, and 0.6%, respectively, with root and shoot length decreasing similarly; the more rapid reduction in shoot length indicates that the above-ground organs are more sensitive to salt stress. The tolerance index correspondingly decreased from 53.7% to 19% (Figure 2). These changes reflect reduced water uptake due to the osmotic effect of salt and the toxicity of Na⁺ ions (Shrivastava

& Kumar, 2015; Rengasamy, 2016). Na₂SO₄ produced a smaller reduction (seedling length 12.1, 9.7, and 6.2 cm; tolerance index 69.9%–35.8%), with root length relatively better maintained, indicating some adaptive capacity of the root system and the weaker toxicity of sulfate ions (Isayenkov & Maathuis, 2019). Overall, the smaller reduction in root length than in shoot length reflects the plant's adaptive mechanisms for acquiring water and nutrients, although at high salt concentrations these mechanisms become insufficient and overall growth is strongly inhibited.

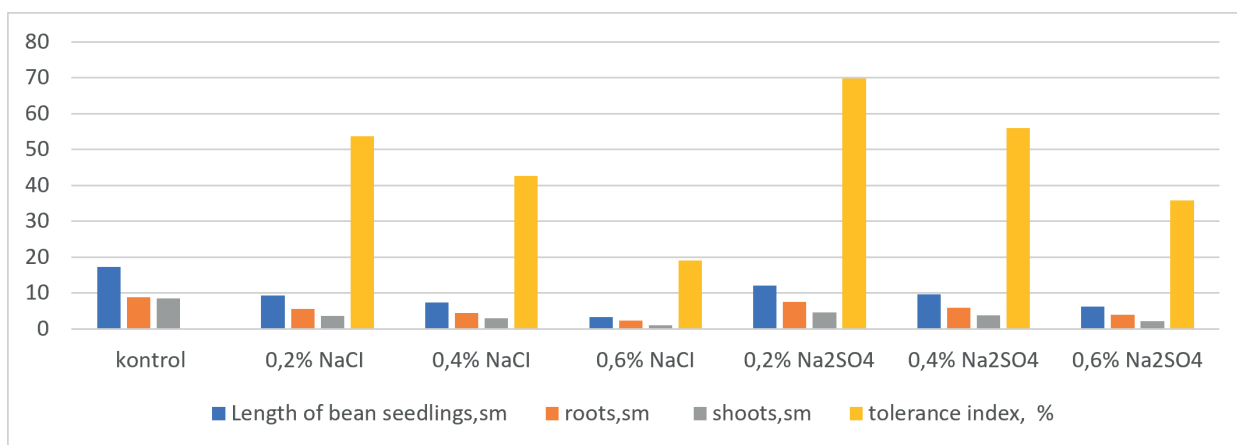


Figure 2. Length of bean seedlings, roots and shoots, and tolerance index under NaCl and Na₂SO₄ stress.



Figure 3. Bean seedlings grown in soil culture under (a) NaCl and (b) Na₂SO₄ salinity.



Figure 4. Bean seedlings grown in soil culture.

Effect of salt stress on dry biomass and moisture content

In the control, the shoot dry matter was 10.85% and the root dry matter 3.17% (Table 4), indicating high metabolic activity under normal conditions. NaCl gradually reduced the dry matter (shoot and root to 10.61% and 2.96% at 0.2%, and to 10.24% and 2.41% at 0.6%), reflecting inhibition of photosynthesis and reduced carbon assimilation (Lal, 2015; Lehmann & Kleber, 2015). Na₂SO₄ also reduced the dry matter, but less (shoot and root 10.57% and 2.99% at 0.2%; shoot 9.36% at 0.6%), confirming the

lower toxicity of sulfate ions (Raza et al., 2020). The moisture content of both shoot and root increased slightly in all treatments; at high salt concentrations this increase is associated with the plant's attempt to maintain osmotic balance by retaining water in the tissues. The higher moisture level of the root reflects its primary role in the direct uptake of water and ions, although with increasing salt stress the water-absorption capacity of the root system decreases and biomass formation is negatively affected (Shen et al., 2018).

Table 4. Dry matter and moisture content of the bean plant (%).

Variant	Dry matter, stem	Dry matter, root	Moisture, stem	Moisture, root
Control	10.85±0.36	3.17±0.16	89.15±3.5	96.83±3.9
0.2% NaCl	10.61±0.36	2.96±0.11	89.39±3.5	97.04±3.9
0.4% NaCl	10.49±0.36	2.74±0.11	89.51±3.5	97.26±3.9
0.6% NaCl	10.24±0.36	2.41±0.1	89.76±3.5	97.59±3.9
0.2% Na ₂ SO ₄	10.57±0.36	2.99±0.11	89.43±3.5	97.01±3.9
0.4% Na ₂ SO ₄	10.19±0.36	2.85±0.11	89.81±3.5	97.15±3.9
0.6% Na ₂ SO ₄	9.36±0.35	2.53±0.11	90.64±3.5	97.47±3.9

* $p < 0.001$.

Effect of salt stress on leaf parameters

In the control, the relative water content (RWC) was 100% with no water deficit, and the leaf area was 23 cm² (Figure 5). NaCl sharply reduced RWC (to 72%, 61%, and 42% at 0.2%, 0.4%, and 0.6%), while the water deficit rose correspondingly from 28% to 58%, reflecting the reduction of soil water potential and the consequent impairment of water uptake (Shrivastava & Kumar, 2015; Rengasamy, 2016). Na₂SO₄ produced weaker effects (RWC 89% and a water deficit of only 11% at 0.2%, and RWC maintained

at 57% at 0.6%), indicating that sulfate ions are relatively less harmful (Isayenkov & Maathuis, 2019). Leaf area decreased in all treatments — more strongly under NaCl (14 cm² → 10 cm²) than under Na₂SO₄ (17.4 cm² → 15 cm²). The increase in water deficit and the reduction in leaf area are adaptive defense responses that limit transpiration and water loss while maintaining metabolic activity (Rengasamy, 2016; Raza et al., 2020).

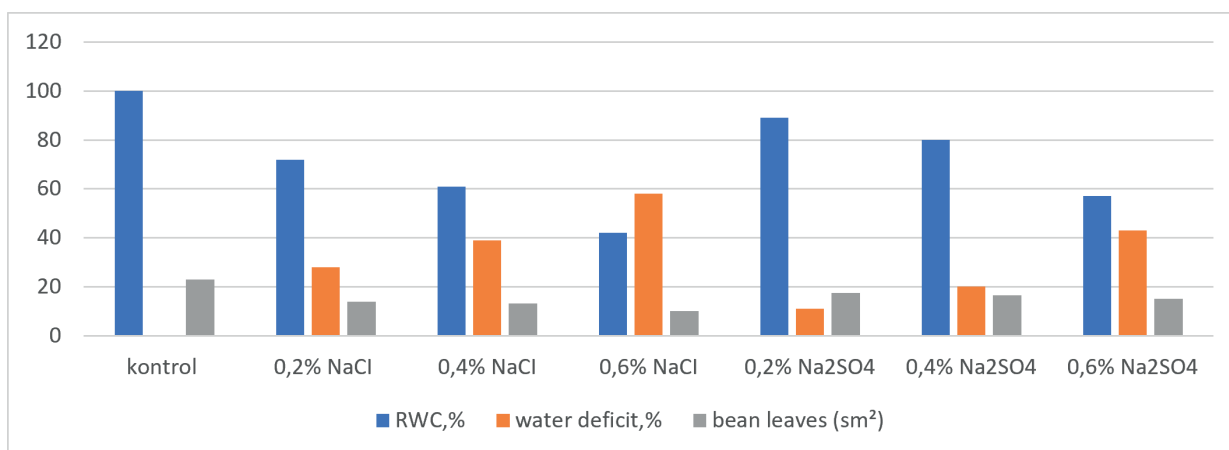


Figure 5. RWC, water deficit, and leaf area of bean leaves under NaCl and Na₂SO₄ stress.

Effect of salt stress on photosynthetic pigments

High salinity reduces chlorophyll synthesis and disrupts the structural integrity of chloroplasts (Zhang H. et al., 2016; Hasanuzzaman et al., 2018). Both NaCl and Na₂SO₄ decreased chlorophyll a, chlorophyll b, and total pigments (Chl a + b) in the seedlings, reflecting reduced photosynthetic activity (Parida & Das, 2005; Olatunbosun et al., 2023) (Table 5). In 7-day-old seedlings, the effect of NaCl was more pronounced; the reduction in chlorophyll a and b is associated with damage to photosystem II (Munns & Tester, 2008), and the faster decline of chlorophyll b increased the Chl a/b ratio, indicating structural changes in the photosynthetic apparatus, while the decrease in carotenoids indicates a weakening of antioxidant defense (Gill & Tuteja, 2017; Jafarzade, 2022). Under Na₂SO₄ the decrease in pigments was smaller, reflecting

the lower toxicity of sulfate ions (Hanin et al., 2016).

In 10-day-old seedlings, the effect of salt stress persisted and the reduction in pigment content became more pronounced; despite the development of certain adaptive mechanisms, the decrease occurred more rapidly under NaCl (Shahbaz & Ashraf, 2020), and the greater sensitivity of chlorophyll b again raised the Chl a/b ratio. In 14-day-old seedlings, partial adaptation was observed after prolonged stress; nevertheless, the reduction in pigments remained more pronounced under NaCl, associated with oxidative damage to chloroplast membranes (Hosain et al., 2015), whereas under Na₂SO₄ the decrease was more stable and relatively mild. These results are consistent with previous studies confirming the negative impact of salt stress on plant physiology (Munns & Tester, 2008; Hasanuzzaman et al., 2018).

Table 5. Effect of salt stress on the content of photosynthetic pigments (Chl a, Chl b, carotenoids; mg/L) in bean seedlings.

Variant	Control	0.2%	0.4%	0.6%
7-day-old seedlings				
Chl a (NaCl)	74.4	59.4	41.7	35.4
Chl b (NaCl)	29.4	21.4	13.7	7.3
Car (NaCl)	41.4	35.4	24.2	15.4
Chl a+b (NaCl)	103.8	80.8	55.4	42.7
Chl a/b (NaCl)	2.53	2.775	3.043	4.849
Chl a (Na ₂ SO ₄)	74.4	62.4	49.3	37.8
Chl b (Na ₂ SO ₄)	29.4	25.1	14.4	11.3

Variant	Control	0.2%	0.4%	0.6%
Car (Na ₂ SO ₄)	41.4	38.9	33.1	25.0
Chl a+b (Na ₂ SO ₄)	103.2	87.5	63.7	49.1
Chl a/b (Na ₂ SO ₄)	2.53	2.486	3.423	3.345
10-day-old seedlings				
Chl a (NaCl)	81.319	65.593	50.36	39.365
Chl b (NaCl)	32.775	28.283	14.36	10.325
Car (NaCl)	49.091	38.869	29.36	20.321
Chl a+b (NaCl)	114.094	93.876	64.72	49.69
Chl a/b (NaCl)	2.481	2.319	3.506	3.812
Chl a (Na ₂ SO ₄)	81.319	69.247	51.215	42.26
Chl b (Na ₂ SO ₄)	32.775	29.396	15.955	13.311
Car (Na ₂ SO ₄)	49.091	41.01	36.715	29.338
Chl a+b (Na ₂ SO ₄)	114.094	98.643	67.17	55.571
Chl a/b (Na ₂ SO ₄)	2.481	2.355	3.209	3.174
14-day-old seedlings				
Chl a (NaCl)	66.2	56.2	47.6	39.2
Chl b (NaCl)	24.6	19.7	17.4	14.4
Car (NaCl)	42.1	38.5	33.3	26.4
Chl a+b (NaCl)	90.8	75.9	65.0	53.6
Chl a/b (NaCl)	2.691	2.852	2.735	2.722
Chl a (Na ₂ SO ₄)	66.2	54.0	53.3	40.5
Chl b (Na ₂ SO ₄)	24.6	23.8	22.4	20.1
Car (Na ₂ SO ₄)	42.1	39.2	35.5	29.0
Chl a+b (Na ₂ SO ₄)	90.8	77.8	75.7	60.6
Chl a/b (Na ₂ SO ₄)	2.691	2.268	2.379	2.014

* Precision index < 3%.

CONCLUSION

- The forest soil possesses high humus reserves and an adequate supply of essential nutrients, confirming its high natural fertility and suitability for crop cultivation.
- The ionic composition of its water-soluble salts shows a low salt content and no salinization risk; the soil is non-saline.
- Increasing salt concentration raises soil electrical conductivity and shifts the pH toward neutrality; NaCl affects soil salinity more strongly than Na₂SO₄.
- Increasing salt stress significantly reduces germination, morphological parameters, and

the tolerance index of bean seeds, with NaCl exerting a stronger negative effect than Na₂SO₄.

- Increasing salt stress reduces the dry biomass of bean plants (NaCl > Na₂SO₄), while a relative increase in moisture content is observed as an adaptive response.
- Salt stress disrupts the leaf water balance, decreasing RWC and increasing water deficit, and thereby reducing leaf area (NaCl > Na₂SO₄).
- Salt stress decreases photosynthetic pigments and photosynthetic efficiency, with NaCl more pronounced than Na₂SO₄, owing to the higher toxicity of chloride ions.

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ARTICLE 6

Comparative Analysis of Biochemical Indicators (Protein, Lysine, and Tryptophan) in Soybean (*Glycine max* (L.) Merr.) Genotypes in the Nakhchivan Autonomous Republic

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ABSTRACT

The provision of the population with plant-based protein is a pressing issue in the Nakhchivan Autonomous Republic. Among the leguminous crops of the world, soybean (*Glycine max* (L.) Merr.) occupies the largest cultivated area and is a valuable technical, fodder, and food plant whose grain and green mass contain a large amount of high-quality protein, closer in amino-acid composition to animal protein than that of most other legumes. The aim of the present research was to identify soybean samples with high productivity and a high content of protein, lysine, and tryptophan for use in future selection work in the region. Sixteen soybean varieties, obtained from the Genetic Resources Institute and the Scientific Research Institute of Agriculture of the Ministry of Science and Education of the Republic of Azerbaijan, were evaluated during 2021–2022 on irrigated gray soils in the experimental field of the Institute of Bioresources (Nakhchivan). The content of grain protein was determined by the Kjeldahl method (nitrogen $\times$ 6.25), lysine by the Muséiko–Sysoyeva method, and tryptophan by the Ermakov–Yarosh method, using an infrared analyzer (LOMO Spektran-119M). Analysis of variance based on the two-year means revealed statistically significant differences among varieties for all three parameters ($p < 0.01$). The protein content varied from 27.0% (CU-4) to 34.3% (Bravo*), the lysine content from 593 to 927 (in the units reported by the authors), and the tryptophan content from 0.50 to 0.80. The varieties Bravo*, Regale*, Regalia, Regale, and Kanata were distinguished by high protein contents. Bravo* also had the highest lysine and tryptophan contents, whereas CU-4 had the lowest values for all three parameters. Cluster analysis of the biochemical indicators grouped the 16 samples into three clusters, with a mean genetic diversity index of 0.482. The varieties Bravo* and Regale* are proposed for use in future soybean selection work in the region.

Keywords: soybean, *Glycine max*, protein, amino acids, lysine, tryptophan, Nakhchivan.

1. INTRODUCTION

Protein is one of the most necessary components of the human diet, and providing the population with adequate protein-rich and high-energy foods is one of the fundamental agricultural and social challenges. Because the growth of the world population outpaces the growth of protein production, the search for high-yielding plant sources of protein, particularly among cereals, legumes and oilseeds, has become increasingly

important.

Among these crops, soybean (*Glycine max* (L.) Merr.) is of primary importance for solving the protein problem, as its grain and green mass contain a large amount of high-quality protein together with vegetable oil. Unlike most cereals and other legumes, its protein is close to animal protein in amino-acid composition and is rich in essential amino acids such as lysine, tryptophan, and methionine (Leshchenko et al., 1987; Soya,

1981). Soybean farms can therefore obtain two valuable products from the same crop, complete protein and vegetable oil, and the protein is highly soluble in water and easily digestible.

Grain legumes also play an important role in the national economy: their cultivation increases grain reserves, helps to solve the problem of protein feed, and enriches the soil by fixing atmospheric nitrogen (Asadova et al., 2016). Rich in protein, fat, vitamins and essential amino acids, legumes have been used in Azerbaijani cuisine as the main ingredient of national dishes since ancient times, so that collecting and preserving both the local and the global gene pool of legumes and selecting promising samples for the relevant regions are important tasks (Amirov et al., 2014). Under the diverse soil and climatic conditions of the Nakhchivan Autonomous Republic, the creation of productive, high-quality, adaptive varieties, resistant to adverse environmental factors, diseases and pests, is particularly relevant (Mirzayev et al., 2014).

The aim of the present research was to identify high-quality samples by determining the grain content of protein, lysine, and tryptophan in local and introduced soybean varieties evaluated in the Nakhchivan Autonomous Republic, in order to select promising material for further selection work.

2. MATERIALS AND METHODS

Sixteen soybean varieties, obtained from the Genetic Resources Institute and the Scientific Research Institute of Agriculture of the Ministry of Science and Education of the Republic of Azerbaijan, were used as research material. The field trials were conducted during 2021–2022 on irrigated gray soils in the experimental field of the Institute of Bioresources of the Ministry of Science and Education of the Republic of Azerbaijan (Nakhchivan).

Biochemical analyses of protein, lysine, and tryptophan in the soybean grain were carried out at the “Biochemical Genetics and Technology” laboratory of the Genetic Resources Institute and at the “Cereal, Legume and Technical Plants” laboratory of the Institute of

Bioresources (Nakhchivan) using an infrared analyzer (LOMO Spektran-119M).

The grain protein content was determined by the Kjeldahl method (Ermakov, 1969). A finely ground sample of 80–100 mg was placed in a Kjeldahl flask, 5–7 mL of concentrated sulfuric acid and 1 g of catalyst were added, and the mixture was digested and then titrated with alkali. The nitrogen content thus determined was multiplied by 6.25 to calculate the crude-protein content.

Lysine was determined by the method of Muséiko and Sysoyeva (Ermakov, 1972): 30 mg of flour was placed in a test tube in duplicate and hydrolyzed in 2% Na₂CO₃ in a water bath at 80 °C for 10 min.

Tryptophan was determined by the method of Ermakov and Yarosh (Ermakov & Yarosh, 1969): 200 mg of powder was taken from the ground sample, transferred to a 100 mL volumetric flask, and hydrolyzed at 40 °C for 18–20 h by adding a 4% gelatin solution dissolved in 25% KOH.

Analysis of variance and cluster analysis of the biochemical indicators were performed on the basis of the two-year means; the precision index of the measurements did not exceed 3%.

3. RESULTS AND DISCUSSION

The content of protein in the soybean samples was measured on plants cultivated under irrigation in 2021–2022. During the biochemical analyses, a number of varieties were found to have higher values of the studied parameters in the grain.

In the first year of the study (2021), the highest protein content was recorded in Bravo* (33.9%), Regale* (33.4%), Regalia (33.1%), and Regale (32.9%), and the lowest in CU-14 (27.9%), CU-1 (27.5%), Kioto (27.9%), and CU-4 (26.5%). The highest lysine content was recorded in Bravo* (924), Regale* (848), and Regale (805), and the lowest in Angelica (598) and CU-4 (586). The highest tryptophan content was recorded in Bravo* (0.82), Regale* (0.80), and Bravo (0.80), and the lowest in Kofu st. (0.57) and CU-4 (0.55).

In the second year (2022), the highest

protein content was recorded in Bravo* (34.7%), Regalia (33.9%), Regale* (33.8%), and Regale (33.7%), and the lowest in CU-14 (28.9%), CU-1 (27.9%), and CU-4 (27.5%). The highest lysine content was recorded in Bravo* (930), Regale* (856), and Regale (815), and the lowest in Angelica (610) and CU-4 (600). The highest tryptophan content was recorded in Bravo* (0.78), Regale* (0.70), and Bravo (0.70), and the lowest

in Kofu st. (0.53) and CU-4 (0.45).

Analysis of variance based on the two-year means revealed statistically significant differences among varieties for protein, lysine, and tryptophan ($p < 0.01$). The highest two-year mean protein content (34.3%) was recorded in Bravo* and the lowest (27.0%) in CU-4, a difference of 7.3 percentage points (Table 1).

Table 1. Two-year means (2021–2022) of protein, lysine, and tryptophan in the soybean samples.

No.	Sample	Protein, %	Lysine	Tryptophan
1	Kofu st.	31.2	604	0.55
2	Bravo	31.6	801	0.75
3	Kanata	33.1	669	0.65
4	Regale	33.3	810	0.75
5	Bravo*	34.3	927	0.80
6	Regale*	33.6	852	0.75
7	Krasnodar-68	32.5	711	0.70
8	Sinara	32.7	776	0.70
9	Angelica	29.4	604	0.60
10	Kofu	30.4	604	0.60
11	Kanata No. 4	29.2	636	0.65
12	CU-4	27.0	593	0.50
13	CU-14	28.4	649	0.65
14	Regalia	33.5	690	0.70
15	CU-1	27.7	615	0.60
16	Kioto	28.4	647	0.60

* Precision index of the measurements does not exceed 3%.

The protein content ranged from 27.0% to 34.3%, the lysine content from 593 to 927, and the tryptophan content from 0.50 to 0.80. These

values are consistent with previously reported data on the biochemical composition of soybean grain.

Table 2. Variation of biochemical parameters in the 16 soybean samples (two-year means).

Biochemical indicator	Min.	Max.	Mean	Standard deviation	Standard error ($\pm$ SE)	Coefficient of variation, %
Protein, %	27.0	34.3	30.65	2.39	± 0.60	5.71
Lysine	593.0	927.0	760.0	1.03	± 2.57	10.65
Tryptophan	0.50	0.80	0.65	0.08	± 0.02	7.0

Cluster analysis of the biochemical parameters divided the 16 samples into three clusters, with a genetic-diversity index of

GD = 0.361–0.624 and a mean value of 0.482 (Figure 1; Table 3). The majority of the samples fell into the first cluster.

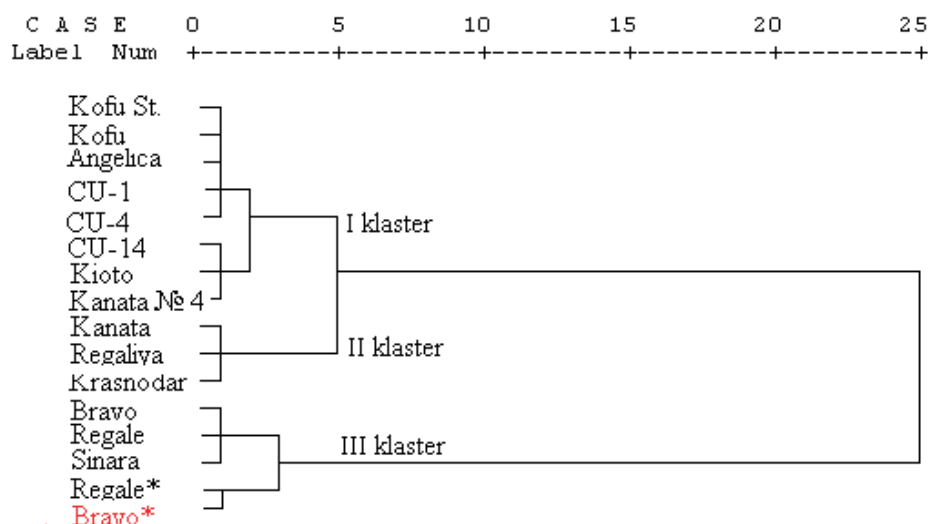


Figure 1. Grouping of the soybean varieties based on the biochemical parameters (cluster dendrogram).

Cluster I contained 8 samples (Kofu st., Angelica, Kofu, Kanata No. 4, CU-4, CU-14, CU-1, and Kioto). In most of these varieties, the protein content was below the mean; only Kofu st. (31.2%) was close to the mean, and the remaining seven varieties had below-mean values. The lysine content was below the mean in all eight varieties. The tryptophan content was close to the mean in CU-14 and Kanata No. 4 and below the mean in the other six varieties.

Cluster II contained 3 samples (Kanata, Regalia, and Krasnodar-68). The protein content in these varieties was above the mean; the lysine

content was below the mean in Kanata and Regalia and close to the mean in Krasnodar-68; the tryptophan content was close to the mean in Kanata and above the mean in Regalia and Krasnodar-68.

Cluster III contained 5 samples (Bravo, Regale, Bravo*, Regale*, and Sinara). In this group, the protein (31.6–34.3%), lysine (776–927), and tryptophan (0.70–0.80) contents were all above the mean. The highest protein content in this group, and in the whole trial, was recorded in Bravo* (34.3%).

Table 3. Cluster analysis of the biochemical indicators.

Cluster	Samples (No. from Table 1)	Protein, %	Lysine	Tryptophan
I	1, 9, 10, 11, 12, 13, 15, 16	27.0–31.2	593–649	0.50–0.65
II	3, 7, 14	32.5–33.5	669–711	0.65–0.70
III	2, 4, 5, 6, 8	31.6–34.3	776–927	0.70–0.80
Mean		30.65	760.0	0.65

4. CONCLUSION

Analysis of variance and cluster analysis of the 16 soybean varieties evaluated in 2021–2022 under irrigated gray-soil conditions in the Nakhchivan Autonomous Republic revealed high variation in the grain contents of protein, lysine, and tryptophan. The varieties Bravo* (34.3%), Regale* (33.6%), Regalia (33.5%),

Regale (33.3%), and Kanata (33.1%) stood out for their high protein contents. The highest lysine value was recorded in Bravo* (927) and the lowest in CU-4 (593); the highest tryptophan value was recorded in Bravo* (0.80) and the lowest in CU-4 (0.50). In both study years, the tryptophan content was highest in Bravo*, Regale*, Regale, and Bravo, and lowest in CU-4

and Kofu st., while Bravo* and Regale* were consistently distinguished by their lysine contents. On the basis of these results, samples with high biochemical indicators have been identified, and it is recommended that the varieties Bravo* and Regale*, together with Regale, Regalia, and Kanata, be used in future selection work aimed at improving the productivity and quality of soybean in the region.

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ARTICLE 7

Floristic and Ethnobotanical Aspects of Sandy and Wetland Phytocoenoses of the Nabran Area, Caspian Coast

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ABSTRACT

This article presents the results of botanical, systematic, floristic, and geobotanical studies carried out along the Caspian coast of the Nabran settlement, Khachmaz district, from 20 to 27 August 2023. In addition, the ethnobotanical characteristics and phytocoenological analysis of the useful plant resources, together with the current state of the species in nature, were investigated. On the basis of the geobotanical research, the current state of the natural phytocoenoses in which the species occur was evaluated, the genera and species were arranged into taxonomic units, the distribution ranges of certain species were clarified, and digital photographs were taken. A taxonomic composition of 104 species belonging to 72 genera and 38 families was determined from the collected herbarium specimens.

The biomorphological analysis indicates that the flora is dominated by perennial and annual herbs (each accounting for 40.4%). The remaining life forms are distributed as follows: trees (5.8%), lianas (2.8%), biennials (2.8%), subshrubs (2.8%), tree-shrubs (1.9%), undershrubs (1.9%), and shrubs (1.0%). Most of the plants occurring in the area possess medicinal, essential-oil, tannin, food, dyeing, and other useful properties.

During the ethnobotanical surveys, valuable data on a number of useful plants used by the local population were obtained. Notably, *Alnus glutinosa* was recorded for the first time for the dendroflora of Azerbaijan, and a new locality of *Samolus valerandi* L. (family Primulaceae Batsch ex Borkh.) was identified in the coastal area of Shimali village. Finally, methods for the conservation and sustainable use of the Caspian coastal flora and vegetation are proposed.

Keywords: Nabran, Caspian coastal flora, vegetation, biomorphological analysis, ethnobotany, useful plants, new floristic records.

1. INTRODUCTION

Situated within the administrative territory of the Khachmaz district, Nabran is one of the most prominent resort and tourism zones in north-eastern Azerbaijan, within the Samur-Yalama lowland. The region lies close to the northern border with the Russian Federation (Dagestan). The defining geographical feature that makes Nabran unique is its flat terrain, where a dense forest belt merges directly with the sandy shores of the Caspian Sea. Globally,

coastal zones in which marine and forest ecosystems intertwine to such an extent are remarkably rare.

The area has a temperate-warm climate, with hot, relatively humid summers and mild winters. This climatic regime favors both the development of rich vegetation and the growth of tourism. The forests of Nabran are remnants of ancient relict woodlands, distinguished by high biodiversity and characteristic endemic plant species. Comprising the Yalama forests, this

primary vegetation cover constitutes a unique ecosystem that is now incorporated into the Samur-Yalama National Park.

The species of these remarkable forests are notable for both their evolutionary antiquity and their cryptic ecological traits. *Quercus castaneifolia*, a relict species listed in the Red Book of the Republic of Azerbaijan, is an ancient, monumental tree and the definitive symbol of the Nabran forests.

Tree species such as hornbeam (*Carpinus*), elm (*Ulmus*), linden (*Tilia*), and beech (*Fagus*) form the principal canopy, ensuring forest density and shade while holding significant ecological value for both apiculture and the local fauna. The large lianas and ivy that dominate the vertical structure of the Nabran forests climb the coastal trees, imparting a distinct microclimate and a dense, rainforest-like character to the area. Furthermore, the regional flora includes a widespread distribution of wild berries and medicinal plants, including wild pomegranate (*Punica granatum* L.), blackberry, wild strawberry, rosehip, and hawthorn.

The present state of the flora and vegetation of the sandy coastal areas from the northern village of Nabran to Khudat, along the Caspian Sea, was investigated. The objective of this study was to identify the plant species and phytocoenoses present in the study area and to assess the potential uses of the useful plant species.

The research was based on generally accepted floristic, geobotanical, bioecological, and phenological methods, using both route and stationary observation techniques. In addition to the materials collected during the field expeditions, relevant literature sources, botanical keys, herbarium collections, and official online databases and other information resources were comprehensively reviewed and analyzed. The methods included classical and modern botanical, floristic (Flora of Azerbaijan, 1950–1961; Asgarov, 2016), ecological, statistical, and widely accepted geobotanical approaches (Field Geobotany, 1959–1976; Yaroshenko, 1961; Ramensky, 1937), with reference to the fundamental works of V. C. Hajiyeve (2004) and A. A.

Grossheim (1945); life forms were determined following I. G. Serebryakov (1964), among others. Rare and endangered species were categorized according to the relevant version of the International Union for Conservation of Nature (IUCN) Red List. The collected herbarium specimens were processed, and the plant species were taxonomically identified.

3. RESULTS AND DISCUSSION

Many plant species in the flora of Azerbaijan are widely used in both scientific and traditional medicine. The majority of these species have been officially listed as medicinal (officinal) plants in the State Pharmacopoeia over the years (Ibadullayeva, 2017; Ibrahimov, 1998; Borodin, 1940; Cherepanov, 1995). Numerous useful plant groups were recorded in the study area, including fodder, melliferous, food, seasoning, dye-producing, ornamental, fibrous, tannin-containing, resinous, medicinal, essential-oil-bearing, glycoside-containing, alkaloid-containing, saponin-rich, flavonoid-rich, vitamin-rich, and cosmetic plants. Globally, medicinal plants are of considerable importance in modern medicine, with over 50% of current pharmaceutical preparations being plant-derived.

During the study, a total of 214 wild and cultivated plant species were documented. Cultivated species occur mainly in home gardens and roadside plantings. The most frequently encountered genera include *Ulmus* L., *Salix* L., *Populus* L., *Ailanthus* Desf., *Quercus* L., and *Cupressus* L. In home gardens, fruit and ornamental plants such as *Cydonia*, *Pirus* L., *Malus* Mill., *Cerasus* Mill., *Punica* L., *Cornus* L., *Rubus* L., and various other fruit and ornamental species are commonly cultivated.

In the flora of the study area, xerophyte, xerohalophyte, halophyte, psammohalophyte, mesophyte, mesoxerophyte, and hydrophyte plant groups have mainly been identified (Huseynova et al., 2016). These form comparable phytocoenoses within distinct plant communities. Such species include *Polygonum aviculare* L., *Persicaria hydropiper* (L.) Delarbre, *Sambucus ebulus* L., *Plantago major* L., *P. lanceolata* L., *Hypericum*

perfoliatum L., *Mentha longifolia* (L.) L., *Xanthium strumarium* L., *X. spinosum* L., *Capparis spinosa* L., *Lotus corniculatus* L., *Malva silvestris* L., *M. neglecta* Wallr., *Cynodon dactylon* (L.) Pers., *Amaranthus retroflexus* L., *Chrozophora tinctoria* (L.) A. Juss., *Atriplex tatarica* L., *Marrubium vulgare* L., *Tamarix ramosissima* Ledeb., and *Acinos arvensis* (Lam.) Dandy, among others.

The productivity and economic value of the plant cover are determined by the abundance of dominant species; the role of edificators, dominants, and subdominants; and the cover of the vegetation layer. In the digital photographs presented below, the dominant plants and the species composition of individual phytocoenoses are clearly visible (Figure 1).

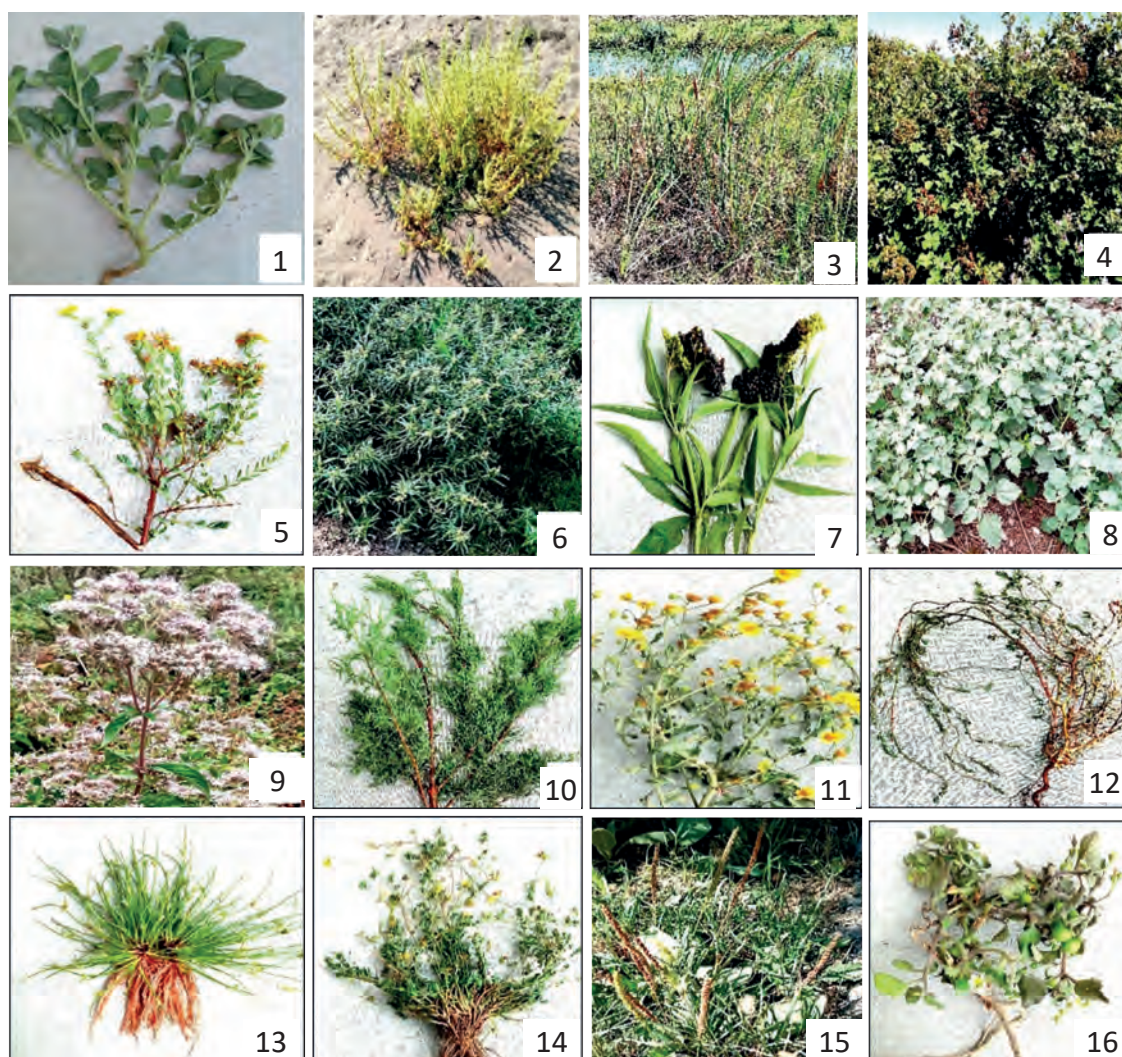


Figure 1. Selected wild plant species of Nabran: (1) *Heliotropium ellipticum* Ledeb.; (2) *Rubus caesius* L.; (3) *Typha angustifolia* L.; (4) *Hypericum perforatum* L.; (5) *Salsola soda* L.; (6) *Xanthium spinosum* L.; (7) *Sambucus ebulus* L.; (8) *Chrozophora tinctoria* (L.) A. Juss.; (9) *Polygonum aviculare* L.; (10) *Eupatorium cannabinum* L.; (11) *Tamarix meyeri* Boiss.; (12) *Pulicaria dysenterica* Bernh.; (13) *Plantago lanceolata* L.; (14) *Solanum villosum* Mill.; (15) *Lotus corniculatus* L.; (16) *Verbena officinalis* L.; (17) *Juncus bufonius* L.; (18) *Cynodon dactylon* (L.) Pers.

In these phytocoenoses, *Pulicaria dysenterica*, *Juncus filiformis* L., *Rubus caesius* L., *Sambucus ebulus* L., and *Chrozophora tinctoria* (L.) Juss. are widely distributed along the coast, occurring repeatedly. Vegetation cover ranges from 75 to 80%, and in some areas the cover

of thickets and shrub patches reaches 80–85%.

At some distance from the sandy (psamphilous) phytocoenoses, marsh and aquatic phytocoenoses occur. These are formed by hydrophilous and hygrophilous plant species (Figure 2).

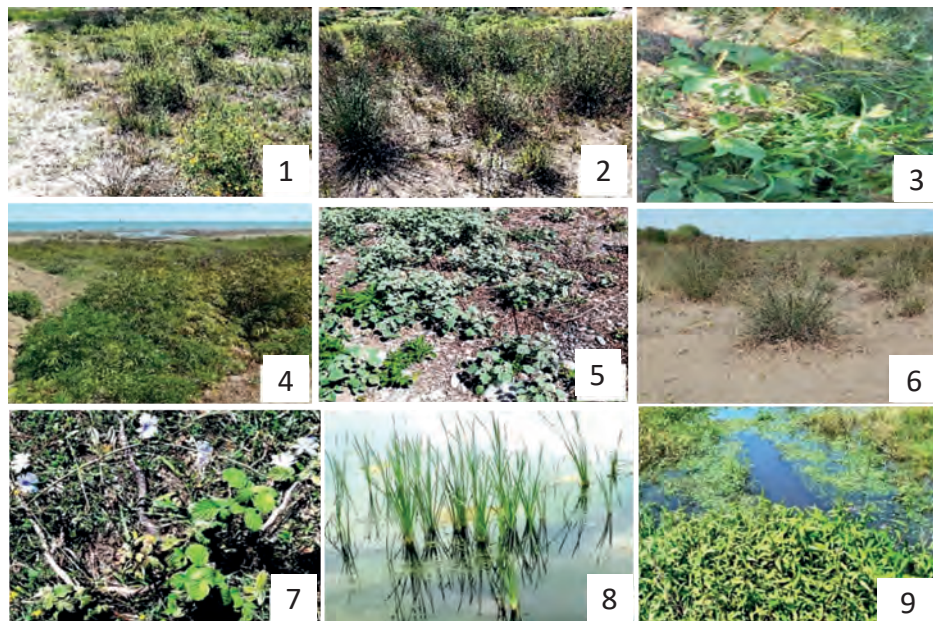


Figure 2. (1) *Pulicaria dysenterica*; (2) *Pulicaria dysenterica* (L.) Bernh. + *Juncus effusus* L.; (3) *Plantago major* L.; (4) *Sambucus ebulus* L. and its associated phytocoenoses; (5) *Chrozophora tinctoria* in pure and mixed phytocoenoses; (6) *Juncus filiformis* L.; (7) *Rubus caesius* L. + *Cichorium intybus*; (8) *Typha angustifolia* L.; (9) *Persicaria hydropiper* + *Veronica anagallis-aquatica* (vegetation cover in these phytocoenoses ranges from 70 to 85%).

In these communities, species such as *Persicaria hydropiper*, *Mentha longifolia* (L.) L., *Typha angustifolia* L., *Bolboschoenus maritimus* (L.) Palla, *Veronica anagallis-aquatica* L., *Eryngium campestre* L., *Xanthium strumarium* L., *Raphanus raphanistrum* L., *Verbascum phlomoides* L., and *Convolvulus persicus* L., among others, are well developed (Figure 3).



Figure 3. (1) *Eryngium campestre* L.; (2) *Veronica anagallis-aquatica* L.; (3) *Xanthium strumarium* L.; (4) *Raphanus raphanistrum* L.; (5) *Verbascum phlomoides* L.; (6) *Convolvulus persicus* L.

The classification of the flora by life form is essential for understanding the ecological structure and adaptive characteristics of the plant community in the study area, allowing a comprehensive evaluation of how the species use the local environmental resources. According to the floristic analysis, the 104 recorded plant species were categorized by life form

(biomorph). The flora is co-dominated by perennial herbs (42 species, 40.4%) and annual herbs (42 species, 40.4%). Among the woody and semi-woody life forms, trees are represented by 6 species (5.8%), followed by lianas and subshrubs with 3 species each (2.8%).

Tree-shrubs and undershrubs account for 2 species each (1.9%), while shrubs constitute 1 species (1.0%). Among the remaining herbaceous groups, biennial herbs comprise 3 species (2.8%) (Figure 4).

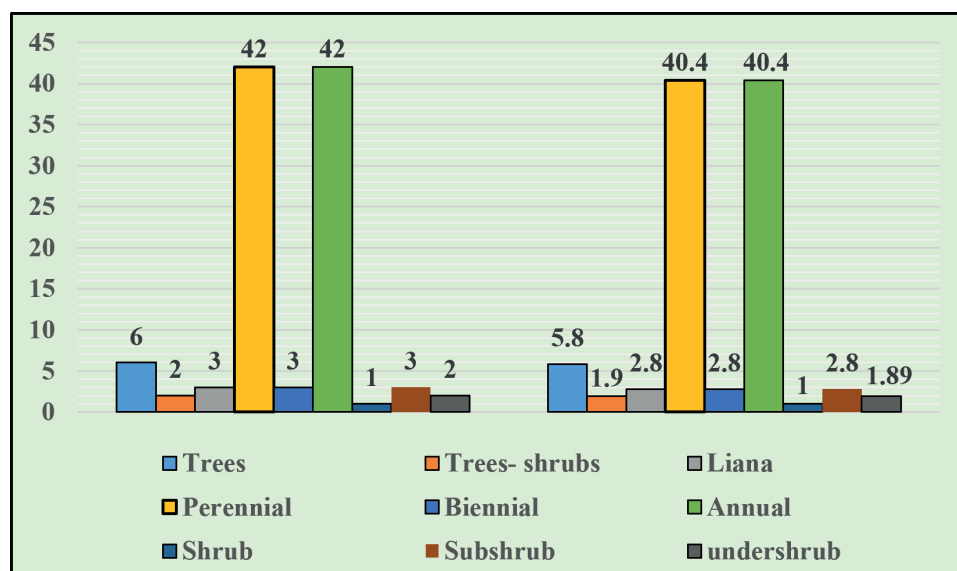


Figure 4. Life-form spectrum of the species.

Azerbaijan's medicinal plant resources represent a rich natural treasure. Preserving and sustainably managing these resources, while using them effectively to improve human health and working capacity, is of paramount importance. Human diseases should ideally be treated not only with synthetic drugs but also with plant-derived medicines rich in the biologically active compounds essential to the body (Ibadullayeva, 2017; Ibrahimov, 1998; Suvorova, 2009).

Plantago major L. – broadleaf plantain. Its constituents include mucilage; bitter compounds such as the aucubin (rhinantin) glycoside; the enzymes invertin and emulsin; polysaccharides; flavonoids; trace alkaloids; phytoncides; bitter principles; carotene; vitamins C (ascorbic acid) and K; citric acid; 4% tannins; and small amounts of saponins. The mucilage contains galactose, arabinose, and rhamnose. Polysaccharides, caffeic-acid derivatives, flavonoids, iridoid glycosides, and terpenoids have been identified in the leaves. In medicine, plantain leaves (Fol. Plantaginis) and aerial parts are used in cough remedies, for

gastrointestinal disorders such as gastritis, and for wound healing, particularly of abscesses and purulent lesions. The species is abundant in the region.

Polygonum aviculare L. – common knotgrass. Its aerial parts contain medicinally important compounds, including quercetin-derived flavonoid glycosides (hyperoside and avicularin), 3% saponins, carotene, vitamin C (160–215 mg%), sugars, and proteins. Avicularin and four flavonoid glycosides (juglanin, avicularin, astragalin, and bermidin) have been isolated from the aerial parts. The flowers are rich in flavones, and the roots contain anthraquinones. The local population uses the dried aerial parts as a hemostatic agent and for kidney stones and as a diuretic (Habibi Roudkenar et al., 2011).

Persicaria hydropiper (L.) Delarbre – an annual herb of the family Polygonaceae, named for its pungent, peppery-tasting leaves and seeds. Globally, the genus contains 200 species; in the Caucasus, 40 species; and in Azerbaijan, 27 species. In the study area, it forms the *Persicarieta hydropiperiae* community, as

well as mixed herbaceous groups (*Persicarieta hydropiperae* formation; *Persicarietetum hydropiperiosum* association) with numerous microgroups. Associated species include wetland and marsh plants such as *Typha latifolia*, *T. angustifolia*, *Juncus filiformis*, *Rumex acetosa* L., *Urtica dioica* L., and *Mentha longifolia* (L.) Huds.

Most of the species observed in the area are perennial herbs. Forest phytocoenoses also include species such as *Quercus macranthera* Fisch. & C. A. Mey., *Acer campestre* L., *Alnus glutinosa* (L.) Gaertn., and *Corylus avellana* L., among others.

In these communities, *Persicaria hydropiper* dominates the first tier, often forming dense, pure thickets that facilitate collection. Its aerial parts are used to prepare spiced salads, soups, and sauces. The leaves contain polygopiperin glycosides, flavonoids, saponins, essential oil, valerianic acid, acetic acid, vitamin K, carotene, and vitamin C. In traditional medicine, it is used as an astringent, analgesic, and hemostatic agent, and for disorders of the liver and kidney, edema, gastric ulcer, eczema, asthma, and thyroid disorders (Ayaz et al., 2020).

In modern medicine, it is used for hemorrhoids, chronic endometritis, uterine fibroids, and menorrhagia. It is also a melliferous species and is abundant in the region.

Malva sylvestris L. – common mallow, of the family Malvaceae. Globally, the genus *Malva* includes 125 species; in the Caucasus, 11 species; and in Azerbaijan, 10 species. Its flowers and leaves are rich in mucilage composed of L-arabinose, D-galactose, L-rhamnose, and D-galacturonic acid, as well as tannins, carotene, ascorbic acid, organic acids, traces of essential oil, mineral salts, sugars, and phytosterols. The anthocyanin glycoside malvin has also been identified in the flowers. Ethnobotanical surveys indicate that local people use the flowers and leaves (Flor. et fol. *Malvae sylvestris*) as demulcents and for bronchial inflammation, respiratory infections, and digestive disorders. The species is abundant in the study area.

Tamarix L. – of the family Tamaricaceae; the genus contains 90 species worldwide and 7 species in the Caucasus and Azerbaijan. In the study area, *Tamarix tetrandra* Pall. ex M. Bieb.

and *T. ramosissima* Ledeb. are widely distributed. These species are known under various local names, such as “God’s tree,” “Hashtarkhan jasmine,” and “cingil,” and are common along the Baku–Nabran road and the Caspian coast. In general, all tamarisks (*Tamarix* spp.), distinguished by their high tolerance of drought and salinity, are well suited to landscaping and greening projects, particularly along highways and in parks and gardens. Owing to their decorative habit, appearance, and range of colors, these plants markedly enhance the visual quality of landscapes. The tamarisk bears long, raceme-like inflorescences of small flowers, predominantly pink and purple. Tamarisk is also a valuable melliferous plant and is widely used by local people, especially as a raw material for small-scale handicrafts, weaving, and carving. It has long been used in folk medicine for various ailments (Bahramsoltani et al., 2022). Leaf infusions are used for rheumatism, spleen disorders, and diarrhea, and as diaphoretic, diuretic, and analgesic agents. Flower infusions are used for gastrointestinal diseases, while the fresh sap is applied to inflammations of the oral mucosa. In the past, in the Caucasus, decoctions of the branches were used as a remedy for infertility, and decoctions of the fruits were used in the treatment of syphilis (Ibrahimov, 1998; Borodin, 1940). A further, anecdotal report on tamarisk was obtained from a biologist who has devoted many years to the study of medicinal plants: according to him, by burning one end of a tamarisk branch and collecting the liquid exuded from the opposite end, this substance can be used to prevent and treat psoriasis.

Juncus filiformis L. – owing to its content of flavonoids, coumarins, steroids, carotenoids, and phenolcarboxylic acids, it has anti-inflammatory, antioxidant, and sedative properties and shows antibacterial activity (Tóth et al., 2016). In the wild, certain animals and birds depend on *Juncus* species for food and habitat, using the plants for shelter and consuming their seeds. Aquatic environments where these species are widespread serve as refuges for various wetland birds and provide valuable spawning grounds for certain fishes.

The local population uses the roots of this plant, known in some regions as “Qindirgha,” for medicinal purposes against colds.

During the study, a tree species new to the

flora of Azerbaijan was identified: *Alnus glutinosa* (L.) Gaertn. (black alder), of the family Betulaceae, genus *Alnus* Mill. (Figure 5).



Figure 5. *Alnus glutinosa* (L.) Gaertn.

The genus comprises four species in the Caucasus and three in Azerbaijan (*A. subcordata* C. A. Mey., *A. incana* (L.) Moench, *A. barbata* C. A. Mey.). One species, *Alnus rotundata* Goepp., has been reported from fossil records in Nakhchivan dating to the Miocene or Pliocene (Mehdiyeva, 2011; Blinova et al., 1990; Gubanov et al., 1976; Gubanov et al., 2003; Kolakovsky, 1973; Talibov et al., 2018), but it is not recorded for the flora of Azerbaijan in the relevant literature (Asgarov, 2016; Mehdiyeva, 2011). *Alnus glutinosa* (L.) Gaertn., Fruct. et Sem. II: 54 (1791) – *Betula alnus* var. *glutinosa* L., Sp. Pl.: 983 (1753) – *A. glutinosa* a) *vulgaris* – Fomin & Voronov II: 61.

The leaves of black alder are 4–9 cm long and 3–7 cm wide, obovate or oblong, sometimes cuneate or rounded at the base. When young, they are sparsely to moderately pubescent along the veins and in the axils on the underside; at maturity they become fully glabrous, or occasionally bearded in the vein axils, and are usually doubly serrate.

The fruiting catkins are broadly ovoid, 12–14 mm long, and generally extend well beyond the peduncle. The species occurs in forests,

along riverbanks, and in swampy areas of the lower and middle mountain belts. Its chorological type is Western Palearctic.

Alder is a source of valuable medicinal compounds. Extracts of its fruits (Fructus Alni) have been used to treat acute respiratory viral infections (Aldrovandi, 2011) and diseases of the gastrointestinal tract (Aldrovandi, 2011). Alder contains natural antioxidants, including polyphenols with pronounced anti-septic properties. Its principal economic value derives from the tannins in its organs: the bark contains 5–9% tannins and the cones up to 14–16%, which are used in leather tanning (Gubanov, 2003; Flora of Azerbaijan, 1950–1961). The bark and catkins yield dyes for leather and wool in yellow, red, and black, and a brown dye is obtained from the buds (Lantratova, 1991; Neishtadt, 1954). In the past, silk fabrics in the Caucasus were dyed with decoctions of alder bark. Alder fruits (Fructus Alni) are used as an astringent medicinal raw material in the treatment of gastrointestinal diseases (Cherepanov, 1995). A decoction of the cones is used for gargling during colds. Extracts of the bark and leaves show anti-inflammatory, antispasmodic, and choleretic effects; aqueous and

alcoholic extracts of the fruits possess antioxidant, membrane-protective, analgesic, exudative, and proliferative properties, as well as antibacterial and antitumor activity. Extracts of the inflorescences and pollen show anti-protozoal activity. Soils where alder grows are highly fertile, as the tree enriches the soil with nitrogen through nitrogen-fixing actinomycetes in its root nodules. Bees collect alder pollen and resinous bud and leaf secretions to produce propolis. Black alder is indispensable for the afforestation of wetlands, marshes, and swampy shorelines. *Alnus glutinosa* is included in the Red Data Books of Kazakhstan, Moldova,

and the Omsk Region, and in the IUCN Red List (IUCN, 2012).

A new locality of *Samolus valerandi* L. (family Primulaceae Batsch ex Borkh.) was identified in the coastal area near Shimali village, along the Caspian Sea. *S. valerandi* is a perennial herb that mainly occupies humid habitats near springs, in marshes, and along seashores. The species has distinctive botanical and traditional-medicinal characteristics, with bright green, fleshy, ovate leaves and small, white flowers arranged in racemes in the upper part of the stem (Wagner et al., 2020).



Figure 6. *Samolus valerandi* L.: (1) in water; (2) on land; (3) at the water's edge.

On the basis of the geobotanical surveys, several hydrophilic and hygrophilic phytocoenoses (associations) in which *Samolus*

valerandi acts as a dominant or subdominant species were identified and classified according to international syntaxonomic rules, as follows:

Table 1. Structure of the identified phytocoenoses.

Syntaxonomic Association (Phytocoenosis)	Geographic-Ecological Environment (Biotope)	Dominant / Subdominant Species
<i>Samolo-Typhetum angustifoliae</i>	Extremely humid coastal areas and marshes	<i>Samolus valerandi</i> , <i>Typha angustifolia</i>
<i>Samolo-Persicarietum</i>	Alluvial and seasonally moist soils	<i>Samolus valerandi</i> , <i>Persicaria</i> spp.
<i>Samolo-Juncetum maritimi</i>	Coastal saline sandy areas	<i>Samolus valerandi</i> , <i>Juncus maritimus</i>
<i>Samolo-Lotetum corniculati</i>	Humid coastal meadows	<i>Samolus valerandi</i> , <i>Lotus corniculatus</i>
<i>Samolo-Carduetum dysenterici</i>	Humid and partially degraded coastal zones	<i>Samolus valerandi</i> , <i>Carduus dysentericus</i>
<i>Samolo-Persicarietum hydropiperis</i>	Margins of stagnant water, extremely damp ecotopes	<i>Samolus valerandi</i> , <i>Persicaria hydropiper</i>

With an almost cosmopolitan distribution, the species occurs in the humid zones of Azerbaijan, particularly on sandy-saline soils and along freshwater margins.

During the field expeditions, plant species occurring in the study area were collected, taxonomically identified, and analyzed. On the basis of the ethnobotanical surveys, the ethnobotanical characteristics of the useful plant species were determined, and information was obtained on the use of resources of particular importance for medicinal, food, fodder, industrial, and agricultural purposes. The collected data were systematized and generalized, and their inclusion in a database for subsequent scientific analyses is envisaged.

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ARTICLE 8

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Analyses on the European Chestnut (*Castanea sativa* Mill.) Distributed in the Sheki-Zagatala Region of AzerbaijanCoshgun I. Mammadov*  | <https://orcid.org/0009-0008-1354-5778>

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ABSTRACT

This study assessed the current condition of *Castanea sativa* Mill. (European chestnut, Fagaceae) stands in the foothill villages, private holdings and natural foothill forests of the Sheki-Zagatala region of Azerbaijan, at altitudes of 600–1,600 m above sea level, following three decades of decline attributed to chestnut blight (*Cryphonectria parasitica* (Murrill) Barr) and root and collar rot (*Phytophthora* spp.). Field expeditions were conducted over two consecutive seasons across 71 villages in seven administrative districts — Balakan, Zagatala, Gakh, Sheki, Oguz, Qabala and Ismayilli — and stand density was assessed following the methodology of Nesterov (1954). Across the surveyed area, approximately 65% of chestnut plants had died as a result of disease, while a further 30% of the surviving plants were infected and undergoing progressive dieback. Severe desiccation affecting 60–65% of the stand was recorded in 54 villages, whereas chestnut stands had been eliminated entirely in 17 villages. Disease severity was consistently greater in villages along the southern margin of the region than in the foothills. Trees older than 50–100 years, which formerly accounted for the largest share of production, were infected at the earliest stage and were the first to die; regional chestnut yield has consequently fallen by 60–70% relative to the 1990s. Field observations further demonstrate that *C. parasitica* alone does not account for the mortality observed: root and collar rot caused by *Phytophthora* was recorded in numerous dead trees, and in some dead trees stem canker was absent entirely. A combined phytopathological approach is therefore required for any restoration programme.

Keywords: *Castanea sativa* Mill.; *Cryphonectria parasitica* (Murrill) Barr; *Phytophthora* spp.; Greater Caucasus; Sheki-Zagatala region; forest decline; conservation

1. INTRODUCTION

The Sheki-Zagatala economic-geographical region is one of the most favourable natural zones of the Greater Caucasus. Its area is 987,689 ha, or 11.4% of the territory of the Republic of Azerbaijan. The region comprises the western parts of the Balakan, Zagatala, Gakh, Sheki, Oguz, Qabala and Ismayilli districts, covering the mountainous belt of the Greater Caucasus and the adjoining Alazan-Ayrichay depression, the

northern slopes of the Shirak and Sheki-Qabala plateaux, the Haftaran river valleys, and the Aji-Nohur and Turud-Saryja plains. Relief ranges from 200 m to 3,500 m above sea level, with the principal settled belt lying at 600–800 m, where a subtropical climate prevails. The region consequently supports high biodiversity.

In the foothill and lowland forests, wild forms of numerous fruit species occur in their natural habitats, including apple (*Malus* Mill.), pear

(*Pyrus communis* L.), medlar (*Mespilus germanica* L.), cornelian cherry (*Cornus mas* L.), hawthorn (*Crataegus* L.), quince (*Cydonia oblonga* Mill.), hazelnut (*Corylus avellana* L.), chestnut (*Castanea sativa* Mill.), walnut (*Juglans regia* L.), cherry plum (*Prunus cerasifera* Ehrh.), sweet cherry (*Prunus avium* (L.) L.) and apricot (*Prunus armeniaca* L.) (Mammadov et al., 2019).

Unlike other regions of Azerbaijan, the Sheki-Zagatala region is distinguished by the cultivation of hazelnut, chestnut, persimmon and tobacco. The chestnut, a highly valuable species that in Azerbaijan grows naturally only in this region and is now threatened with local extinction, plays an important role in the rural economy and traditional livelihoods of the region.

In its natural range, *C. sativa* is distributed across southern Europe, the Mediterranean coast, the Balkan countries, Asia Minor, Italy, western Ukraine and Moldova (Mammadov, 2016). Asian countries lead world production, accounting for 87.5% of the global chestnut harvest, with China, the Republic of Korea, Türkiye and Japan as the principal producers (Ministry of Agriculture of the Republic of Azerbaijan, n.d.).

Of the ten species of *Castanea* Mill. (Fagaceae), a single species — *C. sativa* — occurs naturally in Azerbaijan. The fruit is a nut enclosed in a cupule, the surface of which is smooth or spiny depending on the species (Gurbanov, 2009). In Azerbaijan the species is distributed in the foothills of the Greater Caucasus, particularly in the Sheki-Zagatala region, where individual trees may live for 150–1,200 years.

Chestnut stands are of considerable economic and ecological value. They yield high-quality timber, fuelwood and tannin. Dried chestnut flowers are rich in oil, protein, sugars, antioxidants, polyphenols, minerals and dietary fibre, and are used as natural ingredients in the baking industry (Carocho et al., 2015). Interest in chestnut-derived bee products has also increased in recent years; the antioxidant properties of chestnut bee pollen vary substantially with geographical origin (Karkar et al., 2021).

The species is thus of scientific, economic and practical importance for the development of industrial horticulture in Azerbaijan, and its conservation is directly relevant to the National

Strategy for the Protection and Sustainable Use of Biological Diversity in the Republic of Azerbaijan for 2017–2020 (President of the Republic of Azerbaijan, 2016).

Within the Sheki-Zagatala region, chestnut occurs in the mountainous, foothill and plain zones at altitudes of 600–1,600 m above sea level. Across the Greater and Lesser Caucasus and Karabakh it is found chiefly on lower montane slopes as a component of beech and beech-hornbeam formations, and occasionally as discrete stands (Askerov, 2016). At 500–1,400 m, hazel thickets develop on slopes where chestnut-hornbeam forests are sparse (Mammadov, 2002). Chestnut requires annual precipitation above 800 mm; the semi-humid subtropical climate of the study region provides 729–1,022 mm (Mammadov, 2023).

Over the past three decades, chestnut populations both globally and in Azerbaijan have come under threat of extinction as a consequence of infection by chestnut blight (*Cryphonectria parasitica* (Murrill) Barr) and root and collar rot (*Phytophthora* spp.). The aim of the present study was to document the current condition of chestnut stands throughout the Sheki-Zagatala region and to identify the principal causes of the observed decline.

2. MATERIALS AND METHODS

The study was carried out across 71 villages of the Sheki-Zagatala region over two consecutive field seasons. A census of chestnut plants was conducted in each village, and change in stand size over the preceding 30 years was estimated. Stand density was assessed using the methodology of Nesterov (1954). Trees were classified into two categories on the basis of the proportion of the village stand affected: severe desiccation, in which 60–65% of the plants surveyed showed advanced dieback while living individuals remained; and complete elimination, in which no living chestnut trees remained at the time of survey.

Surveys were conducted in the following villages, by district:

Balakan (6): Pochtchina, Nazimgarey, Mahamalar, Katekh, Gerakli, Mazimchay.

Zagatala (21): Masex, Jar, Meshlesh, Yukhari

Tala, Mukhax, Yukhari Chardakhlar, Sabunchu, Cimcimak, Tarikhler, Akh-akh Dere, Kebeloba, Micgar, Takhtalar, Agdamkelal, Kohne Suvagil, Galal, Azgilli, Alesgar, Gozbarakh, Mamrikh, Gabizdere.

Gakh (8): Lakit, Ilisu, Saribas, Agchay, Qashgachay, Armudlu, Zarne, Gum.

Sheki (9): Bash Shabalid, Bash Layiski, Cunud, Bash Goynuk, Gumukh, Bakhgal, Bash Kaldak, Oraban, Bash Küngüt.

Oguz (5): Bash Dashagil, Mukhas, Jalut, Filfil-i, Khachmaz.

Qabala (20): Zaraghan, Bum, Hamzali, Gamarvan, Tikanli, Abrikh, Yemishenli, Uludash, Mykhlykovag, Nohurgishlag, Vandam, Bunut, Dashlica, Aydingishlag, Boyuk Pireli, Nij, Kichik Pireli, Chukhur Gabala, Soltan Nukha, Mirzabayli.

Is mayilli (2): Sumagalli, Galajig.

3. RESULTS AND DISCUSSION

Across the surveyed area it was determined that approximately 65% of chestnut plants had died as a result of disease, while a further 30% of the surviving plants were infected and undergoing progressive dieback.

3.1 Causal agents of decline

Field observations demonstrate that stem

canker caused by *Cryphonectria parasitica* is not the sole cause of chestnut mortality in the region. In addition to stem canker, root and collar rot caused by *Phytophthora* was recorded in a large proportion of the dead trees, and in some dead trees stem canker was not recorded at all (Figures 1 and 2). A comprehensive phytopathological approach to the problem is therefore essential.

This distinction is epidemiologically important. *C. parasitica* colonises the bark and cambium of stems and branches and does not spread into the root system; infected trees may therefore recover through callus formation and produce new shoots. By contrast, *Phytophthora* attacks the root system and root collar, causing irreversible mortality of the entire plant. In the village of Bash Shabalid (Sheki district), in Lakit and Armudlu (Gakh district), and in Jar, Yukhari Chardakhlar, Kohne Suvagil, Meshlesh and Yukhari Tala (Zagatala district), the stems and branches of many trees infected with stem canker were observed to be recovering and regenerating, yet the trees nevertheless died completely — an outcome consistent with concurrent root infection.



Figure 1. Chestnut (*Castanea sativa* Mill.) tree infected with stem canker (*Cryphonectria parasitica* (Murrill) Barr), Bash Shabalid village, Sheki district.



Figure 2. Chestnut (*Castanea sativa* Mill.) tree infected with root and collar rot (*Phytophthora* spp.), Hamzali village, Qabala district.

3.2 Spatial pattern of decline

Disease severity showed a consistent spatial gradient. Decline was most severe in villages located along the southern margin of the region and comparatively mild in the foothills. Severe desiccation, affecting 60–65% of the stand, was recorded in 54 villages, whereas chestnut stands had been eliminated entirely in 17 villages (Table 1).

Decline was most acute in the Zagatala district, where up to 90% of chestnut trees have died in the villages of Jar, Yukhari Chardakhlar, Yukhari Tala, Masex, Kebeloba and Sabunchu; of these, the stands at Yukhari Tala and Kebeloba have been eliminated entirely, while living individuals persist at Jar, Yukhari Chardakhlar, Masex and Sabunchu (Table 1). Complete stand loss was likewise recorded at Nij, Kichik Pireli, Mirzabayli, Soltan Nukha and Chukhur Gabala in the south of the Qabala district. All of these

populations face local extirpation.

3.3 Age structure and economic consequences

Trees older than 50–100 years, which formerly accounted for the largest share of regional production, were infected at the earliest stage of the epidemic and were the first to die. Regional chestnut yield has consequently declined by 60–70% relative to the 1990s. Twenty-five to thirty years ago, chestnut production was the principal fruit crop attracting external capital into the north-western region and represented a profitable occupation for the rural population; in addition to meeting domestic demand, chestnuts were exported to the Russian Federation and Iran. At present, by contrast, domestic production no longer satisfies the internal market, and chestnuts are imported from China, Georgia and other countries.

Table 1. Current condition of *Castanea sativa* Mill. stands across 71 surveyed villages of the Sheki-Zagatala region.

No.	District	Village	60–65% desiccation	Completely dried out
1	Balakan	Pochtchina		♦
2		Nazimgarey	♦	
3		Mahamalar	♦	

No.	District	Village	60–65% desiccation	Completely dried out
4		Katekh	♦	
5		Gerakli		♦
6		Mazimchay	♦	
	Balakan subtotal: 6 villages		4	2
7	Zagatala	Masex	♦	
8		Jar	♦	
9		Meshlesh	♦	
10		Yukhari Tala		♦
11		Mukhax		♦
12		Yukhari Chardakhlar	♦	
13		Sabunchu	♦	
14		Cimcimak	♦	
15		Tarikhler	♦	
16		Akh-akh Dere	♦	
17		Kebeloba		♦
18		Micgar	♦	
19		Takhtalar	♦	
20		Agdamkelal	♦	
21		Kohne Suvagil	♦	
22		Galal	♦	
23		Azgilli	♦	
24		Alesgar	♦	
25		Gozbarakh	♦	
26		Mamrikh	♦	
27		Gabizdere	♦	
	Zagatala subtotal: 21 villages		18	3
28	Gakh	Lakit	♦	
29		Ilisu		♦
30		Saribas	♦	
31		Agchay		♦
32		Qashgachay	♦	
33		Armudlu	♦	
34		Zarne	♦	
35		Gum	♦	
	Gakh subtotal: 8 villages		6	2
36	Sheki	Bash Shabalid	♦	
37		Bash Layiski		♦
38		Cunud		♦
39		Bash Goynuk		♦
40		Gumukh	♦	
41		Bakghal	♦	
42		Bash Kaldak	♦	

No.	District	Village	60–65% desiccation	Completely dried out
43		Oraban	◆	
44		Bash Küngüt	◆	
	Sheki subtotal: 9 villages		6	3
45	Oguz	Bash Dashagil	◆	
46		Mukhas	◆	
47		Jalut	◆	
48		Filfilli	◆	
49		Khachmaz		◆
	Oguz subtotal: 5 villages		4	1
50	Qabala	Zaraghan		◆
51		Bum	◆	
52		Hamzali	◆	
53		Gamarvan	◆	
54		Tikanli	◆	
55		Abrikh	◆	
56		Yemishenli	◆	
57		Uludash	◆	
58		Mykhlykovag	◆	
59		Nohurgishlag	◆	
60		Vandam	◆	
61		Bunut	◆	
62		Dashlica	◆	
63		Aydingishlag	◆	
64		Boyuk Pireli	◆	
65		Nij		◆
66		Kichik Pireli		◆
67		Chukhur Gabala		◆
68		Soltan Nukha		◆
69		Mirzabayli		◆
	Qabala subtotal: 20 villages		14	6
70	Ismayilli	Sumagalli	◆	
71		Galajig	◆	
	Ismayilli subtotal: 2 villages		2	0
TOTAL: 71 villages surveyed across 7 districts			54	17

Notes:

◆ in the column '60–65% desiccation': advanced dieback recorded in 60–65% of the chestnut plants surveyed in that village, with living individuals remaining; grey shading marks the recorded category.

◆ in the column 'Completely dried out': stand entirely eliminated — no living trees remaining at the time of survey; grey shading marks the recorded category.

Village name components (transliterated from Azerbaijani): Bash = head / principal section; Boyuk = large; Yukhari = upper; Kohne = old; Kichik = small; Chukhur = hollow. No abbreviations are used in this table.

4. CONCLUSIONS

1. The decline of chestnut stands in the

Sheki-Zagatala region cannot be attributed to stem canker (*Cryphonectria parasitica* (Murrill))

Barr) alone. This pathogen does not spread to the root system and does not by itself cause complete mortality of the plant, whereas root and collar rot caused by *Phytophthora* results in the complete death of the tree. In Bash Shabalid (Sheki district), Lakit and Armudlu (Gakh district), and Jar, Yukhari Chardakhtar, Kohne Suvagil, Meshlesh and Yukhari Tala (Zagatala district), the stems and branches of many cancer-infected trees were regenerating, yet the trees died completely.

2. Decline was more severe in villages along the southern margin of the region and comparatively mild in the foothills. Chestnut stands had been eliminated entirely in 17 of the 71 surveyed villages, including Nij, Kichik Pireli, Mirzabayli, Soltan Nukha and Chukhur Gabala in the south of the Qabala district.

3. Trees over 50–100 years of age, which held the largest share of regional production, were infected at the earliest stage and died first. Regional chestnut production has consequently fallen by 60–70% compared with the 1990s.

5. RECOMMENDATION

A dedicated fund and research centre should be established for the conservation and restoration of chestnut genetic diversity across the South Caucasus, the North Caucasus and Türkiye. Given the evidence presented here that *Phytophthora* root and collar rot is a decisive factor in stand mortality, any restoration programme should combine resistance breeding against *C. parasitica* with soil-borne pathogen management and site selection based on drainage characteristics.

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ARTICLE 9

Quantitative Screening of Artemisinin Derivatives in *Artemisia herba-alba* from the Diyala Governorate, Iraq

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ABSTRACT

Artemisia herba-alba Asso (Asteraceae), commonly known as wormwood, is a perennial flowering herb native to the arid and semi-arid zones of the Mediterranean basin and a rich source of phytochemicals, including artemisinin derivatives. These sesquiterpene lactones are of major medicinal importance, and artemisinin-based therapies are used against chloroquine-resistant *Plasmodium falciparum*. Because the yield of these phytochemicals depends on the environmental and cultivation conditions, this study aimed to determine the percentage of artemisinin derivatives in the leaves of *A. herba-alba* collected from the Diyala governorate, Iraq, and to assess whether the local conditions are favorable for the plant. The aerial parts were harvested twice, in spring (April) and summer (July) 2024. The leaves were separated, dried in the dark, and pulverized, and a hexane extract was prepared by cold maceration and evaporated to dryness. The extract was screened for six *Artemisia* terpenoids — artemisinic acid, arteannuin B, dihydroartemisinin, artemisitene, artemisinin, and deoxyartemisinin — by reversed-phase high-performance liquid chromatography (RP-HPLC), and the percentage of each compound in the leaves was calculated from the extraction yield. The extraction yield was 0.85% for the April sample and 3.85% for the July sample, and the leaf content of the measured compounds was markedly higher in the July (summer) sample — approximately eight times that of the April (spring) sample. The summer artemisinin content approached the upper part of the range previously reported for *Artemisia* (0.01–0.8% in *A. annua*). These results indicate that summer is the optimal harvesting season and that *A. herba-alba* grown in the Diyala governorate can serve as a promising source of artemisinin derivatives, warranting measures to improve and exploit its yield.

Keywords: *Artemisia herba-alba*, artemisinin derivatives, terpenoids, RP-HPLC, malaria, phytochemical screening.

1. INTRODUCTION

Artemisia herba-alba, commonly known as wormwood, is a perennial flowering herb of the genus *Artemisia* that grows natively in the arid and semi-arid zones of the Mediterranean basin. It also occurs in Africa and further north, where winters are sunny and moderately cold. Like other *Artemisia* species, the plant prefers circum-neutral to alkaline, loamy soils of moderate moisture; it cannot withstand over-wet or

waterlogged conditions but is drought-resistant and can persist in poorly drained areas (Aljayyash et al., 2018).

Artemisia herba-alba develops in spring and blooms in early summer. It typically grows 20–40 cm tall (Moufid & Eddouks, 2012) and provides cover for grazing animals such as sheep and goats (Hassani et al., 2008), although its aromatic character makes it less palatable. Its characteristic odor is attributed to volatile oil,

while its bitter taste is ascribed to sesquiterpene lactones and alkaloids (Hassani et al., 2008). It may act as a companion plant resistant to pests and diseases (Al-Khayri et al., 2023) but is susceptible to root rot in poorly drained areas (Al-jaiyash et al., 2018).

Artemisia herba-alba has numerous medicinal and non-medicinal uses, including soil improvement, ground cover, companion planting for its pest-repellent properties, and erosion control and soil stabilization in dry areas. Its flowers provide nectar and pollen for pollinators, including bees; its leaves are consumed by various insects and herbivores; and its dense foliage and leaf litter offer shelter and habitat for beneficial insects and other invertebrates (Hassani et al., 2008).

The aerial parts are best harvested in summer to obtain the highest yield of phytochemicals. The plant is rich in phytochemicals with diverse medicinal effects, including volatile oils, sesquiterpenes known as *Artemisia* terpenoids, monoterpene alcohols, alkaloids, flavonoids, and coumarins. Among the volatile oils are cineole, thujone, oxygenated monoterpenes such as camphor, terpinen-ol and borneol, chrysanthenone, and chrysanthenol (El Ouahdani et al., 2021). The sesquiterpene lactones include eudesmanolides and germacranolides (Bourgou et al., 2017). The plant is also enriched with flavonoids occurring as O-glycosides (e.g., hispidulin, cirsilineol) and C-glycosides (e.g., isovitexin, schaftoside, isoschaftoside) (Sewidan et al., 2020). Additional bioactive compounds such as gallic acid, catechin, vanillic acid, caffeic acid, epicatechin, ferulic acid, and sinapic acid have also been identified (Hanae et al., 2023).

This diversity of phytochemicals confers pleiotropic medicinal effects, including antiseptic, vermifuge, antiparasitic, antimalarial, anthelmintic, antitubercular, antiviral, antihyperlipidemic, antiemetic, antidepressant, anticancer, anti-asthmatic, antihypertensive, antidiabetic, anxiolytic, hepatoprotective, gastroprotective, and insecticidal actions. In traditional medicine, the plant is burned and the fumes inhaled to treat coughs and chest, stomach, and muscular pains; the crushed plant is applied to strengthen the hair and to treat cuts and various skin

disorders; and its macerated leaves, mixed with olive oil, are applied to the skin to treat lesions (Mohammed et al., 2021; Ouedrhiri et al., 2022; Alshehri, 2022).

In addition, a tea of *Artemisia herba-alba*, equivalent to the aqueous extract, has been used in Iraqi folk medicine as an antidiabetic remedy and was found effective against alloxan-induced type-I diabetes mellitus and in reducing postprandial glucose in patients with type-2 diabetes (Boudjelal et al., 2015). Preliminary evidence suggests that a water extract of the herb may reduce symptoms and resolve pinworm infections in adults and children after three days of treatment (Ahmed et al., 2020). An essential-oil extract of the aerial parts also shows antibacterial activity against some Gram-positive and Gram-negative bacteria in vitro, with the santolina alcohol constituent apparently responsible (Mohammed et al., 2021).

Because artemisinin derivatives constitute the most important group of medicinal phytochemicals in this plant, the present study aimed to determine the percentage of artemisinin derivatives in the leaves of *Artemisia herba-alba* collected from the Diyala governorate, Iraq.

2. MATERIALS AND METHODS

2.1. Chemicals

Analytical-grade hexane (Sigma-Aldrich, MO, USA) was used as the extraction solvent. Analytical-grade sodium hexanesulfonate (Fisher Scientific, UK) was used together with a mixture of mono- and dihydrogen phosphates (Merck Ltd) as buffering agents. HPLC-grade acetonitrile and deionized water were used as the solvents and organic modifiers for the mobile phase.

2.2. Plant material

A sample of *Artemisia herba-alba* (Asteraceae) was collected from the Diyala governorate, Iraq, twice (in April and July 2024). It was taxonomically authenticated by a specialist at the Faculty of Agriculture (Abu Ghraib, Baghdad). The leaves were then separated, dried for 20 days in the dark, and mechanically pulverized with herb grinders. The powder was packaged in a paper bag and stored in a refrigerator at 4 °C.

2.3. Extraction

A hexane extract was prepared by cold

maceration, mixing 100 g of the pulverized plant with cold hexane for 24 h. The extract was rotary-evaporated to dryness at 40 °C to obtain a residue representing the final extract. The extraction yield was calculated by dividing the weight of the residue by the original amount of raw plant material.

2.4. Quantitative evaluation of the *Artemisia* terpenoid content

The extract was screened for six *Artemisia* terpenoids that are abundant in different *Artemisia* species — artemisinic acid, arteannuin B, dihydroartemisinin, artemisitene, artemisinin, and deoxyartemisinin — by reversed-phase high-performance liquid chromatography (RP-HPLC). The residue was dissolved in 1 L of a mobile phase composed of acetonitrile, water, and methanol (50:30:20). The solution was analyzed on an RP-HPLC system (Shimadzu LC) equipped with a binary delivery pump. The stationary phase was a fast-liquid-chromatography C18 column (50 × 4.6 mm I.D.). The mobile phase was delivered at 0.9 mL/min, and the eluted compounds were detected by UV at 220 nm; the amount of each compound was determined from its retention time. A standard curve of concentration versus peak area was constructed for

each derivative by injecting solutions of known concentration and measuring the area under the curve, and the amount of each derivative was estimated from this curve.

2.5. Calculation of the percentage of the active constituents in the leaves

The percentage of each constituent in the extract was determined and, together with the extraction yield, was used to estimate the amount of each compound in the leaves.

3. RESULTS

The extraction yield was 0.85% for the April sample and 3.85% for the July sample, indicating a higher yield when the plant was kept until the summer season. The percentage of each phytochemical in the leaves of the April and July samples was calculated from the amount determined by HPLC in each extract relative to the raw leaf material used. Each compound was identified from its retention time, and its amount was determined from the area under its peak. The peaks obtained for the April and July samples are shown in Figure 1; the retention times and peak areas are given in Table 1, and the percentages of each compound in the leaves are given in Table 2.

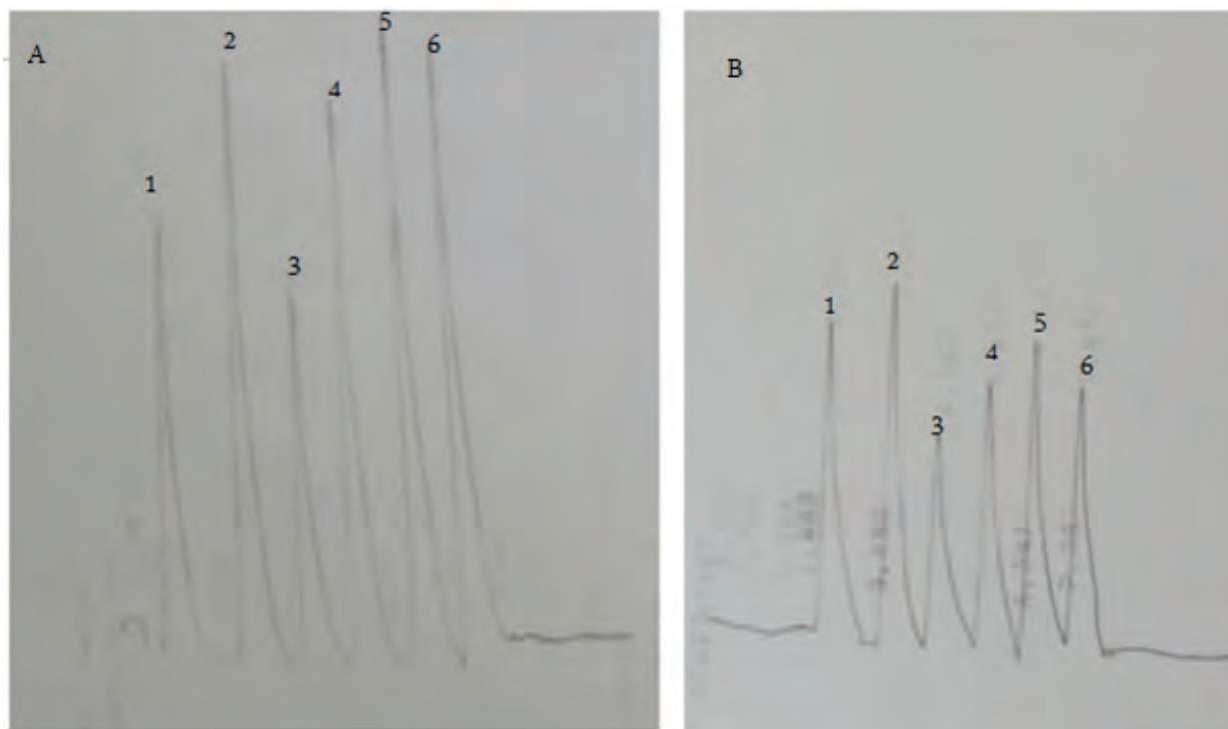


Figure 1. Reconstructed RP-HPLC chromatograms based on the retention times and peak areas reported in Table 1. Peaks 1–6: artemisinic acid, arteannuin B, dihydroartemisinin, artemisitene, artemisinin, and deoxyartemisinin. (A)

July (summer) sample; (B) April (spring) sample.

Table 1. Retention time and area under the curve (AUC) of the six phytochemicals obtained from *Artemisia herba-alba* (Diyala governorate).

Phytochemical	Retention time (min)	AUC, April sample	AUC, July sample
artemisinic acid	2.072	64711	38320
arteannuin B	3.378	96736	42555
dihydroartemisinin	4.555	84564	29853
artemisitenene	5.45	84405	32689
artemisinin	6.625	100548	34606
deoxyartemisinin	7.865	100720	35035

Table 2. Percentage of each phytochemical in the leaves of the April and July samples.

Phytochemical	Percentage in leaves, April	Percentage in leaves, July
artemisinic acid	0.0592	0.505568
arteannuin B	0.0879	0.750666
dihydroartemisinin	0.0686	0.585844
artemisitenene	0.0774	0.660996
artemisinin	0.0787	0.672098
deoxyartemisinin	0.0695	0.59353

4. DISCUSSION

Artemisia herba-alba is rich in medicinally active compounds belonging to different phytochemical classes, such as alkaloids, coumarins, flavonoids, volatile oils, and terpenoids (Lazreg et al., 2025). The most important of these are the artemisinins, sesquiterpene-lactone derivatives of the terpenoids (Wahnou et al., 2025; Abdallah et al., 2025), which are well known for their antimalarial (Cheong et al., 2020) and anticancer (Dai et al., 2021) effects. Their biosynthesis occurs only in *Artemisia* species, and their abundance and quantity in the active parts of the plant depend on environmental conditions such as temperature, humidity, and the nature and moisture of the soil.

The study showed that the phytochemical content of the leaves was higher when the crop was harvested in summer and much lower in the spring samples. Keeping the plant in the soil until summer increased the leaf content of the measured compounds approximately eight-fold, supporting the view that summer is the optimal time for harvesting *A. herba-alba* and indicating that the local soil and environmental conditions were favorable for the plant. The summer

content fell within the range previously reported for the plant (0.01–0.8% in *A. annua*).

Previous studies have indicated that the phytochemicals mentioned have cytotoxic effects; nevertheless, the plant does not pose a major hazard to grazing livestock, which may be attributed to its relatively low content in the active parts and to the limited period during which the phytochemicals are synthesized, as it is a perennial herb that sprouts in spring and blooms in summer, so that animal exposure is insufficient to exert a cytotoxic effect. One toxicology study reported that the plant is non-toxic at low doses and short durations, whereas at higher doses (up to 3 g/kg) and longer exposure, toxicity may occur, with rapid respiration, neurotoxicity, cardiotoxicity, hemotoxicity, genotoxicity, embryotoxicity, or reproductive toxicity (Khneissi et al., 2022). Further in-depth studies of the medicinal uses, clinical efficacy, and safety are needed.

Artemisinins have been approved as antimalarial agents (artemisinin-based therapy) widely used to treat malaria caused by chloroquine-resistant strains of *Plasmodium falciparum*, reflecting the pharmaco-economic importance of this plant and encouraging both its cultivation and

the maintenance of indigenous populations.

Previous studies have reported that the leaves and flowers of *Artemisia* may yield artemisinin at 0.01–0.8% on a dry-weight basis, although that work was conducted on *Artemisia annua*, another *Artemisia* species (Wang et al., 2023). The present study showed that plants

growing in the Diyala governorate, Iraq, may produce artemisinin at a percentage approaching the upper limit of this range, indicating that the plant can serve as a good phytochemical source of artemisinins and encouraging the use of various procedures to improve and exploit the yield.

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ARTICLE 10

Evaluation of the Expression Level of *Di19* Genes in *Gossypium hirsutum* L. Under Salt StressShader Alizade^{1*}  | <https://orcid.org/0000-0002-6059-2330>Kamila Aliyeva¹  | <https://orcid.org/0000-0001-8160-0777>Ruhangiz Mammadova²  | <https://orcid.org/0000-0003-0776-0937>¹ Baku State University, Baku, Azerbaijan² Genetic Resources Institute, Ministry of Science and Education of the Republic of Azerbaijan, Baku, Azerbaijan

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ABSTRACT

Cotton (*Gossypium hirsutum* L.) is a multifunctional crop of immense economic importance, yet its productivity is frequently constrained by abiotic stressors, particularly soil salinity. The Drought-Induced 19 (*Di19*) gene family, which encodes conserved C2H2 zinc-finger transcription factors, has been identified as a critical regulator in plant stress responses. While the roles of *Di19* members vary across species — acting as either positive or negative regulators — molecular characterization of these genes in local cotton germplasm remains limited. In this study, the expression profiles of *GhDi19-1* and *GhDi19-2* genes in 31 diverse cotton cultivars sourced from the National Genebank of Azerbaijan under 100 mM and 200 mM NaCl stress conditions were investigated. Quantitative PCR (qPCR) analysis revealed significant transcriptomic variability between genotypes and stress levels. Notably, no universal expression trend was identified; both genes exhibited divergent up-regulation and down-regulation patterns across salt-tolerant and salt-sensitive varieties, consistent with previous findings regarding the functional diversity of this family. These results underscore the complex regulatory role of *Di19* genes in cotton and provide essential data for future functional studies aimed at improving salinity resilience in cotton through targeted breeding strategies.

Keywords: cotton; salt stress; *Di19*; resistance; qPCR; *Gossypium hirsutum*

1. INTRODUCTION

Cotton is one of the world's most important fiber crops and also a valuable source of seed and oil products, making it central to both the textile industry and agricultural economies (Akparov et al., 2021). Salinity stress is one of the most damaging abiotic stresses in agriculture because it reduces germination, slows growth, disrupts water and nutrient uptake, and ultimately lowers crop yield and quality across many species (Zörb et al., 2019).

Plants respond against salt stress through coordinated mechanisms involving osmolyte accumulation, antioxidant defense, ion homeostasis, hormone regulation, and transporter control (Huang et al., 2020; Alizade, 2022). In plants, *Di19* genes encode a conserved family of C2H2 zinc-finger transcription factors that help

link developmental programs with abiotic-stress signaling, especially drought, salinity, and ABA responses, but their effects can be either positive or negative depending on the species and specific *Di19* member (Qin et al., 2014; Zhao et al., 2022a).

Cotton germplasm held at the National Genebank of Azerbaijan has undergone extensive morphometric assessment; however, molecular genetic characterization regarding stress tolerance remains limited. This study aims to bridge that gap by quantifying the expression of *GhDi19-1* and *GhDi19-2* genes under saline conditions, specifically comparing transcriptomic variations between salt-tolerant and salt-sensitive cotton genotypes.

2. MATERIALS AND METHODS

This study was conducted in the Department of Industrial and Forage Crops at the Institute of Genetic Resources of the Ministry of Science and Education of Azerbaijan. For this investigation, 14 local (Aghdash-3, AP-317, Bayraqdar, Barakat, Ganja-110, Ganja-114, Ganja-160, Ganja-182, Ganja-195, Ganja-200, Kharabakh-11, Kharabakh-12, Zafar) and 17 introduced (Kırqızistan-174, Tashkent-1, Tashkent-2, Tashkent-3, Navai-9, Edessa, Sezener-76, May-344, Beyaz altun-440, CSN-12, PG, Lima, Carisma, Cristina, Assos, Prime, Select) cotton cultivars of the species *Gossypium hirsutum* L. were analyzed, with all seed samples sourced directly from the National Genebank.

For each experimental variety, ten pre-fumigated seeds were sown in plastic containers. All plants were initially irrigated with standard Hoagland's solution (Hoagland & Arnon, 1950) until they reached the first true leaf stage. To induce salinity stress, NaCl concentrations

were gradually increased to final levels of 100 mM and 200 mM. Leaf samples were harvested from both control and salt-treated plants and flash-frozen at -80°C for subsequent RNA extraction. All experiments were conducted in triplicate.

Total RNA was isolated using the RNX-Plus reagent (Cat. No: EX6101). First-strand cDNA synthesis was performed using the SinaClon First Strand cDNA Kit (Cat. No: RT5201) in accordance with the manufacturer's protocols. Specific primers were designed utilizing the Primer3 online platform (<https://primer3.ut.ee/>), with the *GhTUB1* gene (encoding beta-tubulin) serving as an internal reference (Table 1). Relative gene expression was analyzed via quantitative PCR (qPCR) using RT² SYBR Green Mastermix (Qiagen, Cat. No: 330502) on a Rotor-Gene Q 5-plex system (Qiagen, ID: 9001570). Expression levels of *GhDi19-1* and *GhDi19-2* were determined using the $2^{-\Delta\Delta\text{CT}}$ method.

Table 1. Characteristics of gene-specific primers used in the study.

Gene	Primer	Sequence (5'→3')	nmol	Tm (°C)	GC (%)
<i>GhTUB1</i>	Forward	ATGGATCTGGAACCCGGTAC	17.19	59.35	55
	Reverse	AATCGCAATTCTCGGCTTCC	19.16	57.30	50
<i>GhDi19-1</i>	Forward	GCTGATTCATGGAGTGCTCG	16.06	59.35	55
	Reverse	TATCAACGCCACCCTCATT	16.62	57.30	50
<i>GhDi19-2</i>	Forward	TGCAATGAGGGTGGGAGTAG	15.71	59.35	55
	Reverse	CGAAGACAGCAACGGATCAG	16.05	59.35	55

3. RESULTS AND DISCUSSION

Under 100 mM NaCl stress, the expression level of *GhDi19-1* was increased in 17 and decreased in 14 of the 31 analyzed genotypes. At the higher concentration (200 mM NaCl), expression decreased in 15 and increased in 16 genotypes relative to the control (Table 2). Among local genotypes, Bayraqdar showed the highest increase in *GhDi19-1* expression under both 100 mM and 200 mM NaCl stress. Among the introduced genotypes, Navai-9 (Uzbekistan) demonstrated the most pronounced expression increase at both concentrations.

Evaluation of *GhDi19-2* under the same conditions revealed a similarly wide genotypic variation. Under 100 mM NaCl, expression

decreased in 16 samples and increased in 15; under 200 mM NaCl, expression decreased in 19 and increased in 12 samples. Among local genotypes, Kharabakh-11 showed the highest increase in *GhDi19-2* expression at both concentrations, while among the introduced varieties, Navai-9 and Tashkent-2 exhibited the most marked increases at 100 mM and 200 mM NaCl, respectively.

These results revealed no clear directional trend distinguishing salt-tolerant from salt-sensitive varieties — both up-regulation and down-regulation were observed among cultivars of each category, at both concentrations. This is consistent with previous reports on *GhDi19-3* and *GhDi19-4*, which act as negative regulators

of salt tolerance (Zhao et al., 2022a), and with findings in soybean (*GmDi19-5*: Yunhua et al., 2023) and wheat (*TaDi19-7*: Linying et al., 2023),

which similarly demonstrated the functional diversity of the *Di19* family across species.

Table 2. Changes in the expression level of drought-induced-19 genes in cotton plants

Genotype	NaCl concentration	<i>GhDi19-1</i>	<i>GhDi19-2</i>	Genotype	NaCl concentration	<i>GhDi19-1</i>	<i>GhDi19-2</i>
Aghdash-3	100 mM	↓0.354	↓0.31	Tashkent-3	100 mM	↑2.848	↑3.506
	200 mM	↓0.46	↓0.624		200 mM	↓0.049	↓0.006
AP-317	100 mM	↑3.972	↓0.693	Navai-9	100 mM	↑729.1	↑139.1
	200 mM	↑5.657	↓0.49		200 mM	↑18	↑79.89
Bayraqdar	100 mM	↑138.1	↑2.014	Edessa	100 mM	↓0.019	↓0.12
	200 mM	↑82.14	↓0.019		200 mM	↓0.074	↓0.115
Barakat	100 mM	↓0.029	↓0.268	Sezener-76	100 mM	↑282.1	↑1.79
	200 mM	↓0.012	↓0.003		200 mM	↓0.599	↓0.005
Ganja-110	100 mM	↑52.35	↑2.204	May-344	100 mM	↑1.434	↓0.006
	200 mM	↑3.63	↓0.01		200 mM	↑1.625	↓0.435
Ganja-114	100 mM	↑10.7	↓0.005	Beyaz altun-440	100 mM	↓0.979	↓0.015
	200 mM	↑25.11	↓0.006		200 mM	↓0.002	↑1.693
Ganja-160	100 mM	↓0.137	↓0.005	CSN-12	100 mM	↑38.32	↑46.53
	200 mM	↑1.24	↓0.722		200 mM	↑1.424	↑45.25
Ganja-182	100 mM	↓0.036	↓0.113	PG	100 mM	↑29.24	↓0.196
	200 mM	↑39.4	↓0.325		200 mM	↓0.005	↓0.027
Ganja-195	100 mM	↑39.4	↑12.64	Flash	100 mM	↓0.052	↓0.187
	200 mM	↓0.624	↑7.111		200 mM	↓0.018	↓0.002
Ganja-200	100 mM	↑6.964	↑4.228	Lima	100 mM	↑26.72	↑74.03
	200 mM	↓0.415	↓0.035		200 mM	↑12.55	↑54.57
Kharabakh-11	100 mM	↓0.002	↑20.68	Carisma	100 mM	↓0.013	↓0.796
	200 mM	↑67.18	↑17.27		200 mM	↓0.086	↓0.029
Kharabakh-12	100 mM	↑1.087	↑2.204	Cristina	100 mM	↓0.409	↑6.021
	200 mM	↑4.563	↑5.579		200 mM	↓0.426	↑2.639
Zafar	100 mM	↑18.51	↓0.042	Assos	100 mM	↓0.261	↓0.002
	200 mM	↓0.006	↑8.574		200 mM	↑1.347	↓0.467
Kırqızistan-174	100 mM	↑398.9	↑1.102	Prime	100 mM	↓0.019	↓0.007
	200 mM	↓0.031	↓0.006		200 mM	↑10.27	↑1.385
Tashkent-1	100 mM	↓0.015	↓0.012	Select	100 mM	↑8	↑1.042
	200 mM	↓0.177	↓0.06		200 mM	↑10.34	↑1.959
Tashkent-2	100 mM	↓0.004	↑5.315				
	200 mM	↑8.938	↑129.8				

4. CONCLUSION

This study examined the expression profiles of *GhDi19-1* and *GhDi19-2* genes in 31 cotton genotypes under salt stress. Results demonstrated significant, genotype-dependent variations in transcriptional response at both 100 mM and 200 mM NaCl, with no consistent pattern distinguishing salt-tolerant from salt-sensitive varieties. High-performing genotypes, such

as Bayraqdar, Navai-9, and Kharabakh-11, exhibited pronounced expression increases, highlighting the functional complexity and variability of the *Di19* family in cotton. These findings suggest that *GhDi19* genes act as dynamic transcriptional regulators, and that their role in salinity tolerance is context-dependent rather than a uniform indicator of resistance.

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