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Comparative Anatomical and Morphological Study of Three Populations of *Salvia aethiopis* L. Growing in the Southern Balkhash Region

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ABSTRACT

The manuscript presents the results of morpho-anatomical structure of vegetative organs of *Salvia aethiopis* L. *S. aethiopis* L. is a promising medicinal plant of flora of Kazakhstan, which is able to grow under certain soil and climatic conditions. According to its anatomical and morphological features, *S. aethiopis* L. is a drought-resistant plant that does not tolerate excess moisture. The aim of the study was to investigate the morpho-anatomical structure of *S.aethiopis* L. plants growing in three different populations of the South Balkhash region of the country. As a result of the study of the morpho-anatomical structure of vegetative organs of the studied plants, the following features of xeromorphic structure of *S.aethiopis* L. were noted: strong pubescence of all above-ground parts of plants by simple, globular and glandular hairs, well expressed cuticle with small spinules, presence of cuticular thickening of guard cells, large number of stomatal apparatus on lower epidermis. A comparative analysis of the structure of plants of three different populations showed that in the stem of plants of population № 1 the primary cortex and the diameter of the central cylinder are more pronounced in comparison with plants growing in populations № 2 and № 3, which is due to the growing conditions of these populations. In plants of population № 1, an increase in the thickness of the leaf blade as well as inclusions of essential oils, which are localized in single or paired essential oil passages, were noted. In plants of populations № 1 and № 2, the more essential oil passages were observed. The studied plants of population № 1 have the most pronounced thickness of the primary bark than plants of populations № 2 and № 3. The revealed changes in the morpho-anatomical structure of plants are associated with the influence of various environmental and anthropogenic factors depending on the location of the studied plants.

Keywords: plant, association, morphology, anatomy, epidermis, exoderm.

INTRODUCTION

Currently, the use of plant resources is becoming increasingly relevant in connection with the development of the economy of the Republic of Kazakhstan. Work is actively underway to study the resource characteristics of economically valuable plants of the Balkhash region (Gemedzhieva et al., 2017). The study and practical application of useful plants is of great importance in the national economy, medicine, since plants characteristic only of our territory are a cheap and easily renewable source of raw materials for agriculture, food, perfumery, cosmetics and pharmaceutical industries. Essential oils used in the cosmetic, perfumery, food and pharmaceutical industries play an important role among useful plants of general use. In recent decades, the use of traditional medicines, especially herbal medicines, has increased significantly (Popovic et al., 2016). Since ancient

times, people have used medicinal plants for treatment and other medical purposes, probably because of their wide availability, as well as the high cost and inaccessibility of medicines in some regions of the world. Their use also represents the preservation of cultural heritage, considering the practice of traditional medicine as part of the culture of peoples (Van Wyk and Wink, 2018).

Geobotanical, phytochemical and morpho-anatomical studies of promising medicinal plants are being conducted in Kazakhstan (Kurbatova et al., 2003; Ydyrys et al., 2013; Atabayeva et al., 2016; Bekbolatova et al., 2018; Kuprianov et al., 2020; Childibayeva et al., 2022; Özek et al., 2022; Atabayeva et al., 2022). There are works on the study of representatives of medicinal plants from the *Lamiaceae* family under study, such as *Marrubium vulgare* and *M. alternidens* (Kurbatova et al., 2016), *Ziziphora Bungeana Juz.* (Zhaparkulova et al., 2016). One of these plants is *Salvia aethiopis* L. with promising medicinal value. Morpho-anatomical studies of promising medicinal plants growing in the South Balkhash region have been carried out, and for the first time geobotanical, morpho-anatomical studies of the plant *S.aethiopis* L. Therefore, our study on the study of *S. aethiopis* L. at the population level, it is completely new and covers issues related to the problems of geobotany, morphology and anatomy of plants, as well as the ecology of this plant. Sage is one of the most important genera of medicinal plants of the *Lamiaceae* family and includes about 1000 species worldwide (Walker et al., 2004). Several types of sage have very interesting pharmacological properties, namely antioxidant (Esmaeili and Sonboli, 2010), antidiabetic (Flores-Bocanegra et al., 2017), analgesic (Simon-Arceo et al., 2017), anti-inflammatory activity in vitro / in vivo (Akram et al., 2015), and effects against Alzheimer's (Senol et al., 2017), which in some cases are attributed to secondary metabolites, especially phenolic compounds.

In different species of the genus *Salvia* L. about 300 biologically active substances have been established. The composition of plants rich in essential oils, some industrial areas, in particular the food, cosmetics and perfume industries, show economic interest in these species. They are also used in the pharmaceutical industry due to the biologically active phytochemicals contained in them (Baykova et al., 2002). There are about 6000 higher plant species in the flora of Kazakhstan, in which there are also enough plant species containing 10 essential oils (Kukenov, 1990). Distribution in Kazakhstan, found in the mountains of Chu-Ili, Karatau, Dzungarian Alatau. There are 8 species of the genus *Salvia* L. in Kazakhstan: *S. trautvetteri* Rgl., *S. macrosiphon* Boiss., *S. sclarea* L., *S. aethiopis* L., *S. stepposa* Schost., *S. virgate* Jacq., *S. deserta* Schang., *S. verticillata* L. *Salvia aethiopis* L. grows in steppes and on meadow slopes of steppe mountains, in dry rocky and clay places, on chalk and calcareous outcrops, on arable land and pastures.

According to morphological characteristics, *Salvia aethiopis* L. is a perennial from 50 to 120 cm in height, with a thick, ribbed, densely, felt, hairy, simple stem, strongly branching only in the inflorescence; the basal and lower stem leaves are ovate, large from 6 to 25 cm in length and up to 15 cm in width, with equal on the plate with petioles, along the edge bluntly notched-toothed, wrinkled, tangled, felt-pubescent, especially strongly along the veins, the upper leaves gradually decrease, the uppermost of them are "sessile" and triangular-lanceolate in outline; the inflorescence is strongly branched, with numerous, 6-10-flowered whorls, the nuts are round-ovate, greenish brown, glabrous (Flora of Kazakhstan, 1964).

Scientists from Turkey studied the anatomical structure of medicinal plants *S. aethiopis* L., *S. virgata* Jack and *S. dichroantha* Stapf. In herbaceous stems, the bark is collenchymal and parenchymal in all studied species. Vascular system consisting of a hollow cylinder of xylem surrounded by phloem. The integumentary hairs are simple and unicellular or multicellular (Eröz Poyraz et al., 2007). There are other data on the study of morphological and anatomical features of Salvia aethiopis. The species belonging to the glandular and simple hairs for this species was determined, which played an important role in the classification. When studying morphological features, it was found that the species has a rectangular stem with basal leaves and a scaly corolla. During anatomical studies, it was noticed that the primary rays located in the root and stem are heterogeneous. It is described that the species has a diacytic stomata type and isolateral leaves (Özkan et al. 2006).

Also, there are data on the introduction of sage: *S. aethiopis* L., *S. virgata* Jacq., *S. dichroantha* Stapf.. These species pass the introduction tests well. Anatomical features of the root, stem and leaf were revealed. The features of the structure of glandular, segmented, head-shaped

hairs are noted, diagnostic signs of the species are determined (Eroz et al., 2007). Anatomical and micro-morphological characteristics of Salvia vermifolia from Central Anatolia are available in the literature. The study revealed common anatomical features, namely the features of the stem, leaf blade and petiole, and also described micro-morphological features, trichomes, pollen and nuts of Salvia vermifolia Hedge, which were examined using light microscopy (LM) and scanning electron microscopy (SEM). The results obtained during anatomical studies of S. Vermifolia show that the stem consists of 4–8 layers of collenchyma cells and 1–3 layers of chlorenchyma cells. The leaf blade is amphistomatic, two-sided, with 2-3-tiered palisade cells. The petiole has three large central bundles and six small additional bundles. Pilate glandular, glandular, glandular and non-glandular trichomes are present in *S. vermifolia*. Glandular trichomes are present in large numbers on the calyx (Bagherpour et al., 2010).

MATERIAL AND METHODS

Using traditional and new methods of botanical research, the state of distribution of this promising medicinal plant in modern nature is assessed and thus the further preservation of natural populations is taken into account. Anatomical and morphological features of vegetative organs (root, stem, leaf) were studied of the above species from three populations. In the manufacture and description of preparations, methods of plant anatomy were used (Prozina, 1960; Permyakov, 1988; Barykina and Veselova, 2004; Barykina et al., 2004; Lotova, 2007).

Microscopic studies were carried out on plant material, which was fixed in a mixture of alcohol, glycerin and water in a ratio of 1:1:1. To study the stems, their segments were boiled in a 5% sodium hydroxide solution, thoroughly washed with water, the epidermis was removed with a scalpel, examined from the surface and diagnostic signs were determined first at low, then at high magnification. Anatomical preparations were made using a microtome with a freezing device OL-ZSO (Inmedprom, Russia) (Prozina, 1960; Permyakov, 1988; Barykina and Veselova, 2004; Barykina et al., 2004; Lotova, 2007). For quantitative analysis, morphometric indicators were measured using an eyepiece micrometer MOV-1-15 (with a lens ×10, magnification ×40, 10, 7). Micrographs of anatomical sections were taken on a microscope MC 300 (Micros, Austria) with a video camera CAM V400/1.3M (JProbe, Japan). The description of external signs is carried out in accordance with the requirements of GF XI (State Pharmacopoeia of the USSR, 1987a; 1990b).

RESULTS AND DISCUSSION

Anatomical and morphological structure of the roots of *Salvia aethiopis* L.

The object of our study was *Salvia aethiopis* L. The species is confined to certain soil and climatic environmental conditions. *Salvia aethiopis* L. tends to grow on road banks, in open steppe valleys of the Southern Balkhash region. The current state of normal vegetation growth, widespread in the natural conditions of the Southern Balkhash region under the influence of various environmental factors, is considered.

In 2021, samples of plant raw materials of Ethiopian sage (*Salvia aethiopis* L.) from 3 populations were collected. The first population (P1) of *Salvia aethiopis* L. was collected on the territory from the right bank of the Kapshagai-Bakanas highway towards the Almaty region, from an altitude of 709 m above sea level, an open steppe zone of a grassy community. The second population (P2) was found in the Kerbulak district of the Almaty region in an artificial forest belt of the ostroplodny loch and the elm on the right side of the Kapshagai-Sary-Ozek highway beyond the village Shengeldy. Coordinates N 44° 7’23.49”, E 77°35’22.95”, altitude 694 m above sea level.

The third population (P3) was found on the right bank of the Kapchagai-Bakanas highway, in the valley of the open steppes of the Bakpaktinsky rural district on pastures of significant herbaceous plants of various grasses. The climate of the described territory is temperate continental. The soil is sandy-dusty-loose. Coordinates N: 44.06.04°; E:77.03.48 ° altitude above sea level 788 m. On a cross-section of *Salvia aethiopis* L. (Fig. 1), at low magnification (×10), a two-layer periderm (a dark brown layer of cells) is traced on the surface of the root, forming rows of loosely closed, loose peripheral cells. Small, polygonal cells are tightly connected and strongly elongated in the radial direction. Under it, the storage parenchyma of the primary bark with starch grains are located in more or less concentric layers. The

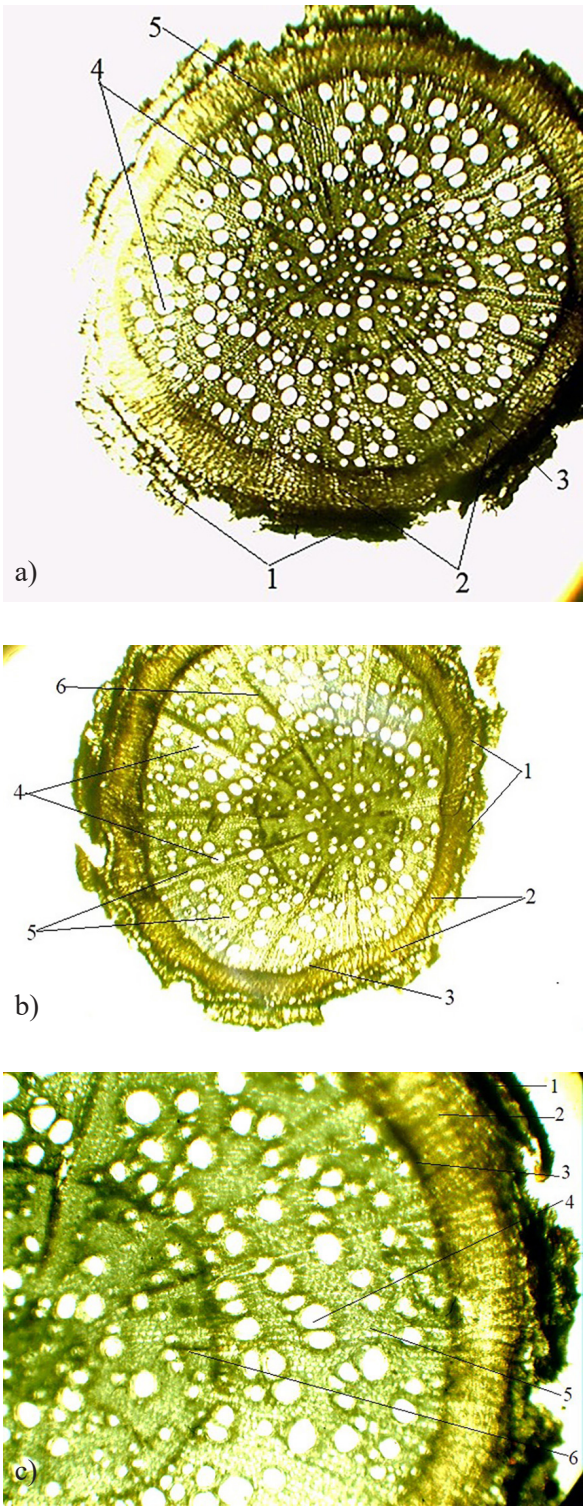

Fig. 1. Anatomical structure of *Salvia aethiopis* L. root (×10): a – population, 1 – periderm, 2 – primary cortex (cortical parenchyma), 3 – cambium, 4 – xylem vessels, 5 – phloem, 6 – core rays

cells are rounded with slightly thickened walls with sparse intercellular spaces. There are single inclusions in the cells. The inner layer is represented by tightly closed cambium cells, which borders on the central cylinder. The pericycle is represented by a single layer of cells. The phloem is located in small areas between the vessels of the xylem. The primary xylem is represented by 5–6 radial chains of narrow-light small vessels converging to one wider vessel of the metaxylem. The secondary xylem occupies the central part of the root. In the center, around the vessels of the secondary xylem, cells of mechanical tissue are localized. Morphometric parameters of the *Salvia aethiopis* L root are presented in Table 1.

Based on the results of Table 1, it should be noted that the thickness of the primary bark in plants with P1 is most pronounced (76.150 microns) than in plants with P3 (56.595 microns), the diameter of the central cylinder has a similar pattern, i.e. in plants with P1 it is larger (330.696 microns) than in plants with P2 and P3 (316.263/310.55 microns). With regard to the area of the xylem elements, a slight decrease in the area of the vessels and the total area of the conducting beams can be noted.

Anatomical and morphological structure of the stems of *Salvia aethiopis L.*

The structure of the stem of *Salvia aethiopis* L. (Fig. 2) is typical for representatives of the *Lamiaceae* Lindl family. The stem on the cross section is tetrahedral, consists of a bark and a central cylinder. The cortical zone is represented by one layer of the epidermis. Under the epidermis there are bundles of angular collenchyma, which completely fills the ribs. The facets fill in small quantities, about one or two layers of cells. The cortical zone ends with a single-layer endoderm. At the periphery, the conducting beams have a sclerenchymal lining. Endoderms are represented by single intermittent cells. The central cylinder is represented by endoderm cells, a conduction zone with radially arranged bundles. Rounded core cells are traced in the center. The vessels of the xylem and the sieve tubes of the phloem are differentiated in the central cylinder. The main part of the parenchymal tissue of the core is represented by cells with very thin shells, and the cells themselves are quite large. The conductive beams in the edge are much larger, almost reaching the size of the conductive beams of the face. When studying the elements of the xylem, it can be concluded that the origin of the conducting beam of the edge is by merging two, small sizes. There is a cavity inside the stem and due to its

Table 1. Morphometric parameters of the *Salvia aethiopis* L. root

Population (P)	The thickness of the primary crust, microns	Diameter of the central cylinder, microns	Area of conducting beams, x10^{-3}мм^2	Xylem area, x10^{-3}мм^2
1	78,059 76,247 74,145	341,324 324,894 325,871	116,697 115,027 112,851	37,149 35,498 29,123
Average value	76,150	330,696	114,858	33,923
2	65,879 63,854 62,659	319,567 315,751 313,471	119,541 114,981 109,247	35,147 32,541 29,124
Average value	64,131	316,263	114,589	32,271
3	57,842 56,487 55,457	311,484 310,754 309,412	114,547 111,741 107,841	33,451 32,115 28,471
Average value	56,595	310,55	111,376	31,346

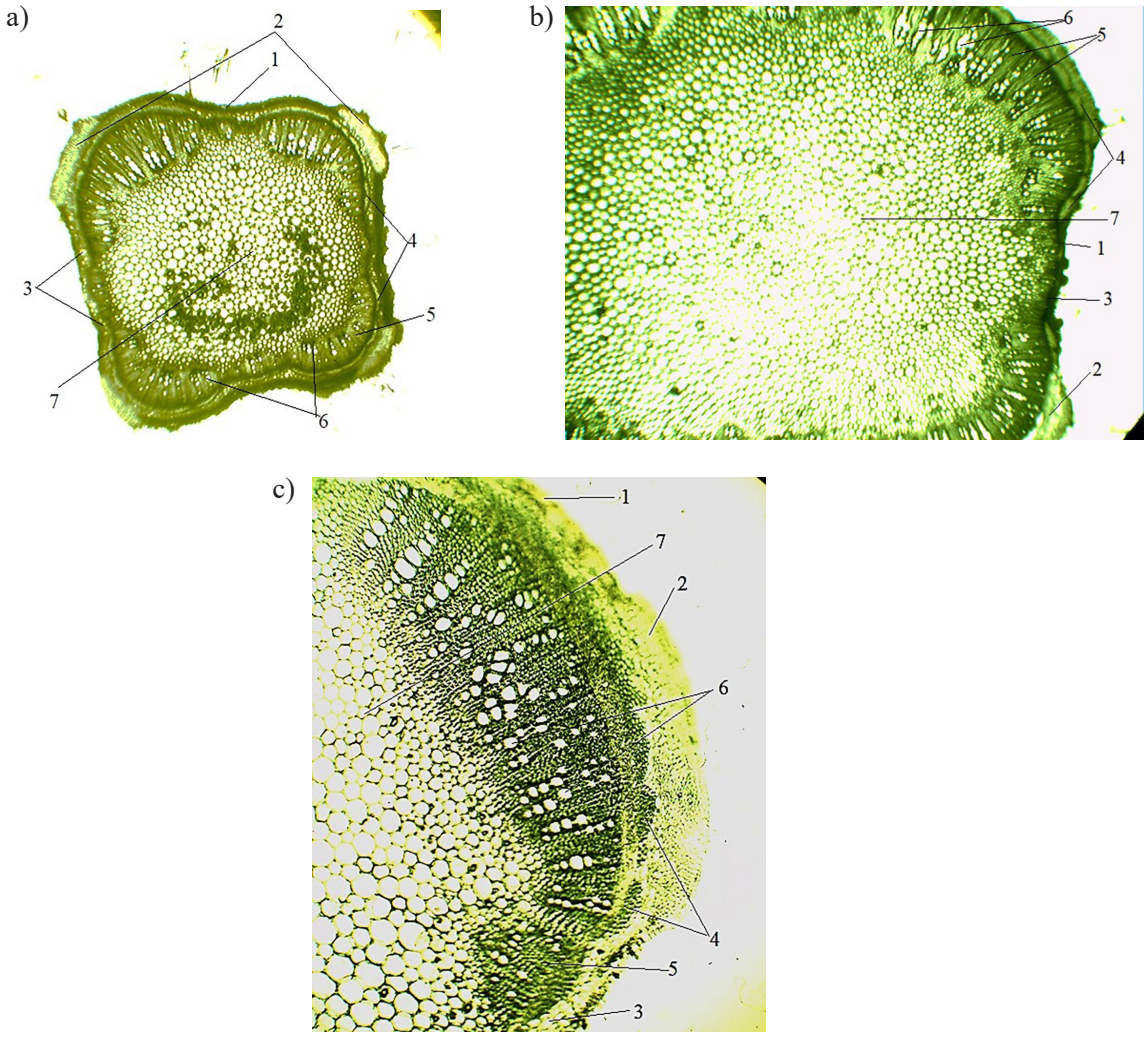

Fig. 2. Anatomical structure of the stem of *Salvia aethiopis* L. (×40): a – population, 1 – epidermis, 2 – angular collenchyma, 3 – parenchyma of the primary cortex, 4 – sclerenchyma, 5 – phloem, 6 – xylem vessels, 7 – core with single inclusions

increase in the parenchyma there is a process of accumulation of salt crystals. The interstitial cambium consists of 4–5 layers of cells, depositing a strong weight of sclerenchyma. Morphometric parameters of the Salvia aethiopis L stem are presented in Table 2. Based on the results of Table 2,

Table 2. Morphometric parameters of the stem of Salvia aethiopis L.

Population (P)	The thickness of the primary crust, microns	Diameter of the central cylinder, microns	Area of conducting beams, x10^{-3}мм^2	Xylem area, x10^{-3}мм^2
1	68.478 64.534 64.991	401.523 399.456 399.852	119.231 109.483 94.587	11.214 9.823 7.586
Average value	66.001	400.277	107.767	9.541
2	62.343 61.876 61.527	315.489 314.563 314.389	212.456 209.567 200.481	12.185 9.841 7.951
Average value	61.915	314.814	207.501	9.646
3	53.941 52.645 52.314	245.843 241.347 239.821	213.457 119.786 63.451	18.729 14.127 6.911
Average value	52.967	242.337	132.231	13.257

Table 3. Morphometric parameters leaf of Salvia aethiopis L.

Population (P)	Sheet thickness, microns	Thickness of the epidermis, microns		Area of conducting beams, x10^{-3} mm^2
		Upper	Lower	
1	757,112 748,547 741,879	14,641 13,895 12,997	11,231 10,009 9,912	305,554 299,456 281,983
Average value	749,179	13,844	10,384	295,664
2	689,125 661,743 658,186	11, 217 10,653 10,005	9,993 9,405 8,894	287,247 282,543 279,991
Average value	669,684	10,625	9,431	283,260
3	501,427 500,645 489,982	9,987 9,678 9,185	7,673 7,545 7,008	219,631 218,834 218,009
Average value	497,351	9,617	7,409	218,825

it should be noted that the thickness of the primary bark in plants with P1 is more pronounced (66,001 microns) than in plants with P2 (61,915 microns) and P3 (52,967 microns), a similar pattern can be traced in the diameter of the central cylinder. The area of conducting beams is better expressed in P2 (207,501×10^{-3} mm^2), worse in P3 – 132,231×10^{-3} mm^2. The area of xylem vessels has the highest index in P3 of 13.257×10^{-3} m^2, and the lowest index in plants of P1 (9.541×10^{-3} mm^2), which is due to the growing conditions of these populations.

Anatomical and morphological structure of *Salvia aethiopis* L. leaves

In the structure of the epidermis of the leaves of Ethiopian sage, the characteristic features are the following: the walls of the cells of the epidermis are tortuous, and the tortuosity is less pronounced on the upper epidermis than on the lower one, they differ somewhat in size. In the cross section, the cells of both the upper and lower epidermis have a shape elongated in the horizontal plane, their outer walls are slightly thickened. The cuticle is well expressed. The epidermis cells in the veins (supra-spider cells) are straight-walled, elongated along the veins, they are long, narrow in the lower epidermis, and shorter and wider in the upper epidermis.

Stomata are present on both sides of the leaf (amphistomatous type of leaf), and there are much more of them on the lower epidermis. Stomata are arranged in no particular order. By the nature of the location of the periosteal (side) cells, the type of stomatal apparatus is diacetic (cross-cellular or karyophylloid) – with two accompanying cells located transversely to the stomatal cleft, while one of the periosteal cells is smaller than the other. Glandular trichomes are of the glandular and peltate type. The obtained data on the anatomical structure of the leaves of *S. aethiopis* L. confirm the studies of İlham Eröz Poyraz and Fehmiye Koca, who also found some differences in the glandular and non-glandular hairs and medullary rays of the studied species. The leaves of all studied species are dorsoventral and

amphistomatic. The integumentary and glandular hairs of the leaves are similar to the hairs on the stems (Poyraz et al., 2007).

Trichomic formations on both epidermis are represented by simple single, glandular and glandular hairs. Simple single hairs are long, thin, two- or three-celled, simple hairs are three-five-ray unicellular. At the base of the hairs there are two or three epidermal cells, which differ in shape from other cells of the epidermis - they have a rounded shape. On the lower epidermis, the hairs are longer and thicker. They are scattered on the upper epidermis. Glandular hairs are of two types: some have two-celled and four-celled heads on a short unicellular pedicle. Essential oil glands consist of 4–6 cells, found only on the lower epidermis. The leaves of *Salvia aethiopis* L. have a thick covering of covering linear unicellular trichomes on both sides, while the tip of the trichomes is bent in the form of a hook. All trichomes are of completely different lengths, but long ones are predominant. Glandular trichomes consist of a bicellular pedicle and a unicellular head, and they are much smaller.

When considering the cross-section with an increase of x70, the following features were noted: there is a thin layer of cuticle on the surface of the cells of the upper and lower epidermis. The cells of the epidermis are small, have a rounded, slightly oblong shape. The thickness of the cells of the upper epidermis layer is smaller, and the thickness of the lower epidermis is larger.

The mesophyll occupies the entire space between the upper and lower epidermis. Mesophyll cells from the upper epidermis are represented by 2–3 rows, have a uniform rounded shape, are densely arranged relative to each other, and the cells of the spongy mesophyll are rounded, larger, densely arranged. Spongy parenchyma represents the bulk of the cells of the leaf blade. The

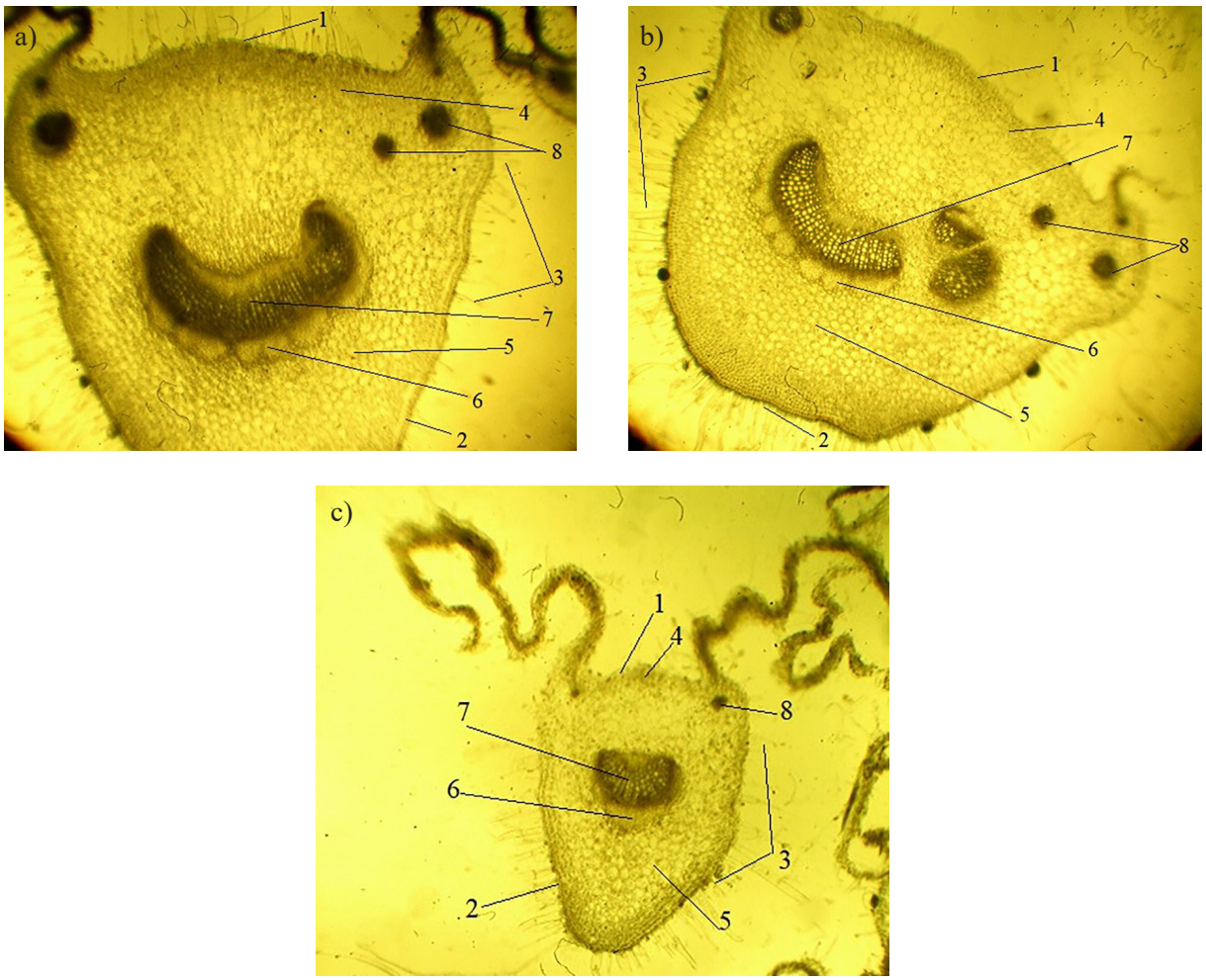

Fig. 3. Anatomical structure of the leaf blade *Salvia aethiopis* L. (x 40): a – population, 1 – upper epidermis, 2 – lower epidermis, 3 – epidermal formations, 4 – mesophyll, 5 – spongy mesophyll, 6 – sclerenchyme, 7 – conducting bundle, 8 – essential oil passages

central conducting bundle, located in the central part of the leaf blade, is surrounded by sclerenchyma, whose cells are elongated along the bundle. Smaller conductive bundles are located in the thickness of the leaf blade, along the perimeter. The bundles are collaterally closed, represented by sieve-shaped phloem tubes and xylem vessels.

Epidermal formations occur in the form of glabrous, simple hairs, as well as glands. In the structure of sage leaves, inclusions of essential oils were noted, which are localized in single or paired essential oil passages. In plants P1 and P2, twice as many essential oil passages were noted. Morphometric parameters of the *Salvia aethiopis* L. leaf are presented in Table 3.

Based on the results of table 3, it should be noted that the thickness of the lower and upper epidermis of leaves in plants is not the same. The thickness of the upper epidermis is greater than the lower one by an average of 1–2 mkm. The thickness of the leaf blade in plants with P1 is greater (749,179 mkm) than in plants with P2 (669,684 mkm) and P3 (497,351 mkm). With respect to the area of the beams, the results show a decrease in the area of the conducting beams from P1 to P3. Also, the identification of morphological features of this species growing in three populations was carried out.

Morphological structure seeds of *Salvia aethiopis* L.

The fruit consists of 4 brownish-brown nuts. Nut size: length 2 = 0.046 mm, width 1 = 0.012 mm. Each nut is covered with a dense dark brown peel on the outside. The structure of the peel is dense, rough, uneven. On the nuts, there is a notch in the central part at the attachment points (Figure 4). The morphological structure of *Salvia aethiopis* L. plants is considered in a comparative aspect (Figure 5). The morphological structure of the leaves (Figure 6), stems (Figure 7) and roots (Figure 8) of *Salvia aethiopis* L. is considered in a comparative aspect. The stems have identical structural features regardless of the growing conditions: tetrahedral, simple, thick enough, slightly ribbed, with thickly felt pubescence, strongly branching only in the inflorescence. The root system is rod-shaped. The structure is identical: the roots are slightly branching, the lateral roots in P1 and P2 are most pronounced, thick, straight with a narrowed tip of the main root.

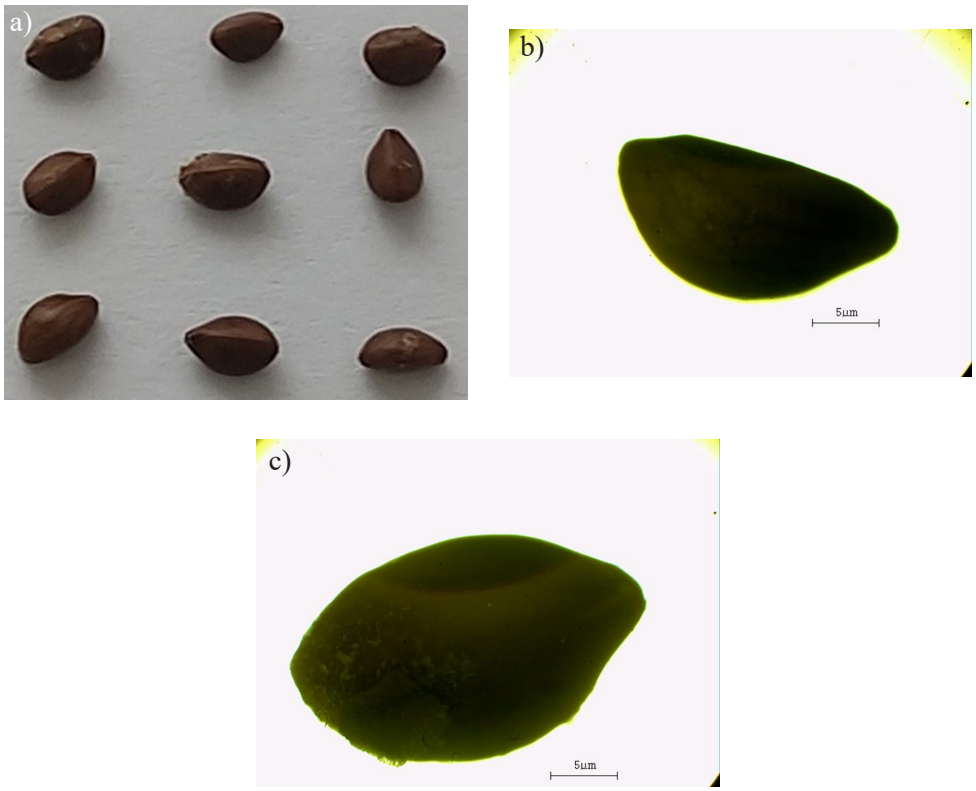

Fig. 4. Seeds of *Salvia aethiopis* L.

Fig. 5. Morphological structure of *Salvia aethiopis* L. (a) 1 population – from the main shoot, 20–25 medium-sized lateral shoots with numerous strongly branching inflorescences depart; (b) 2 population – from the main shoot, 8 to 10 lateral shoots depart, the lower part of which is slightly pubescent, inflorescences branching slightly at the ends of the shoots; (c) 3 population – 6–8 shoots, thicker with sparse, sparse inflorescences, depart from the main shoot.

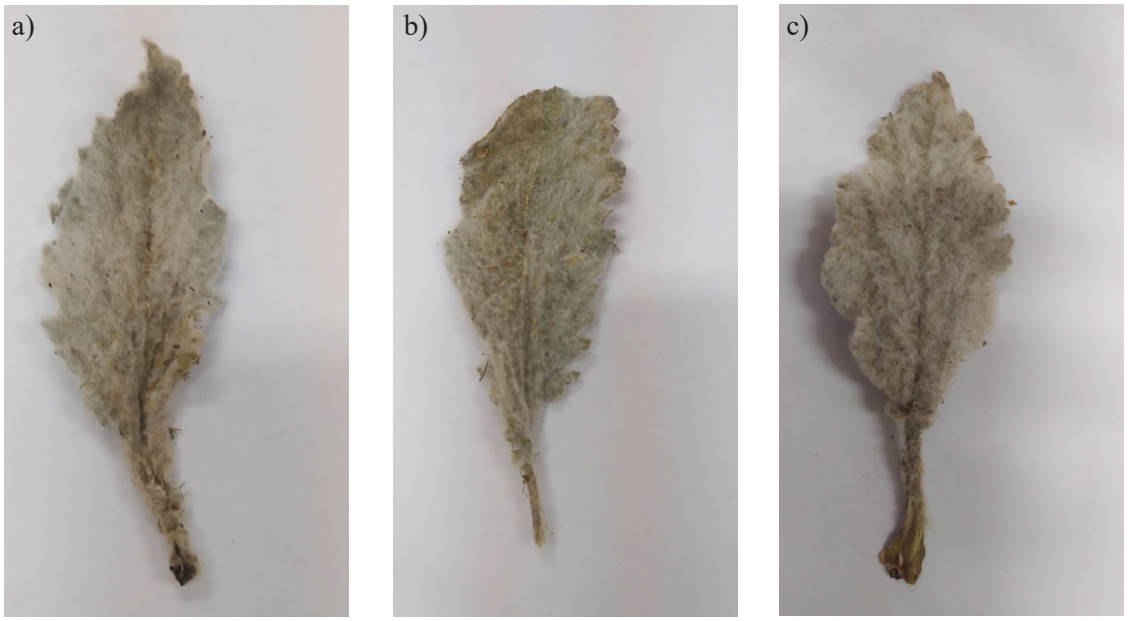

Fig. 6. Morphological structure leaves of *Salvia aethiopis* L. 1 population. The leaf is elongated-oblong with uneven, wavy edges, strongly pubescent, the petiole is weakly pronounced

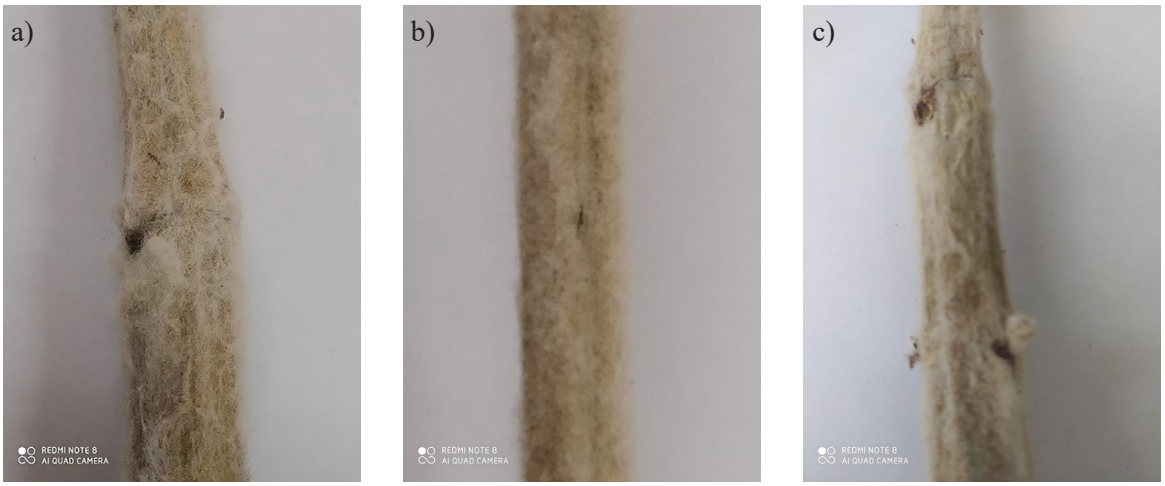

Fig. 7. Morphological structure stems of *Salvia aethiopis* L.: (a) population 1; (b) population 2; (c) population 3

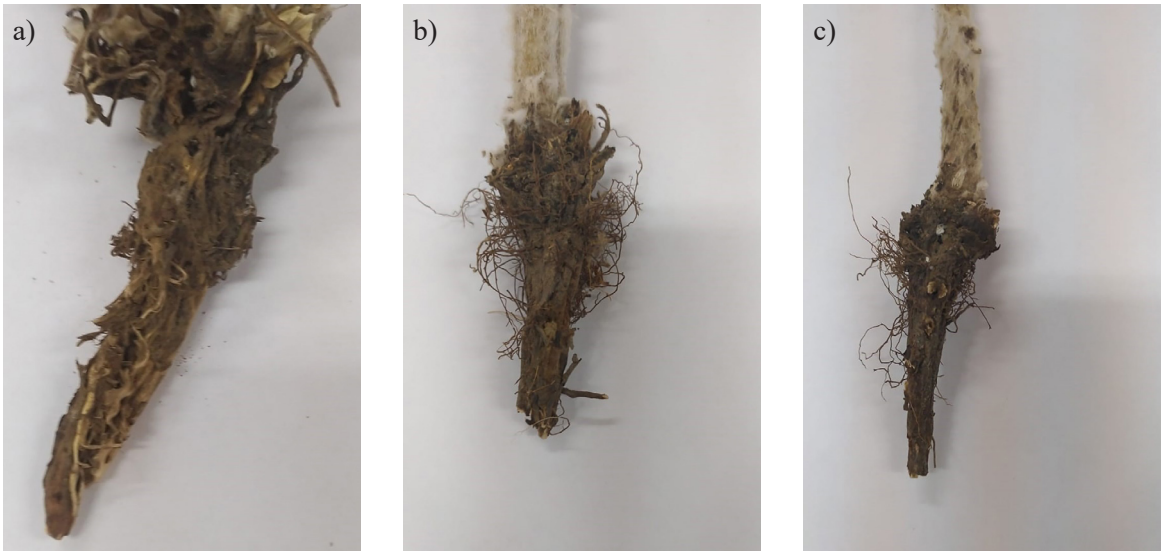

Fig. 8. Morphological structure of the roots of *Salvia aethiopis* L.:
(a) population 1; (b) population 2; (c) population 3

CONCLUSION

Thus, the species *Salvia aethiopis* L. by anatomical and morphological features should be attributed to drought-resistant, which does not tolerate excess moisture. The species is confined to the most xerophytic grass communities: meadow and real steppes, settled meadows. In the anatomical structure of vegetative oranges *Salvia aethiopis* L. features of the xeromorphic structure are noted: strong pubescence of all aboveground parts of the plant with simple, glandular and glandular hairs, a well-defined cuticle with small spikes, the presence of cuticular thickenings of the closing cells, a large number of stomatal apparatuses on the lower epidermis. The structure of the root of the studied species from P1, P2, P3 also has a number of similar features of the organization - the secondary structure.

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