

IRSTI 34.29.00

^{1*}M.S. Kurmanbayeva, ¹N.M. Mukhitdinov, ¹A.D. Serbayeva,
²G. Sramko, ¹M.K. Makhambet

¹Laboratory of plant ecomorphology, Almaty, Kazakhstan

²University of Debrecen, Debrecen, Hungary

*e-mail: Meruyert.Kurmanbayeva@kaznu.kz

Anatomical features of the rare species of *Erysimum croceum* M.Pop. from Trans-Ili Alatau

Abstract: *Erysimum croceum* M. Pop. (*Brassicaceae*) is an endemic species, growing only in Trans-Ili Alatau. It is included into Kazakhstan Red Data Book. The aim of this study is to compare two populations of *Erysimum croceum* M.Pop identified in Trans-Ili Alatau region, for which morphological and ecological analysis was undertaken. Plant communities of the 1st population growing in Small Almaty gorge (N43° 06.316', E077° 04.184') are dominated by indigenous plants with a significant proportion of endemic taxa, while the 2nd population from the Big Almaty gorge (N43° 04.790', E076° 59.512') is growing in subruderal positions with the presence of other plants. Statistical analysis of the morphological traits revealed significant differences between the two populations. In this article summary on comparative analysis of the main features of morphological and anatomical structures of endemic species *Erysimum croceum* is given. To identify the cause of its disappearance we studied seed germination and growth of seedlings. Seed germination is low and only reaches 18 %. Morphological structure of seedlings on the 45th day showed overall length of sprouts 6.67±3.84 cm, hypocotyl length 1.12±0.06 cm, root length 5.49±3.57 cm, leaves length 0.39±0.63 cm, and leaves width 0.2±0.2 cm. The anatomical features of *Erysimum croceum* were defined. Stem diameter in *Erysimum croceum* collected from the 1st population equals to 1052.92±11.7 µm, epidermis thickness – 25±0.9 µm, primary cortex thickness – 96.24±0.6 µm, core diameter – 796.51±5.9 µm. Stem diameter in *Erysimum croceum* collected from the 2nd population equals to 996.38±9.8 µm, epidermis thickness – 21±0.7 µm, primary cortex thickness – 80.98±0.4 µm, center circle diameter – 701.59±8.7 µm. Anatomical sections of rhizome and leaf were compared on virginal and generative stages, while the stem was studied only at generative stage. Main specification into the inner structure is designated by accumulation of sclerenchyma between conducting bundles in central cylinder and alternative location of small and large bundles.

Key words: *Erysimum croceum* M. Pop., seed, anatomy, stem, sclerenchyma, xylem.

Introduction

There are more than 13 thousand species of valuable plants in Kazakhstani flora, among which 5754 species are widely spread, 1820 species are endemic. *Erysimum (E.) croceum* plants are met in northeastern part of Trans-Ili Alatau, where its range is constantly reduced. For that reason, it was included into the Kazakhstan Red Data Book, containing rare species of cabbage samples, which needs special protection. The flowering period of this species is from April to May. It is propagated by seeds.

Erysimum is widespread at the Northern hemisphere, rich in morphological features and taxon complexity, with main distribution of *Erysimum* allied species in Europe, where 150-350 species are

encountered. Several methods of phylogenetic relations were used for its study, including the patterns of morphologic evolutionary indicators and application of variability of the related genus.

Internal transcribed spacer DNA sequences from c. 85% of the species (117 for the first time), representing the full range of morphological variation and geographical distribution was performed with several approaches to reconstruct phylogenetic relationships, dating of diversification and patterns of evolution of morphological characters in the genus. Ancestral-state reconstructions of four morphological diagnostic characters were performed using maximum parsimony, maximum likelihood and Bayesian methods. This phylogenetic framework strongly supports the monophyly of *Erysimum* and recovers some well-

supported clades that are geographically, rather than morphologically, correlated. The study confirms the placement of *Erysimum* in lineage I and reveals two *Malcolmia* (*M.*) spp. (*M. maritima* and *M. orsiniana*) as its sister taxa. The results suggest that the biennial duration and caespitose habit (vs. annual or perennial duration and herbaceous or woody habit) and large, yellow, glabrous (vs. small, non-yellow, pubescent) petals are ancestral in *Erysimum*. The ancestral-state reconstruction results show that annual vs. perennial and woody vs. herbaceous features have been independently derived several times. The dating analyses suggest an early radiation of *Erysimum* during the late Pliocene or early Pleistocene [1].

This tribe is distinguished by exclusively sessile, stellate and/or malpighiaceae (two-armed T-shaped) trichomes, yellow flowers and multi-seeds siliques [2]. Since it is extremely difficult to distinguish between morphological features, in 14 different Central European species of *Erysimum* relatives special attention was given to the study of the carotene and molecular volatility in the field, with largest differences observed in *E. cheiri* and *E. crepidifolium*. At the same time, it was noted that the exact set of taxon is quite similar to that of the cornea, only slightly different from *E. vergatum*. The molecular methods, including the total physical response, have increased the density of rDNA, while the genetic features of all *E.* relatives were established using random amplified polymorphic DNA. As a result, complexity of five out of six taxon problematic species: *E. hungaricum*, *E. pieninicum*, *E. wahlenbergii*, *E. vergatum*, *E. durum* and *E. hieracifolium* was noted [3].

In order to examine the systematic application of seed-coat characters in *Erysimum* (*Brassicaceae*) distributed in Northeast of Iran, Khorassan province, the seeds of nine species with dense grain (14 populations) were examined with application of light (LM) and scanning electron microscopy (SEM). According to results of the LM, diagnostic signs include the shape of the seed, the width of the wing, epidermal cell-wall shape, and seed-surface sculpture. The SEM at high magnifications reveal seven types of seed-surface sculpture pattern, including: 1) reticulate, the basic type; 2) ocellate; 3) papillate; 4) reticulate-papillate; 5) scaliform; 6) ribbed; 7) reticulate-ocellate. The seed coat typically consists of four layers, including of the epidermis, subepidermis, sclerotic (or palisade) and the parenchymatous layer. Some species may not have some types of these layers [4].

E. capitatum seeds were planted alongside the alpine and low altitudes in order to study plants adaptability to location and climate. Plants exhibited

home-size advantage in the form of higher survival at their native sites, especially for plants from high altitude. High- and low-altitude populations differed in seed germination, growth and morphology. Seeds from alpine *E. capitatum* tended to germinate to higher levels than those from low altitude populations, especially under alpine conditions. The production of multiple rosettes, a characteristic morphology of alpine plants was negatively associated with survival at low altitude. Plants at low altitude suffered higher mortality, but showed faster growth and reproduction of those that survived. Thus, the differential plant performance observed in natural populations across altitude is attributable to direct environmental effects (plasticity) as well as population differentiation caused by genetic differentiation and/or maternal environmental effects [5].

Differences in the reproductive success of the two species *E. pieninicum* and *E. odoratum* can be considered among the potential causes for their contrasting distribution success, although their seed biological characteristics (preserved viability in seed gene banks for long time and sudden germination without the need of dormancy breaking treatments) show them as promising candidates for nature conservation and habitat restoration projects [6].

N, P and K concentration in aerial parts of *E. amasianum* is generally high in vegetative period. In generative period, however there is a decline. This situation can be explained with that in aerial parts of the plant, physiological actions are dense and elements are carried into the aerial parts of the plant. In generative phase the aerial parts complete their development. The elements are carried into the underground parts in order that the plant can live until next vegetative period. The reason why N, P and K concentration increases in generative phase is this. Similar results can be seen in researches that were done to the plants such as *Asphodelus aestivus* Brot., *Iris sari* Schott ex Baker and *Alkanna haussknechtii* Bornm. [7].

Although establishing the adaptive significance of polyploidy to explain the geographic distribution of cytotypes is challenging, the occurrence of different cytotypes in different ecological niches may suggest an adaptive role of genome duplication. Climate variables, population elevation and soil properties to model ecological niches for the different cytotypes of *E. mediohispanicum* was used in order to study the effect that ploidy level has on the floral phenotype. A clear geographic pattern in the distribution of cytotypes, with diploid individuals occurring in the southernmost part of the distribution range was

noted, while tetraploids were found in the northern area. A contact (mosaic) zone between both cytotypes was identified, but diploids and tetraploids occur in sympatry in only one population (although in a highly unbalanced proportion). Gene flow between different cytotypes seems to be negligible, as evident from an almost complete absence of triploids and other minority cytotypes. Niches occupied by both cytotypes showed subtle, but significant differences, even in the contact zone. Precipitation was higher in regions occupied by tetraploid individuals, which present wider corolla tubes and thinner but taller stalks than diploids. Findings highlight the potential role of polyploidy in the ecological adaptation of *E. mediohispanicum* to both abiotic factors and biotic interactions [8].

With recent advances in genome sequencing, transcript profiling, plant transformation, transient expression assays, and plant metabolite analysis, another *Erysimum* representative, *E. cheiranthoides* (wormseed wallflower), a rapid-cycling, self-pollinating species with a relatively small, diploid genome, which characteristically produces cardiac glycosides as defensive metabolites, is proposed as a suitable model system to advance research on the biosynthesis of cardiac glycosides in plants [9].

In order to detect the cause of rare species of various populations encountered in Kazakhstan, the reproductive, morphological and anatomical structure of the seed requires a thorough investigation, and a comprehensive study is needed to preserve this species in nature.

Materials and methods

Study of morphological structure of *Erysimum croceum* M. Pop. (*E. croceum*) plants was carried out using the methods of common structural analysis. Morphological growth, germination, vegetation, diameter, root count, number of leaves, its shape, size were studied by the methodology of Serebryakov I.G. (1964), Fedorov A.A. (1956). Anatomical structure was studied using the generally accepted methodology, by methods of structural analysis and temporary preparations [12-16].

Measurements and microphotographs were produced by Micros MCx100 video microscope camera 519 CU5.0M CMOS.

Morphology-geographical method was used in carrying out the systematic analysis. Type determination was based on detection of the basic morphological features in a blossoming phase. For the purpose of a further morphologic-anatomic research of *E.*

croceum plants were collected as herbarium during the expeditions to Trans-Ili Alatau. Stereoscopic binocular MBS-10 was used in the study of the objects morphological structure. Studies were conducted at the Laboratory of plant ecomorphology, Department of Biodiversity and Bioresources, al-Farabi Kazakh National University, Almaty, Kazakhstan.

Conservation of plant material was carried out by a technique of Strasburger and Fleming. Anatomic preparations were produced according to the standard techniques of Prozina M.N. (1960), Permyakov A.I. (1988) and Barykina R.P. (2004).

Possibility to use optic microscope Leica DM 6000 M with the high-resolution digital camera and software for the microscopic study of anatomic structure of reproductive organs of plants was kindly provided by our colleagues from the national nanotechnological laboratory of public type at al-Farabi Kazakh National University.

Results and discussion

The reason for the disappearance of endangered plant species is directly related to seed germination. In order to define sprout intensiveness 400 *E. croceum* plant seeds were placed into four Petri dishes (by 100 seeds in each).

Germinating capacity of seeds was defined on the 25th day according to the generally accepted international system. As can be seen from the Figure 1 *E. croceum* plant seeds did not germinate in noticed 25 days, germinating capacity 0%.

On the 30th-31st days, changes started appearing. On the 45th day plants got measured with overall length: 6.67 cm, hypocotyls overall length: 1.12 cm, roots overall length: 5.49 cm, overall length of leaves: 0.39 cm, leaves overall width: 0.2 cm (Figure 2).

According to the results from the Small Almaty and Big Almaty gorges *E. croceum* plants height is 30-60 cm, stem grows straightly. Leaves are long or like forage grasses, mitering corners; lowers are pedicellate, uppers are pliant. Flowers are reddish yellow or red, panicle settled in bouquet. They flower in May to July and give fruits in July to August. Fruit is tetrahedral pods. Plants can be used as decorative plants and may be grown in culture.

Study of morphological structures showed that *E. croceum* plants are biennial. No stem is developed during the 1st year (virginal period), only starting from 2nd year (generative period) stem will start growing, overall length in the 1st population is 45.9±18 cm, in the 2nd population is 49.8±3.6 cm, morphological structure published [16].

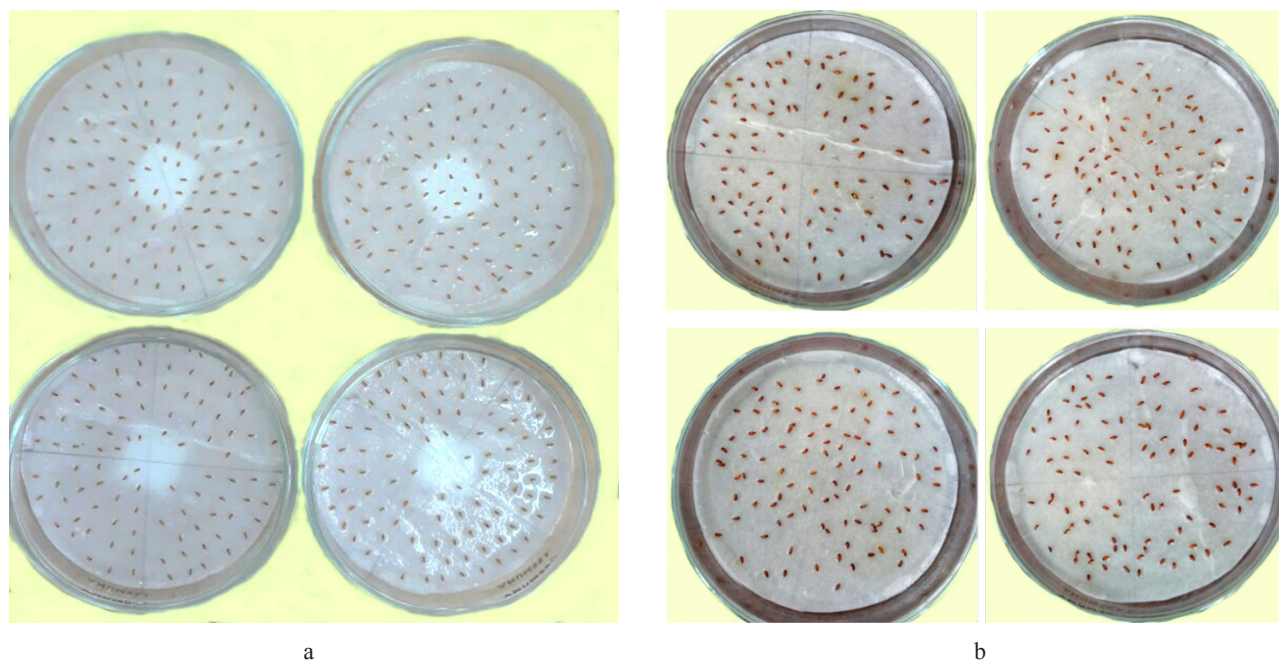


Figure 1 – *E. croceum* plant seeds germinating capacity on the 1st (A) and 25th (B) day



Figure 2 – *E. croceum* plant length, cm

The population structure of *E. croceum* plants is well defined in Small Almaty gorge, since this place is its growing areal; this species were found and characterized there for the first time.

Fixed material of *E. croceum* plants from Big and Small Almaty gorges of Trans-Ili Alatau was used in order to conduct comparable analysis of the index of anatomical structure.

Study of the anatomical structure of the stem of *E. croceum* plants from the 1st population (generative period) revealed its tetrahedral structure and well-developed trichomes in epidermis cells. Under epider-

mis primary cortex decreased. It consists from parenchyma and collenchyma. Primary cortex parenchyma cells located in 5-6 lines, conducting bundles in center circle located in a certain line, formed collateral open bundles. In center circle conducting bundles located over cambium circle located between collateral open xylem and phloem. Sclerenchyma is clearly developed and gathered in conducting bundles and in between two conducting bundles. Core expanded, parenchymas are consists of similar many-sided cells, which takes main part of stem. Core parenchyma is well developed (Figure 3).

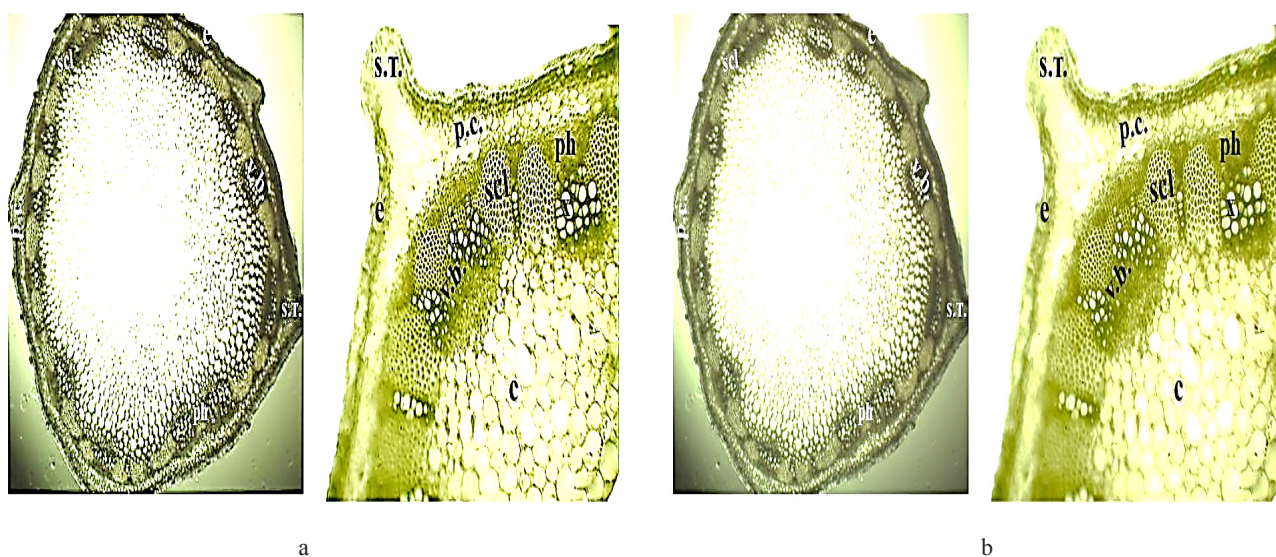


Note: e – epidermis, p.c. – primary cortex; v.b. – vascular bundle, c – core, scl – sclerenchyma surrounding bundle; x – xylem, ph – phloem, s.t. – simple trichome

Figure 3 – Anatomical structure of *E. croceum* stem, generative period, 1st population

Study of the anatomical indexes of the stem of *E. croceum* plants from the 2nd population (generative period) reveals the tetrahedral structure of its trichomes, similarity of epidermis cells along the stem and emergence of glandular fibers. Hederals expand and collenchyma decreases, primary cortex parenchyma consists of colorless cells. Well-defined growth of sclerenchyma located between each centered conducting bundle is revealed. Sclerenchyma located after large conducting bundle might be interchanged with small conducting bundle. In large bundle 10-15 lines of xylem are in row, and in small bundle, we can notice only one line of xylem. In large conducting bundle, sclerenchyma can be clearly observed. In core parenchyma, some unknown matters are present (Figure 4).

Comparative analysis of the morphometric specification of *E. croceum* stem shows higher index in the 1st population over the 2nd population (Table 1).



Note: e – epidermis, p.c. – primary cortex; v.b. – vascular bundle, c – core, scl – sclerenchyma surrounding bundle; x – xylem, ph – phloem, s.t. – simple trichome

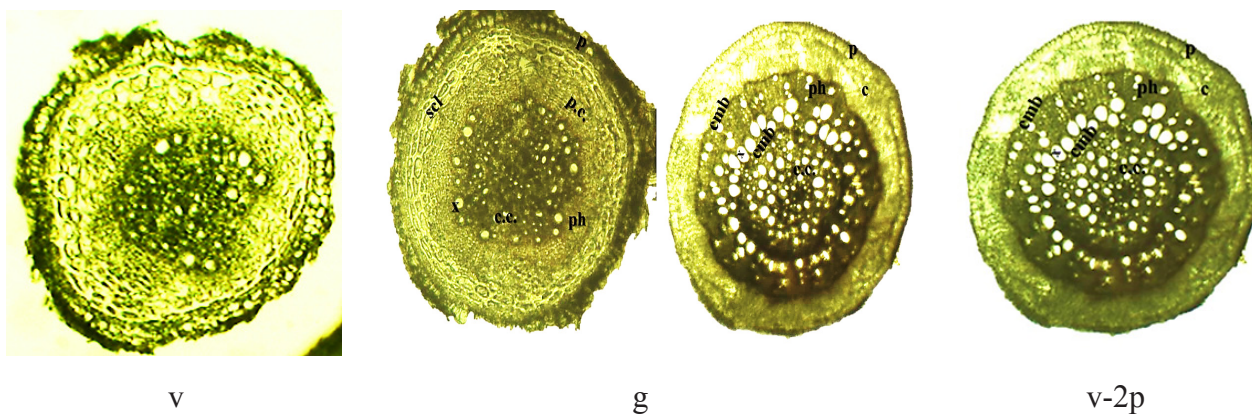
Figure 4 – Anatomical structure of *E. croceum* stem, generative period, 2nd population

Table 1 – Morphometric indexes in two populations of *E. croceum* plants

Generative period	Primary cortex thickness, μm	Center cylinder diameter, μm	Central conducting bundle diameter, μm
1 st population	1280.62±13.9	932.55±9.4	96.00±0.7
2 nd population	1125.53±12.4	856.24±8.3	72.11±0.4

In the 1st population stem diameter is 1052.92±11.7 μm, epidermis thickness is 25±0.9μm, primary cortex thickness is 96.24±0.6μm, and center circle diameter is 796.51±5.9μm. Morphometric in-

dex of the 2nd population is lower, with stem diameter of 996.38±9.8 μm, epidermis thickness of 21±0.7 μm, primary cortex thickness of 80.98±0.4 μm, center circle diameter of 701.59± 8.7 μm (Figure 5).

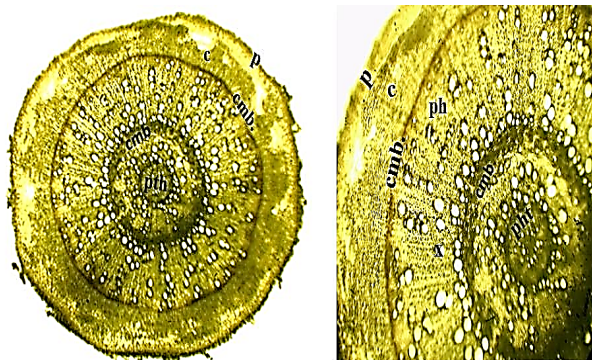


Note: p – periderm, p.c. – primary cortex, c.c. – central cylinder, cmb – cambium, c – cortex, scl – sclerenchyma, ph – phloem, x – xylem

Figure 5 – Anatomical structure of *E. croceum* roots in virginal (v) and generative (g) periods, 1st population, and virginal period (v-2p), 2nd population

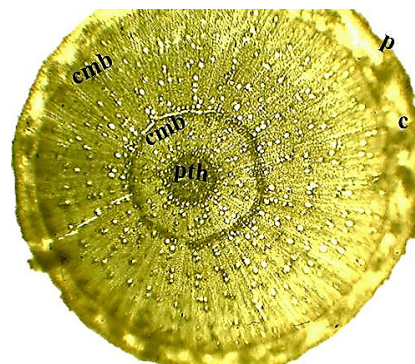
Anatomical structure of roots was studied in virginal and generative periods. In virginal period root is covered with second roofing tissue periderm. Con-

sequent expansion of the core with enlarged xylem tubes along with decrease in primary cortex thickness is noticed in generative period (Figure 6).



Note: p – periderm, p.c. – primary cortex, pth – pith, cmb – cambium, c – cortex, ph – phloem, x – xylem

Figure 6 – Anatomical structure of *E. croceum* rhizomes, virginal period, 2nd population, close-up



Note: p – periderm, pth – pith, cmb – cambium, c – cortex

Figure 7 – Anatomical structure of *E. croceum* rhizomes, generative period, 2nd population

Study on anatomical structure of root reveals rhizome; a two-year ring is clearly visible. Study of *E. croceum* plant rhizomes anatomical structure at virginal and generative periods reveals rhizome covered periderm, clearly defined lenticels (Figure 7).

Results of the comparative analysis of morphometric indexes of *E. croceum* rhizomes at virginal and generative periods are presented in Table 2, indicating higher general level defined at virginal period, even though diameter of central cylinder in generative period is larger.

Table 2 – Morphometric indexes of *E. croceum* rhizomes, virginal and generative periods, 2nd population

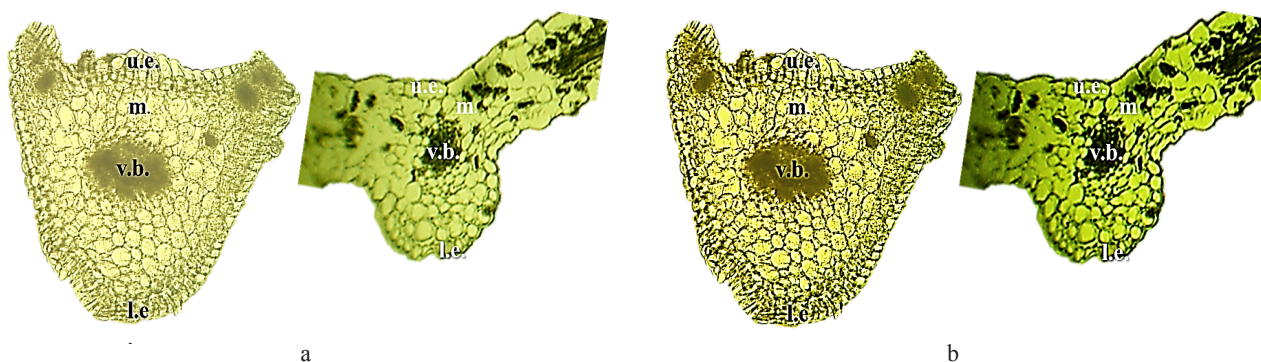
Virginal period		Generative period	
Measure the area	Length, μm	Measure the area	Length, μm
Overall rhizome diameter	625.89	Overall rhizome diameter	599.58
Primary cortex thickness	99.14	Primary cortex thickness	46.75
Diameter of central cylinder	427.61	Diameter of central cylinder	506.08

Morphometric indexes of *E. croceum* rhizomes in the 1st population are higher in comparison with the 2nd population.

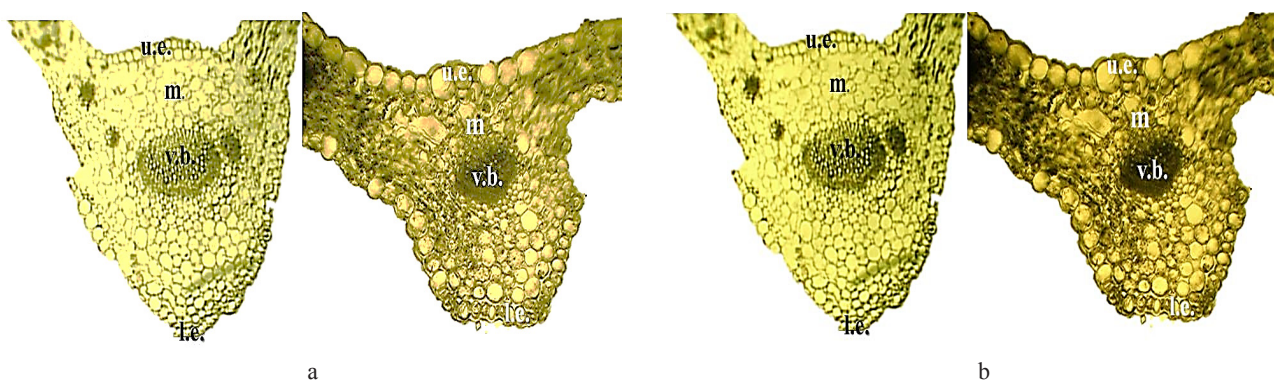
Anatomical structure of *E. croceum* leaf at virginal period clearly shows that upper and lower epidermis cells consists from round shaped cells with defined trichomas. Conducting bundle consists directed to collateral upper epidermis xylem and directed to lower epidermis phloem. Large bundle is located at the core, over it small bundles are located; mesophyll

is divided into column and lacunar cells. Core chlorenchyma is presented by clearly seen varying round shaped cells (Figures 8 and 9, Table 3).

Study of *E. croceum* leaf anatomical structure reveals larger conducting bundles in the 1st population in comparison with the 2nd population (Figure 8). In the 2nd population core is located under the upper epidermis, parenchyma is diffused. There is a defined air cavity at the core upper side of conducting bundle. In lower epidermis, trichomas are well developed.



Note: u.e. – upper epidermis, l.e. – lower epidermis, m. – leaf mesophyll, v.b. – vascular bundle

Figure 8 – *E. croceum* leaf anatomical structure, virginal (A) and generative (B) periods, 1st population

Note: u.e. – upper epidermis, l.e. – lower epidermis, m. – leaf mesophyll, v.b. – vascular bundle

Figure 9 – *E. croceum* leaf anatomical structure, virginal (A) and generative (B) periods, 2nd population

Results of the comparative analysis of *E. croceum* leaf morphometric indexes in the 1st and 2nd populations are presented in Table 3.

Plants from the 1st population were collected at the humid slopes of Trans-Ili Alatau, which had positive influence at *E. croceum* leaf morphometric indexes. For instance, core thickness in the 1st popu-

lation is 689.08 µm, and in the 2nd population, it decreases to 601.79 µm.

Thus, comparative analysis of anatomical and morphometric parameters of two populations shows higher indexes of each part (stem, rhizome, leaf) of *E. croceum* in Big Almaty gorge of Trans-Ili Alatau.

Table 3 – Morphometric indexes of *E. croceum* leaf in the 1st and 2nd populations

1 st population		2 nd population	
Measured unit	Size, µm	Measured unit	Size, µm
Core thickness	689.08	Core thickness	601.79
Mesophyll thickness	259.75	Mesophyll thickness	209.38

Conclusion

Erysimum croceum M. Pop. plant seeds germination capacity is low, it comprises only 18%. In this regard, there is a risk of seed loss due to poor germination and measures should be taken in order to improve seeds germination. Plants do not form stem during the first year, stem started to grow only at the generative period of the second year.

Some features were defined during the study of anatomical structures. Stem is tetrahedral with well-developed trichomes in epidermis cells. Parenchyma cells stay only at sides of the primary cortex, in center circle, expanding conducting bundles are located in a certain order, and collateral open bundles are formed. The main noticed difference is that sclerenchyma formes bundles between the two bundles. Pith widenes, parenchyme is formed from the same cells, which define its basic position in the stem.

Comparative analysis of *E. croceum* M. Pop. plant rhizome morphometric indexes showed higher values at the virginal than in the generative period. In the structure of the rhizome, the biennial ring is clearly visible and in the center is the pith.

There is a defined air cavity at the core upper side of conducting bundle in leaves. In lower epidermis, trichomas are well defined.

References

1. Moazzeni H., Zarre S., Pfeil B.E., Bertrand J.K., German D.A., Al-Shehbaz I.A., Mummenhoff K., Oxelman B. (2014) Phylogenetic perspectives on diversification and character evolution in the species-rich genus *Erysimum* (Erysimeae; Brassicaceae)

based on a densely sampled ITS approach. *Bot J Linn Soc.*, vol. 175, no. 4, pp. 497-522.

2. Gostin I.N. (2009) Anatomical and micromorphological particularities of vegetative organs in endemic *Erysimum wittmanii* Zaw. ssp. *Wittmanii*. *Anal Univ Oradea, Fasc Biol.*, vol. 16, no. 2, pp. 74-79.

3. Czarna A., Gawronska B., Nowinska R., Morozowska M., Kosinski P. (2016) Morphological and molecular variation in selected species of *Erysimum* (Brassicaceae) from Central Europe and their taxonomic significance. *Flora*, vol. 22, pp. 68-85.

4. Csontos P., Rucinska A., Puchalski J.T. (2010) Germination of *Erysimum pienicum* and *Erysimum odoratum* seeds after various storage conditions. *Táj Lapok*, vol. 8, no. 3, pp. 389-394.

5. Ghaempanah S., Ejtehadi H., Vaezi J., Farsi M. (2013) Seed-coat anatomy and microsculpturing of the genus *Erysimum* (Brassicaceae) in Northeast of Iran. *Phytotaxa*, vol. 150, no. 1, pp. 41-53.

6. Kim E., Donohue K. (2013) Local adaptation and plasticity of *Erysimum capitatum* to altitude: its implications for responses to climate change. *J Ecol.*, vol. 101, no. 3, pp. 796-805.

7. Cansaran A., Ergen Akçin Ö., Kandemir N. (2007) A study on the morphology, anatomy and autecology of *Erysimum amasianum* Hausskn. & Bornm. (Brassicaceae) distributed in Central Black sea region (Amasya-Turkey). *Int J Sci Tech.*, vol. 2, no. 1, pp. 13-24.

8. Muñoz-Pajares A.J., Perfectti F., Loureiro J., Abdelaziz M., Biella P., Castro M., Castro S., Gómez J.M. (2018) Niche differences may explain the geographic distribution of cytotypes in *Erysimum mediohispanicum*. *Plant Biol.*, suppl. 1, pp. 139-147.

9. Züst T., Mirzaei M., Jander G. (2018) *Erysimum cheiranthoides*, an ecological research system with potential as a genetic and genomic model for studying cardiac glycoside biosynthesis. *Phytochem Rev.*, pp. 1-13.
10. Serebrjakov I.G. (1964) Zhiznennye formy vysshih rastenij i ih izuchenie // Polevaja geobotanika [Life forms of higher plants and their study // Field geobotany]. *L.: Nauka*, vol. 3, pp. 146-205.
11. Fedorov A.I., Kirpichnikov M.Je., Artjushenko Z.T. (1956) Atlas po opisatel'noj morfologii vysshih rastenij [Atlas on descriptive morphology of higher plants]. *Izd-vo AN SSSR*, p. 303.
12. Prozina M.N. (1960) Botanicheskaja microtehnika [Botanical microtechnology]. *M.: Vyssh. shk.*, p. 206.
13. Permyakov A.I. (1988) Microtehnika [Microtechnology]. *M.: MGU*, p. 58.
14. Barykina R.P. et al. (2004) Spravochnik po botanicheskoj microtehnike, osnovy i metody [Reference book on botanical microtechnology, fundamentals and methods]. *M.: MGU*, p. 312.
15. Lakin G.F. (1990) Biometrija [Biometrics]. *M.: Vyssh. shk.*, p. 352.
16. Abidkulova K.T., Mukhitdinov N.M., A.A. Ivashchenko, A. Ametov, Serbayeva A.D. (2017) Morphological characteristics of a rare endemic species *Erysimum croceum* M. Pop. (*Brassicaceae*) from Trans-Ili Alatau, Kazakhstan. *Modern Phytomorph.*, vol. 11, pp. 131-138.