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### Справка

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# 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. Rare species *Erysimum croceum* included into Kazakhstan Red Data Book. The aim of this study is to compare 2 populations of *Erysimum croceum* M.Pop identified in Trans-Ili Alatau. To study the differences between both sites, a morphological and ecological analysis was undertaken on 2 populations growing in Trans-Ili Alatau. Ecological communities where is growing in 1 population Small Almaty canyon (N43\* 06.316',E077\* 04.184') are dominated by indigenous plants with a significant proportion of endemic taxa, while in 2- population Big Almaty canyon (N43\* 04.790',E076\* 59.512') are growing in subruderal positions with the presence of other plants. Statistical analysis of the morphological traits revealed significant differences between the 2 populations. In this article was given comparatively morphological and anatomical structures features of endemic species *Erysimum croceum*. To identify the cause of the disappearance was researched

the seed germination and growth of seedlings. Seed germination was low and only 18%. Morphological structure seedlings in 45 days showed overall length of sprouts  $6.67\pm3.84$ cm, hypocotyl length  $1.12\pm0.06$  cm, root length  $5.49\pm3.57$ cm, leaves length  $0.39\pm0.63$ cm, leaves width  $0.2\pm0.2$ cm. Trans-IIi Alatau 1-population Small Almaty canyon and 2-population Big Almaty canyon. The anatomical features *Erysimum croceum* are defined. *Erysimum croceum* collected from 1-population showed in morphometric index of stem comparing with plants from 2-population, in 1-population stem diameter was  $1052.92\pm11.7\mu$ m, epidermis thickness  $25\pm0.9\mu$ m, primary cortex thickness  $96.24\pm0.6\mu$ m, center circle diameter  $796,51\pm5.9\mu$ m. *Erysimum croceum* from 2-population the stem diameter was  $996.38\pm9.8\mu$ m, epidermis thickness  $21\pm0.7\mu$ m, primary cortex thickness  $80.98\pm0.4\mu$ m, center circle diameter  $701.59\pm8.7\mu$ m, 2-population had low indexes. Anatomical sections of rhizome and leave compared in virginal and generative periods, and the stem researched only at generative period, the main specification into stems inner structure is that in central cylinder between conducting bundles accumulated sclerenchyma and small bundle with big bundle are alternately located.

Key words: Erysimum croceum M.Pop., seed, anatomical, conducting bundle, sclerenchyma, xylem.

#### Introduction

There are more than 13 thousand species of valuable plants in the flora of Kazakhstan. Including high level -5754 species, 14% species are endemic, and relict species are more. *Erysimum croceum* plant encountered in North-East of Zaiyilisk and Big Almaty, it is a specie which areal is shortening. It has entered in Kazakhstan Red Data Book, rare species of cabbage samples, which needs special protection. This plant multiplies with breeds.

In 14 different species of *Erysimum* relatives in Central Europe, special attention has been paid to the study of the carotene and molecular volatility in the field, which is extremely difficult to distinguish between morphological features. (*E. hungaricurn, E. pieninicum, E. wahlenbergii, E. vergatum, E. durum* and *E. hieracifolium*). The largest difference is *E. cheiri* and *E. crepidifolium*, and the exact set of taxon is quite similar to that of the cornea, only slightly different from *E. vergatum*. The molecular methods, including the TPR (Total physical response), have increased the density of pHDD and the genetic peculiarities of all *Erysimum* relatives of RAPD (Random Amplified Polymorphic DNA) have been determined. As a result, *Erysimum* demonstrated the complexity of five of the six taxon problematic species of related species [1].

*Erysimum* is widespread at the Northern hemisphere. The center of *Erysimum* allied species is Europe, there encountered over 150-350 species. It is famous for its morphological features and its taxon complexity. *Erysimum* related species using inner DNA chain transcription 85% (see also 117) of the species represented by the first sample of phylogenetic propagation shows the full sector of morphological flow and geographical division. Used several methods of phylogenetic communation, including the evolutional allience morphological signs and difference of related species. Phylogenetic substantiation are not sheltered on morphological, but geographical. The research made to nursery plant of related *Erysimum*. Findings proved that *Erysimum* area on 1 line *Malcolmia* 2 specie (*M. maritima* and *M. orsiniana*) massed up as taxon. Findings defined that biennial and big green, *Erysimum* bare farinas can be used [2].

To study the systematic use of seeds *Erysimum (Brassicaceae)* in Northeast of Iran (province of Khorasan) dense species of seed (14 population), used light microscope (LM) and scanner electron microscope (SEM). According to the results of the study with light microscope diagnostic signs shows the shape of the seed, the width of the wound, the epidermis cell line and the shape of the seed surface. Increasing in a high level with the help of scanner electron microscope (SEM) shows that on the surface of the seed of seven species has differences: 1) mesh, main specie; 2) eye; 3) granulose haustorium; 4) cancellate- granulose; 5) scalar; 6) grainer; 7) cancellate- eye. Seedlings usually contain four layers of epidermis, subepidemia, sclerosis and parenchymosis. Some species may not have some types of these membranes [3].

*Erysimum capitatum* plant seeds and seedlings were planted alongside the alpine and low altitudes in order to adapt to the current climate change and shrinkage. The epidemic of *E. Capitatum*, which grows in an adorable habitaciometer, grows in the lower and lower altitudes, as follows:

diversity of production, size and morphological features. The reproduction of the seeds in the area of their habitats is high [4].

In plants protection, some 32% of plants were submerged under subpopulation, various impacts, and the temperature was increased to 1000%. The decrease in population is associated with a decrease in the number of herbs, which does not depend on the aureus and its diapozone. Expansion of the genetic diversity allowed to increase the number of residents in the area, which, in turn, had a negative impact on vegetation density. The average volume of the plant depends on the reproductive index of the population [5].

The ultrastructure of plants that survive on the seeds *Brasicaceae* (*Erisimum cheirii*) identified. Adaccial epidermal cells are a suction type. The only epithelium of the adaxial epidermis. Pigmentation can be noticed in epidermal and mesophilic cells. These pigmented vesicles of the cornea will be visible under the microscope [6].

To know more about the biological features of the plants, it has become known to know the peculiarities of the morphological and anatomical structure of individual cycles. Specific features of the plant directly relate to the soil of that area.

Alpine plants grown at high level, but they showed a high mortality rate when cultivating them in low level. Growing at low altitudes had a negative impact to alpine *E. capitatum* plant. It was expected that climatic changes would be in accordance with the morphology of non-high-altitude plants in alpine plants. But adaptive alpine *E. capitatum* may indicate high index and a decrease in seed yield. Climatic changes has great damage to alpine plants. To observe the influence of climatic changes to alpine plants it is important to observe in different heights. [4,7-14]. In order to keep the fence surrounding and protect the plants spreading to adapting, rhythmicity of *Erysimum* (*Brassicaceae*) plant important step to growth special attention has been paid to reproduction of seeds and seedlings, also made researches [15-26].

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 through investigation, and a comprehensive study is needed to preserve this species in nature.

#### Materials and methods

Research of morphological structure of *Erysimum croceum* M. Pop. plant done through common structural choosing methods. Morphological growth, germination, vegetation, diameter, root count, number of leaves, its shape, size lead by the methodology of I.G. Serebryakov, A.A. Fedorov. In order to investigate anatomical structure used generally accepted methodology, structural analysis methods and temporary preparations [26-30]. Provisions and photomicrograph are made by video microscope Micros Austria MCX100 camera 519 CU5.0M CMOS.

The morphologic-geographical method was used in carrying out the systematic analysis. In case of the type determination there were considered the main morphological features in a blossoming phase. During the expedition for the purpose of a further morphologic-anatomic research of *Erysimum croceum* M. Pop. plants were collected in a herbarium. During the research of the morphological structure of the object, stereoscopic binocular "MBS-10" was used, researches were conducted at department of the Biodiversity and bioresources, KazNU named after al-Farabi. In vitro plant material for the purpose of saving a lifetime status was recorded. Conservation of plants was carried out by a technique of Strasburger-Flemminga. Anatomic medicines were made according to standard techniques of Prozina M.N. (1960), Permyakov A. I. (1988), Barykina R. P. (2004). For a microscopic study of an anatomic structure of reproductive organs of plants were conducted in the national nanotechnological laboratory of public type KazNU named after al-Farabi with use of optic microscope Leica DM 6000 M with the high-allowing digital cameras and the software for the analysis and saving images.

#### **Results and their discussion**

The reason for the disappearance of endangered plant species is directly related to seed germination, in this respect *Erysimum croceum* M. Pop. plant seed in order to define sprout intensiveness set 400 seed in Petri plate and investigated, Figure 1.

Germinating capacity of seed will be defined at 25<sup>th</sup> day according to accepted international system. *Erysimum croceum* plant seed did not germinate in noticed 25 days. Germinating capacity: showed 0%.

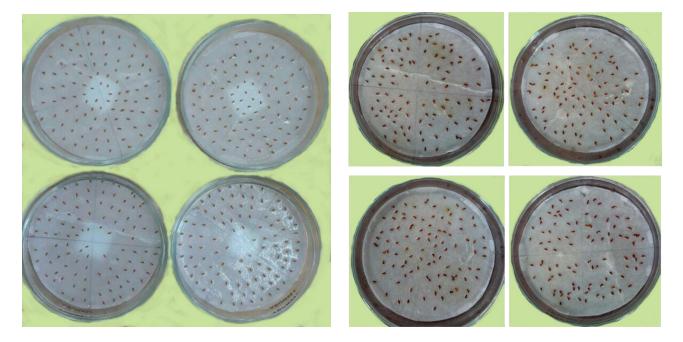


Figure 1 - Erysimum croceum plant seed germinating capacity: A - 1 day; D - 25 day

The results were at controll after 25 day. At 30-31 days there started changings and they strike roots. In laboratory conditions in Petri plate *Erysimum croceum* plant seed in 45<sup>th</sup> day took to measure (Figure - 2). In 45<sup>th</sup> day after seeding 18 plant from 400 seeds overall length: 6,67 cm, hypocotyls overall length: 1,12cm, roots overall length: 5,49 cm, overall length of leaves: 0,39 cm, leaves overall width: 0,2 cm.



Figure 2 - Erysimum croceum plant length, cm

Ginger sisymbrium is protected as relict because of rare occurrence, entered into Kazakhstan Red Data Book. In order to comparable controlling of *Erysimum croceum* plant morphological specification plants of 2 population collected from Small Almaty and Big Almaty canyon with the employees of the Department of Biodiversity and Bioresources in the project frame researched at virginal and generative growing periods.

*Erysimum croceum* plant 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 yelow or red, panicle settled in bouquet. Increased from seed. In May-July expand, in July-August fructification. Fruit is – tetrahedral pods. Adornment plant, can be grown in a cultural way.

In the results of investigating morphological structure, as plant was biennial, at first year stem wouldn't develop, in virginal period there no stem, only starting from second year at generative period stem will start growing, overall length in 1-population 45.9±18 cm, 2-population 49.8±3.6 cm, morphological structure publicized [31].

In the result of our research it is defined that in Small Almaty ravine well developed, because this place is growing areal, this species was first found and characterized there.

In order to control comparable analysis index of anatomical structure of *Erysimum croceum* plant from Big and Small Almaty ravine collected from Trans-Ili Alatau, made to fixed *Erysimum croceum*.

Generative period of 1-population *Erysimum Croceum* stem on anatomical structure defined polyhedral and trichome well developed in epidermis cells. There collenchymas in hedrals. Under epidermis primary cortex decreased, consist 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 which between collateral open xylem and phloem. In conducting bundles and between two bundles sclerenchyma is clearly developed and gathered. Core expanded, parenchymas are consists of similar many-sided cells, which takes main part of stem. Core parenchyma is well developed. (Figure 4).

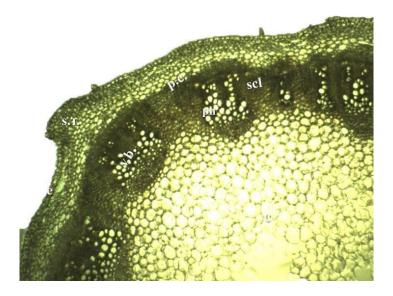


Figure 4 – *Erysimum croceum* stem`s anatomical structure generative period (1<sup>st</sup> population); where: e – epidermis (epiderma), p.c. – primary cortex; v.b. – vascular bundle, c – core, scl – sclerenchyma surrounding bundle; x – xylem, ph – phloem, s.t. – simple trichome

In generative period anatomical indexes of Erysimum croceum plant stem taken from 2population defined that trichomes are tetrahedral, epidermis cells are similar in stem, gland fibers. Hedrals are expansed and collenchyma decreased, primary cortex parenchyma consists of colorless cells selection. Between center circles, conducting bundle defined well growth of sclerenchyma. Sclerenchyma located interchangingly with big and small bundles, sclerenchyma located between each bundle. After big conducting bundle located sclerenchyma and its interchanged with small

conducting bundle. In conducting bundle between big and small sclerenchyma is clearly developed, in big bundle 10-15 xylem are in row, and in small bundle we can notice only 1 line xylem. In big conducting bundle, sclerenchyma had clearly observed. In core parenchyma, there are unknown matters, Figure 5.

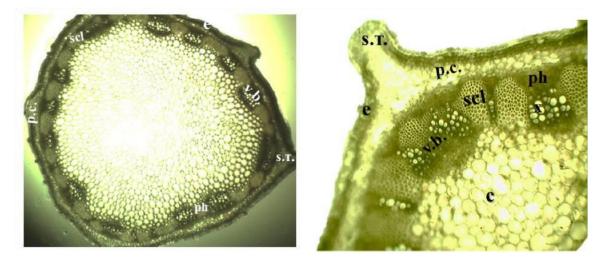


Figure 5– *Erysimum croceum* stem`s anatomical structure generative period, 2<sup>nd</sup> population, x100); where: e – epidermis (epiderma), p.c. – primary cortex; v.b. – vascular bundle, c – core, scl – sclerenchyma surrounding bundle; x – xylem, ph – phloem, s.t. – simple trichome

During comparing of morphometrical specification of 2<sup>nd</sup> population *Erysimum croceum* plant stem 1<sup>st</sup> population had higher index, Table 1.

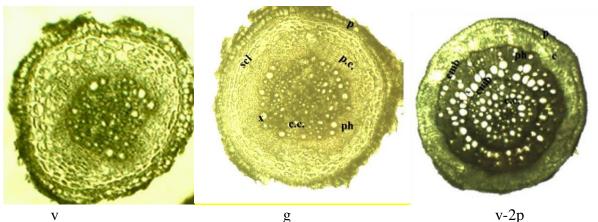
**Table 1** –  $2^{nd}$  population *Erysimum croceum* plant morphometric indexes, µm

| Generative | Primary       | cortex | Center cylinder | Central conducting  |
|------------|---------------|--------|-----------------|---------------------|
| period     | thickness, µm |        | diameter, µm    | bundle diameter, µm |
|            |               |        |                 |                     |
|            |               |        |                 |                     |
|            |               |        |                 |                     |
| 1          | 1280.62±13,9  |        | 932.55±9,4      | 96.00±0,7           |
| 2          | 1125.53±12,4  |        | 856.24±8,3      | 72.11±0,4           |
|            | ,             |        | 1               | ,                   |

Collected from 1-population *Erysimum croceum* plant stem`s morphometric index comparing with 2-population, in 1-population stem diameter was  $1052.92\pm11.7\mu$ m, epidermis thickness  $25\pm0.9\mu$ m, primary cortex thickness  $96.24\pm0.6\mu$ m, center circle diameter  $796.51\pm5.9\mu$ m, in 2population stem diameter was  $996.38\pm9.8\mu$ m, epidermis thickness  $21\pm0.7\mu$ m, primary cortex thickness  $80.98\pm0.4\mu$ m, center circle diameter  $701.59\pm8.7\mu$ m, had low indexes.

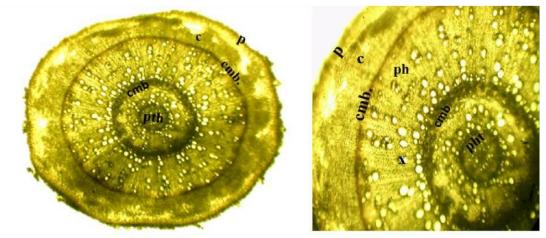
During comparing of morphometric indexes of *Erysimum croceum* plant stem defined that 1<sup>st</sup> population had higher index. According to 1<sup>st</sup> population collected Trans-Ili Alatau, defined that the Trans-Ili Alatau condition had positive influence to *Erysimum croceum* plant stem`s anatomical index.

Roots anatomical structures are virginal and generative periods are researched. In virginal period root covered with second roofing tissue periderm. Center circle expanced comparing with immaterial period, accordingly primary cortex thickness decreased. In center circle xylem tubes enlarged, at 2table we can notice 1<sup>st</sup> population indexes are higher (Figure 6).



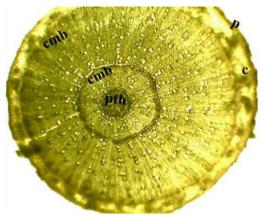
v g v-2p p – periderm, p.c.- primary cortex, c.c. – central cylinder, cmb – cambium, c-cortex, scl – sclerenchyma, ph – phloem, x – xylem

Figure 6- *Erysimum croceum* roots anatomical structures virginal and generative periods (1<sup>st</sup> population), v-2p- virginal period (2<sup>nd</sup> -population)



P-periderm, p.c.- primary cortex, pth - pith, cmb - cambium, c - cortex, ph - phloem, x - xylem

Figure 7- Erysimum croceum rhizomes anatomical structure virginal period (2<sup>nd</sup> -population)



p – periderm, p - pith, cmb – cambium, c-cortex, Figure 8- *Erysimum croceum* rhizomes anatomical structures generative period (2<sup>nd</sup> -population)

Study on anatomical structures revealed that plant has a rhizome, which can be distinguished in anatomical structure, a two-year ring is clearly visible. In the research of *Erysimum Croceum* M.Pop. plant rhizomes anatomical structure at virginal and generative periods, rhizome covered periderm, clearly defined lenticels. On 2-table we can notice comparable anatomical index by 2- periods. In the

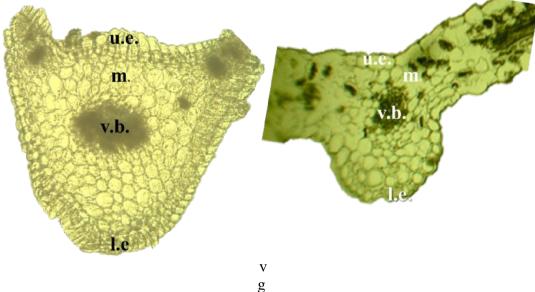
results of research defined that in virginal period anatomical indexes were higher, but diameter of central cylinder in generative period was higher.

| Virginal period              |             | Gene                         | Generative period |  |
|------------------------------|-------------|------------------------------|-------------------|--|
| Measure the area             | Length (µm) | Measure the area             | Length (µm)       |  |
| Overall rhizome<br>diameter  | 625.89      | Overall rhizome<br>diameter  | 599.58            |  |
| Primary cortex<br>thickness  | 99.14       | Primary cortex<br>thickness  | 46.75             |  |
| Diameter of central cylinder | 427.61      | Diameter of central cylinder | 506,08            |  |

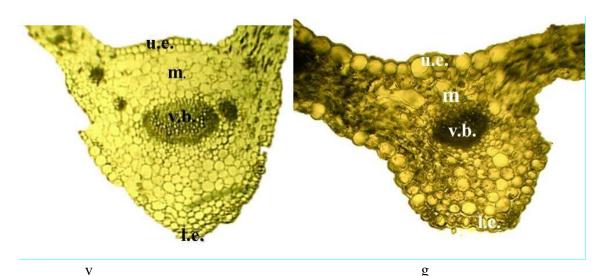
2-table Morphometric indexes of *Erysimum croceum* M.Pop. plant rhizomes, µm 2<sup>nd</sup> population)

During comparing *Erysimum croceum M.Pop.* plant rhizomes morphometric indexes in 1<sup>st</sup> population defined that it had high index. In 1<sup>st</sup> population from Small Almaty according to the humidity quantity more than Big Almaty, xylem formed big tubes in rhizome. Noticed that Trans-Ili Alatau condition had positive influence to *Erysimum croceum* plant rhizome`s anatomical index.

At virginal period *Erysimum croceum* M.Pop. leav's anatomical structure, clearly noticed that upper and lower epidermis cells consists from round shaped cells. Defined trichomas. Conducting bundle consists directed to collateral closed upper epidermis xylem and directed to lower epidermis phloem. At central nervous system located big bundle, over them small bundles, mesophyll divided into column cell and lacunar. Central nervous system chlorenchyma are formed from clear various round shaped cells (Figure 9-10, table - 3).



u.e. – upper epidermis, l.e. – lower epidermis, m. – leave mesophyll, v.b. – vascular bundle Figure 9 - *Erysimum croceum* leave`s anatomical structures virginal and generative periods (1<sup>st</sup> population)



u.e. – upper epidermis, l.e. – lower epidermis, m. – leave mesophyll, v.b. – vascular bundle Figure 10- *Erysimum croceum* leave`s anatomical structures virginal and generative periods (2<sup>nd</sup> population)

During the reserch leave's anatomical structure in central nervous system at 1<sup>st</sup> population (Figure 9) defined that conducting bundle is bigger than 2<sup>nd</sup> population. Central nervous system at 2<sup>nd</sup> population under upper epidermis the parenchyma is diffused. In leave's structure central nervous system at upper side of conducting bundle defined air cavity. In lower epidermis, trichomas are well developed. Trichomas specified with it's three star.

3-table *Erysimum croceum* M.Pop. plant's leave morphometric indexes, µm (1<sup>st</sup> and 2<sup>nd</sup> population)

| 1 <sup>st</sup> population          |        | 2 <sup>nd</sup> population                 | 2 <sup>nd</sup> population |  |
|-------------------------------------|--------|--|----------------------------|--|
| Measure the area                    | Length | Measure the area Length                    |                            |  |
| Central nervous system<br>thickness | 689.08 | Central nervous 601.79<br>system thickness |                            |  |
| Mesophyll thickness                 | 259.75 | Mesophyll thickness 209.38                 |                            |  |

During comparing *Erysimum croceum* plant leave's morphometric indexes (4-table) in No1 population central nervous system thickness 689.08  $\mu$ m, in 2<sup>nd</sup> population decreased to 601.79  $\mu$ m. 1<sup>st</sup> population in Trans-IIi Alatau humidity sides mesophyll thickness is 259.75  $\mu$ m. Humidity quantity is more than Big Almaty, according to that conducting bundle had higher indexes in leaves of No1 population. The condition in Trans-IIi Alatau had positive influence to *Erysimum Croceum* plant leave's anatomical index.

Comparing of two population's anatomical and morphological side, noticed that *Erysimum Croceum* plant which encountered in Trans-Ili Alatau, each part (stem, rhizome, leave) had higher indexes than Big Almaty *Erysimum Croceum* plant.

# Conclusion

Investigated *Croceum* plant seeds germination capacity is bad, it composes only 18%, there is a risk of the loss of seed due to poor germination, in this regard, measures should be taken to improve

the germination of seeds. When considering from morphological structure, the plant didn`t form stem in first year, only in second year at generative period stem started to grow.

And during investigation of anatomical structure, there defined some features. *Erysimum Croceum* plant stem anatomical structure many-sided, in epidermis cells there developed trichomes. Primary cortex parenchyma cells are saved only at sides, in center circle in a certain order located expanding conducting bundles, formed collateral open bundles. The main difference is that noticed sclerenchyma which formed bundles between two bundles. Pith widened, paranchymes formed from same cells, defined that it took main place of stem.

During comparing *Erysimum croceum* plant rhizome morphometric indexes defined, that at virginal periods had higher indexes than generative plants. In the structure of the rhizome, the biennial ring was clearly visible and in the center is the pith.

In leave structure at central nervous system the conducting bundle's upper side the air cavity was detected. In lower epidermis there well developed three star trichomes.

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