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# АНАЛИЗ МОРФОМЕТРИЧЕСКИХ И ОПТИЧЕСКИХ ПАРАМЕТРОВ СЕМЯН ПОДРОДА CEPA (*ALLIUM* L., ALLIACEAE) МЕТОДОМ ЦИФРОВОГО СКАНИРОВАНИЯ

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Представлены результаты исследования морфологии семян из подрода Cepa: секции Cepa (Mill.) Prokh. – *Allium fistulosum* L., *A. altaicum* Pall., *A. galanthum* Kar. & Kir., *A. oschaninii* O. Fedtsch., *A. pskemense* B. Fedtsch.; секции *Schoenoprasum* Dum. – *A. altyncolicum*, *A. ledebourianum*, *A. oliganthum*, *A. schoenoprasum* L.; секции *Condensatum* N. Friesen – *A. condensatum.* Морфологические признаки семян могут быть использованы в качестве дополнительных таксономических показателей в идентификации и различения таксонов в пределах подрода Cepa рода *Allium*. Семена имели длину 2,74–3,50 мм, ширину 1,33–2,14 мм. Измерение морфометрических и оптических параметров семянок осуществляли путем анализа изображений с помощью программного обеспечения. Цифровые изображения семянок получены с использованием цифрового планшетного сканера HP Scanjet 200, разрешение 600 dpi, формат файлов JPG. Определены морфометрические и оптические параметры семян, в том числе площадь проекции (см$^2$), длина, ширина, периметр, средний размер (мм), средний диаметр Фере, факторы округлости, удлиненности, эллипса, изрезанности (отн. ед.), параметры яркости, тональности, насыщенности цвета (отн. ед.). По результатам исследования составлены ряды распределения видов в порядке убывания по каждому из изученных признаков. В пределах секции Cepa максимальные линейные размеры, периметр и площадь сечения имели семена *A. pskemense*. Среди представителей секции *Schoenoprasum* максимальную длину имели семянки *A. altyncolicum*. Максимальные ширина, периметр, площадь сечения, средний диаметр Фере семян зафиксированы у *A. ledebourianum*. В секции Cepa среднее значение RGB в порядке убывания составило ряд: *A. pskemense* > *A. galanthum* > *A. fistulosum* > altaicum > *A. oschaninii*. В секции *Schoenoprasum* этот ряд имеет вид: *A. schoenoprasum* > *A. ledebourianum* > *A. altyncolicum* > *A. oliganthum*.

**Ключевые слова**: *Allium* L., морфология, цифровой анализ изображений, цветовые признаки в системе RGB

# ANALYSIS OF MORPHOMETRIC AND OPTICAL PARAMETERS OF SEEDS OF THE SUBGENUS CEPA (*ALLIUM* L., ALLIACEAE) BY DIGITAL SCANNING

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The results of the study of seed morphology from the subgenus Cepa: section Cepa (Mill.) Prokh. - *Allium fistulosum* L., *A. altaicum* Pall., *A. galanthum* Kar. & Kir., *A. oschaninii* O. Fedtsch., *A. pskemense* B. Fedtsch.; section *Schoenoprasum* Dum. - *A. altyncolicum*, *A. ledebourianum*, *A.*

*oliganthum*, *A. schoenoprasum* L.; section *Condensatum* N. Friesen - *A. condensatum* are presented. Morphological characters of seeds can be used as additional taxonomic indicators in the identification and distinction of taxa within the subgenus Cepa of the genus *Allium*. The seeds were 2.74-3.50 mm long and 1.33-2.14 mm wide. The morphometric and optical parameters of seeds were measured by analyzing images using software. Digital images of seeds were obtained using an HP Scanjet 200 digital flatbed scanner, 600 dpi resolution, JPG file format. Morphometric and optical parameters of seeds were determined, including projection area (cm$^2$), length, width, perimeter, mean size (mm), average feret diameter, factors of roundness, elongation, ellipse, indentation (relative units), parameters of brightness, tonality, color saturation (relative units). According to the results of the study, a series of distribution of species in descending order for each of the studied traits are formed. Within the Cepa section, *A. pskemense* seeds had the maximum linear size, perimeter, and cross-sectional area. Among the representatives of *Schoenoprasum* section, the maximum length of the seeds was found in *A. altyncolicum*. Maximum width, perimeter, cross-sectional area, average feret diameter of the seeds were recorded in *A. ledebourianum.* In the Cepa section, the average RGB value in descending order was as follows*: A. pskemense > A. galanthum > A. fistulosum > altaicum > A. oschaninii.* In the *Schoenoprasum* section this series has the form*: A. schoenoprasum > A. ledebourianum > A. altyncolicum > A. oliganthum.*

**Keywords:** *Allium* L., seeds, morphology, digital image analysis, color signs in the RGB system

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## INTRODUCTION

Imaging methods in agriculture and plant science have been used to analyze images captured by remote sensing techniques involving aircraft, satellites, and at close range, which have then been processed and analyzed using computers. With new technological advances in image capture and data processing, imaging methods address a variety of practical problems in biology, medicine, and agriculture. Different types of imaging methods, such as thermal imaging, fluorescence imaging, hyperspectral imaging, and photometric imaging, have contributed significantly to the advancement of various aspects of plant phenotyping. One of these is colorimetry, or RGB-based imaging, because of its dependence on color changes in various biological objects. In recent years, significant progress in the application of RGB-based imaging has been noted in various areas of agriculture and plant science. RGB analysis has been successfully used for identifying weed plants [1], mapping the weediness of crops [2], assessing the condition of lawns [3], analyzing physiological processes in leaves [4], and testing seeds for the degree of maturation [5].

The modern level of scientific knowledge in the study of seeds of agricultural crops requires the use of innovative instrumental methods characterized by high informativeness and speed of execution. The introscopic methods of seed quality assessment associated with the peculiarities of the internal structure of seeds are successfully used [6, 7]. Computer analysis technologies of seed images are actively used [8].

Morphometric parameters determine the shape of seeds, which, in turn, characterizes their viability and, ultimately, the productivity and quality of crop yield. Seed counting and "manual" morphometry are time-consuming and labor-intensive to perform. In this regard, various effective approaches of computer seed morphometry using image processing techniques have been proposed [9]. Most of these approaches are implemented using desktop PC software to analyze images of seeds on a light background obtained with a digital camera or scanner [10]. These approaches allow users to estimate a large number of morphometric parameters of the seed describing shape and color [11], which provide improved methods for variety identification using seed images, seed moisture content determination, and yield prediction [12].

Image processing methods for seed morphometry and classification have been used since the 1980s. Updates of these methods occur constantly, including in recent years [13]. Various optical sensing methods are being developed to assess seed quality and safety [14], and describe complex seed shapes using 2D images [15]. Revolutionary 3D imaging technology and robotics [16] or X-ray computer tomography [17] can be implemented to accurately assess seed shape. However, there is still a need for seed phenotyping using simple and accessible tools with high throughput [18].

Dimensional characteristics determining the shape of seeds, shades of their surface color suitable for further processing make modern imaging methods highly adaptable. The biomorphological properties of seeds can be analyzed using computer image analysis systems, and the data can be quickly processed and saved, and plotted on the graph [19].

Argus-BIO company (St. Petersburg) has developed a new morphometric method for the analysis of digital scanned images of seeds using the serial software "VideoTestMorphology". The new program is completely devoid of subjectivity, eliminates operator errors, significantly speeds up the analysis time and adds new parameters for evaluation of the examined material.

*Allium L.* is a large, diverse and taxonomically complex genus of monocotyledons. The genus includes more than 800 species belonging to 15 subgenera and more than 70 sections [20], many cultivated species - mainly vegetable and ornamental plants, some with medicinal properties [21]. A high level of morphological diversity of seed coat in the genus *Allium* was established, whose details are clearly visible under a scanning electron microscope. Differences in seed size, shape, color and cell structure of the seed coat served as taxonomic and/ or phylogenetic markers [22].

The purpose of the study was to study the geometric parameters and optical characters of seeds of the subgenus Cepa (*Allium* L., Alliaceae) from the biocollection of the All-Russian Research Institute of Vegetable Growing (VNIIO), a branch of the Federal Scientific Vegetable Center, by digital scanning.

## MATERIAL AND METHODS

Seeds of *Allium* L. from the subgenus Cepa: section Cepa (Mill.) Prokh. - *A. fistulosum* L., *A. altaicum* Pall., *A. galanthum* Kar. & Kir., *A. oschaninii* O. Fedtsch., *A. pskemense* B. Fedtsch.; *Schoenoprasum* Dum. section - *A. altyncolicum*, *A. ledebourianum*, *A. oliganthum*, *A. schoenoprasum* L.; *Condensatum* N. Friesen section - *A. condensatum* from the biocollection of VNIIO (Moscow Region) were the object of research. Plants were 4-5 years old.

Image analysis software allows automatic and manual measurements from images. Measurement of morphometric and optical parameters of the seeds was performed by image analysis using "VideoTest-Morphology" software developed by LLC "Argus-BIO" (St. Petersburg). Digital images of seeds were obtained using a digital flatbed scanner HP Scanjet 200, resolution 600 dpi, file format JPG. The program measures linear parameters of seeds with high accuracy (up to 1/1000 fractions of mm). The number of seeds of each species is 100 pcs.

The program is able to distinguish objects of interest (from the background) by pixel values in the RGB and HSB color systems (color hue, saturation, lightness). The RGB color model was used to describe the chromaticity when analyzing digital images. This color model allows up to 28 (256) gradations of brightness for each of the three base colors. The brightness in any of the channels of the digital image at a given point reflects the intensity of the light in the red, green and blue regions of the spectrum hitting the matrix of the scanner's recording device. According to the color model R, G and B can take absolute values from 0 to 255.

Average RGB value (R value + G value + B value) / 3.

The following morphometric parameters of seeds were determined:

– the projected area, $cm^2$;

– linear dimensions: length, width, perimeter, average size, mm;

– factors of roundness, elongation, ellipse, indentation, relative units;

– brightness parameters: brightness, tonality, saturation, relative units;

The roundness factor (r.u.) is the ratio of the perimeter of a seed to the perimeter of a circle with the same area. For a circle, the index is close to one. The elongation factor (r.u.) shows the ratio of the overall length of the seed to the overall width. The projection of an image of a seed not shaped like a circle, measured as the distance between the tangents to the contour of the image drawn parallel to the chosen direction (average diameter of Fere).

Confidence intervals of the mean values of the studied attributes with a significance level of 0.01 were calculated [22].

## RESULTS AND DISCUSSION

*Allium* is one of the largest monocotyledonous genera. The subgenus Cepa includes five sections. In the present study, we describe the seeds of 10 species from three sections. The genus *Allium* is characterized by umbrella-shaped inflorescences, with a veil at the base. The ovary is monocotyledonous with six or numerous seed buds. The fruit is a boll, which explodes in dissepiments. The fruit contains up to six achenes; rare species have more than 10.

Seed polyvariaty is a necessary condition for the adaptive strategy of plant species in native phytocenoses, but in agrocenoses, its manifestation is limited by the level of applied technologies of crop cultivation. The variation of morphometric parameters of seeds, as well as any other traits, is genospecific, hence selective significant [23].

The seeds of *A. fistulosum*, *A. altaicum*, *A. galanthum*, *A. oschaninii*, and *A. pskemense* were analyzed from the Cepa section. Within the section, the achenes of *A. pskemense* were the longest (3.09 mm) and the shortest was that of *A. fistulosum* (2.88 mm). The maximum width of the achene was recorded for *A. pskemense* (2.14 mm), the minimum for *A. altaicum* (1.89 mm). The distribution of the average size and area of the achene within the section was in descending order: *A. pskemense* > *A. oschaninii* > *A. galanthum* > *A. fistulosum* > *A. altaicum*. Seeds of all the species studied are elliptical: the ellipse factor is 0.99 r.u., in *A. altaicum* it is 0.98 r.u., which has the lowest roundness (0.61 r.u.) and the highest elongation (1.57 r.u.). The distribution of the perimeter and the seed angularity within the section was in the descending order: *A. pskemense* > *A. oschaninii* > *A. galanthum* > *A. altaicum* > *A. fistulosum* (see Table 1).

The seeds of *A. altyncolicum*, *A. ledebourianum*, *A. oliganthum*, and *A. schoenoprasum* were studied in the *Schoenoprasum* section. Within the section, the achenes of *A. altyncolicum* were the longest (3.26 mm) and the shortest were those of *A. oliganthum* (2.74 mm). The maximum width of the achene was recorded for *A. ledebourianum* (1.89 mm), the minimum for *A. oliganthum* (1.33 mm). The distribution of the area, perimeter, width, average size, and average diameter of the achene within a section was in the descending order: *A. ledebourianum* > *A. altyncolicum* > *A. schoenoprasum* > *A. oliganthum*.

The distribution of the length and maximum Fere diameter of the achene within the section was in the descending order: *A. altyncolicum* > *A. ledebourianum* > *A. schoenoprasum* > *A. oliganthum*. Seed elongation was 2.08 p.u. in *A. oliganthum* and *A. schoenoprasum* species, 2.05 in *A. altyncolicum*, and 1.64 p.u. - in *A. ledebourianum*. Seeds of all the studied species are elliptical: ellipse factor 0.99 r.u., indentation 0.01 r.u.

In our studies, the *Condensatum* section was represented by the *A. condensatum* species. The achene length was 3.5 mm, the width was 1.95 mm, and the average size was 2.73 mm. The perimeter was recorded at 8.86 mm, and the indentation was 0.03 r.u. The seeds were elliptic: ellipse factor 0.99 r.u.

The analysis of seed indentation of the studied *Allium* species from the three sections showed that the value of this trait is maximum (0.03 r.u.) in *A. pskemense* and *A. codensatum* species. The confidence intervals of the mean values of the trait "elongation" for the sections Cepa (1.46-1.50), *Schoenoprasum* (1.93-1.99) and *Condensatum* (1.77-1.87) did not overlap, therefore, seed elongation can be the primary marker trait for the section identification.

The software can also extract and export color information from an image. Color analysis has become very important in the field of plants in recent years. It allows identifying variations in the accumulation of different pigments, diagnosing plant diseases, comparing mutant phenotypes and taxonomic variations [23].

Based on the intensity of all individual color components and the average RGB value, differences in values between sections and species were determined (see Table 2). In the Cepa section, the average RGB value in descending order was as follows: *A. pskemense* > *A. galanthum* > *A. fistulosum* > *A. altaicum* > *A. oschaninii*. In the *Schoenoprasum* section this series is as follows: *A. schoenoprasum* > *A. ledebourianum* > *A. altyncolicum* > *A. oliganthum*. The highest average RGB value was found for *A. schoenoprasum* (63.31 luminance units) and *A. condensatum* (63.07 luminance units).

A series of RGB values in descending order was revealed: in the Cepa section and in the *A. condensatum* section, R > G > B. In the *Schoenoprasum* section these values are ambiguous: in *A. altyncolicum*, *A. oliganthum,* and *A. schoenoprasum* - B > R > G, in *A. ledebourianum* - R > G > B. The maximum tonality of achenes was found in the *Schoenoprasum* section: in *A. altyncolicum* - 0.73 r.u., in *A. schoenoprasum* - 0.72 r.u.

In the Cepa section, *A. altaicum* showed high values of maximum brightness (241.68 units of brightness) and saturation (0.04 relative units). However, in the *Schoenoprasum* section the opposite regularity was found: in *A. schoenoprasum* at the lowest value of maximum brightness (226.75 units of brightness), high saturation (0.04 relative units) was observed.

## CONCLUSION

The geometrical parameters and optical characters of seeds of the subgenus Cepa (*Allium* L., *Alliaceae*) from the biocollection of the All-Russian Research Institute of Vegetable Breeding (VNIIO), a branch of the Federal Scientific Center of Vegetable Breeding, were studied by digital scanning. Within the Cepa section, the seeds of *A. pskemense* had the maximum linear size, perimeter, and cross-sectional area. Among the representatives of the *Schoenoprasum* section, the seeds of *A. altyncolicum* had the maximum length. The maximum width, perimeter, cross-sectional area, and average diameter of Fere seeds were recorded for *A. ledebourianum*. In the Cepa section, the average RGB value in descending order was as follows: *A. pskemense* > *A. galanthum* > *A. fistulosum* > *A. altaicum* > *A. oschaninii*. In the *Schoenoprasum* section this series is as follows: *A. schoenoprasum* > *A. ledebourianum* > *A. altyncolicum* > *A. oliganthum*.

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**Табл. 1.** Морфометрический анализ семянок некоторых видов *Allium* L. ($n$ = 100)
**Table 1.** Morphometric analysis of the seeds of some species of *Allium* L. ($n$ = 100)

| Parameter | Cepa (Mill.) Prokh. section | | | | | *Schoenoprasum* Dum. section | | | | *Condensatum* N. Friesen section |
|---|---|---|---|---|---|---|---|---|---|---|
| | *A. fistulosum* | *A. altaicum* | *A. galanthum* | *A. oschaninii* | *A. pskemense* | *A. altyncolicum* | *A. ledebourianum* | *A. oliganthum* | *A. schoenoprasum* | *A. condensatum* |
| Surface area, mm$^2$ | 4,10/0,07 | 4,01/0,08 | 4,19/0,07 | 4,57/0,15 | 4,72/0,08 | 3,58/0,06 | 4,34/0,08 | 2,53/0,06 | 3,06/0,06 | 4,77/0,10 |
| Perimeter, mm | 7,71/0,06 | 7,73/0,08 | 7,80/0,07 | 8,11/0,14 | 8,42/0,08 | 7,66/0,06 | 8,03/0,07 | 6,45/0,07 | 7,15/0,06 | 8,86/0,14 |
| Length, mm | 2,88/0,02 | 2,95/0,03 | 2,90/0,02 | 3,02/0,06 | 3,09/0,03 | 3,26/0,02 | 3,06/0,02 | 2,74/0,03 | 3,02/0,02 | 3,50/0,05 |
| Width, mm | 1,98/0,02 | 1,89/0,02 | 1,99/0,02 | 2,06/0,03 | 2,14/0,02 | 1,60/0,02 | 1,89/0,03 | 1,33/0,02 | 1,47/0,02 | 1,95/0,03 |
| Average size, mm | 2,43/0,02 | 2,42/0,02 | 2,45/0,02 | 2,54/0,04 | 2,61/0,02 | 2,43/0,02 | 2,47/0,02 | 2,04/0,02 | 2,24/0,02 | 2,73/0,03 |
| Fere maximum diameter, mm | 2,84/0,02 | 2,90/0,03 | 2,86/0,02 | 2,97/0,06 | 3,04/0,03 | 3,19/0,02 | 3,02/0,02 | 2,67/0,03 | 2,95/0,02 | 3,45/0,05 |
| Fere average diameter, mm | 2,43/0,02 | 2,44/0,02 | 2,44/0,02 | 2,54/0,04 | 2,61/0,02 | 2,43/0,02 | 2,53/0,02 | 2,04/0,02 | 2,24/0,02 | 2,75/0,03 |
| Circle factor, relative units | 0,86/0,00 | 0,84/0,01 | 0,86/0,01 | 0,87/0,01 | 0,84/0,01 | 0,77/0,01 | 0,84/0,01 | 0,76/0,01 | 0,75/0,01 | 0,77/0,02 |
| Ellipse factor, relative units | 0,99/0,00 | 0,98/0,00 | 0,99/0,00 | 0,99/0,00 | 0,99/0,00 | 0,99/0,00 | 0,99/0,00 | 0,99/0,00 | 0,99/0,00 | 0,99/0,00 |
| Roundness, relative units | 0,65/0,01 | 0,61/0,01 | 0,65/0,01 | 0,66/0,01 | 0,65/0,01 | 0,45/0,01 | 0,60/0,01 | 0,45/0,01 | 0,45/0,01 | 0,52/0,01 |
| Elongation, relative units | 1,47/0,02 | 1,57/0,02 | 1,47/0,02 | 1,46/0,02 | 1,45/0,02 | 2,05/0,03 | 1,64/0,03 | 2,08/0,03 | 2,08/0,03 | 1,82/0,05 |
| Indentation, relative units | 0,01/0,00 | 0,01/0,00 | 0,02/0,00 | 0,02/0,00 | 0,03/0,00 | 0,01/0,00 | 0,01/0,00 | 0,01/0,00 | 0,01/0,00 | 0,03/0,01 |

Note. Value / - confidence interval.

**Табл. 2.** Величины цветовых составляющих семянок некоторых видов *Allium* L. по модели RGB ($n$ = 100)
**Table 2.** Values of color components of the seeds of some species of *Allium* L. by RGB model ($n$ = 100)

| Parameter | Cepa (Mill.) Prokh.section | | | | | *Schoenoprasum* Dum. section | | | | *Condensatum* N. Friesen section |
|---|---|---|---|---|---|---|---|---|---|---|
| | *A. fistulosum* | *A. altaicum* | *A. galanthum* | *A. oschaninii* | *A. pskemense* | *A. altyncolicum* | *A. ledebourianum* | *A. oliganthum* | *A. schoenoprasum* | *A. condensatum* |
| Average brightness, unit of brightness | 58,12/0,56 | 56,62/0,84 | 58,35/0,82 | 54,17/0,64 | 61,83/0,88 | 52,80/0,78 | 61,64/0,79 | 51,99/0,61 | 61,59/0,75 | 62,61/1,11 |
| Brightness deviation, unit of brightness | 26,44/0,26 | 27,60/0,35 | 27,04/0,31 | 25,00/0,34 | 29,62/0,47 | 31,82/0,34 | 32,31/0,30 | 32,30/0,35 | 40,39/0,29 | 30,13/0,39 |
| Minimum brightness, unit of brightness | 9,70/0,46 | 9,80/0,56 | 10,10/0,54 | 10,35/0,59 | 9,59/0,54 | 6,00/0,60 | 7,71/0,47 | 7,90/0,58 | 9,86/0,48 | 8,01/0,63 |
| Maximum brightness, unit of brightness | 239,00/0,20 | 241,68/0,22 | 239,56/0,23 | 237,43/0,17 | 238,55/0,29 | 242,24/0,30 | 243,45/0,25 | 243,22/0,23 | 226,75/0,08 | 240,98/0,47 |
| Red, unit of brightness | 60,37/0,62 | 59,88/0,97 | 60,71/0,86 | 56,76/0,71 | 63,35/0,90 | 53,45/0,81 | 64,69/0,80 | 52,19/0,63 | 62,40/0,80 | 64,62/1,18 |
| Green, unit of brightness | 57,84/0,55 | 55,94/0,82 | 58,00/0,82 | 53,68/0,62 | 61,79/0,88 | 52,75/0,77 | 60,95/0,79 | 52,08/0,61 | 61,08/0,74 | 62,34/1,09 |
| Blue, unit of brightness | 57,20/0,51 | 55,18/0,72 | 57,60/0,77 | 53,54/0,57 | 61,63/0,86 | 55,24/0,77 | 60,93/0,81 | 55,17/0,63 | 66,44/0,70 | 62,24/1,02 |
| Average value of RGB, unit of brightness | 58,47/0,56 | 57,00/0,84 | 58,77/0,82 | 54,66/0,63 | 62,26/0,88 | 53,81/0,78 | 62,19/0,80 | 53,15/0,62 | 63,31/0,75 | 63,07/1,10 |
| Tone range, relative units | 0,27/0,04 | 0,29/0,06 | 0,40/0,05 | 0,46/0,07 | 0,41/0,05 | 0,73/0,01 | 0,46/0,06 | 0,68/0,01 | 0,72/0,01 | 0,46/0,07 |
| Saturation, relative units | 0,03/0,00 | 0,04/0,00 | 0,03/0,00 | 0,03/0,00 | 0,02/0,00 | 0,02/0,00 | 0,03/0,00 | 0,03/0,00 | 0,04/0,00 | 0,02/0,00 |

Note. Value / - confidence interval.

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