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## The comparative morphometric analysis of seeds representatives of the genus *Paeonia* L. (*Paeoniaceae* Rudolphi) of the Kazakh Altai's flora, introduced in the Altai Botanical Garden

The article provides data on the study of the external structure, morphometric and weight parameters of seeds of the complex of *Paeonia anomala* L., *P. hybrida* Pall., *P. intermedia* C.A. Mey., grown in the conditions of the mountain-taiga zone of the Kazakhstan Altai, and clarification of the possibility of using these characters to distinguish between species. The primary analysis of the seed material revealed differences in the length, width and thickness of the seed in the cultivated samples from different ecological and geographical conditions of the habitats. A comparative study of such morphometric and weight parameters as the external structure, length, width, and thickness of seeds of the samples of *Paeonia anomala*, *P. hybrida*, *P. intermedia*, introduced from different ecological and geographical habitats of Kazakhstan Altai, revealed a certain variation in the selected parameters in the species. When comparing the external morphological structure of *P. intermedia* and *P. anomala*, the differences were practically not established, due to the close relationship of the species. When comparing the average metric and weight parameters of seeds in the *Paeonia anomala* specimens taken from ridge Ivanovsky, did not reveal significant differences. This happened despite the interpopulation difference in quality of the planting material with respect to ecology and location above sea level. This indicates the evenness of the length, width, and thickness of the seed material of the cultivated *Paeonia anomala* specimens. In cultivate specimens of *P. hybrida* from Narym ridge and Azutau in the ratio of the length, width and thickness of the seed, as well as the weight of 1000 pieces, seed differences were observed. Despite the low level of variability of morphological parameters of seeds, it is premature to recommend them for differentiation of species due to the short duration of the study period.

**Keywords:** *Paeonia* L., carpology, Kazakhstan Altai, introduction, category of rarity.

### Introduction

The conservation of the Earth's biological diversity is one of the most important problems of modern biology. Its effective solution is possible only with a comprehensive study of the ecological-biological features of each species of plants. This is especially relevant for rare species, since research on their ecology and biology is not only of theoretical interest, but also necessary for the development of ways to protect these species [1]. The main reasons for the reduction of species of the genus *Paeonia* L. are anthropogenic effects on natural ecosystems and the collection of plants for medicinal and ornamental purposes [2].

Genus *Paeonia* L., includes 36 species [3], belongs to the number of systematically complex taxa of flowering plants [4]. Representatives of the genus are perennial herbs with thickened spindle-shaped roots and difficult palm-dissected leaves or tree-like shrubs (8 species) [5].

The fruit of the peony is apocarps leafy. A multi-leaflet is a collection of non-growing leaflets, each of which arises from a separate carpel. A separate leaflet can be called a fruit. The fruit is multi-seeded, opening suturally (along the abdominal suture). The pericarp is dry, the number of seeds is usually up to 10 or slightly more, but always less than the number of embedded seed bugs [6].

High morphological variability creates great difficulties in establishing boundaries between species [7]. There are controversial points in taxonomy and systematics of the genus [8]. Especially between *P. anomala*, *P. intermedia* and *P. hybrida*, forming a single complex of Siberian-Central Asian herbaceous species, characterized by twice tripartite strongly segmented leaves, covered with bristles on top of the veins. All species of complex are diploids [9]. Analysis of literary data on the state of study of the topic showed that information on the morphology of species of the genus *Paeonia* is clearly not enough to conduct a comparative morphometric analysis of seeds of the studied species [10–14].

The purpose of this work was to study the external structure, morphometric and weight indices of seeds *Paeonia anomala* L., *Paeonia hybrida* Pall., *Paeonia intermedia* C.A. Mey., grown under the conditions of culture, and find out the possibility of using these features to distinguish species.

### Materials and Methods

The object of the study was the seed material of three rare species of peonies: *Paeonia anomala* L., *Paeonia hybrida* Pall., *Paeonia intermedia* C.A. Mey, included to the introduction from various habitats of Kazakhstan Altai and grown in the exposition of the natural flora of the Altai Botanical Garden.

*Paeonia anomala* L. is boreal, mainly Siberian species, mesophyte. It distributes in the northern regions of European Russia, in Western Siberia, Baikal region, on Tien Shan, Mongolia. In Kazakhstan, it grows in the northern, eastern and southeastern regions: Caspian (Volga region), Kokchetau, Zaisan, Altai, Tarbagatai, Dzungar, Transili, Ketmen, Kyrgyz Alatau and on Western Tien Shan [5]. It prefers moistened meadows and fairly well-warmed and clarified forest glades. Throughout the range it occurs discretely, is intensively shrinking. It is included in the Red Book of Kazakhstan [15], in the regional Red Books of the Russian Federation [16–22].

*Paeonia hybrida* Pall. is the Central Asian mesoxerophyte species. The general distribution of the species is Western Siberia and Western China [23]. In Kazakhstan, it grows on the steppe slopes of foothills and shallow slopes, on steppe meadows, open rocky slopes of the lower mountain belt in geographical areas: Turkestan, Altai, Tarbagatai, Dzungar, Transili, Ketmen, Kyrgyz Alatau, Chu-Ili mountains (Krasnogorsk) and in Tien Shan. In 1991 it was found in the Republic of Bashkortostan [24]. It is included in the Red Book of Kazakhstan [15], the Red Book of Uzbekistan [25], the Red Book of the Altai Territory [26].

*Paeonia intermedia* C.A. Mey. is mesoxerophyte species, area covers Western Siberia, Chinese and Mongolian Altai [27]. In Kazakhstan, it is found in Kazakhstan Altai [28], Saur, Tarbagatai, Dzungar Alatau, Western Tien Shan [27]. The species is included in the Red Book of Tajikistan [29].

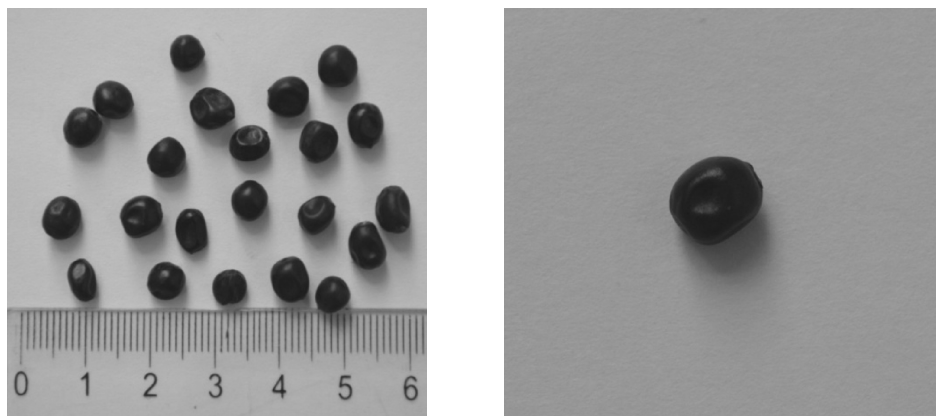
In climatic terms, the research area (Ridder, East Kazakhstan, geographical region: South-West Altai) is characterized by cold long winters, warm and humid summers, sharp temperature and humidity drops during the year, seasons of the day. According to the Ridder Weather Station, the area of introduction belongs to a moderately cool, moderately moistened agro-climatic zone. The climate is sharply continental, with an average annual air temperature of +1.8 °C. The average monthly air temperature of the winter months ranges from –5 °C to –16 °C with an absolute minimum of –46,7 °C, summer months from +17 °C to +25 °C with an absolute maximum of +41.5 °C. The average annual rainfall ranges from 432 to 937 mm with a summer maximum [30].

During describing the morphological features of seeds, special attention was paid to the size and structure of the surface of the seed, to a lesser extent to the shape and color. Well-made seeds were selected for analysis. The seeds were collected after the flyer flaps diverged when the seeds acquired a light brown or dark blue color [31]. Morphological characteristics of seeds were compiled using terminology presented in the works of Z.T. Artyushenko [32] and M.G. Nikolaeva et al. [31]. Morphometric measurements are made with a micrometer MK-25 in 20–25 times repetition for each parameter. The color palette of seeds is determined on the basis of the color scale of A.S. Bondartsev [33] and the color scale of the English Royal Society of Flower Growers [34]. The statistical analysis calculated the mean values of the analyzed indices (length, width, thickness of the seed with deviation from the mean and correlation coefficient) [35]. Latin plant names were verified according to S.K. Czerepanov [36]. The circuit map is made in QGIS 3.14.15 Pi.

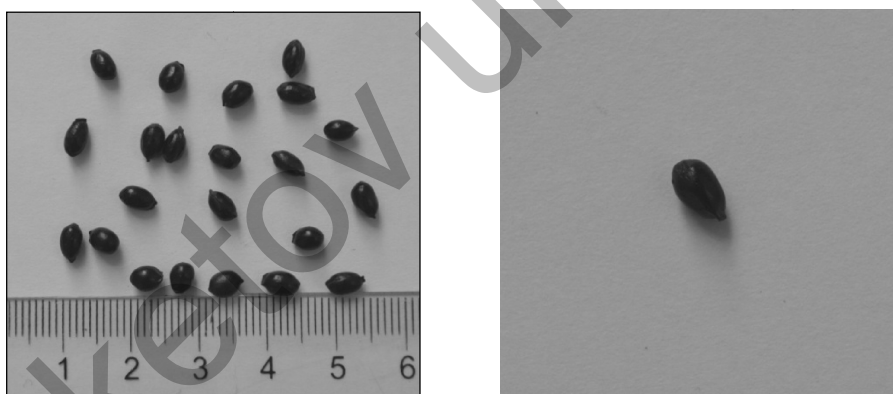
### Results and Discussion

As a result of the cameral treatment, the description of the external structure of the seeds *Paeonia anomala* L., *Paeonia hybrida* Pall., *Paeonia intermedia* C.A. Mey was carried out at 2019 samples of the collection site of the Altai Botanical Garden.

*Paeonia anomala*: seeds large, rounded, slightly flattened (Fig. 1, 2). Length: 6.15–7.25 (average 6.52) mm, width: 4.82–6.15 (average 5.47) mm, thickness 2.99–5.04 (average 4.46) mm. The mass of 1000 seeds is from 67 to 140 g. The color is from brilliantly black to matte black, sometimes grayish in the lateral and chalazal parts. The color is mainly glossy. The shape of the seeds is spherical, sometimes slightly flattened in the back. The dough is rough, sometimes almost smooth; the surface is fine in places, always bare. The peel is dense, thick enough. In the abdominal part there are two unequal parallel recesses, oval in shape, forming a longitudinal protrusion between them. Often small circular depressions are found on the sides of the seed. Micropile is overgrown, invisible. The chalazal end narrows slightly to the base, usually smooth, rarely longitudinally wrinkled. Seed scar of medium size, linear type, longitudinally ellipsoidal in shape, sometimes almost oval, noticeably protruding; located in the basal part, less often in the lower part of the abdominal side. Rafe is absent.

Figure 1 and 2. Seeds of *Paeonia anomala* L.

*Paeonia hybrid*: straight-type seeds, elongated (Fig. 3, 4). Length: 4.4–6.2 (average 5.41) mm, width: 3.1–3.8 (average 3.38) mm, thickness: 2.9–3.5 (average 3.18) mm. The mass of 1000 pieces of seeds is from 28 to 37 g. The color of the seeds is from brown-brown to dark brown. Seeds have a characteristic gloss. The seed peel is smooth, rarely with small roughness, sometimes finely chopped. The surface of the seeds is bare, without pubescence. The dough is very dense, strong. Often, the surface of the seeds can be longitudinally rigid in the abdominal and lateral parts, with characteristic clearly pronounced cellular gyros. The shape of the seeds is barrel-shaped, ellipsoidal in shape, noticeably thickened in the middle part. The micropile is overgrown, clearly pronounced formed by external integrations, longitudinally slit-shaped, elongated into a small well-distinguished spout. The halazal end is noticeably narrowed, passing into a seed scar. The medium-sized seed scar is longitudinally slit-shaped, basal, protruding. Rafe is clearly pronounced, narrow, and clearly distinguishable to micropiles.

Figure 3 and 4. Seeds of *Paeonia hybrida*

*Paeonia intermedia*: seeds are large, rounded; length: 6.78–8.33 (average 7.17) mm, width 4.93–6.52 (average 5.41) mm, thickness 4.41–5.26 (average 4.82) mm; The mass of 1000 pieces of seeds is 96.74 g. The color gamut of the seeds varies from gray-slate to coal-black. The color is mainly matte, sometimes glossy. Slightly lighter in the thickened part, ovate or oblong-ovate in shape, noticeably volumetric and convex in the back. The seed peel is quite dense, strong and thick. The surface of the seeds is almost smooth, sometimes slightly rough, finely grained, glabrous; without any pubescence. In the abdominal part of the seed there is a significant notch, less often two, oval or oblong-ellipsoidal in shape. In the dorsal part of the seed there are sometimes weakly expressed intrusions. The micropilar entrance is completely overgrown, located in a characteristic small rounded decrease; located in a smoothly tapering end of the seed. The halazal end is aligned, usually has a smooth surface, sometimes slightly thickened, sharply tapering to the seed scar. Seed scar of rather large size, basal, linear type, quadrangular shape, short, slightly protruding, longitudinal location. There is always no rafe (Fig. 5, 6).

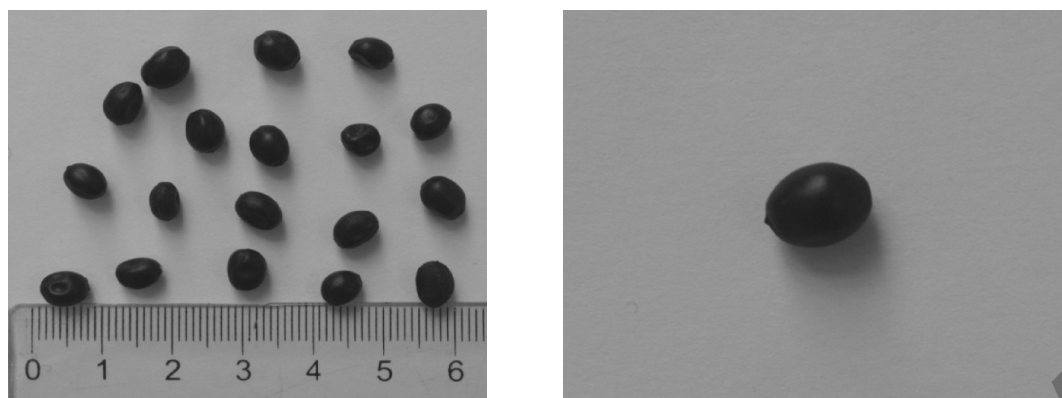


Figure 5 and 6. Seeds of *Paeonia intermedia* C.A. Mey

It is noted that *P. intermedia* and *P. anomala* seeds are almost identical in external structure. This is probably directly related to the close relationship of these species, which were previously assigned to one species. For comparative analysis of morphometric and weight indices we carried out statistical processing of seeds (collection 2016) of studied species *Paeonia anomala* and *Paeonia hybrida*, attracted to introduction in different periods from different ecological and geographical habitats of Kazakhstan Altai (Fig. 7).

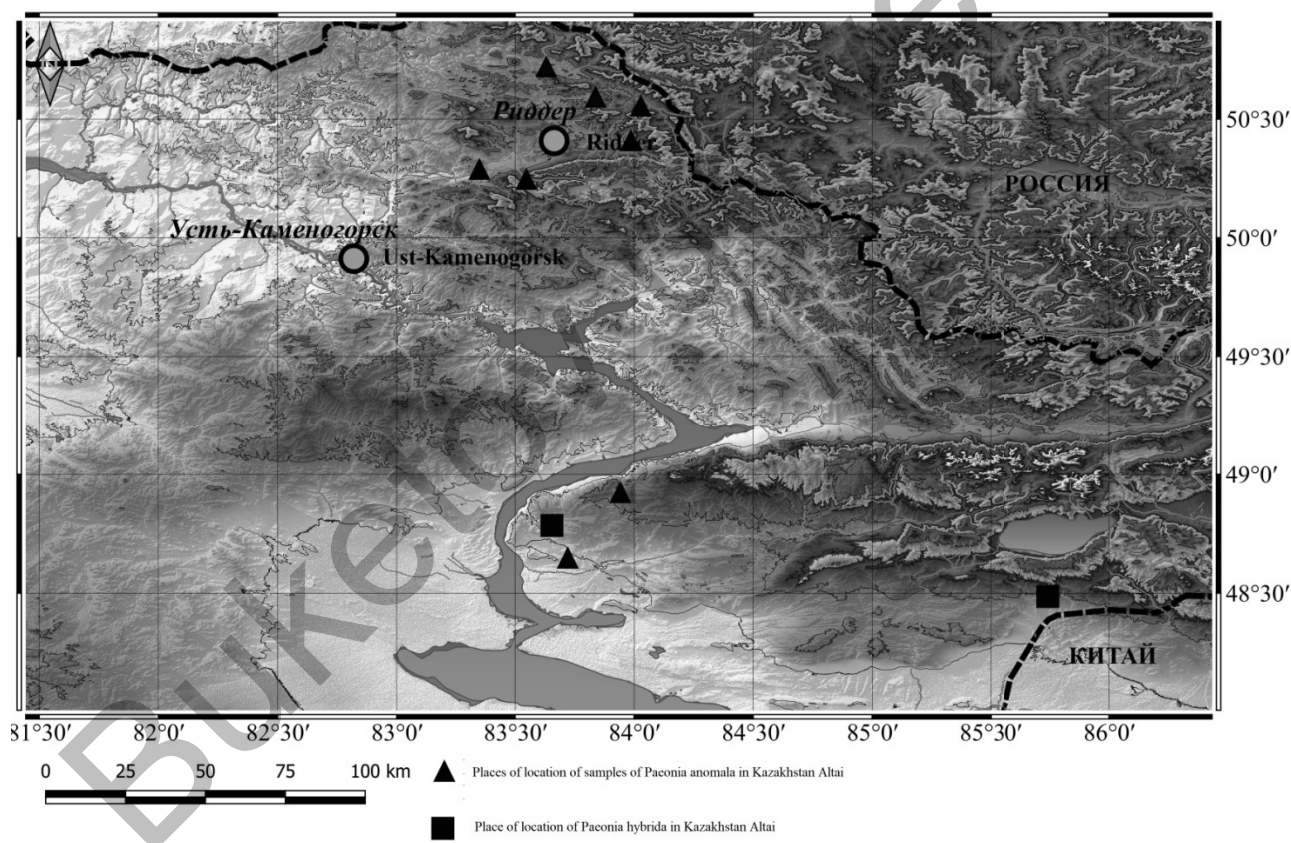


Figure 7. Scheme — map of ecological and geographical habitats of different samples of species *Paeonia anomala* and *Paeonia hybrida* in the territory of Kazakhstan Altai

Data for the comparative analysis is presented in Table 1.

**Biometric indicators of seeds of *Paeonia anomala* and *Paeonia hybrida* in introduction from different ecological-geographical habitats of Kazakhstan Altai**

Sample origin	Seed length, mm		Seed width, mm		Seed thickness, mm		Mass of 1000 seeds, g
	$\frac{M \pm m}{\text{Min-Max}}$	Cv, %	$\frac{M \pm m}{\text{Min-Max}}$	Cv, %	$\frac{M \pm m}{\text{Min-Max}}$	Cv, %	
<b><i>Paeonia anomala</i></b>							
Ivanovsky ridge, tract Broad Log, 1400 m above sea level	$\frac{6.52 \pm 0.16}{6.15-7.25}$	4.43	$\frac{5.47 \pm 0.19}{4.82-6.15}$	7.02	$\frac{4.46 \pm 0.28}{2.99 \pm 5.04}$	11.53	85.26
Ridge Ivanovsky, the upper reaches of the Kazachikha River, eastern slope, sparse cedar, 1900 m above sea level	$\frac{6.64 \pm 0.26}{5.84-7.7}$	7.15	$\frac{4.97 \pm 0.19}{4.5-5.7}$	7.12	$\frac{3.79 \pm 0.21}{3.24-4.3}$	10.1	73.61
Ridge Ivanovsky, gorge «Gray Meadow», north-western slope, coniferous forest, 1200 m above sea level	$\frac{6.54 \pm 0.25}{5.8-7.1}$	6.94	$\frac{5.41 \pm 0.23}{4.7-6.1}$	8.17	$\frac{4.52 \pm 0.28}{3.6-5.2}$	11.1	82.18
Ridge Ivanovsky, «Passage» below, north-western slope, 1600 m above sea level	$\frac{6.61 \pm 0.21}{5.7-7.3}$	6.02	$\frac{5.03 \pm 0.2}{4.5-5.8}$	7.6	$\frac{4.15 \pm 0.29}{3.1-5.1}$	12.8	74.29
Ridge Ivanovsky, northern spur, tract «Haircut Pit», 1100 m above sea level, fir forest with an admixture of birch	$\frac{6.79 \pm 0.17}{6.3-7.3}$	4.5	$\frac{5.06 \pm 0.19}{4.3-5.6}$	7.16	$\frac{4.04 \pm 0.33}{2.9-5.0}$	15.1	77.77
Ridge Ubinsky, southeastern spurs, Kozlushka Mount, surrounding Butakovo, 710 m above sea level	$\frac{6.72 \pm 0.3}{6.19-7.87}$	8.12	$\frac{4.92 \pm 0.29}{4.19-5.75}$	11.1	$\frac{3.87 \pm 0.29}{2.61-5.01}$	13.9	67.99
Ridge Kurchumsky, northern slope, surrounding Kurchum, 503 m above sea level	$\frac{7.83 \pm 0.22}{7.1-8.4}$	5.14	$\frac{6.21 \pm 0.13}{5.7-6.5}$	3.92	$\frac{5.75 \pm 0.21}{5.3-6.6}$	6.5	139.59
Ridge Narymsky, gorge Akbulak, north-western crushed slope, 940 m above sea level	$\frac{7.23 \pm 0.25}{5.8-7.7}$	6.39	$\frac{5.11 \pm 0.22}{4.5-5.6}$	7.71	$\frac{4.27 \pm 0.19}{3.6-5.1}$	9.13	86.55
<b><i>P. hybrida</i></b>							
Narymsky ridge, Tolebay tract, north-western crushed slope, 740 m above sea level	$\frac{5.41 \pm 0.24}{4.4-6.2}$	8.02	$\frac{3.38 \pm 0.12}{3.1-3.8}$	6.72	$\frac{3.18 \pm 0.09}{2.9 \pm 3.5}$	5.67	28.22
Ridge Azutau, Koytas tract, south-western slope, 777 m above sea level	$\frac{5.55 \pm 0.21}{5.1-5.9}$	5.25	$\frac{3.51 \pm 0.13}{3.2-3.7}$	5.02	$\frac{3.3 \pm 0.11}{3.1-3.6}$	4.67	36.11

Note. in numerator:  $M \pm m$  — average value  $\pm$  permissible limits; in denominator: Min-Max — minimum and maximum value.

Primary analysis of *Paeonia anomala* seeds revealed differences in the length, width and thickness of the seed in cultivated samples from different ecological-geographical habitats. The largest seeds are collected from a sample imported from range Kurchumsky, 503 m above sea level, in which the mass of 1000 seeds is 139.59 g, dimensions: length —  $7.83 \pm 0.22$  mm, width —  $6.21 \pm 0.13$  mm. Large seeds produce individuals originating from range Narymsky (gorge Akbulak, 940 m above sea level), in which the mass of 1000 seeds is 86.55 g, dimensions: length —  $7.23 \pm 0.25$  mm, width —  $5.11 \pm 0.22$  mm. Seed material from samples from the Ubinsky ridge (Kozlushka Mount, 710 m above sea level) has the smallest mass of 1000 seeds — 67.99 g and intermediate parameters of the length and width of the seeds compared to *Paeonia anomala* samples collected from range Ivanovsky, Narymsky and Kurchumsky.

Study in culture of average biometric and weight indices of seeds in *Paeonia anomala* samples collected with range Ivanovsky did not reveal significant differences, despite the inter-population diversity of the planting material relative to ecology and location above sea level.

The coefficients of variation in the length, width and thickness of the seed showed that the studied features have similar variation amplitudes in all samples and correspond to a low level of variability. This indi-

cates that the length, width and thickness of the seed material of the cultivated *Paeonia anomala* samples are aligned.

Similar results during the statistical processing of morphometric and weight indices were found for *P. hybrida* seeds. In cultivated samples of the species with range Narymsky and Azutau showed slight differences in the ratio of the length, width and thickness of the seed, as well as the mass of 1000 seeds. It was also found that for both species, the coefficients of variation of these indicators have a low degree of variability.

### Conclusion

A comparative study of such morphometric and weight parameters as external structure, length, width, seed thickness of samples *Paeonia anomala*, *P. hybrida*, *P. intermedia*, collected from different ecological and geographical habitats of Kazakhstan Altai, revealed a certain variation of selected indicators in species. Apparently, this is related to the origin of material from geographically distant populations. However, when comparing the external structure of *P. intermedia* and *P. anomala* seeds, differences are practically not established. There is probably a close relationship between these species, previously attributed to the same species *P. anomala*. After comparing average metric and weight values of seeds in *Paeonia anomala* samples attracted from range Ivanovsky did not reveal significant differences, despite the inter-population diversity of the planting material relative to ecology and location above sea level.

Despite the low level of variability of morphological indicators of seeds, it is premature to recommend them for differentiation of species due to the short-term period of research.

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## Алтай Ботаникалық бағына жерсіндірілген Қазақстан Алтайының флорасының *Paeonia* L. (*Paeoniaceae* Rudolphi) туыстасының өкілдерінің тұқымдарының морфометриялық салыстырмасы

Мақалада *Paeonia anomala* L., *P. hybrida* Pall., *P. intermedia* С.А. Меу түрлер тұқымдарының сыртқы құрылымын, морфометриялық және салмақтық параметрлерін кешенді зерттеу туралы мәліметтер келтірілген. Және де Қазақстан Алтайының таулы-тайга зонасы жағдайында өсірілген және түрлерді ажырату үшін осы танбаларды қолдану мүмкіндігін нақтылау сипатталған. Тұқым материалының алғашқы талдауында әр түрлі экологиялық-географиялық жағдайлардан алынған өсімді үлгілеріндегі тұқымның ұзындығы, ені мен қалыңдығындағы айырмашылықтар анықталды. *Paeonia anomala*, *P. hybrida*, *P. intermedia* үлгілерінің тұқымдарының сыртқы құрылымы, ұзындығы, ені және қалыңдығы сияқты тұқымдардың морфометриялық және салмақтық параметрлерін салыстырмалы түрде зерттеу барысында Алтайдың әр түрлі экологиялық-географиялық тіршілік ету орталарынан енгізілгені бола тұрып, таңдалған түрдегі параметрлердің белгілі бір ауытқуын анықтады. *P. intermedia* мен *P. anomala*-ның сыртқы морфологиялық құрылымын салыстыру кезінде бұл түрлердің тығыз байланысына байланысты айырмашылықтар іс жүзінде анықталмады. Тұқымдардың орташа метрикалық және салмақтық параметрлерін Ивановский жотасынан алынған *P. anomala* үлгілерімен салыстыру кезінде, экологияға және отырғызу материалының сапасының популяциялар арасындағы әртүрлілігіне және теңіз деңгейінен жоғары орналасуына қарамастан айтарлықтай айырмашылықтарды анықтаған жоқ. Бұл өсірілген *P. anomala* үлгілерінің тұқым материалының ұзындығының, енінің және қалыңдығының біркелкілігін көрсетеді. Нарын мен Азутау жоталарынан алынған *P. hybrida* түрлерінің өсірілген үлгілерінде тұқымның ұзындығына, еніне және қалыңдығына, сондай-ақ 1000 дана салмағына тұқымдық айырмашылықтар байқалды. Тұқымдардың морфологиялық параметрлерінің өзгерістік деңгейінің төмендігіне қарамастан, оларды зерттеу кезеңінің қысқа болуына байланысты түрлерді саралауға ұсыну ерте.

*Кілт сөздер:* *Paeonia* L., карпология, Қазақстандағы Алтай, жерсіндіру, сирек категориясы.

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## Сравнительная морфометрия семян представителей рода *Paeonia* L. (*Paeoniaceae* Rudolphi) флоры Казахстанского Алтая, интродуцированных в Алтайском ботаническом саду

В статье приведены данные по изучению внешнего строения, морфометрических и весовых показателей семян комплекса видов *Paeonia anomala* L., *P. hybrida* Pall., *P. intermedia* C.A. Mey., выращиваемых в условиях горно-таежной зоны Казахстанского Алтая, и выявлены возможности использования данных признаков для разграничения видов. Первичный анализ семенного материала показал отличия по длине, ширине и толщине семени у культивируемых образцов из разных эколого-географических условий местообитаний. Сравнительное изучение таких морфометрических и весовых параметров, как внешнее строение, длина, ширина, толщина семян образцов *Paeonia anomala*, *P. hybrida*, *P. intermedia*, интродуцированных из разных эколого-географических местообитаний Казахстанского Алтая, выявило определенное варьирование выбранных показателей у видов. При сравнении внешнего морфологического строения семян *P. intermedia* и *P. anomala* различия практически не установлены ввиду близкого родства видов. Сравнение средних метрических и весовых показателей семян у образцов *Paeonia anomala*, привлеченных с хр. Ивановский, не дало значительных различий, несмотря на межпопуляционную разнокачественность посадочного материала относительно экологии и расположения над уровнем моря, что свидетельствует о выровненности показателей длины, ширины и толщины семенного материала культивируемых образцов *Paeonia anomala*. У культивируемых образцов *P. hybrida* вида с хр. Нарынский и Азутау в соотношении показателей длины, ширины и толщины семени, а также массы 1000 шт. семян наблюдались незначительные различия. Несмотря на низкий уровень изменчивости морфологических показателей семян, рекомендовать их для разграничения видов преждевременно из-за краткосрочности периода исследований.

*Ключевые слова:* *Paeonia* L., карпология, Казахстанский Алтай, интродукция, категория редкости.

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