Chromosomal mechanisms in adapting Allium hymenorhizum Ledeb. under introduction conditions

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Abstract. The paper presents a karyotypic description of the rare relict species Allium hymenorhizum Ledeb. illustrated by three samples introduced to the South Ural Botanical Garden-Institute from the Bashkir Cis-Ural and South Ural regions. It was found out that the chromosome number in somatic tissue of this species is $2n=16$; chromosomes are of medium size ($6.05\pm0.15$ to $9.35\pm0.22$ \(\mu\)m) and belong to the metacentric and submetacentric types. The variability of chromosomal morphometric parameters is characterized by the extremely low, low and average values of the coefficients of variation. The samples introduced from different climatic conditions vary in the number and frequency of secondary constrictions in the chromosomal complements, this evidently being associated with the processes of their adaptation to new growing conditions.

1. Introduction
Allium hymenorhizum Ledeb. of the family Alliaceae is a rare Pleistocene relict plant of the Asian origin grown in the South Ural and Cis-Ural regions. Relictual populations of this species in the Republic of Bashkortostan (RB) are very small and endangered [1]. Therefore, the species is included into the "Red Data Book of the Republic of Bashkortostan" [2] and falls into category 1 (species in danger of extinction). It belongs to wild relatives of domesticated crops [3] and is of much interest as a promising ornamental [4, 5], melliferous and food plant [6].

The development of measures to protect A. hymenorhizum necessitates further integrated investigations of this species, including the use of karyological analysis. Karyological research studies of A. hymenorhizum were conducted within the Pamir-Alay [7-9], Kazakhstan [10], Altai region [11, 12] and Tajikistan [13]. In Bashkortostan no karyological studies of A. hymenorhizum have been performed until now. In this regard, they seem to be especially topical.

Karyological investigations of species belonging to the genus Allium are undertaken by researchers of many countries in different habitats. They determine the chromosome number in somatic and generative tissues, the ploidy level of chromosomal complements, chromosome sizes and morphological types and the presence of satellite and B chromosomes [14-21]. All this has undoubtedly great practical value in plant breeding and is a strong argument when dealing with the issues of taxonomy and evolution of this genus.
2. Materials and Methods

Investigative materials were collected in 2017 at the introduction nursery of South Ural rare and endangered plants (Ufa Institute of Biology) located on the territory of the South Ural Botanical Garden-Institute (Ufa, Russia). The introduction nursery lies within the forest-steppe zone of the Bashkir Cis-Urals (grey forest heavy loam soil). The samples originate from: 1) RB, Blagovarsky district, right bank of the Cheramyan river, 4 km to the north-east from the village of Novokonstantinovka, introduced with living plants in 2014; 2) RB, Baymaksky district, right bank of the Tanalyk river, 3 km to the south from the village of Bakhtigareevo, introduced with living plants in 2008; 3) RB, Baymaksky district, right bank of the Tanalyk river, 3 km to the south from the village of Bogachevo, introduced with living plants in 1996. Sample 1 originates from the Bashkir Cis-Urals (slightly salinated bottomland meadows and thin birch forests), samples 2 and 3 are from the South Urals (slightly salinated bottomland meadows). For the sake of convenience the samples (populations) will further be named after the places of their origin (Novokonstantinovka, Bakhtigareevo, Begichevo). Seeds for karyological analysis were obtained via self-pollination and artificial cross pollination inside each population using isolators (figure 1).

![Figure 1. Plants Allium hymenorhizum under study (introduction nursery, June 2017)](image1)

![Figure 2. Microphotograph of Allium hymenorhizum metaphase plate (Bakhtigareevo). The scale equals 10 μm.](image2)

Karyological investigations were conducted using the meristematic tissue of seedling root tips as the research material. The colouring of the materials was done in 1 % aqueous solution of acetohematoxylin [22]. Microspecimens were pressed flat and studied with a microscope BIMAM R-13 using the x40 objective lens, the x7 ocular lens and the head x2.5. Each population was tested with 30 metaphase plates.

Karyological analysis was performed according to Turkov et al. [23], morphometric types were determined according to the classification proposed by Grif and Agapova [24]. Basic statistical characteristics were calculated according to standard methods [25]. The degree of variation was assessed for the traits under study according to Marnaev’s scale [26].

3. Results and discussion

The somatic number of chromosomes in A. hymenorhizum plants of the populations in question was 2n=16; chromosomes were of medium size (6.05±0.15 to 9.35±0.22 μm). Microphotograph of the metaphase plate is shown in Figure 2. Statistically processed results on chromosome morphometric parameters for 30 metaphase plates in each population are given in table 1. The average overall length of the diploid chromosomal complement is presented in table 2. Karyotype ideograms of the population under study are shown in Figure 3. It was revealed that all chromosome pairs in the Novokonstantinovka and Bogachevo populations were of the metacentric type (Ic>40); chromosome pairs I-VII in the Bakhtigareevo population were of the metacentric type while chromosome pair VIII
was of the submetacentric type (30<Ic<40 %). The variability of chromosomal metamorphic parameters is characterized by very low (CV<7 %), low (CV=8-12 %) and average (CV=13-20 %) values of the coefficient of variation. The highest level of variability is determined by the absolute chromosome length, the lowest one by the relative chromosome length (table 2).

Table 1. Morphometric parameters for Allium hymenorhizum chromosomes in the samples (populations) under study.

| Chromosome pairs | Absolute length (L), μm | Relative length (L'), % | Centrometric index (P), % |
|------------------|-------------------------|-------------------------|--------------------------|
|                  | M±m CV, % | M±m CV, % | M±m CV, % |
|                  | Novokonstantinovka |                       | Bakhtigareevo |                          | Bogachevo |
| I                | 9.24±0.21 17.84 | 7.70±0.05 4.65 | 47.11±0.23 3.78 |
| II               | 8.46±0.20 18.65 | 7.07±0.06 6.11 | 46.53±0.30 4.93 |
| III              | 7.90±0.19 18.56 | 6.58±0.03 3.11 | 46.14±0.29 4.86 |
| IV               | 7.49±0.18 18.27 | 6.24±0.03 3.36 | 46.68±0.26 4.33 |
| V                | 7.25±0.17 17.72 | 6.05±0.03 3.27 | 46.45±0.27 4.46 |
| VI               | 6.98±0.16 18.01 | 5.82±0.03 3.74 | 45.43±0.41 6.96 |
| VII              | 6.59±0.16 18.23 | 5.49±0.03 4.35 | 43.88±0.56 9.86 |
| VIII             | 6.05±0.15 18.75 | 5.05±0.05 8.27 | 40.87±0.67 12.75 |

Bakhtigareevo

| I                | 9.28±0.17 14.02 | 7.52±0.04 4.18 | 46.44±0.32 5.39 |
| II               | 8.64±0.16 14.38 | 7.01±0.03 3.87 | 45.56±0.47 7.96 |
| III              | 8.06±0.15 13.98 | 6.54±0.03 3.05 | 46.08±0.28 4.76 |
| IV               | 7.78±0.15 14.67 | 6.30±0.02 2.50 | 46.01±0.38 6.39 |
| V                | 7.51±0.14 14.19 | 6.09±0.02 3.03 | 44.81±0.51 8.80 |
| VI               | 7.23±0.13 14.26 | 5.85±0.02 3.19 | 45.30±0.35 6.05 |
| VII              | 6.81±0.13 14.93 | 5.51±0.02 3.48 | 43.46±0.68 12.20 |
| VIII             | 6.39±0.12 14.10 | 5.18±0.03 4.70 | 39.73±0.80 15.67 |

Bogachevo

| I                | 9.35±0.22 18.20 | 7.56±0.06 6.01 | 46.86±0.21 3.53 |
| II               | 8.58±0.19 16.83 | 6.95±0.03 3.31 | 47.11±0.16 2.68 |
| III              | 8.11±0.18 17.31 | 6.56±0.03 3.65 | 46.77±0.25 4.10 |
| IV               | 7.81±0.17 16.86 | 6.33±0.02 2.69 | 46.56±0.22 3.63 |
| V                | 7.57±0.17 17.05 | 6.12±0.02 2.37 | 46.31±0.21 3.53 |
| VI               | 7.20±0.16 17.39 | 5.83±0.02 3.44 | 46.10±0.28 4.80 |
| VII              | 6.86±0.16 17.72 | 5.55±0.03 4.92 | 45.61±0.33 5.65 |
| VIII             | 6.26±0.12 15.56 | 5.09±0.05 7.62 | 42.33±0.54 9.83 |

There are some differences among the populations regarding the number and frequency of occurrence of the secondary constrictions in the chromosomal complements. The highest values of these indicators were observed in the populations Bakhtigareevo and Bogachevo, and the lowest ones in the population Novokonstantinovka (table 3, figure 3). This can probably be associated with the adaptation processes of the populations under study to new growing conditions.

In the opinion of many researchers, nucleolus organizers localized on chromosomes in the regions of secondary constrictions have functions peculiar to adaptive systems, since they participate in the vitally important function of the organism, i.e. the mechanism of protein synthesis. The intensification of any biosynthetic processes is based on the change in genome activity, including its part responsible for rRNA synthesis [27-29]. The following fact should also be noted. It is precisely nucleolus activity that turned out to be the most indicative cytological criterion in assessing the impact of stress on the organism when the sensitivity scale was developed for ecological monitoring [30].

It is an interesting fact in our research that the samples were brought to the introduction nursery from different climatic conditions, namely, sample 1 (Novokonstantinovka) from the Cis-Urals to conditions of the same region, whereas samples 2 (Bakhtigareevo) and 3 (Bogachevo) from the South Urals to the conditions of the Cis-Urals. Consequently, the change in the growing conditions of...
samples 2 and 3 is quite evident. That is perhaps why we observe a greater number and frequency of occurrence of the secondary constrictions in the chromosomal complements of samples 2 and 3, which testifies to higher nucleolus activity of their genomes induced by the contrasting change in the growing conditions.

**Table 2.** Characteristics of Allium hymenorhizum variability according to karyological indices in the samples under study (populations).

| Population          | Chromosome absolute length (L<sub>a</sub>) | Chromosome relative length (L<sub>r</sub>) | Chromosome centrometric index (I<sub>c</sub>) | Overall length of chromosomal complements (2n) | Variation range, μm | M±m | CV   |
|---------------------|--------------------------------------------|------------------------------------------|-----------------------------------------------|------------------------------------------------|--------------------|-----|------|
| Novokonstantinovka  | 17.72-18.75                                | 18.25                                    | 4.61                                          | 3.78-12.75                                    | 6.49               | 119.92±3.91 | 17.85 |
| Bakhtigareevo       | 13.98-14.93                                | 14.32                                    | 3.50                                          | 4.76-15.67                                    | 8.40               | 123.37±3.17 | 14.06 |
| Bogachevo           | 15.56-18.20                                | 17.11                                    | 4.25                                          | 2.68-9.83                                     | 4.72               | 123.52±3.76 | 16.65 |

**Table 3.** Occurrence of secondary constrictions in karyotypes of the samples (populations) under study.

| Population          | Number of secondary constrictions per one metaphase plate | Number of secondary constrictions per karyotype | Chromosome pairs with constrictions | Location of constrictions (sc), % |
|---------------------|------------------------------------------------------------|-----------------------------------------------|------------------------------------|----------------------------------|
|                     | total| stable| unstabl e |                                      | short arm | long arm |
| Novokonstantinovka  | 0.10 | 1     | 0         | VIII                               | 50.00     | -        |
| Bakhtigareevo       | 0.33 | 4     | 1         | II, III, VII, VIII                 | 63.31; 54.5; 55.5; 40.15 | -        |
| Bogachevo           | 0.37 | 3     | 1         | IV, VII, VIII                      | 54.54; 54.54; 46.62 | -        |

Note: Constrictions with less than 10% frequency of occurrence were considered unstable, and those with more than 10% frequency were considered stable.

**Figure 3.** Karyotype ideograms of the samples (populations): a – Novokonstantinovka, b – Bakhtigareevo, c – Bogachevo. The scale equals 10 μm.
4. Conclusions

Karyological analysis showed that the chromosome number in somatic tissue of Allium hymenorhizum is 2n=16; chromosomes are of medium size (6.05±0.15 to 9.35±0.22 μm) and belong to the netacentric and submetacentric types.

The variability of chromosomal morphometric parameters is characterized by the extremely low, low and average values of the coefficients of variation. The highest level of variability is determined by the absolute chromosome length, the lowest one by the relative chromosome length.

The samples introduced from different climatic conditions vary in the number and frequency of secondary constrictions in the chromosomal complements, this evidently being associated with the processes of their adaptation to new growing conditions.

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