Analysis of the genetic diversity of Russian common oat varieties using alleles of avenin-coding loci

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Abstract. Alleles of avenin-coding loci were identified in 24 varieties of common oat origin from 6 different breeding centers of the Russian Federation. It was found that 33% of the studied varieties are homogeneous. Heterogeneous varieties contain from 2 to 10 biotypes. The analysis revealed 67 different genotypes, ten of which are found in the spectra of several varieties at once. Despite the presence of matching genotypes, all the studied varieties differ in the genetic formulas of avenin, the number and frequency of occurrence of biotypes. This makes it possible to effectively identify and distinguish oat varieties sown by Russian breeding using the method of prolamin electrophoresis. The analyzed groups of varieties are characterized by high values of genetic (0.54-0.79) and intra-population diversity, which indicates the stability of populations. The exception is varieties originating from the Irkutsk region, low values of genetic and intra-population diversity in the population of which may indicate the process of genetic erosion. Analysis of the frequency distribution of alleles of avenin-coding loci does not allow to determine whether oat varieties belong to certain breeding centers, which is associated with the introduction of the same genotypes into the breeding programs of different regions.

1 Introduction

Oat (Avena sativa L.) is a grain crop of Asian origin. Sown oat is used for the production of human food, as well as for animal feed, especially birds and horses [1, 2]. Unlike other grain crops, oat can produce high yields even at low temperatures and is not demanding of soil fertility. The main sown areas of oat are between 40° and 60° north and south latitude

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in Europe, Asia, Africa, America, and Australia. In the last decade, interest in oat has increased. This is due to the dietary properties of its grain – functional food products produced from it can have a beneficial effect on the human body due to the balanced amino acid composition, as well as the presence of unsaturated acids, pectins, phytoestrogens and β-glucans [3]. Also, oat products can be eaten by people suffering from celiac disease – a hereditary eating disorder associated with allergies to wheat, rye and barley gluten proteins [4, 5].

At present, the problem of reducing the genetic diversity of agricultural crop species is acute. Many researchers see the reason for this in the fact that during the twentieth century, most of the old-local varieties were replaced by modern ones. At the same time, to obtain new, high-yielding varieties of an intensive type, the same genotypes were often introduced into crosses [6, 7]. The assessment of the genetic diversity of cultivated species, including oat, is carried out using a diversity of marker systems [8-11]. One of the most accessible markers is the prolamin-coding loci. Oat prolamins - avenins - are inherited by groups, and their synthesis is controlled by three avenin-coding loci - Avn A, Avn B and Avn C. The analysis of the frequency of occurrence and distribution of alleles of avenin-coding loci allows to assess the intraspecific genetic diversity and characterize the genetic transformations occurring in the population [12-15].

On the territory of the Russian Federation, oat is cultivated almost everywhere. In this regard, the study and comparison of oat varieties originating from different breeding centers of the country is of particular interest.

The purpose of our research was to study the genetic diversity of varieties of Russian-bred oat using alleles of avenin-coding loci to assess the genetic structure of populations of varieties of different ecological and geographical origin.

### 2 Materials and methods

The research was carried out in the laboratory of varietal identification of seeds of the Institute of Applied Agricultural Research and Development of the State Agrarian University of the Northern Trans-Urals in 2020-2021. 24 oat varieties were studied, originating from 6 different breeding centers of the Russian Federation (Table 1, Fig. 1). The research material was provided by originators from breeding centers and from the collection of the Federal Research Center N. I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR).

| Variety     | Institution-originator                      | Region of origin                  |
|-------------|---------------------------------------------|-----------------------------------|
| Dogoy       | Buryat Research Institute of Agriculture    | The Republic of Buryatia, Ulan-Ude |
| Mergen      |                                             |                                   |
| Geser       |                                             |                                   |
| Assol       | Agrostandart LLC                            | Krasnodar region, Krasnodar       |
| Desant      |                                             |                                   |
| Petrovich   |                                             |                                   |
| Ekspress    |                                             |                                   |
| Tigrovy     | Far Eastern Agricultural Research Institute | Khabarovsk region, Vostochnoe    |
| Marshal     |                                             |                                   |
| Premyer     |                                             |                                   |
| Oven        |                                             |                                   |
| Egorych     | Irkutsk Agricultural Research Institute*    | Irkutsk region, Pivovarikha      |
| Tulunskiy 19|                                             |                                   |
| Tyumenskiy  | Scientific Research Institute of Tyumen     | Tyumen region, Tyumen            |

Table 1. Characteristics of plant material.
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The gene diversity per locus (H) was calculated for varieties originating from different breeding centers separately according to the following formula (1):

$$H = \frac{n}{n-1} \times \left(1 - \sum_{i=1}^{k} p_i^2\right),$$

where $p_i$ is the population frequency of the $i$-th allele, $k$ is the number of locus alleles, $n$ is the sample size [16]. To calculate the average gene diversity ($\bar{H}$), the number of alleles per locus was averaged for all loci. The calculations were performed using the Arlequin Ver 3.5.2.2 program (Copyright 2015 L. Excoffier. CMPG, University of Berne).

| Variety    | Institution-originator Region of origin                  |
|------------|----------------------------------------------------------|
| golozereny  | Agriculture of the Northern Trans-Urals – a branch of the Federal Research Center of the Tyumen Scientific Center of the Siberian Branch of the Russian Academy of Sciences |
| Megion     | Siberian Federal Scientific Center of Agrobiotechnology of the Russian Academy of Sciences * |
| Talisman   | Novosibirsk region, Krasnoobsk                           |
| Otrada     |                                                          |
| Foma       |                                                          |
| Tobolyak   |                                                          |
| Mustang    |                                                          |
| Kreol      |                                                          |
| Gavrosh    |                                                          |
| Pomor      |                                                          |
| SIG        |                                                          |

Note: * - Plant material was provided from the collection of the Federal Research Center N. I. Vavilov All-Russian Institute of Plant Genetic Resources.

Fig. 1. Regions of origin of the studied varieties of common oat. (http://russia-karta.ru/).
The indicators of intrapopulation diversity ($\mu$) and the proportion of rare morphs ($h$) were calculated using formulas (2) and (3), respectively:

$$\mu = \left(\sqrt{p_1} + \sqrt{p_2} + \sqrt{p_3} + \cdots + \sqrt{p_m}\right)^2,$$

$$h = 1 - \frac{\mu}{m},$$

where $p_1$, $p_2$, ... $p_m$ are the frequencies of alleles in fractions of one, $m$ is the number of variations of the trait identified in this sample [17].

The Statistika 12 software package was used to cluster groups of varieties.

### 3 Results and discussion

The electrophoretic spectra of avenin were analyzed for 24 studied varieties and alleles of avenin-coding loci were determined. A total of 31 alleles were identified for three loci. At the same time, 19 alleles were discovered for the first time and are absent from the catalog of genetic nomenclature (Table 2).

#### Table 2. Alleles of the avenin-coding loci of the studied varieties of common oat.

| Variety          | Number of biotypes | Alleles of the avenin-coding locus | $Avn\ A$ | $Avn\ B$ | $Avn\ C$ |
|------------------|--------------------|------------------------------------|----------|----------|----------|
| Dogoy            | 1                  |                                    | 2        | 1        | 2        |
| Mergen           | 4                  | 2+2+new13+2                       | 4+4+5+1  | 2+6+2+6  |
| Geser            | 2                  | 2                                  | 1        | 2+4      |
| Assol            | 1                  | 4                                  | 1        | new9     |
| Desant           | 2                  | 2+4                                | 8+1      | 5+new9   |
| Petrovich        | 1                  | 2                                  | 8        | 5        |
| Ekspress         | 10                 | 2+2+2+4+new11+2+new9+4+4+new11    | 1+4+1+4+4+1+1+8+new10+4 | 6+new10+new10+3+3+2+1+1+new10+1 |
| Tigravy          | 6                  | 2+new14+2+4+new1+5+new15          | new7+5+4+new7+5+4        | 2+3+2+2+4+3         |
| Marshal          | 5                  | 4+4+4+2+4                        | new7+5+4+new7+5+4        | 2+3+2+2+4+3         |
| Premyer          | 7                  | 2+new14+2+4+2+2+2+2              | 1+4+4+4+new7+1+1+new12  | 2+2+2+5+6+3         |
| Oven             | 2                  | 2                                  | 8+new13  | 2+3      |
| Egorych          | 4                  | new11+new11+2+2                   | 8        | 1+2+1+2  |
| Tulsanskii y 19  | 2                  | new11+2                           | 8        | 1        |
| Tyumenskiy golozernyy | 1           | 2                                  | new6     | 3        |
| Megion           | 2                  | 2+new11                           | new6     | 5        |
| Talisman         | 1                  | 4                                  | 4        | 2        |
| Otrada           | 2                  | new10+4                           | 4        | 1        |
| Fona             | 1                  | 4                                  | new7     | 1        |
| Tobolya          | 1                  | 4                                  | 8        | 2        |
It was found that 33% of the studied varieties are homogeneous in the component composition of avenins. The remaining varieties contain from 2 to 10 biotypes. The largest number of biotypes was found in the oat varieties of the Far Eastern Agricultural Research Institute seed selection. According to some authors, the presence of several biotypes increases the adaptive potential of varieties, especially in regions unfavorable for growth [18, 19]. The analysis revealed 67 different genotypes, ten of which are found in the spectra of several varieties at once. This genotype 2.1.2 common to varieties Dogoy, Geser (biotype I), Ekspress (biotype VI), Premyer (biotype I) and Kreqel (biotype III); genotypes 2.4.2 and 2.1.6 were shared among biotypes of varieties Mergen, Ekspress and Tigrovyy; genotype 2. new8.2 is found in biotypes of varieties Oven, Egorych and SIG. 6 more identical genotypes occur in varieties originating from a single breeding center: genotypes 2. new8.5 (biotype I of varieties Desant, Petrovich) and 4.1. new10 (biotype II of varieties Desant, Assol) are found only in the cultivars originated in the Krasnodar Region; genotypes 2.4. new10 and 4. new7.2 matched groups of varieties originated in the Khabarovsk Region (biotypes of varieties Ekspress, Marshal and Tigrovyy, Premyer, respectively); and the genotypes new11. new8.1 and 2. new8.1 are common for varieties Egorych and Tulunskiy 19 of the Irkutsk region. It was found that 57 (85%) of the detected genotypes are variety specific. Avenins are characterized by a lower level of polymorphism than prolamins of wheat and barley, and, in case of a coincidence of electrophoretic spectra, it is recommended to use other marker systems for the differentiation of samples [13]. Nevertheless, despite the presence of identical spectrum types, all the studied varieties differ in the genetic formulas of avenin, the number and frequency of occurrence of biotypes. Even in case of a match for one or more genotypes, differences between varieties can be identified at the expense of other biotypes.

The appearance of varieties with identical alleles of prolamin-coding loci in varieties is associated with the involvement of the same genotypes in breeding programs [7, 15, 18]. This statement is partially consistent with the data we have received. For example, the biotypes of the varieties Oven, Egorych and SIG have a common genotype (2. new8.2). The variety Egorych was obtained by crossing the varieties Oven × Rovesnik, which explains the presence of identical spectra in the varieties Oven and Egorych. The common oat variety SIG was bred in another breeding center, but it has common ancestors with the Egorych variety (Fig. 2). The SIG oat variety was obtained from a hybrid population (Orel (=Ornhafer) × Tayozhnik) × Selma; at the same time, the Tayozhnik variety is also included in the parentage of the Egorych variety: one of its parent varieties – Rovesnik – was obtained by chemical mutagenesis of the Novosibirsk 88 variety, derived by hybridization of the H67 × Tayozhnik line. It is known that the avenin components during crossing are inherited by blocks, within which the frequency of recombination is extremely low. As a result, hybrids will have various combinations of the same alleles of polyamine-coding loci as in the parent individuals, and sometimes completely coincide with them.
Nevertheless, the avenin spectra also coincide in varieties that do not have common ancestors: for example, the Assol and Desant varieties were obtained by individual selection from the Krasnodarsky 73 and Magne varieties, respectively. A.Yu. Novoselskaya-Dragovich and colleagues analyzed alleles of gliadine-coding loci of soft wheat varieties of Omsk and Saratov breeding, came to the conclusion that the genetic differentiation of varieties is associated with the natural and climatic conditions of their growth, and gliadine-coding loci represent gene associations that provide the adaptive potential of plants, or mark such associations [7]. The same conclusions were reached by many authors who studied the distribution of alleles of prolamin-coding loci of wheat and barley [20, 21, 22]. V.A. Portyanko, having studied European varieties of oat, noted that the frequency of occurrence of alleles and their combinations are not random and are associated with geographical zoning [23]. Thus, the coincidence of alleles of avenin-coding loci in varieties that do not have common ancestors may be due to the fact that these alleles are or mark gene associations associated with economically valuable and adaptively significant traits for this region.

The presence of varieties with matching alleles of prolamin-coding loci may indicate a decrease in intraspecific genetic diversity. To evaluate and compare the genetic structure of populations of oat varieties from different breeding centers, we calculated the frequency of occurrence of alleles of avenin-coding loci (Fig. 3).

Fig. 2. Related relationships of the common oat varieties Oven, Egorych and SIG. Above the name of the variety, its avenin protein formula is indicated.

The frequency of occurrence of alleles in the locus $Avn\ A$, %
Fig. 2. Related relationships of the common oat varieties Oven, Egorych and SIG. Above the name of the variety, its avenin protein formula is indicated. Nevertheless, the avenin spectra also coincide in varieties that do not have common ancestors: for example, the Assol and Desant varieties were obtained by individual selection from the Krasnodarsky 73 and Magne varieties, respectively. A.Yu. Novoselskaya-Dragovich and colleagues analyzed alleles of gliadine-coding loci of soft wheat varieties of Omsk and Saratov breeding, came to the conclusion that the genetic differentiation of varieties is associated with the natural and climatic conditions of their growth, and gliadine-coding loci represent gene associations that provide the adaptive potential of plants, or mark such associations [7]. The same conclusions were reached by many authors who studied the distribution of alleles of prolaminie-coding loci of wheat and barley [20, 21, 22]. V.A. Portyanko, having studied European varieties of oat, noted that the frequency of occurrence of alleles and their combinations are not random and are associated with geographical zoning [23]. Thus, the coincidence of alleles of avenin-coding loci in varieties that do not have common ancestors may be due to the fact that these alleles are or mark gene associations associated with economically valuable and adaptively significant traits for this region.

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As can be seen from Figure 3, several alleles are found in almost all groups of varieties – alleles A2, A4, B1, B4, Bnew8, C1 and C2. However, in general, groups of varieties originating from different breeding centers differ in the set and frequency of occurrence of alleles. In each group alleles of avenin-coding loci not found in other regions were identified: only in Novosibirsk varieties of detected alleles Anew16, Anew17, B3, Bnew14; in Tyumen ones – alleles Anew10, Bnew6; the varieties of Irkutsk region – Bnew13; from the Khabarovsk Region – Anew14, Anew15, Bnew10, Bnew11, Bnew12, Cnew10; from Krasnodar varieties the allele Cnew9 was identified, and from the Buryat – Anew13. These alleles are of interest for further study since they can mark gene associations responsible for adaptation to certain natural and climatic conditions.

Based on the data on the frequency of occurrence of alleles of avenin-coding loci, we have constructed a dendrogram characterizing the genetic relation of varieties (Fig. 4).
As a result of clustering, all varieties were divided into three large clusters. At the same time, some varieties originating from the same breeding center entered different clusters. Thus, the cluster C includes varieties Petrovich (the Krasnodar Region); Oven, Egorych and Tulunskiy 19 (Irkutsk region); Pomor and SIG (Novosibirsk region), and varieties originating from the Tyumen region - Tyumenskiy golozernyy and Megion. Cluster B combines all varieties from the Khabarovsk Region, as well as from the Tyumen region and Buryatia (Mergen). In the work of A.Yu. Novoselskaya-Dragovich as a result of clustering based on the frequency of occurrence of alleles of gliadine-coding loci, soft wheat varieties were divided into two clusters corresponding to Omsk and Saratov varieties, which indicates the formation of two populations with different genetic pool [7]. In our case, there is no clear connection between the differentiation of varieties and the conditions of their growth. In our opinion, the commonality of their origin, reflected in a similar set of alleles of avenin-coding loci, had a greater influence on the distribution of varieties into clusters. Due to the fact that in many regions the same oat varieties are introduced into breeding programs, there is no formation of separate genetic pools characteristic of different breeding centers.

To assess the structure of populations of oat varieties from different breeding centers, the values of intraspecific genetic diversity (H), intrapopulation diversity (µ) and the proportion of rare morphs (h) were calculated based on the distribution of allele frequencies (Table 3).
### Table 3. Genetic diversity, intrapopulation diversity and the share of rare morphs in populations of oat varieties originating from different regions.

| Indicator | Avenin coding locus | Region of origin |                |                |                |                |                |
|-----------|---------------------|------------------|----------------|----------------|----------------|----------------|----------------|
|           |                     | The Republic of Buryatia | Krasnodar region | Khabarovsk region | Irkutsk region | Tyumen region | Novosibirsk region |
| Genetic diversity, N | Avn A | 0.28±0.20 | 0.67±0.20 | 0.72±0.06 | 0.56±0.12 | 0.75±0.14 | 0.79±0.09 |
|           | Avn B | 0.67±0.16 | 0.67±0.20 | 0.79±0.06 | 0.25±0.18 | 0.79±0.11 | 0.73±0.11 |
|           | Avn C | 0.67±0.16 | 0.67±0.20 | 0.84±0.03 | 0.68±0.12 | 0.82±0.10 | 0.83±0.07 |
| Average gene diversity for three loci, B | Avn A | 0.54 | 0.67 | 0.78 | 0.49 | 0.79 | 0.78 |
| Number of trait variations in the sample, m | Avn A | 2 | 2 | 6 | 2 | 4 | 5 |
|           | Avn B | 3 | 2 | 8 | 2 | 4 | 5 |
|           | Avn C | 3 | 2 | 7 | 3 | 4 | 5 |
| Intrapopulation diversity, µ | Avn A | 1.70±0.27 | 2.00±0.00 | 4.89±0.44 | 1.97±0.99 | 3.66±0.39 | 4.54±0.42 |
|           | Avn B | 2.78±0.29 | 2.00±0.00 | 6.53±0.59 | 1.66±0.27 | 3.73±0.35 | 4.30±0.50 |
|           | Avn C | 2.78±0.29 | 2.00±0.00 | 6.44±0.36 | 2.80±0.27 | 3.86±0.26 | 4.76±0.31 |
| Share of rare morphs, h | Avn A | 0.15±0.13 | 0.00±0.00 | 0.18±0.07 | 0.02±0.04 | 0.08±0.10 | 0.09±0.08 |
|           | Avn B | 0.07±0.10 | 0.00±0.00 | 0.18±0.07 | 0.17±0.13 | 0.07±0.09 | 0.17±0.10 |
|           | Avn C | 0.07±0.10 | 0.00±0.00 | 0.08±0.05 | 0.07±0.09 | 0.03±0.06 | 0.05±0.06 |

The highest values of genetic diversity are typical for varieties originating from the Tyumen and Novosibirsk regions, as well as the Khabarovsk Region – from 0.79 to 0.78. This is a high indicator. For example, for winter durum wheat varieties of Russian breeding, this indicator is 0.52 [20], for soft wheat varieties from Uregionne – 0.5-0.6 [24]. At the same time, the maximum contribution to the value of the average gene diversity is made by the Avn C locus. Only the varieties from the Irkutsk region are characterized by low genetic diversity – 0.49.

The indicator of intrapopulation diversity (µ) allows to assess the degree of heterogeneity of individuals in the population. The more evenly distributed the alleles are, the closer the value of µ is to the value of m. As a result of our research, it was found that only varieties originating from the Krasnodar Region have a uniform distribution of alleles. The indicator of the share of rare morphs for this region is 0. For groups of varieties from other regions, the intra-population diversity also has quite high values. The exception is the relatively low values of µ for the Avn A locus for varieties from the Buryatia, Khabarovsk Region, and Irkutsk region, which is associated with a significant predominance of genotypes with the A2 allele. For varieties from the Irkutsk region, a low value of µ for the Avn B locus was also noted, due to the high frequency of occurrence of the Bnew8 allele.

The proportion of rare morphs in these cases increases, but only slightly. For all groups of varieties, the value of h, far from one, is characteristic. According to I.E. Trofimov (2008), h values increase with a decrease in genotypic diversity under unfavorable conditions and indicate the adaptive abilities of the population to certain unfavorable environmental factors. In our case, the values of intrapopulation diversity and the shares of rare morphs indicate a uniform distribution of allele frequencies of avenin-coding loci and indicate the stability of the studied populations of varieties. Only a group of varieties from the Irkutsk region is distinguished, which is characterized by low values of genetic and intrapopulation diversity, which may indicate the process of genetic erosion. This may lead to a decrease in the resistance of the population of oat varieties from this region to unfavorable biotic and abiotic factors.
4 Conclusion

The varieties originating from different breeding centers are characterized by high values of genetic (0.54-0.79) and intra-population diversity, which indicates the stability of the studied populations. The exception is oat varieties originating from the Irkutsk region, low values of genetic and intra-population diversity in the population of which may indicate the process of genetic erosion.

Analysis of the frequency distribution of alleles of avenin-coding loci does not allow to determine whether common oat varieties belong to separate genetic pools characteristic of different breeding centers, which is caused by the introduction of the same genotypes into the breeding programs of different regions. Alleles of avenin-coding loci found in varieties originating from a certain region were identified. Alleles \textit{Anew16, Anew17, B3, Bnew14} were found only in varieties from the Novosibirsk region; alleles \textit{Anew10, Bnew6} were found from the Tyumen region; \textit{Bnew13} - from the Irkutsk region; \textit{Anew15, Bnew10, Bnew11, Bnew12, Cnew10} - from the Khabarovsk Region; only the Krasnodar varieties had an allele \textit{Cnew9}, the Buryat – \textit{Anew13}. These alleles are valuable as markers of associations of genes responsible for adaptation to certain natural and climatic conditions.

The results of studies of the allelic composition of avenin-coding loci and the genotypic structure of varieties allow to effectively identify and distinguish from each other varieties of Russian-bred oat using the method of prolamin electrophoresis.

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