Prolamin electrophoresis method for assessing the varietal qualities of oat seeds

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Abstract. The oat is one of the most widely cultivated crops in Western Siberia. One of the factors for obtaining stable grain crops is the use of high-quality seeds for sowing. To control the varietal purity and constancy of the biotypic composition of cereal varieties, the method of electrophoresis of storage proteins - prolamins is successfully used. The aim of the study was to assess the effectiveness of the prolamin electrophoresis method for laboratory varietal control of oat using the example of varieties cultivated in the Tyumen region. Eighteen varieties of oats were analyzed, which are included in the State register of selection achievements in the region from 1929 to 2019. It was found that of the 26 genotypes, only 11 were sort-specific. Seven groups of varieties with identical types of prolamin spectra were found. The number of samples with individual types of avenin spectra at different time periods was not the same. Until 1970, from 25.0 to 33.3% of genotypes were characterized by variety-specific spectra. Later, with the advent of new varieties in the region, the number of genotypes with individual types of spectra increased and reached 100.0% by 2019. This makes it possible to accurately distinguish modern varieties of oats from each other, to carry out varietal identification and assessment of varietal purity. It is necessary to have up-to-date databases of reference spectra of varieties cultivated in the region and, in the case of varieties with identical types of spectra, assess varietal affiliation and varietal purity using other marker systems.

1. Introduction
The oat is one of the main cultivated crops of the Northern Trans-Urals. A great influence on its distribution was exerted by the ability of this culture to grow and produce stably high yields in a wide variety of climatic conditions - from dry steppes to taiga. Starting from the 30s of the XX century, 18 varieties of spring oat were included in the State register of selection achievements in the Tyumen region. Since 1993, varieties of selection of the SRIA for NTUR – Branch of Tyumen Scientific Centre SB RAS have appeared in the region, the share of which in the varietal crops of the region was constantly growing and reached 100% by 2019 [1].

For the successful introduction of new varieties into production and their effective use, improving breeding and seed production, the use of high-quality seeds for sowing, and maintaining the constancy of the biotypic composition of varieties and their varietal purity are of great importance. To control the varietal qualities of seeds, field testing and soil varietal control based on morphological characteristics are widely used. However, the application of these methods is complicated by the fact that many
cultivated varieties belong to the same variety and are practically indistinguishable externally. Another difficulty is the presence of heterogeneous varieties, which are more difficult to maintain during seed production due to the presence of several biotypes. In this regard, laboratory varietal control, which is based on the electrophoresis of storage alcohol-soluble proteins - prolams, is increasingly being used. An analysis of the electrophoretic spectra of prolams allows detecting varietal impurity in cases when it is impossible to do it by morphological characteristics, and also makes it possible to control and maintain the constancy of the biotypic composition of heterogeneous varieties [2, 3]. The advantages of using prolams include the ease of isolation and identification, specificity, polymorphism and the absence of pleiotropy effect. The use of storage proteins as a marker system makes it possible to work with individual seeds, and the codominant type of prolamin inheritance allows the detection of heterozygotes. Both the methods adopted in Russia and international varietal identification methods are based on the electrophoretic spectra of prolams. An analysis of the electrophoretic spectra of prolams allows detecting varietal impurity in cases when it is impossible to do it by morphological characteristics, and also makes it possible to control and maintain the constancy of the biotypic composition of heterogeneous varieties [2, 3]. The advantages of using prolams include the ease of isolation and identification, specificity, polymorphism and the absence of pleiotropy effect. The use of storage proteins as a marker system makes it possible to work with individual seeds, and the codominant type of prolamin inheritance allows the detection of heterozygotes. Both the methods adopted in Russia and international varietal identification methods are based on the electrophoretic spectra of prolams. To analyze the genetic diversity of oats, prolams - avenins - have been successfully used. Due to the significant polymorphism in molecular weight and number of components, each variety or biotype is characterized by a specific composition of prolamin components. The components of the electrophoretic spectra of avenin are inherited by groups and are controlled by three independent loci Avn A, Avn B, Avn C [4].

However, many authors note that in recent years, the number of varieties with similar or even identical types of prolamin spectrum has increased. Most often this is observed in those varieties that have a common origin [5, 6]. In the study of gliadin-coding loci of common wheat varieties of Omsk and Saratov breeding, A.Yu. Novoselskaya- Dragovich et al. have established that, knowing the genetic formula of gliadin and the biotypic composition of the sample, it is possible to determine its belonging to a specific breeding center [7]. The appearance of a large number of varieties with identical spectra may indicate a decrease in genetic diversity in the population, which can lead to genetic erosion of the species.

The presence of identical spectra makes it difficult to use the electrophoresis method for laboratory variety control. The level of avenin polymorphism is lower than that of prolamins of wheat and barley [8]. As a result, the probability of coincidence of the spectra of oats varieties increases. The aim of our study was to assess the effectiveness of the prolamin electrophoresis method for laboratory varietal control of oats using the example of varieties cultivated in the Tyumen region.

2. Materials and methods

The studies were carried out on the basis of the laboratory for varietal seed identification of the Agrobiotechnological Center of the State Agrarian University of Northern Trans-Urals and the laboratory for the selection of grain crops of the Research Institute of Agriculture, a branch of the Tyumen Scientific Center of the Siberian Branch of the Russian Academy of Sciences, in 2018-2019. The research material was 18 oat varieties included in the State register of selection achievements in the Tyumen Region from 1929 to 2019. (tab. 1).

Table 1. Oat varieties zoned in the Tyumen region.

| № catalog of VIR | Variety      | Origin       | Years in regionalization |
|------------------|--------------|--------------|--------------------------|
| 7965             | Seger        | Sweden       | 1929-1963                |
| 7947             | Golden rain  | Sweden       | 1929-1976                |
| 8494             | Onhafer      | Sweden       | 1939-1982                |
| 8256             | Udarnik 883 | Krasnoyarsk region | 1957-1960 |
| 2874             | Nidar        | Norway       | 1957-1963                |
| 11132            | Severyanin   | Arkhangelsk region | 1966-1974 |
| 11717            | Skorospelyj  | Kirov region | 1974-1981                |
| 11122            | Narymskij 943 | Tomsk region | 1975-1996                |
| 12245            | Tayozhnik    | Tomsk region | 1977-2001                |
Plant material was provided from the collection of the Federal Research Center N.I. Vavilov Institute of Plant Genetic Resources (VIR) and the institution-originator of varieties - the Scientific Research Institute of Agriculture of the Northern Trans-Ural s - a branch of the Federal Research Center of the Tyumen Scientific Center of the Siberian Branch of the Russian Academy of Sciences.

For laboratory analysis, 100 seeds of each variety selected by random sampling were used. For one-dimensional electrophoresis of avenins, the published method \[8\] was used with modifications. Proteins were extracted from individual crushed seeds by adding 90 μl of 70% ethanol. The obtained extract was centrifuged and 300 μl of methylene green dye were added to it. Protein extract (22 μl) was added to the polyacrylamide gel. Gel composition: 13.17 g of acrylamide, 0.66 g of N, N'-methylene-bis-acrylamide, 7.17 g of urea, 2.0 mg of iron sulfate (III), 80.0 mg of ascorbic acid, and 0.26 g of aluminum lactate. All reagents were dissolved in 100 ml aluminum-lactate buffer (pH 3.1). Acrylamide polymerization was initiated by adding 25 μl of 15% hydrogen peroxide to 75 ml of a gel solution. Electrophoresis was carried out in vertical electrophoretic chambers with dimensions of the formed plates of 17.8×17.8×0.15 cm (VE-20, Helicon, Russia) for 4.0-4.5 hours at a constant voltage of 500 V. For fixing and staining gel, a 10% solution of trichloroacetic acid with the addition of 0.05% Coomassie Brilliant Blue R-250 in ethanol was used.

Based on the obtained electrophoretic spectra of prolamin, a source data matrix was compiled in which the presence of the component was marked 1 and the absence was marked 0. The protein fractions were distinguished from each other based on the speed of their movement in the gel carrier. To identify the degree of genetic differentiation of the samples, the data of the obtained matrix were processed by the method of cluster analysis. The Dice coefficient was used as the similarity index:

\[
S = \frac{2n_{ab}}{n_a + n_b},
\]

where \( n_a \) and \( n_b \) – the number of components present in spectra A and B, respectively, and \( n_{ab} \) – the number of components common to two spectra [9]. For clustering, the Unweighted Pair-Group Method with Arithmetic Mean (UPGMA) was used. The dendrogram was built using the TREECON 1.3b program for Windows with bootstrap analysis for 100 replicates.

3. Results
As a result of the studies, it was revealed that out of 18 analyzed varieties, 8 consisted of two biotypes - Seger, Golden rain, Omhafer, Severyanin, Narimskij 943, Tayozhnik, Megion, Otrada. Later, each biotype was considered by us as a separate sample. A total of 26 samples were studied.

Figure 1 shows the dendrogram obtained as a result of clustering. As a result of clustering, some varieties were combined into groups with a Dice genetic distance of 0. This indicates the identity of their storage protein spectra.
Figure 1. Dendrogram of oat varieties, based on data on the composition of avenin. The scale shows the Dice genetic distance. Numbers 1 and 2 indicate the numbers of biotypes.

In total, 7 groups of varieties with matching spectra of avenin were revealed: 1 - Golden rain (biotype I), Nidar; 2 - Seger (biotype I), Golden rain (biotype II); 3 - Seger (biotype II), Omhafer (biotype II); 4 - Novosibirskij 88, Narymskij 943 (biotype II), Astor; 5 - Perona, Talisman; 6 - Severyanin (biotype II), Skorospelyj; 7 - Udarnik 883, Severyanin (biotype I).

Only 11 (42.31%) of the 26 studied genotypes were characterized by variety-specific spectra - these were varieties Omhafer (biotype I), Foma, Narymskij 943 (biotype I), Selma, Tyumenskij golozynnyj, and all biotypes of the varieties Tayozhnik, Otrada and Megion.

Since the varieties under study were cultivated at different times, it was important to establish what is the proportion of varieties with matching spectra among cultivated plants in the same period. For this, all varieties were grouped. One group included varieties cultivated in the same ten-year period.

Based on the frequency of occurrence of the spectrum types, a diagram was built (Fig. 2) that illustrate the relationship of varieties with variety-specific and matching types of spectra at different time periods.
Figure 2. The number of oat varieties with identical and variety-specific spectra of storage proteins in different years of cultivation, where: 1 - variety-specific genotypes; 2 - matching genotypes.

Until 1970, the number of varieties with identical spectra varied from 66.7 to 75.0%. Only 2–3 genotypes could be reliably distinguished from the others by the prolamin electrophoresis method in a given period of time. Since the 1970s, the number of variety-specific genotypes has sharply increased and ranged from 67.7% in 1970-1980 up to 75.5% between 2000 and 2018. In 2018, the last variety of foreign selection, Perona, whose spectrum was identical to that of Talisman, was excluded from the State register of selection achievements in the Tyumen region. Since 2019, all varieties cultivated in the region are characterized by individual spectra of storage proteins and can be easily differentiated using laboratory variety control.

4. Discussion
As a result of our analysis, it was found that out of 26 studied genotypes, 15 had the same spectrum types. The matching of the types of prolamin spectrum can be caused by a number of reasons. Firstly, it is the common origin of the varieties. It is known that avenins are inherited in blocks, and their synthesis is controlled by three independent loci Avn A, Avn B and Avn C located in three homeologous chromosomes of group A (corresponds to genetic group 1 Triticeae) [4, 8]. The blocks of prolamin components are characterized by high stability: when crossed, they are inherited without changes, the recombination frequency inside the block is extremely low. As a result, the varieties obtained by crossing will be characterized by the same set of blocks of prolamin components as the parent varieties, and in some cases, their spectra may completely match with the spectrum of one of the parent varieties. In our case, this happened with the varieties Golden rain, Seger and Omhafer. Golden rain and Seger varieties were bred from Milton oat. The same variety is present in the breeding record of the Omhafer variety. The biotypes of these samples, as well as the Nidar variety, were combined into one cluster and formed three groups with identical avenin spectra.

However, not all matches of the spectra can be explained by their common origin. The reason for the identity of the spectra of prolamin may be the selection in the process of creating varieties of ecological types that are most adapted to certain environmental conditions [10]. Samples from other groups with matching spectra have different origins and were bred in different regions or even countries, for example, Perona and Talisman varieties. It is known that allelic variants of prolamin component blocks have rigidly determined relationships with adaptive properties of genotypes. For example, when studying gliadin-coding loci of common wheat varieties of Omsk and Saratov
selection, it was found that based on the gliadin’s genetic formula and the biotypic composition of the sample, it can be determined that it belongs to a specific plant breeding center. At the same time, 5% of varieties of Saratov and 4.4% of varieties of Omsk selection had identical gliadin spectra [7].

V.A. Portyanko et al. revealed geographic zoning in the occurrence of alleles in European varieties of oat. It was found that the combination of allelic variants in genotypes is nonrandom. This indicates that alleles of avenin-coding clusters or loci linked to them differ in their adaptive and selection value [4].

Thus, samples with an identical component composition of avenin, which don’t have a common origin, may have similar or even identical economically valuable traits and adaptive properties. This allowed many of these varieties to be cultivated in the region for quite a long time. For example, Perona cultivated in the region for 33 years, and Talisman variety identical to it in the spectrum of avenin has been cultivated in the region for 24 years; Astor and Narymskij 943 varieties matching by the types of spectra were cultivated for 22 and 21 years, respectively.

The effectiveness of using the prolamin electrophoresis method for laboratory variety control depends on the number of genotypes with matching spectral types. Until 1970, the application of this method to assess the varietal purity of most varieties would have been ineffective due to the too large percentage of varieties with non-specific types of spectrum. In this case, it is recommended to use other marker systems for differentiating genotypes, including PCR analysis methods [11]. The ISTA Rules (International Seed Testing Association) use SDS electrophoresis to distinguish between these varieties. Subsequently, the number of varieties with individual spectra of avenin increased, but did not reach 100%. The appearance of varieties with new types of spectrum of storage proteins is associated with active variety exchange. Varieties of foreign selection were replaced by domestic, characterized by a different composition of the storage proteins. Since 2019, only varieties of local selection having individual prolamin spectra have been cultivated in the Tyumen region. This makes it possible to accurately distinguish varieties from each other, to carry out varietal identification and assessment of varietal purity. The presence of varieties with individual types of protein spectra indicates effective plant breeding work with oat in the region, including the use of genetically diverse breeding material in the selection process.

5. Conclusion
Thus, oat varieties included in the State register of selection achievements in the Tyumen region from 1929 to 2019 are characterized by low levels of avenin polymorphism. Of the 26 analyzed spectra, 42.31% were sort-specific. The number of varieties with individual types of spectra in different periods of time varied from 25.0 to 75.5% and reached 100% by 2019. The effectiveness of the prolamin electrophoresis method for controlling the varietal qualities of oat seeds depends on the set of cultivated varieties in a specific period of time. Avenins are characterized by a low level of polymorphism and, accordingly, varieties with identical spectra are more common. It is necessary to have up-to-date databases of reference spectra of varieties cultivated in the region and, in the case of varieties with identical types of spectra, assess varietal affiliation and varietal purity using other marker systems.

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References
[1] Lyubimova A, Eremin D 2018 Bulletin applied botani, genetics and plant breeding 179 2 pp 85-95 DOI: 10.30901/2227-8834-2018-2-85-95
[2] Yakubyshina L, Kazak A, Loginov Y 2018 Ecology, environment and conservation 24 2 pp 1001-1007
[3] Utebauev M, Dashkevich S, Babkenov A et al 2016 Acta Physiologiae Plantarum 38 204
https://link.springer.com/article/10.1007/s11738-016-2209-4

[4] Portyanko V, Pomortsev A, Kalashnik N, Bogachkov V, Sozinov A 1987 Russian Journal of Genetics 23 5 pp 845-853

[5] Lyubimova, Eremin D 2018 MATEC Web of Conferences 170 pp 04015 https://doi.org/10.1051/mateconf/201817004015

[6] Kudryavtsev V, Dedova V, Melnik A 2014 Russian journal of genetics 50 5 pp 483-488 DOI: 10.1134/S1022795414050093

[7] Novoselskaya- Dragovich A, Fisenko A, Puhal'skii V 2013 Russian Journal of Genetics 49 5 pp 487-496 DOI: 10.1134/S1022795413020087.

[8] Portyanko V, Sharopova N, Sozinov A 1998 Euphytica 102 pp 15-27 DOI: 10.1023/A:1018399919953

[9] Nei M, Li W 1979 Proc. Natl. Acad. Sci. USA 76 pp 5269-5273

[10] Zobova N, Surin N, Gerasimov S, Chuslin A, Onufrienok T 2018 Dostizheniya nauki i tekhniki APK 32 5 pp 45-47 DOI: 10.24411/0235-2451-2018-10511

[11] Wight Ch, Yan W, Fetch J M, Deyl J, Tinker N 2010 Crop Science 50 pp 1207-1218 DOI: 10.2135/cropsci2009.09.0474