Advances in Engineering Research, volume 151
International Conference on Smart Solutions for Agriculture (Agro-SMART 2018)

Component composition of oat prolamins in A. strigosa Schreb., A. abyssinica Hochst., A. byzantina C. Koch. *and* A. sativa L.

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***Abstract*—Modern selection and seed development widely use biotechnology methods for creation of new intense cultivars and subsequent monitoring of biotypical composition. Among those methods is using storage proteins (prolamins) for analysis of genetic diversity. Prolamins and avenins are used for assessment of genetic diversity of oat, however, a lack of a common approach to interpretation and registering of avenin spectra impedes application of available data on component composition of these proteins when assessing the oat-related source material. The aim of the research was to analyze the component composition of avenin in oat cultivars of various ploidity for subsequent use in marker selection when assessing the oat source material. As a result of the research, 173 protein components were identified, 65.8 % of them are species-specific. It has been established, that cultivated oat species positively differ from each other by the mean number of protein components in the spectra of the samples. The highest average number of the avenin protein components has been found in the common oat spectra, at 8.5±0.05. The lowest average number of the avenin protein components has been found for the lopsided oat and the Ethiopian oat at – 4.2±0.32 and 4.6±0.47 respectively. The maximum number of various avenin components has been discovered in the common oat spectra, 160 units, while for the Ethiopian oat there were only 7 units identified. This is an evidence of a high level of genetic diversity in the studied collection of A. sativa and a high probability to identify a large number of selection-valuable genotypes when conducting marker-mediated selection of the common oat.**

Keywords— cultivated oat species, A. strigosa Schreb., A. abyssinica Hochst., A. sativa L., A. byzantina C. Koch, avenin, electrophoretic spectrum.

I. INTRODUCTION

Intraspecies genetic diversity is important for increase of crop yields, improvement of seed quality and increasing the resistance of plants against both biotic and abiotic environmental factors [1, 2, 3]. A large number of markers is employed for the diversity assessment, generally, they are divided into three classes: morphological, biochemical and molecular. The biochemical markers are various proteins, in particular, alcohol-soluble storage proteins of seeds and their metabolites. Among the undoubted advantages of these markers in comparison to molecular ones are relative ease and low cost of analysis, as well as swiftness of research. Composition of the storage proteins is stable and independent of plant growing conditions [4, 5].

Recently, more interest is seen in oat as an agricultural species, both in Russia and abroad. Oat grain is a valuable source of plant protein, fat and starch; also, it has anti-allergy properties due to its low prolamin content. Genus *Avena* L. includes 26 annual plants, which are represented by groups with a various number of chromosomes 2*n* = 14, 28, 42. Each poliploidity group includes cultivated species. Among them are lopsided oat (*A. strigosa* Schreb., 2n=14), Ethiopian oat (*A. abyssinica* Hochst., 2n=28), Byzantine oat (*A. byzantina* C. Koch, 2n=42) and common oat (*A. sativa* L., 2n=42).

Species *A. strigosa* Schreb. (Fig.1A) is common in the countries of Western Europe and in the north-west of Russia, as well as in Belorussia and Estonia. It has the *As* genome.

A. abyssinica Hochst. (Fig.1B) is endemic to Ethiopia, Erithrea and Yemen. It is a weed in crops, but is considered cultured species as its seeds do not fall. Currently, Ethiopian oat is used in selection for graded inter-species crossing of species of different ploidity. The Ethiopian oat genome *AB* has appeared due to duplication of the number of chromosomes in one of diploid species, or as a result of hybridization of two closely related diploid species. Lopsided and Ethiopian oat are in the group of species that did not participate in formation of hexaploid oat species.

Species *A. sativa* L. and *A. byzantina* C. Koch. (Fig.1C, D) are cultivated hexaploids with an *ACD* genome. They take up significant areas, incomparable to other groups of species, due to their allopolyploidity and, possibly due to a presence of two modified genomes *A* [6, 7].

Fig. 1 – External appearance of cultivated species in genus Avena L. A) A. strigosa Schreb., Local (K-4914); B) A. abyssinica Hochst., CAV 2901 (K-14823); C) A. byzantina C. Koch., Palini (K-14836); D) A. sativa L., Local (K-4075).

Not all domesticated species of oat has been cultivated recently. Lopsided and Ethiopian oat are grain field and ruderal weeds, however, they are successfully used in breeding on par with cultivated hexaploid species, including in interspecies breeding [6].

Modern selection and seed development widely use biotechnological methods for creation of new intense breeds and subsequent control of their biotypical composition. Among those methods is using storage proteins (prolamins) for analysis of genetic diversity. [8, 9].

Avenins are characterized by a significant variability in number and mobility of the protein components. Studies dedicated to assessing the heterogeneity of oat prolamins have been conducted since 1960s. Studying of the intraspecies diversity of oat through the avenin spectra, various authors identified from 16 to 34 protein components [6, 10, 11, 12, 13, 14]. Allelic variants of prolamin component units are characterized with strictly deterministic link to agricultural and adaptive properties of the genotypes [15, 16]. It gives an ability to speed up the selection process by more efficiently selecting the parent forms and goal-oriented deriving of genotypes with a desired complex of attributes and properties. The literature on the subject contains very sparse data on the link between the variability of allelic diversity of avenin-coding loci of oat and natural environmental conditions. For instance, E. Souza and M.E. Sorrells, while studying the nature of prolamin inheritance, discovered four loci: *Av1, Av2, Av3* and *Av4,* coding alternative alleles, some of them with geographic zoning of distribution [13]. V.A. Portyanko et al have noted similar dependency; however, they identified only three independent avenin-coding loci: *Avn* A*, Avn* B and *Avn* C [12]. Lack of a common approach to interpretation and registration of the avenin spectra impedes application of available data on component composition of these proteins to assessment of oat-related source materials. In the Laboratory of Cultivar Identification of Seeds of the Northern Trans-Ural State Agricultural University, the research in polymorphism of avenin is conducted based on a catalog of unit component allelic variants, developed by V.A. Portyanko et al [12]. However, further research is complicated by lack of description for many unit components that have been found during the previous research, as well as by the fact that it covers only the common oat. All that begs for research of avenin polymorphism in various oat species for subsequent assessment of heredity of separate components and supplementing the existing catalog of the unit components.

Research objective: Analysis of the component composition of avenine in oat species of various ploidity for subsequent use in marker selection when assessing the oat source material.

II. Subjects and Methods

The research has been conducted in the Laboratory of Cultivar Identification of Seeds of the Northern Trans-Ural State Agricultural University during 2013-2015. The studies used 250 samples of cultivated species of oat of three levels of ploidity: *Avena strigosa* Schreb. (2n=14) – 11 samples, *Avena abyssinica* Hochst. (4n=28) –5 samples, *Avena byzantina* C. Koch. (6n=42) – 12 samples and *Avena sativa* L. (6n=42) – 222 samples, originated from Russia and 37 other countries, including the US, Canada, China, Japan, countries of Eastern and Western Europe, Ethiopia and Australia. Plant material for the research has been provided by R&D Institute of Agricultural Research of the Northern Trans-Ural, a branch of Tyumen Scientific Center of the Siberian branch of the Russian Academy of Sciences and by the Federal Research

Center of the All-Russian Institute of Plant Genetic Resources named after N.I. Vavilov (VIR).

The analysis used 20 randomly selected caryopses of each species. One-dimensional electrophoresis of the oat storage proteins followed the standard method given in [17], with some modifications. From each specimen, 100 seeds were randomly chosen for the analysis. The proteins were extracted from the flour of various individual caryopses by adding 90 µl of 70% ethanol with subsequent incubation at 40 ˚C for 40 minutes. The obtained extract was centrifuged for 4 minutes at 10,000 rpm. The supernanant then was moved to new Eppendorf test tubes, 300 µl of methylene green coloring solution was added (60 g of sucrose, 0.1 g of methylene green, 100 g of carbamide and 100 ml of aluminum-lactate buffer). Protein extract (23 µl) was put in polyacrylamide gel and separated by electrophoresis. The polyacrylamide gel contained: 13.17 g of acrylamide, 0.66 g of N,N'-methylenebisacrylamide, 7.17 g of carbamide, 2 mg of $Fe_2(SO_4)_3\times$ $9H_2O$, 80 mg of ascorbic acid and 0.26 g of aluminum lactate. All the reagents were dissolved in 100 mg of aluminum-lactate buffer. For polymerization, 25 µ of 15% hydrogen peroxide were added to 100 ml of gel solution. For electrophoresis, VE-20 chambers were used, manufactured by Helicon, Russia, which formed the plates sized 178×175×1.5 mm. The electrophoresis was conducted at a constant voltage of 500V during 3.5-4.0 hours. Fixation and coloring of the gel plates were performed in the 10% solution of trichloracetic acid with addition of 0.05% coomassie brilliant blue R-250 in ethanol for 8 hours. Caryopses of common oat cultivar Astor (*Avn* A2 B4 C2) were used as a standard. The protein components were differentiated by differential speed of their movement in the neutral carrier.

Statistic processing of the obtained data was performed in STATISTICA 10.

III. RESULTS

Studies of electrophoretic spectra of caryopses have shown that 46.7% of the analyzed samples are homogenous for the component composition of avenin. Other samples had varied number of biotypes, from 2 to 12. Biotypes of heterogenous genotypes were considered as separate samples for the more comprehensive studies of the avenin component composition. As a result, the total number of studied samples attained 577.

It has been established that different species of oat differed in the number of protein components in their electrophoretic spectra (Fig. 2).

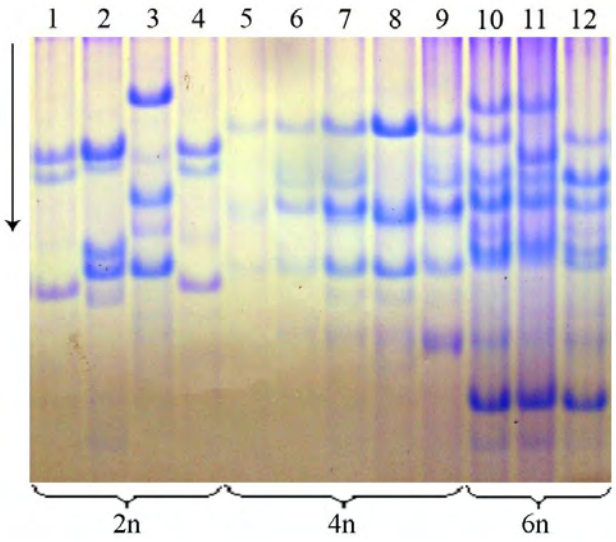

Fig. 2 – Electrophoretic spectra of avenin from cultivated species of genus Avena L.: A. strigosa Schreb.: 1 – Local (K-9285), 2 – Local (K-5200), 3 – Local (K-5287), 4 – GA44 (K-14568); A. abyssinica Hochst.: 5 – Local (K-5075), 6 – CAV 2901 (K-14823), 7 – Local (K-5105), 8 – Local (K-4971), 9 – CAV 3241 (K-14814); A. sativa L.: 10 – Astor (K-11379), 11 – Palu (K-13478), 12 – Saxo (K-11839).

The arrow indicates the direction of protein migration during the electrophoresis. 2n, 4n, 6n show ploidity of the sample.

The lowest mean values of the number of protein fractions were determined in spectra of lopsided and Ethiopian oat, 4.2±0.32 and 4.6±0.47, respectively (Table. 1).

From 6 to ten spectral bands were identified in electrophoregramms of the byzantine oats specimens. The highest mean number of component was identified in the spectra of the common oat (8.5±0.05). In total, the electrophoregramic analysis revealed 173 components differing by the speed of migration in the polyacrylamide gel.

TABLE I. CHARACTERISTICS OF AVENIN SPECTRA OF CULTIVATED SPECIES OF OAT

	Species			
Number of components in the spectrum (off)	**A. strigosa Schreb**	**A. abyssinica Hochst.**	**A. byzantina C. Koch.**	**A. sativa L.**
Total number of components	24	7	59	160
min	3	3	6	5
max	5	6	10	11
M±m	4.2±0.32	4.6±0.47	8.2±0,13	8.5±0.05
Number of non-specific components	4	-	5	101

M is the mean, m is the mean deviation, F=62.607; p<0.05.

It has been established that 65.8% of identified protein fractions were ever found in samples from the same species of oat, indicating their species-specificity. There were no components common for all four of the species studied, however, 12 protein components were found in spectra of three different species. Nine components are common for A.

sativa, A. byzantina and A. strigosa , 2 components are common for A.sativa, A. byzantina and A. abyssinica , one component is common for A. sativa, A. abyssinica и A. strigosa. It is known, that poliploid plants resulting from interspecific crossing are capable of producing proteins that are homologous to those of the initial forms [6]. Appearance of the same protein components in the spectra of specimens from different species may be a result of relatedness between the species and the genomes. The component composition of the analyzed species includes the A genome. We hold by an opinion that the identified common components may be used as markers of this genome.

The studied species of oat were different in the total number of avenin component instances identified in their spectra and in electrophoretic mobility of these components. Mobility of the protein components increased with ploidity of the species. Spectra of A. strigosa completely lacked fast prolamins, while spectra of the common oat had the largest number of them, 24 units.

As a result of analysis of storage protein spectra of A. strigosa , 24 different protein components were identified, including 4 species-specific ones (Table 1).

Information on species-specific avenin fractions has significant prospects for being used in marker-mediated selection. Such components may mark the genome of a corresponding species of oat, as well as certain valuable agricultural and adaptive features important for selection.

Spectra of the Ethiopian oat has shown 7 different components, each of which has analogs in the electrophoregrams of various other species. I.G. Loskutov et al identified 34 protein components while studying diversity of wild species in the genus Avena through avenin spectra. At that, spectra of diploid species contained 15 components on average, spectra of tetraploid species had on average 16 components [6]. In comparison with these values, the collection of the Ethiopian oat specimens that we studied has a low level of avenin polymorphism. This, as well as lack of species-specific components of A. abyssinica speaks for low genetic diversity of the analyzed collection. Autotetraploid genesis of the species and low intra-species diversity of the Ethiopian oat may be among the causes of this uniformity of the avenin spectra.

Hexaploid species of oat were characterized with a higher level of prolamin polymorphism compared to the collection of wild hexaploids, where I.G. Loskutov et al identified 18 different components [6]. By analyzing the electrophoregrams of byzantine oat specimens, we identified 59 protein components, including 5 species-specific ones. As we noted previously, the common oat showed the highest level of avenin polymorphism. Analysis of electrophoregrams of storage proteins extracted from this species allowed identifying 160 different components, 63.1% were species-specific. It has been established that 84.7% of the components identified in electrophoregrams of the byzantine oat may also be found in specimens of A. sativa. Presence of a large number of the same protein fractions in the spectra of the species may be a result of their genesis from a common parent form: A. sterilis L.

The large number of avenin spectrum types and components may be due to a broad geographic range of both common and byzantine oat [6]. The authors hold, that the higher level of genetic variability of these species in comparison with lopsided oat and Ethiopian oat, as well as compared to the collection of wild species previously studied by I.G. Loskutov, may be a result of selection work, which these species have been undergoing for a long time.

IV. CONCLUSION

The electrophoretic analysis of avenins from cultivated species of oat identified 173 protein components, 65.8% of which are species-specific; 12 components, which the authors hold are the markers of the *A* genome, have been found in the spectra of specimens from three different species. The data obtained may serve as a foundation for identification of avenin components that mark some agriculturally important and adaptive features of the genotypes.

The cultivated oat species positively differ from each other by the mean number of protein components in the spectra of the samples. The highest average number of the avenin protein components has been found in the cultivated oat (*A. sativa L.*) spectra, at 8.5±0.05. The lowest average number of the avenin protein components has been found for the lopsided oat (*A. strigosa Schreb*) and the Ethiopian oat (*A. abyssinica Hochst.*) at – 4.2±0.32 and 4.6±0.47 respectively.

The level of prolamin polymorphism in the studied species of oat allows for assessment of their genetic diversity. The maximum variability of avenin spectra and the number of avenin components has been discovered in the common oat spectra, 160 units, while for the Ethiopian oat there were only 7 units identified. This is an evidence that there is a high probability to identify a large number of selection-valuable genotypes when conducting marker-mediated selection of the common oat.

Acknowledgment

The research has been conducted under the government order (Research priority X.10.4 Program X.10.4.150 Project X.10.4.150).

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