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PLANT GENETICS

Comparative Analysis of Diploid Species of *Avena* L. Using Cytogenetic and Biochemical Markers: *Avena canariensis* Baum et Fedak and *A. longiglumis* Dur.

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Abstract—The diploid oat species containing the A genome of two types (Al and Ac) were studied by electrophoresis of grain storage proteins (avenins), chromosome C-banding, and in situ hybridization with probes pTa71 and pTa794. The karyotypes of the studied species displayed similar C-banding patterns but differed in size and morphology of several chromosomes, presumably, resulting from structural rearrangements that took place during the divergence of A genomes from a common ancestor. In situ hybridization demonstrated an identical location of the 45S and 5S rRNA gene loci in *Avena canariensis* and *A. longiglumis* similar to that in the *A. strigosa* genome. However, the 5S rDNA locus in *A. longiglumis* (*5S rDNA1*) was considerably decreased in the chromosome 3Al long arm. The analysis demonstrated that these oat species were similar in the avenin component composition, although individual accessions differed in the electrophoretic mobilities of certain components. A considerable similarity of *A. canariensis* and *A. longiglumis* to the *Avena* diploid species carrying the As genome variant was demonstrated.

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INTRODUCTION

The genus *Avena* L. includes 12 diploid species, 8 of which carry various variants of the A genome: As, Al, Ac, Ap, and Ad [1, 2]. The As genome is present in four species: cultivated *Avena strigosa* Schreb. and wild *A. hirtula* Lagas., *A. wiestii* Steud., and *A. atlantica* Baum. The second variant, Al, was identified in diploid species *A. longiglumis* Dur. [3]. The divergence of this species from the As genome species group was first discovered when analyzing the chromosome pairing in the hybrids between *A. strigosa* and *A. longiglumis* [4–6]. Assay of the grain storage proteins [7, 8] and karyological analysis of *A. longiglumis* [9] also confirmed the genome modification of this species. Study of chromosome meiotic pairing suggested that *A. strigosa* differs from *A. longiglumis* by the presence of at least five chromosome rearrangements [10, 11]. The data of AFLP, RFLP, and RAPD analyses also confirm the phylogenetic divergence of *A. longiglumis* from the remaining species constituting the A genome group [12, 13].

Avena canariensis Baum as a new biological species was first described by Baum et al. [14]. The same authors showed that this species carried a specific variant of the A genome, which was designated Ac. Further study of *A. canariensis* has demonstrated its high intraspecies variation in a number of morphological traits and isozymes as well as in the number of satellite chromosomes and chromosome rearrangements [15]. According to morphological traits, *A. canariensis* is more similar to the tetraploid oat species *A. magna* Murphy et Terr. and *A. murphyi* Ladiz. and the hexaploid species *A. sterilis* L. then the remaining diploid species of the A genome group [16]. Based on the karyotype analysis of *A. canariensis*, Murphy [17] assumed that it could have been the donor of the A genome for hexaploid oat and, possibly, tetraploid species, i.e., *A. canariensis* can be regarded as a putative ancestor of the main commercial species of this culture [14].

In this work, we used biochemical (grain storage proteins) and cytogenetic (C-banding and in situ hybridization) methods for a comprehensive comparative description of the Al and Ac genomes of *Avena* L. species and evolutionary analysis of the diploid oat species in question.

MATERIALS AND METHODS

Ten accessions of two diploid oat species representing different variants of the Ac and Al genomes of various geographic origins were studied by chromosome C-banding (Table 1). The plant material was obtained from the collection of the Vavilov Institute of Plant Industry (VIR; St. Petersburg, Russia). Two typical accessions representing different variants of the A genome were selected for in situ hybridization as well

Table 1. Studied oat accessions and their origins

Species	VIR catalog no.	Origin and collection site
A. canariensis	k-1917	Canary Islands
	k-1916	Canary Islands, Fuerteventura Island, Tiscamanitaa
	k-1915	Canary Islands, Fuerteventura Island, Vista de Toto
	k-1914	Canary Islands, Fuerteventura Island, Cassillas del Angel
	k-293*	Canary Islands, Fuerteventura Island, Betancuria
	k-292	Canary Islands
	k-2077	Canary Islands
A. longiglumis	k-1874	Morocco, 10 km south of Kenitra
	k-1811	Morocco
	k-1810*	Morocco
	k-87	Israel
	k-1881	Origin unknown; obtained from United States
	k-1912	Israel

* Accessions used for in situ hybridization.

as one accession of *A. strigosa*, carrying the As genome. A comparative study of grain storage protein patterns involved 24 accessions belonging to five species from the A genome group, namely, *A. strigosa*, *A. wiestii*, and *A. hirtula* (the As genome, 16 accessions), *A. longiglumis* (the Al genome, 4 accessions), and *A. canariensis* (the Ac genome, 6 accessions).

DNA probes and in situ hybridization. Two cloned sequences of 45S and 5S rRNA genes were used for in situ hybridization; pTa794 is a *Bam*HI fragment of wheat 5S rDNA cloned in the plasmid pBR322 [18], and pTa71 is a 9-kb *Eco*RI fragment of 45S rRNA isolated from wheat and subcloned in the plasmid pUC19 [19]. The probes were labeled with biotin or digoxigenin by nick transcription according to manufacturer's (Roche, Germany) recommendations. In situ hybridization was conducted according to the protocol described in [20] with minor modifications.

C-banding method. The C-banding protocol developed earlier for wheat chromosomes [21] was used for chromosome preparations and differential staining. The preparations were analyzed using a Leitz Wetzlar microscope; selected metaphase plates were photographed at a magnification of 100× with a Leica DFC 280 digital camera. The images were processed using Adobe Photoshop 7.0. The chromosomes of *A. longiglumis* and *A. canariensis* were classified according to the similarity to *A. strigosa* chromosomes [22].

Polyacrylamide gel electrophoresis. One-directional electrophoretic fractionation of avenins was conducted according to a standard protocol [23] with certain modifications. Avenins were extracted with 70% ethanol (60–90 ml) from the flour produced from individual grains with subsequent incubation at 40°C for 30 min. Then the supernatant was supplemented with aluminum lactate buffer containing methylene green, 80% sucrose, and 2 M urea. The samples were centrifuged for 5 min at 12000 rpm. Electrophoresis was conducted in vertical plates of 13% polyacrylamide gel ($150 \times 150 \times 1$ mm) in 0.005 M aluminum lactate buffer (pH 3.1) for 5 h at a constant voltage of 580 V. On completion of the electrophoretic fractionations, the gels were fixed with 10% trichloroacetic acid for 15 min and stained overnight with Coomassie R-250.

RESULTS

Chromosome C-banding

C-banding was used for studying the species representing two variants of the A genome, namely, Ac (*A. canariensis*) and Al (*A. longiglumis*). Totally, ten accessions were studied by this method.

Avena longiglumis Dur. is considered a typical representative of the western Mediterranean. In Europe, this species is rarely met in the south of Spain and Portugal, in Greece, and Italy. It is found in Syria, Libya, Algeria, and Israel. This is a rather typical plant in Morocco, where it frequently occurs on the coast and near the city of Rabat. *A. longiglumis* frequently grows in the community with *A. atlantica* Baum and *A. agadiriana* Baum et Fed. and is widespread in the valleys of Jordan, being a segetal and ruderal plant [2].

A specific feature of *A. longiglumis* is a pronounced symmetry of the karyotype; in addition, all the six studied accessions lacked the acrocentric chromosome characteristic of the As genome [22]. Two satellite (SAT) chromosomes (2Al and 3Al) were detected in *A. longiglumis;* their morphology corresponds to that of the SAT chromosomes of the species from the As genome group (Fig. 1). A characteristic feature of the Al genome is the presence of very large dark colored

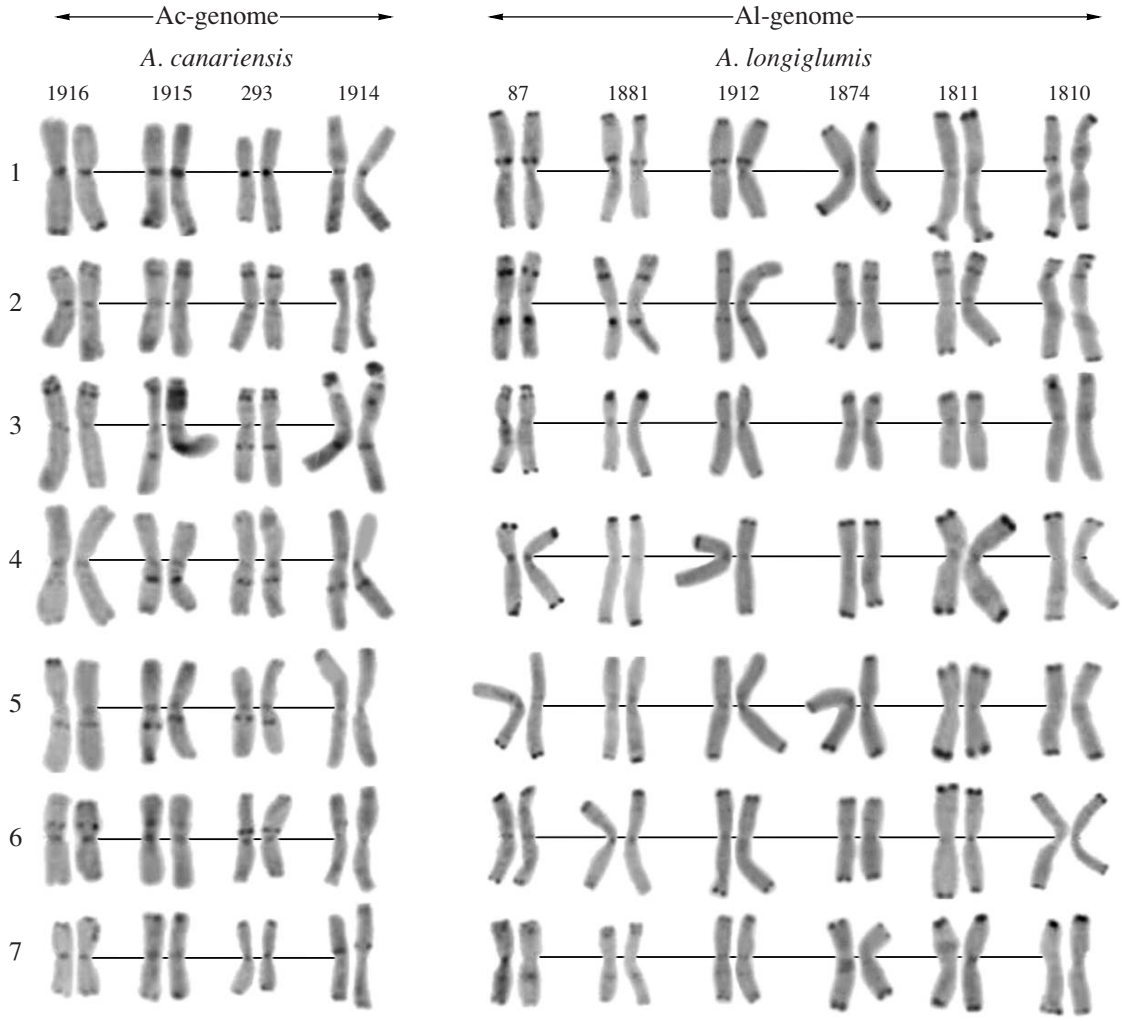

Fig. 1. Chromosome differential banding patterns of the diploid oat species with the Ac and Al genomes. Numbers of accessions are shown at the top and chromosome numbers, to the left.

telomeric heterochromatin (HC) blocks in the long and short arms of the majority of chromosomes. In addition to the telomeric C-bands, the one chromosome of accessions k-87, k-1810, k-1881, and k-1912 carried also a large marker block in the proximal region of the long arm. Considerable intraspecies polymorphism in the C-banding pattern of the *A. longiglumis* 2Al chromosome was detected. Characteristic of this chromosome in three accessions from the western Mediterranean (k-87, k-1881, and k-1912) were a very large C-segment in the proximal long arm region and the absence of telomeric blocks. On the contrary, the 2Al chromosome of three Moroccan accessions contained intensive telomeric blocks yet lacked the marker interstitial band in the long arm. The Moroccan forms displayed a weaker stained block of pericentromeric HC as compared to the remaining accessions. The near-satellite C-block in the 3Al chromosome short arm was of approximately the same size in all the accessions studied; in addition, the Moroccan *A. longiglumis* population had a characteristic small telomeric C-segment in the long arm of this chromosome. The 4Al and 6Al chromosomes display a similar morphology and C-banding pattern; they differed only by the presence of a small marker band located approximately in the middle of the short arm. The 5Al chromosome of the Moroccan *A. longiglumis* accessions contained the telomeric C-blocks in both arms, whereas the telomeric block in the rest accessions was detected only in the long arm; in addition, this chromosome carried a weakly stained band in the subtelomeric region of the long arm. The Moroccan and western Mediterranean populations most pronouncedly differed in the 7Al chromosome. In the three Moroccan accessions, this chromosome was small and approximately metacentric with two large telomeric blocks and virtually lacked intercalary HC. In the rest accessions, 7Al was a small metacentric chromosome containing distinct interstitial bands in the distal parts of the short arm and the proximal part of the long arm. The western Mediterranean accessions also displayed the polymor-

phism in the presence and size of telomeric blocks (Fig. 1). Presumably, the differences in the karyotype structure and C-banding patterns between these two *A. longiglumis* populations are determined by one or several structural chromosome rearrangements. Thus, the conducted chromosome analysis has detected a distinct intraspecies differentiation of *A. longiglumis* according to the geographical origin. However, analysis of larger number of *A. longiglumis* accessions from various countries is required to confirm this hypothesis.

Avena canariensis Baum et Fedak is an endemic species of the Canary Islands (Spain) and is widely spread, in particular, on the Fuerteventura and Lanzarote Islands. It is widespread at an altitude of 200 m above sea level sometimes rising to 550 m and grows in undisturbed associations with *A. barbata, A. occidentalis* Dur., and *A. sterilis* [2].

All the *A. canariensis* chromosomes are submetacentrics somewhat differing in the centromeric index (Fig. 1). Chromosomes 2Ac and 3Ac carry satellites. Unlike the other species constituting the A genome group, the submetacentric SAT chromosome 3Ac is noticeably shorter than the other, metacentric SAT chromosome 2Ac. In addition, the satellite of 2Ac is essentially smaller in size as compared with the homeologous chromosomes of the diploid oat species carrying other A genome variants. Unlike *A. longiglumis,* the majority of *A. canariensis* chromosomes lack pronounced telomeric HC except for the 1Ac chromosome, carrying a large telomeric C-band in the long arm. A bright centromeric band in the 1Ac chromosome as well as distinct dark-colored HC bands in the proximal third of the 3Ac, 4Ac, and 5Ac long arms and the proximal quarter of the 6Ac short arm can be considered the main CH blocks. In addition, many chromosomes contained weakly stained interstitial and more rarely, telomeric, C-bands. Virtually all these blocks were polymorphic in their size. In this species, accession k-1914 stand apart among the other *A. canariensis* representatives due to a considerable decrease in the size of the marker interstitial C-bands in the 5Ac and 6Ac chromosomes and, on the contrary, the presence of a noticeable pericentromeric C-band on the 7Ac short arm, absent in the other accessions (Fig. 1). Presumably, this accession differs from the rest by one or several translocations.

In Situ Hybridization

To characterize the A genomes of oat diploid species, we conducted in situ hybridization with the probes of 5S and 45S rRNA genes. Comparison of the three species with different A genome variants (As, Al, and Ac) has demonstrated that the hybridization patterns obtained with rDNA probes are identical (Fig. 2). All these patterns displayed two pairs of large signals with the probe pTa71, corresponding to the two major 45S rDNA loci, located in the region of secondary constrictions of both SAT chromosomes (*45S rDNA1* and *45S rDNA2*). Hybridization of the metaphase chromosome preparations with the probe pTa794 gave four distinct signals. They were located in a pairwise manner in one of the SAT chromosomes, which corresponded to the 3A chromosome according to its centromeric index. One of the sites (*5S rDNA2*) is localized to the satellite and the other (*5S rDNA1*), to the long arm of the same chromosome; note that these loci of *A. strigosa* and *A. canariensis* differed considerably in size, whereas they were approximately equal in signal intensity in *A. longiglumis.*

Electrophoretic Analysis of Grain Storage Proteins (Avenins)

To study the intra- and interspecies oat polymorphisms, the loci controlling prolamines (avenins) of oat grain were used as genetic markers. It is known that the electrophoretic components of the hexaploid oat *A. sativa* avenins are inherited in groups (blocks) and controlled by three highly polymorphic loci—*AvnA* (*Ave1*), *AvnB* (*Ave2*), and *AvnC* (*Ave3*), localized to three homeologous chromosomes of A group (corresponds to genetic group 1 of Triticeae) [23–26]. In this work, we have analyzed the polymorphism of the *AvnA* avenin-coding loci.

PAGE was used to obtain the avenin patterns of 24 accessions, containing three to eight components of various intensities and electrophoretic (EP) mobilities (Fig. 3). For comparison, the corresponding pattern of the cultivated hexaploid oat *A. sativa* cultivar Astor contained about 10 components. Totally, we have detected 23 variants of avenin EP patterns (14 variants in the As genome group; 4, in the Al genome group; and 5, in the Ac genome group). The pattern variants detected for the species with the As genome were designated A100–A113; with the Al genome, A200–A203; and with the Ac genome, A300–A304.

Only 6 of the 24 studied accessions appeared homogenous, namely, *A. longiglumis* (k-1881), *A. canariensis* (k-292 and k-1916), *A. hirtula* (k-94), and *A. strigosa* (k-4481 and k-14944), whereas the majority of accessions displayed two or three variants of the EP pattern (biotype) (Table 2). In some cases, the biotypes of the same accession differed only in the presence of an additional minor component (for example, A106 and A107 of *A. strigosa* k-3063), whereas in the other cases, the patterns differed essentially (for example, A200 and A202 of *A. longiglumis* k-87). The heterogeneity of this type can result from either the presence of mechanical impurities or the heterogeneity of initial population of the accession.

Totally, we have identified 14 variants of avenin patterns differing in the number, mobility, and intensity of individual components in the species with the As genome (Fig. 3). The frequency of individual variants varied from 1 to 16 (Table 2); note that identical patterns were detectable in both different accessions of the same species (for example, A100 in five accessions of

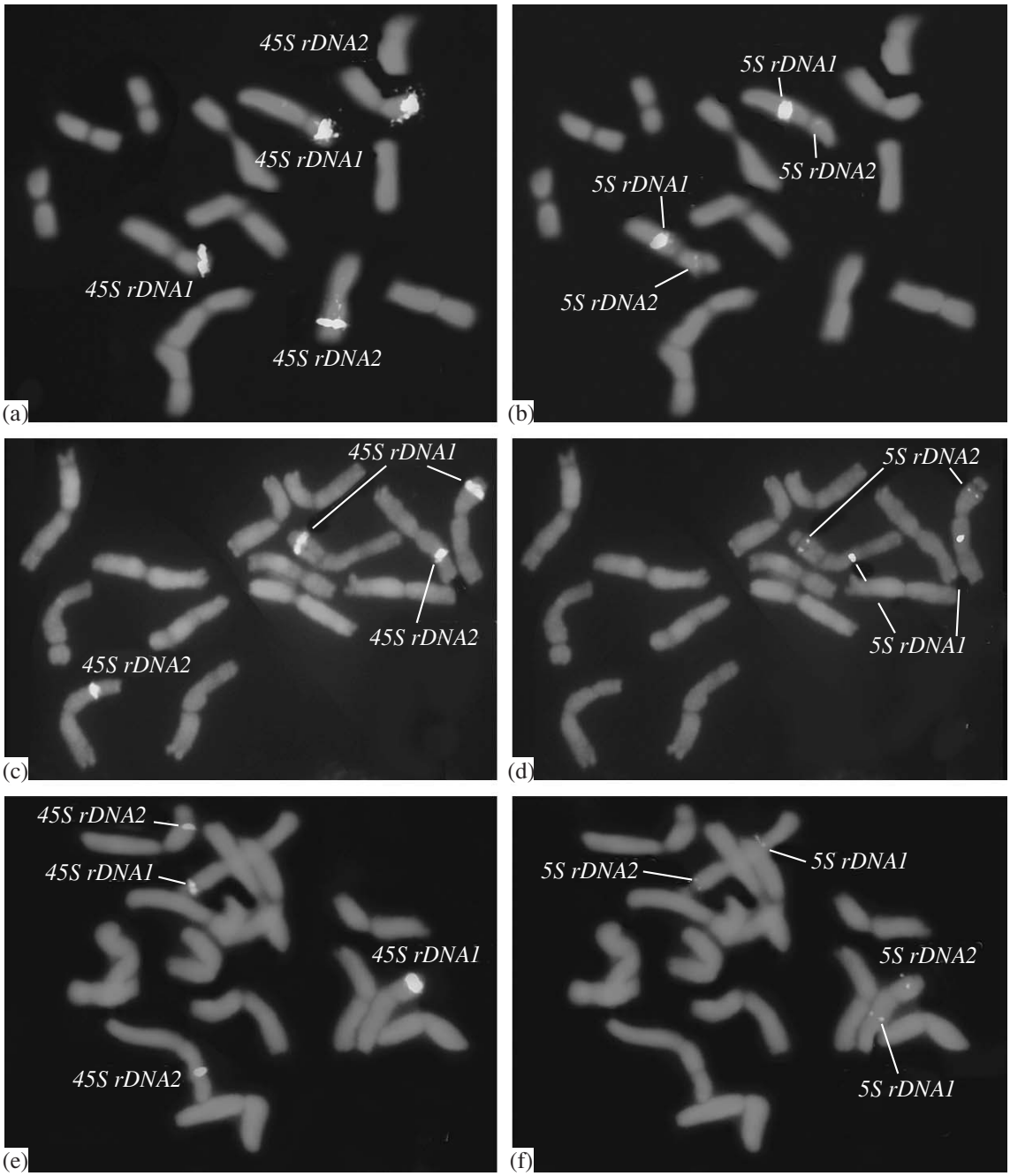

Fig. 2. In situ hybridization with probes pIa71 (a, c, d) and pIa794 (b, d, f) on chromosomes of *A. canariensis* k = 293 (a, b), *A. strigosa* k = 4485 (c, d), and *A. longiglumis* k = 1810 (e, f). Loci of rRNA genes are marked by arrows and designated *5S rDNA1-5S rDNA4* and *45S rDNA1-45S rDNA4*.

A. strigosa with different geographic origins and A109 in three *A. strigosa* accessions) and different species. The variants of the latter type, in particular, A100 and A105, belong to the patterns most widespread in the sample studied. Despite a diversity of the patterns, a number of similar traits in distribution of the bands in EP patterns suggest that all the detected variants are closely related, dederived from the common ancestor protein via mutations in individual gene copies of the *AvnA* cluster. Thus, variants A100–A113 can belong to the common protein family as, for example, is assumed for the barley hordeins [23].

Analysis of the four *A. longiglumis* (Al genome) accessions gave four variants of avenin patterns (A200–A203), which differed from the patterns determined for the species with the As genome (Fig. 3). Among them, variant A202, found in 60% of the *A. longiglumis* grains, was predominant; variant A201 was met sig-

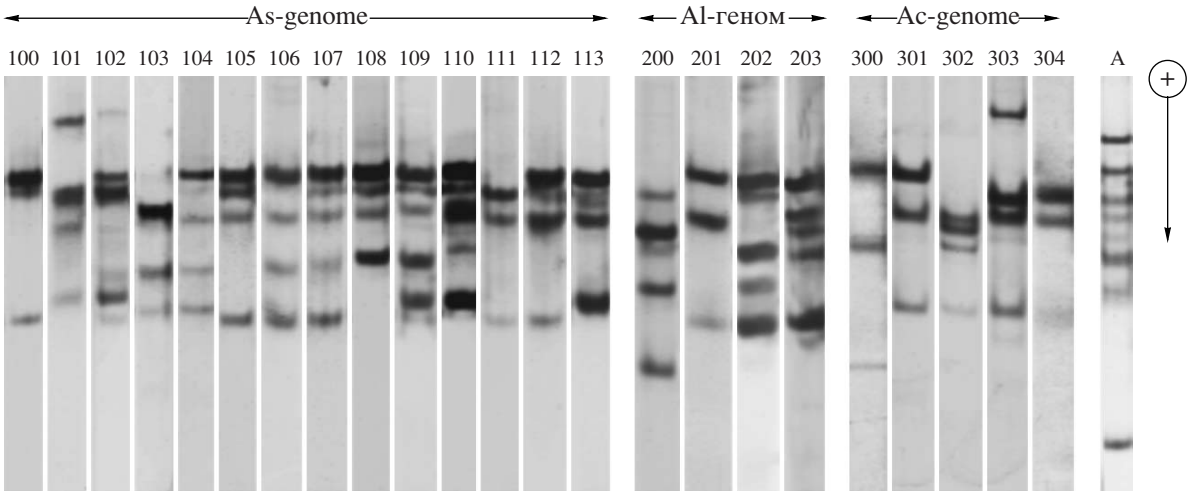

Fig. 3. Electrophoregrams of different accessions of diploid oat species with A-genomes. Spectrum variants are designated by numerals above the lanes.

Table 2. Variants of avenin electrophoretic patterns detected in diploid *Avena* species (numbers of patterns as in Fig. 3)

No.	Genome	Pattern variant	Frequency, %	Species	Accession
1	As	A100	26.23	*A. strigosa*	k-4485; k-5229; k-5184; k-5196; k-14439
				A. hirtula	k-3
2		A101	1.64	*A. hirtula*	k-3
3		A102	1.64	*A. hirtula*	k-3
4		A103	1.64	*A. strigosa*	k-4485
5		A104	1.64	*A. hirtula*	k-2032
6		A105	19.67	*A. strigosa*	k-4035; k-14944; k-14439
				A. wiestii	k-95
7		A106	4.92	*A. strigosa*	k-3063
8		A107	1.64	*A. strigosa*	k-3063
9		A108	4.92	*A. strigosa*	k-5229
10		A109	18.03	*A. strigosa*	k-4485; k-4481; k-5184
11		A110	1.64	*A. strigosa*	k-5244
12		A111	8.20	*A. wiestii*	k-94
13		A112	3.28	*A. wiestii*	k-95
14		A113	6.57	*A. strigosa*	k-5244
15	Al	A200	10	*A. longiglumis*	k-87
16		A201	25	*A. longiglumis*	k-1811; k-1810
17		A202	60	*A. longiglumis*	k-87; k-1810; k-1881
18		A203	5	*A. longiglumis*	k-1811
19	Ac	A300	3.57	*A. canariensis*	k-1914
20		A301	53.57	*A. canariensis*	k-293; k-292; k-1916; k-1917
21		A302	14.29	*A. canariensis*	k-2077
22		A303	25	*A. canariensis*	k-1917; k-1916; k-293
23		A304	3.57	*A. canariensis*	k-2077

Note: The frequency of biotype was calculated for each genome type (As, Al, and Ac, respectively).

nificantly rarer; and the rest variants were observed in single grains. Variants A201–203 of avenin EP patterns were similar to the patterns detected in *A. strigosa.* Presumably, they belong to the same family of component blocks. However, pronounced distinctions in the component distribution of A200 variant is likely to suggest that it belongs to another new family of avenin components.

Among six accessions of *A. canariensis,* five variants of grain storage protein pattern were detected. The most abundant was variant A301, recorded for over half of the analyzed grains belonging to six studied *A. canariensis* accessions (Fig. 3, Table 2). Variant A303, the second in its frequency (25% of *A. canariensis* grains), was detected in three accessions, and variant A302 (14% of the grains), predominant in k-2077, was not found in any other accession. The remaining avenin pattern variants were found in single grains. According to the total patterns, the avenin components identified in *A. canariensis* were similar to the variants of EP patterns detected in other A genome species.

DISCUSSION

The analysis of diploid *Avena* species using various marker types and comparison with the results obtained earlier [22] have demonstrated similarity between various types of A genomes, although each displays distinct specific features of the chromosome structure and C-banding patterns [9]. Thus, our results confirm the common origin of the A genome species. First and foremost, this is suggested by the similarity in distribution of rRNA gene loci on the chromosomes of *A. strigosa, A. canariensis*, and *A. longiglumis* (Fig. 2, see also [27, 28]). Earlier studies of members of the genera *Aegilops* [29], *Hordeum* [30, 31], and *Vicia* [32] as well as some other plant species have demonstrated that this trait is a highly conserved characteristic of genomes and can be used as an indicator of their evolutionary relatedness.

Second, the genetic similarity of the diploids with the A genome follows from the similarity of their karyotypes, morphology of SAT chromosomes, and HC content and distribution. Note that of the three representatives with the A genomes, *A. canariensis* according to this trait was more remote from *A. strigosa* and *A. longiglumis* than these two from one another (see also [9]), although the data of molecular studies does not comply with this assumption [13]. On the whole, *A. longiglumis* displays the most "primitive" karyotype: the majority of its chromosomes are metacentrics or submetacentrics. On the other hand, accepting the hypothesis of Levitsky [33], stating that the more ancient species have more symmetric karyotypes, it would be logical to assume that it is the *A. longiglumis* that had diverged from the common phylogenetic tree earlier than the rest species with the A genome. This agrees with the view of other authors, who consider this species the most ancient among the diploids [34].

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