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PLANT GENETICS

Comparative Analysis of Diploid Species of *Avena* L. Using Cytogenetic and Biochemical Markers: *Avena pilosa* M. B. and *A. clauda* Dur.

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Abstract—The diploid oat species containing the Cp genome—*Avena pilosa* and *A. clauda*—were studied using C-banding,fluorescence in situ hybridization with probes pTa71 and pTa794, and electrophoresis of grain storage proteins (avenins). Species with the C genome differed considerably from the species of the A genome group in the karyotype structure, heterochromatin type and distribution, relative positions of the 45S and 5S rRNA gene loci, and avenin patterns. These facts confirmed that the C genome had diverged from the ancestral genome before the radiation of the various A genome. Presumably, further evolution of the A- and C-genome species occurred separately.

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INTRODUCTION

Of 12 diploid oat species described so far, only 4—*Avena clauda* Dur., *A. pilosa*, M. B., *A. ventricosa* Balan., and *A. bruhnsiana* Grun contain the C genome. [1]. Numerous studies have demonstrated that that the C genome is considerably diverged genetically from the A genomes. This is evidenced by abnormal meiotic chromosome pairing in the hybrids between the species with A and C genomes [2–5] and theiressential differences in karyotype structure [6, 7], chromosome C-banding patterns [8], arrangement of rRNA gene loci [9], sequences of ITS1 and ITS2 regions and 5.8S rRNA genes [10], and chloroplast and mitochondrial DNA [11, 12], as well as the result of RAPD and AFLP analyses of nuclear DNA [13].

Two variants of the C genome have been described. The first, designated Cp, is found in *A. clauda* and *A. pilosa* and the second, Cv, in *A. ventricosa* and *A. bruhnsiana* [8, 14, 15]. The hybrids between the species carrying the Cp and Cv genome variants are sterile [16], thereby demonstrating their divergence. This is confirmed by karyotype analysis [6, 8, 15, 17, 18] and DNA assay with AFLP and RAPD markers [13, 19]. *A. ventricosa* and *A. bruhnsiana* are similar in plant morphology and their hybrids are fertile; this based on many researchers consider these species as geographically isolated *A. ventricosa* subspecies [20, 21]. It is assumed that their divergence can be connected with a pericentric inversion in one chromosome [17]. The karyotypes of *A. pilosa* and *A. clauda* display many common specific features; however, the differences in characteristics of several chromosomes show that they are closely related yet distinctly diverged species [22].

Further comprehensive study of the intra- and interspecies polymorphisms using various types of markers is necessary to determine in more detail the phylogenetic relationships between the diploid species of the genus *Avena*. In this work, we used biochemical (grain storage proteins) and cytogenetic (C-banding and in situ hybridization) methods for this purpose.

MATERIALS AND METHODS

Ten representatives of the diploid oat species carrying the Cp genome—*A. pilosa* (synonym, *A. eriantha* Durieu k-210) and *A. clauda* (k-200), obtained from the collection of the Institute of Plant Industry (St. Petersburg, Russia)—were studied using chromosome C-banding and electrophoresis of grain storage proteins. Both accessions were collected in Azerbaijan. One *A. clauda* accession, k-200, was studied by in situ hybridization.

Two DNA probes—pTa794 (5S rDNA) and pTa71 (18S–5.8S–26S rRNA) were used for in situ hybridization. The probes were labeled with biotin or digoxigenin by nick transcription according to manufacturer's recommendations (Roche, Germany). The in situ hybridization followed the protocol described in [23] with minor modifications.

The C-banding protocol described earlier [24] was used for differential staining. The metaphase plates were photographed at a magnification of 100× with a

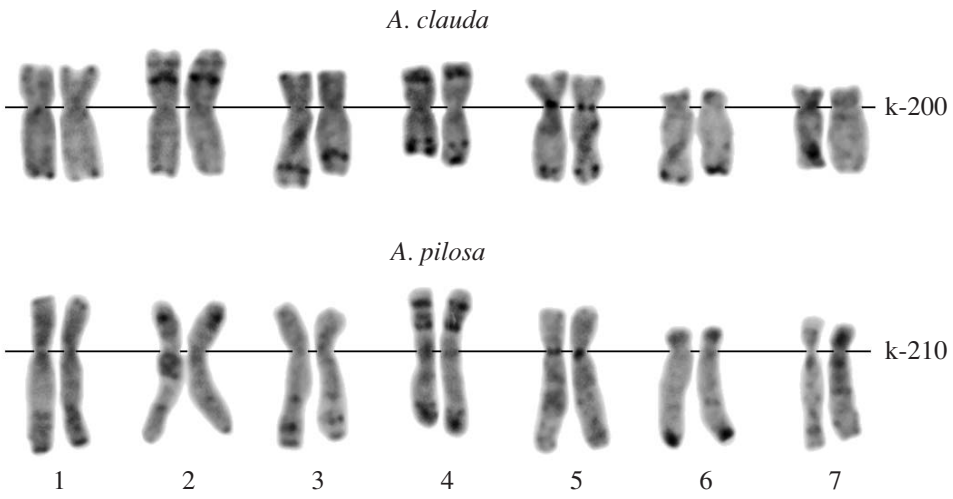

Fig. 1. Differential chromosome staining patterns of the diploid oat accessions carrying the C genome (*A. clauda* k-200 and *A. pilosa* k-210).

Leica DFC 280 digital camera. The images were processed using Adobe Photoshop 7.0. The chromosomes of the *A. pilosa* and *A. clauda* Cp genomes were designated according to the cytological nomenclature [8]. The grain storage proteins were analyzed according to the protocol described in [25].

RESULTS

Avena pilosa and *A. clauda* grow on steppe mountain passes of the western Transcaucasia and more rarely, in the semidesert and desert zones and mountain regions of the Central Asia (Uzbekistan). In Greece (Thrace and Macedonia), *A. pilos*a usually grows together with *A. clauda*. Both species are met in Asia Minor, Iran, Turkish and Iraqi Kurdistan, Lebanon, and Syria. In Turkey, the populations were found in the regions Chardak and Cheilapinar and on the coast of the Aegean Sea. A small population was found in Jordanian highlands and in Algeria near the towns of Oran and Batna. In Europe, *A. clauda* was recorded in Bulgaria and Greece near Athens, Attica, Macedonia, and Thrace as well as on the Crete Island. *Avena. pilosa* also occurs in various parts of Spain. Populations of *A. clauda* and *A. pilosa* were found in Morocco in the foothills of the Middle Atlas Mountains near the town of Azrou. *Avena clauda* contaminates wheat and barley and irrigated alfalfa fields. Together with *A. barbata* and *A. pilosa*, it grows along the roads and near various buildings. *A. clauda* is mainly a segetal and ruderal plant. *A. pilosa* is abundant in the maquis among oaks and pistachio trees, in the cenoses of abandoned pastures, on limestone slopes, and in the narrow cracks on mountain slopes [1].

Analysis of *A. clauda* and *A. pilosa* by C-banding demonstrates their considerable difference from the A genome species. Characteristic specific features of both Cp genome diploids are pronounced asymmetry of the karyotype; the presence of diffuse heterochromatin (HC), making their chromosomes darker colored than those of the A genomes; and predominantly interstitial location of HC blocks (Fig. 1). It has been shown earlier that the presence of diffuse HC and interstitial location of C-bands are characteristic of both the diploid and polyploid oat species [8, 26–29].

The *A. clauda* karyotype displays only one pair of small metacentric chromosomes, 4Cp. The two largest chromosomes, 1Cp and 2Np, are submetacentrics according to their centromeric index; the 3Cp and 5Cp chromosomes are acrocentrics; and 6Cp and 7Cp, subtelocentrics.

The 1Cp chromosome contains small telomeric blocks (of larger size in the long arm) and small interstitial bands in the distal regions of the short and long arms. The short arm of 2Cp chromosome contains a large satellite with characteristic bright HC blocks localized to its central part, separated from the chromosome arm by a bright block of pericentromeric HC. In addition, the 2Cp chromosome contains weakly stained C-bands localized to the telomere and the proximal part of the long arm. The 3Cp chromosome is similar to the 5Cp chromosome in size, morphology differing from the latter by the absence of large pericentromeric C-block and the marker block localized to the distal part of the long arm. The 4Cp chromosome carries a very small satellite in the short arm, containing a bright block of pericentromeric HC. The long arm carries a very bright colored telomeric block and a distinct subtelomeric C-segment. The 6Cp chromosome is a telocentric; it carries a large intensively stained telomeric block in the long arm and weaker C-bands approximately in the middle. Another subtelocentric chromosome, 7Cp, contains no distinct HC blocks; only two–three pairs of very weak C-bands are observable in its long arm (Fig. 1).

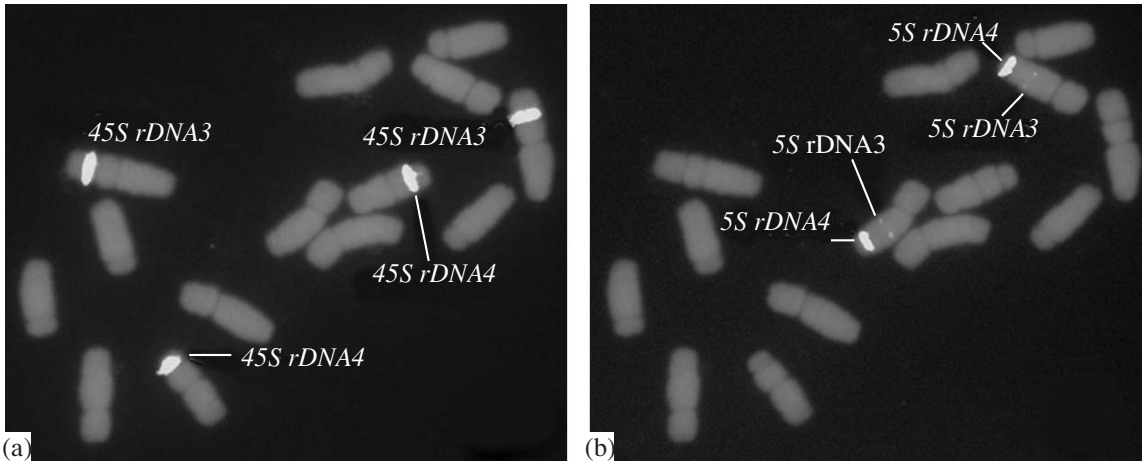

Fig. 2. In situ hybridization with the probes (a) pTa71 and (b) pTa794 on the chromosomes of *A. clauda* k-200. Arrows indicate the rRNA gene loci *5S rDNA3, 5S rDNA4, 45S rDNA3*, and *45S rDNA4.*

The studied *A. pilosa* accession is in general similar to *A. clauda* in the karyotype structure and HC distribution. Two chromosomes, 2Cp and 4Cp, contain satellites with large C-blocks in the regions of secondary constrictions. However, the C-banding pattern of the *A. pilosa* 2Cp short arm resembles the pattern of the *A. clauda* 4Cp and vice versa, the short arm of *A. pilosa* 4Cp chromosome is similar in the location of HC blocks to the *A. clauda* 2Cp short arm. This suggests a possible reciprocal translocation 2Cp/4Cp, which was likely to occur in the *A. clauda* accession, as is demonstrated by comparing our data with the results of Fominaya et al. [8]: the morphology of the satellite chromosomes in the *A. clauda* accession that they studied was identical to that of the 210 *A. pilosa* accession k-210. In addition to the reciprocal translocation, these two species differ in the size of the marker C-bands in the 3Cp and 5Cp long arms and by the presence of small interstitial bands on the 1Cp, 3Cp, and 5Cp chromosomes.

The in situ hybridization using pTa71 probe gave four major signals on the *A. clauda* chromosomes, which correspond to the to major 45S rDNA loci (*45S rDNA3* and *45S rDNA4*), located on the two pairs of satellite chromosomes (Fig. 2); note that the *45S rDNA1* and *45S rDNA2* loci are mapped on the chromosomes of diploid A genome species. The hybridization with pTa794 probe gave two paired signals localized to the long arms of one of the pairs of subacrocentric chromosomes. Smaller signals (*5S rDNA3*) were localized approximately to the middle of the arm and the larger (*5S rDNA4*), to the subtelomeric region. Note that unlike the A genome diploids [25], the 45S rRNA and 5S rRNA gene loci of *A. clauda* are located in different chromosomes.

PAGE detected three variants of grain storage patterns, C100–C102, in the studied *A. pilosa* and *A. clauda* accessions; the variants differed in the number and mobilities of the rapidly migrating minor components (Fig. 3). Despite that all the three variants were present in each species, they differed in frequency. In particular, C100 variant was predominant in *A. clauda* and C101, in *A. pilosa*; the other pattern variants were found only in single grains. The total patterns detected in the species with the Cp genome and controlled by the *AvnN* locus differed essentially from the patterns displayed by the diploids with the A genome [25].

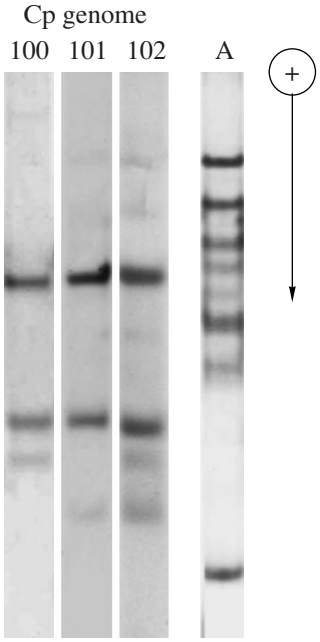

Fig. 3. Electrophoretic patterns of the diploid oat accessions with the Cp genome: 100–102, the variants of *A. clauda* and *A. pilosa* patterns and A, the pattern of hexaploid oat *A. sativa* cultivar *Astor.*

DISCUSSION

Our results confirmed a considerable divergence between the oat species carrying the C genome and the A genome species [25]. This appeared at the levels of both the overall karyotype (symmetric/asymmetric [30]) and individual chromosomes. Clear differences were also observed in the HC content and distribution [8], morphology of SAT chromosomes, and relative arrangement of the 5S and 45S rRNA gene loci. The divergence of the C genome group was recorded also at the level of grain storage proteins. The patterns of avenins encoded by the *AnvA* and *AvnC* loci differed considerably in the character and distribution of the components, presumably, reflecting pronounced genetic differences between the loci themselves. Analysis of the ITS1, ITS2, and 5.8S rRNA [10] genes also showed considerable divergence between the oat A and C genomes.

The fact that the diploids with the C genome displayed marked karyotype asymmetry suggests that their evolution was accompanied by numerous chromosome rearrangements. This is also confirmed by considerable abnormalities in chromosome pairing observed in the hybrids *A. strigosa* × *A. eriantha* (*A. clauda*) [2, 3, 5]. An indirect evidence in favor of this assumption is that the major NOR and 5S rRNA gene loci in the members of these two diploid groups are localized different chromosomes, although the hybridization patterns obtained with the probes pTa71 and pTa794 in the *A. clauda* accession, studied in this and another work [9], were somewhat different. In particular, Linares et al. [9, 31] found only one subtelomeric 5D rDNA site, while the second minor locus was absent. However, they detected a minor pTa71 signal in this chromosome arm, which was undetectable in 200 (*A. clauda*) accession k-200. Such distinctions are explainable by intraspecies polymorphism described earlier in the species belonging to the genus *Aegilops* [32].

Interestingly, the distribution of rDNA loci on the C genome chromosomes in the oat AC genome tetraploids [9, 29] and hexaploid oats [9] differed from both Cp genome species studied. Taking into account this fact and the essential differences in the chromosome structure, it seems unlikely that these Cp diploids were direct ancestors of the C genome in polyploid oats. To resolve this question, it is necessary to study at least one more diploid species, *A. ventricosa*, which carries another variant of the C genome, Cv.

Formation of diploid species comprising the C genome group was accompanied by an appearance of diffuse HC on the chromosomes, which was reported by many authors. Polyploid oats retained this specific feature [8, 9, 26, 29, 33, 34]. Note that the diffuse HC has not yet been detected in any other cereal species except oat. On the other hand, repeated DNA sequences characteristic of C, A, and D genomes have been isolated from *Avena* species, cloned, and characterized [9, 35–40]. Note that the first of these sequences—RS1 [36], pAm1 [9, 37, 41, 42], and AvsC88–137 [35]—as well as the tandem sequence pSc119.2 [43], widely spread in *Triticeae* species, displayed a disperse distribution pattern in *Avena*. It is quite possible that the emergence and amplification of a certain sequence (or several sequences of different types) at the initial stages of oat evolution was the particular factor that determined the appearance of diffuse HC in the C genome species.

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