Phyton-
International Journal of
Experimental Botany

Tech Science Press

http://doi.org/10.32604/phyton.2026.077294

ARTICLE

Identification of Informative Microsatellite Markers in the *Avena* Chloroplast Genome Provides New Insights into Oat Phylogeny

Svetlana Goryunova[1,2,*], Margarita Lebedeva[1], Aya Trifonova[1], Denis Goryunov[2,3], Anastasia Sivolapova[2], Aleksey Troitsky[3], Igor Loskutov[4] and Vitalii Pukhalskiy[1]

[1]Laboratory of Plant Genetics, Vavilov Institute of General Genetics, Russian Academy of Science, Moscow, Russia
[2]Laboratory of Cell and Genomic Technologies, Russian Potato Research Center, Kraskovo, Russia
[3]Department of Evolutional Biochemistry, Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow, Russia
[4]Department Genetic Resources of Oat, Barley, Rye, Federal Research Center N. I. Vavilov All-Russian Research Institute of Plant Genetic Resources (VIR), St. Petersburg, Russia
*Corresponding Author: Svetlana Goryunova. Email: svetlana.v.goryunova@gmail.com

Received: 06 December 2025; Accepted: 12 March 2026; Published: 29 June 2026

ABSTRACT: Twenty-six cultivated and wild oat species with genomes of varying ploidy levels are currently known worldwide. The search for informative markers, as well as the analysis of variability and phylogeny of oat species, represents a key research directions with both fundamental and applied significance. Chloroplast microsatellites are promising markers for studying groups of closely related species, particularly in the context of allopolyploid origin analyses. The transferability of chloroplast microsatellite markers among species belonging to different "core pooids" supertribes within the Pooideae subfamily of Poaceae has been demonstrated. Following preliminary screening, twelve primer pairs were selected for further analysis. Using these markers, 70 samples representing 25 *Avena* species were evaluated. The number of alleles per locus ranged from 2 to 9, with an average genetic diversity value (H) of 0.479. Based on allele length variation, 45 haplotypes were distinguished. Considerable differences in gene diversity were observed among the oat species studied. The highest levels of polymorphism were detected in the diploid species *A. eriantha* and *A. ventricosa* (C-genome), one diploid species with the As-genome (*A. atlantica*), and the tetraploid species *A. insularis* (AC-genome) and *A. agadiriana* (AaBa-genome). The absence of 50 bp-deletion in the intergenic region *ndhF–rpl32* suggests that *A. insularis* is unlikely to be the maternal progenitor of hexaploid oats. Overall, this study enabled the identification of novel informative markers for the analysis of the *Avena* chloroplast genome and contributed to refining current understanding of phylogenetic relationships among oat species.

KEYWORDS: Chloroplast microsatellite; phylogeny; *Avena*; intraspecific variability

1 Introduction

Avena L. is a genus within the Poaceae family that comprises 26 cultivated and wild species exhibiting different ploidy levels (diploid, tetraploid, and hexaploid) [1]. Oat is an important food and forage crop known since ancient times. Recently, the crop has come under increasing scrutiny as a source of functional nutrition due to its numerous health benefits [2–6]. Oat's grains have an interesting nutritional profile that includes high-quality protein, unsaturated fats, soluble fiber, polyphenolic compounds, and micronutrients [7]. Additionally, oats (*Avena sativa* L.) are used as therapeutic plants, particularly in dermatology [8]. Because

Copyright © 2026 The Authors. Published by Tech Science Press. This work is licensed under a Creative Commons Attribution 4.0 International License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

of that, they serve as subjects of multifarious studies [9–15]. Significant efforts have been applied to oat breeding research [16–18].

Development of novel crop varieties requires accurate understanding of phylogenetic relationships as well as the introduction of wild relatives to breeding programs as donors of new traits. Therefore, the investigation of wild species and *Avena* phylogeny has not only theoretical but also important applied concerns. Avena genus species possess four primary genomes: A, B, C, and D. Currently, known oat species include diploids with A- and C-genomes, tetraploids with AB- and AC-genomes, and hexaploids with ACD-genome [19]. Recently, the role of genomic approaches in the investigation of *Avena* species has increased [20–22]. Nevertheless, the phylogenetic relationships among oat species remain unresolved. Therefore, the search for informative molecular markers and the analysis of variability within Avena species are still important.

Chloroplast loci are widely used in plant evolutionary studies; yet identifying informative plastid markers suitable for detecting polymorphism at low taxonomic levels can be challenging [23–26]. Recent research highlights a growing interest in the development of highly informative chloroplast microsatellite systems for various plant taxa, including woody perennials and ecologically specialized species. For example, Guo et al. (2022) developed and characterized cpSSR markers for tree peony, underscoring their utility in population-level analyses. Similarly, Hoang et al. (2025) successfully generated chloroplast microsatellite markers for *Bruguiera hainesii*, demonstrating the applicability of cpSSR approaches in non-model tropical plant species [27,28].

Chloroplast microsatellites, however, can effectively address this issue and often exhibit high levels of variability across different plant groups [24,29–31]. Moreover, chloroplast simple sequence repeat (cpSSR) markers possess several important characteristics, including haploidy, lack of recombination, and uniparental inheritance [32]. These features make cpSSRs promising markers for studying groups of closely related species, including analyses of allopolyploid origins. Their effectiveness in resolving fine-scale population structure has been demonstrated in several recent works; in particular, Xiong et al. (2022) applied chloroplast DNA sequences and cpSSR markers to elucidate phylogeographic patterns and intraspecific divergence in *Elymus sibiricus*, confirming the value of cpSSR markers for studies at both inter- and intrapopulation levels [33].

Due to the overall low substitution rate in the chloroplast genome, cpSSR markers can often be transferred across related species. Typically, they are transferable among species of the same genus or closely related genera [34–36]. However, there are examples of successful application of chloroplast microsatellite markers to more distantly related taxa. For instance, Diekmann et al. [37] developed cpSSR markers based on chloroplast genome sequences from multiple grass species (Gramineae) representing different subfamiliesand successfully applied these markers to the "core pooids" within the Pooideae subfamily. Similar cross-taxon transferability of cpSSR loci has been reported in other plant groups, further supporting the potential of plastid microsatellites for broad phylogenetic applications [27].

Therefore, in the present study, we evaluated the possibility of applying microsatellite markers previously developed for the chloroplast genome analysis in wheat [38] to investigate intraspecific variability and clarify phylogenetic relationships within the genus *Avena*.

2 Materials and Methods

A total of 70 samples representing 25 species of the genus *Avena* were selected for analysis (Table 1). Only the narrowly distributed narrow-range species *A. bruhnsiana*, which is closely relatedto *A. ventricosa* and shares the same genome type (Cv), was not included in the study. The samples were obtained from

the collection of the N.I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR, St. Petersburg) and the Genbank of Plant Resources, Canada (PGRC, Saskatoon). Four accessions of different *Avena* species representing different genome types—*A. damascena* (Ad), *A. hirtula* (As), *A. sativa* (ACD), and *A. clauda* (Cp)—were used to test the primer pairs.

Table 1: *Avena* accessions studied and their haplotypes.

***Avena* Species**	**Genome**	**Accession Code**	**Geographic Origin**	**VIR/PGRC Catalogue Number**	**Haplotype**
A. longiglumis Durieu.	Al	lg551		WIR 1881	1
		lg596		WIR1771	2
		lg534		WIR 87	1
A. damascena Raih. et Baum	Ad	dm55	Syria	CN19458	3
		dm56	Syria	CN19459	3
		dm57	Syria	CN19457	3
A. prostrata Ladiz.	Ap	pr86		WIR 2055	4
A. canariensis Baum et Fedak	Ac	cn121		WIR 292	5
		cn538		WIR 1916	6
		cn134		WIR 2077	7
A. wiestii Steud.	As	ws16		WIR 215	8
		ws550		WIR 94	9
		ws511		WIR 95	8
A. hirtula Lagas.	As	hr531		WIR 3	8
		hr139		WIR 2032	10
		hr594		WIR 2034	11
A. atlantica Baum	As	atl33	Morocco	CN25877	12
		atl51	Morocco	CN25895	13
		atr75	Morocco	CN25848	14
A. strigosa Schreb.	As	str77	Portugal	CN25767	15
		str127		WIR 5196	15
		str129		WIR 5244	16
A. barbata Pott.	AB	br588		WIR 1745	8
		br589		WIR 1883	8
		br591		WIR 6	11
A. vaviloviana Mordv.	AB	v514		WIR 4	8
		v522		WIR 10	9
		v600		WIR 755	8

Table 1: *Cont.*

Avena Species	Genome	Accession Code	Geographic Origin	VIR/PGRC Catalogue Number	Haplotype
A. abyssinica Hoch.	AB	ab512		WIR 14826	8
		ab529		WIR 4972	8
		ab541		WIR 11678	8
A. agadiriana Baum et Fedak	AaBa	ag530	Morocco	WIR 2074	17
		ag70	Morocco	CN25868	18
		ag69	Morocco	CN25824	19
A. magna Murph. et Terr.	AC	mg64		WIR 1786	20
		mg123		WIR 1852	21
		mg545		WIR 2100	20
A. murphyi Ladiz.	AC	mr542		WIR 2088	22
		mr507		WIR 1986	23
A. insularis Ladiz.	AC	ins138	Italy, Sicily	original sampling	24
		ins136	Italy, Sicily	original sampling	25
		ins141_2	Italy, Sicily	original sampling	26
A. fatua L.	ACD	ft50	Canarias, Spain	CN25529	27
		ft95	Iraq	CN19345	28
		ft96	Eşfahān, Iran	CN21200	29
A. occidentalis Durieu.	ACD	oc88		WIR 1968	30
		oc89		WIR 1966	31
		oc32		WIR 1785	32
A. sterilis L.	ACD	ste90		WIR 511	33
		ste91		WIR 980	29
		ste92		WIR 142	33
A. ludoviciana Durieu.	ACD	lud94		WIR 461	33
		lud34		WIR 2006	29
		lud554	Russia, Krasnodar	original sampling	34
A. byzantina C. Koch	ACD	bz540		WIR 11103	35
		bz65		WIR 13351	36
		bz521_2		WIR 4633	29

Table 1: *Cont.*

Avena Species	Genome	Accession Code	Geographic Origin	VIR/PGRC Catalogue Number	Haplotype
A. sativa L.	ACD	sat515		WIR 6934	37
		sat569		WIR 1694	34
		sat570		WIR 5947	34
A. clauda Durieu.	Cp	cl71	Iran	CN19207	38
		cl72	Iran	CN19217	39
		cl73	Nīnawá, Iraq	CN19222	39
A. eriantha Durieu.	Cp	er68	Algeria	CN19329	40
		er97	Madrid, Spain	CN73755	41
		er148	Iran	CN19249	42
A. ventricosa Balansa ex Coss.	Cv	vr52	Algeria	CN21405	43
		vr53	Cyprus	CN21992	44
		vr54	Azerbaijan	CN39706	44
A. macrostachya Balansa ex Coss. et Durieu	CmCm	m142		WIR 1856	45

Total DNA was extracted from individual plants using a modified CTAB protocol [39]. A total of 18 cpSSR markers from the study by Ishii et al. [38] were used for the analysis (Table 2). PCR analysis was performedllowing the approach of Ishii et al. [38] with minor modifications. Each PCR reaction (15 μL) contained 100 ng of template DNA, 1× reaction buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 μM of each primer, and 0.3 U of Taq polymerase (Dialat LTD). The thermal cycling conditions were as follows: initial denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and elongation at 72°C for 2 min; followed by a final extension at 72°C for 7 min.

Amplified products were separated on 6% denaturing polyacrylamide gels using a 38 × 50 cm Bio-Rad SequiGen GT cell according to the manufacturer's instructions. The Invitrogen™ 10 bp DNA Ladder (Thermo Fisher Scientific Inc.) was used to estimate fragment sizes. DNA fragments were visualized by silver staining [40]. Analysis of samples representing different alleles for each marker was performed with replications to confirm allele scoring. Nei's genetic diversity index (H) was calculated for each locus [41]. Multidimensional scaling (MDS) based on the Dice pairwise genetic similarity coefficient was performed using PAST software [42].

3 Results

3.1 Diversity of Chloroplast SSR Loci

Initially, 18 primer pairs developed by T. Ishii and colleagues [38] were tested using DNA templates from four oat species representing different genome types. PCR amplification with 12 of the 18 primer pairs revealed polymorphism among the oat samples analyzed (Table 2). These twelve primer pairs were subsequently used to assess chloroplast genome variability in 70 samples representing 25 *Avena* species.

Table 2: Chloroplast microsatellite markers used in the study.

Marker	Chloroplast Genome Region	Polymorphism
WCt_1	Intergenic region *matK-5′ trnK*	Detected
WCt_2	Intergenic region *psbI-trnS*	No
WCt_3	Intergenic region *psbC-trnS*	Detected
WCt_4	Intergenic region *5′ trnG-tmT*	Detected
WCt_5	Intergenic region *petN-trnC*	Detected
WCt_6	Intergenic region *trnC-rpoB*	Detected
WCt_9	Intergenic region *atpI-atpH*	Detected
WCt_10	Intron *atpF*	Detected
WCt_11	Intron *atpF*	No
WCt_12	Intron *ycf3*	Detected
WCt_13	Intergenic region *trnF-ndhJ*	Detected
WCt_15	Intergenic region *psbE-petL*	No
WCt_16	Intergenic region *psbE-petL*	Detected
WCt_17	Intergenic region *psbE-petL*	Detected
WCt_19	Intergenic region *rpl36-infA*	No
WCt_22	Intergenic region *rps8-rpl14*	No
WCt_23	Intergenic region *rpl14-rpl16*	No
WCt_24	Intergenic region *ndhF-rpl32*	Detected

Across the 12 analyzed loci, the number of alleles identified among the oat samples ranged from 2 to 9 per locus. The lowest allele numbers were detected for the intron sequences of *atp*F (WCt_10), and inf170ycf3 (WCt_12), which exhibited 2 and 3 alleles, respectively. The highest number of alleles (9) was observed at three loci: *matK−5′ trnK* (WCt_1), *petN−trnC* (WCt_5), and *ndhF−rpl32* (WCt_24). The gene diversity index (H) for individual loci ranged from 0.136 to 0.738, with an average value of 0.479. The lowest H value was recorded for the *psbC−trnS* locus (WCt_3), while the highest was found at the *ndhF−rpl32* locus (WCt_24) (Table 3).

The *Avena* species differed markedly in their levels of genetic diversity. For *A. prostrata* and *A. macrostachya*, only one sample per species was available; therefore, the gene diversity index was not calculated. The highest diversity was observed in the C-genome diploid species *A. ventricosa* and *A. eriantha* (H = 0.33). In contrast, another C-genome species, *A. clauda*, displayed considerably lower gene diversity (H = 0.04) (Table 4).

Among A-genome species, *A. atlantica* (As-genome type) exhibited the highest gene diversity (H = 0.32). The lowest diversity values were detected in *A. damascena* (Adgenome), for which all three samples shared the same haplotype, and in *A. strigosa* and *A. wiestii* (each H = 0.04; As-genome). In *A. canariensis* (As-genome), the H value was relatively low (0.07), whereas in *A. hirtula* (As-genome) it reached 0.19.

All AB-genome species were substantially less polymorphic than *A. agadiriana* (AaBa-genome). The genetic diversity index for *A. agadiriana* was 0.30, while *A. barbata* and *A. vaviloviana* (both AB-genome) showed values of 0.07 and 0.04, respectively. No polymorphism was detected among the analyzed samples of *A. abyssinica* (AB-genome).

Table 3: Genetic diversity of investigated Avena species within 12 chloroplast microsatellite loci.

		Marker											
	Species	**WCt1**	**WCt3**	**WCt4**	**WCt5**	**WCt6**	**WCt9**	**WCt10**	**WCt12**	**WCt13**	**WCt16**	**WCt17**	**WCt24**
Genetic diversity, H	*A. longiglumis*	0.44	0.00	0.00	0.44	0.00	0.44	0.00	0.00	0.00	0.00	0.44	0.00
	A. damascena	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	A. prostrata	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	A. canariensis	0.00	0.00	0.00	0.44	0.00	0.00	0.00	0.00	0.00	0.00	0.44	0.00
	A. wiestii	0.00	0.00	0.44	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	A. hirtula	0.44	0.00	0.00	0.44	0.44	0.44	0.00	0.00	0.00	0.00	0.44	0.00
	A. atlantica	0.44	0.00	0.00	0.67	0.67	0.67	0.00	0.44	0.00	0.00	0.44	0.44
	A. strigosa	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.44	0.00
	A. barbata	0.44	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.44	0.00
	A. vaviloviana	0.00	0.00	0.44	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	A. abyssinica	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	A. agadiriana	0.44	0.00	0.44	0.67	0.44	0.67	0.00	0.00	0.00	0.00	0.44	0.44
	A. magna	0.00	0.00	0.00	0.44	0.00	0.44	0.00	0.00	0.00	0.00	0.44	0.00
	A. murphyi	0.50	0.00	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	A. insularis	0.44	0.00	0.44	0.44	0.44	0.44	0.00	0.00	0.00	0.00	0.44	0.00
	A. fatua	0.44	0.00	0.00	0.44	0.44	0.67	0.00	0.00	0.00	0.00	0.00	0.00
	A. occidentalis	0.00	0.00	0.44	0.44	0.44	0.44	0.00	0.00	0.00	0.00	0.44	0.00
	A. sterilis	0.00	0.00	0.00	0.00	0.44	0.00	0.00	0.00	0.00	0.00	0.44	0.00
	A. ludoviciana	0.44	0.00	0.00	0.44	0.44	0.44	0.00	0.00	0.00	0.00	0.44	0.00
	A. byzantina	0.67	0.00	0.00	0.00	0.44	0.44	0.00	0.00	0.00	0.00	0.44	0.00
	A. sativa	0.44	0.00	0.00	0.44	0.00	0.44	0.00	0.00	0.00	0.00	0.44	0.00
	A. clauda	0.00	0.00	0.00	0.00	0.44	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	A. eriantha	0.44	0.00	0.44	0.44	0.44	0.44	0.00	0.00	0.44	0.44	0.44	0.44
	A. ventricosa	0.44	0.44	0.00	0.44	0.44	0.44	0.00	0.00	0.44	0.44	0.44	0.44
	A. macrostachya	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Total	0.66	0.14	0.21	0.65	0.60	0.70	0.25	0.21	0.26	0.59	0.49	0.74
Number of alleles per marker		9	4	5	9	7	6	2	3	6	6	5	9

NA = Not Available.

For *A. insularis* (AC-genome), the H value was 0.22, whereas *A. magna* and *A. murphyi*, which share the same genome type, exhibited lower values of 0.11 and 0.08, respectively. Among hexaploids, *A. sterilis* showed the lowest diversity (H = 0.07); in the remaining species, H ranged from 0.15 to 0.19 (Table 4).

Based on allele length variation, a total of 45 haplotypes were identified among the 70 samples based on allele length variation. Most samples were therefore characterized by unique haplotypes (Table 1). Identical haplotypes were detected in several cases: among samples of the same species (e.g., *A. damascena*, *A. longiglumis*, *A. magna*, *A. clauda*, *A. ventricosa*, *A. strigosa*); among species sharing the same genome type (haplotype 8 in *A. wiestii* and *A. hirtula*, both As-genome; haplotypes 29, 33, 34 in hexaploid ACD-genome

species); and between polyploid species and their diploid progenitors (haplotypes 8, 9, 11 shared by diploid As-genome species and AB-genome tetraploids).

Table 4: Genetic diversity of *Avena* species.

Species	Number of Accessions	Number of Haplotypes	Average Gene Diversity, H
A. longiglumis	3	2	0.148
A. damascena	3	1	0
A. prostrata	1	1	NA
A. canariensis	3	3	0.074
A. wiestii	3	2	0.037
A. hirtula	3	3	0.185
A. atlantica	3	3	0.315
A. strigosa	3	2	0.037
A. barbata	3	2	0.074
A. vaviloviana	3	2	0.037
A. abyssinica	3	1	0
A. agadiriana	3	3	0.296
A. magna	3	2	0.111
A. murphyi	2	2	0.083
A. insularis	3	3	0.222
A. fatua	3	3	0.167
A. occidentalis	3	3	0.185
A. sterilis	3	2	0.074
A. ludoviciana	3	3	0.185
A. byzantina	3	3	0.167
A. sativa	3	2	0.148
A. clauda	3	2	0.037
A. eriantha	3	3	0.333
A. ventricosa	3	2	0.333
A. macrostachya	1	1	NA

NA = Not Available.

3.2 Genetic Relationship Inferred from cpSSR Markers

Multidimensional scaling (MDS) was performed, and a scatter plot was constructed (Fig. 1). The resulting clustering corresponded well to the genomic composition of the species. Three major groups were distinguishable:1—a single sample of *A. macrostachya* (CmCm-genome); 2—a group of diploid C-genome species; 3—a group comprising diploid species of various A-genome types, tetraploid species with AB- and AC-genomes, and hexaploid species with the ACDgenome.

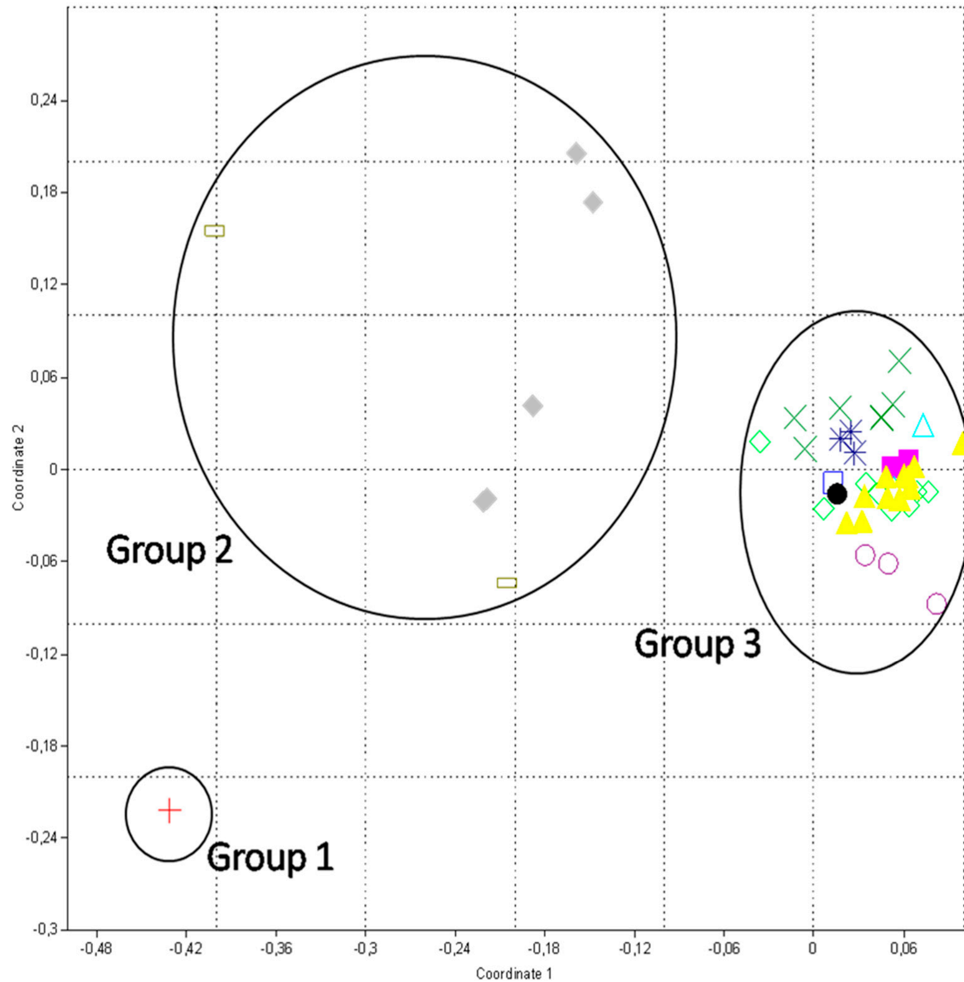

Figure 1: A multidimensional scaling plot of Avena cpSRR data. Symbols: red plus—*A. macrostachya* accession (CmCmgenome); filled gray diamonds—Cp-genome species accessions; gray rectangles—accessions of *A. ventricosa* (Cv-genome); blue triangles—accessions of *A. longiglumis* (Al-genome); black dots—accessions of *A. damascena* (Ad-genome); blue square—*A. prostrata* (Ap-genome) accession; filled pink squares—accessions of *A. canariensis* (Ac-genome); green Xs—As-genome species accessions; blue stars—AB-genome species accessions; violet rings—accessions of *A. agadiriana* (AaBa-genome); light green diamonds—AC-genome species accessions; filled yellow triangles—ACD-genome species accessions.

For a more detailed assessment of "the third group", it was analyzed separately. As shown in Fig. 2, samples of *A. agadiriana* (AaBa-genome) (Group 3-1) form the most distinct cluster. The remaining samples separate into two groups. The first includes diploid species with the As-genome and tetraploid species *A. barbata*, *A. vaviloviana*, and *A. abyssinica* with the AB-genome (Group 3-2).

The last group—Group 3-3-comprises diploid species *A. prostrata* (Ap-genome), *A. damascena* (Ad-genome), *A. longiglumis* (Al-genome), *A. canariensis* (Ac-genome), as well as tetraploid species with the AC-genome and hexaploid species with the ACD-genome (Fig. 2).

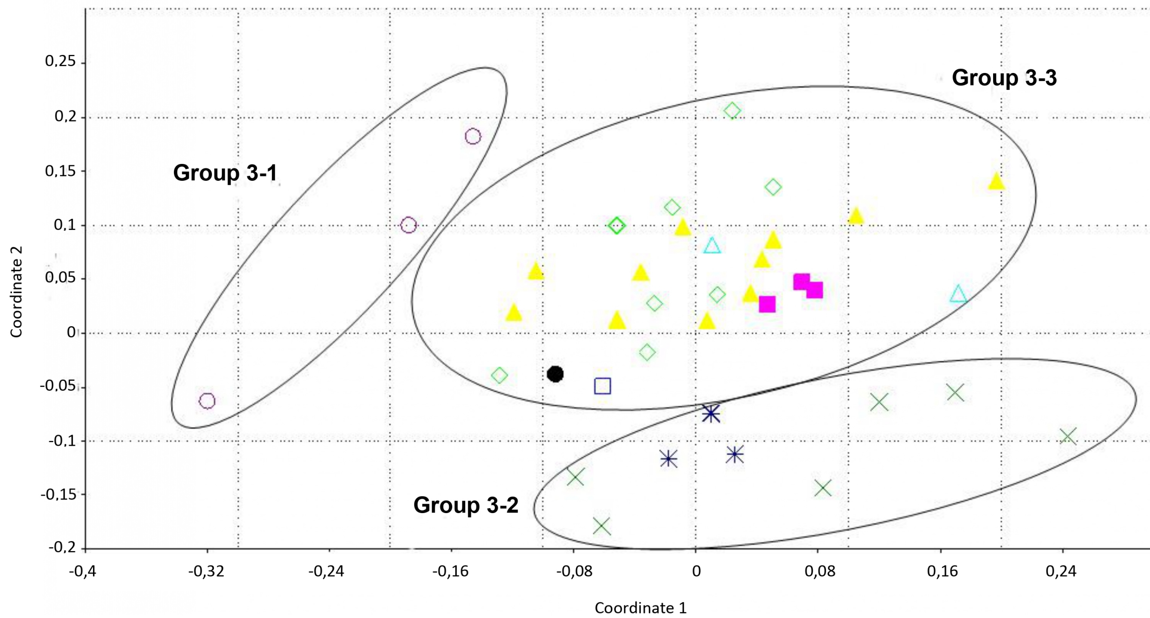

Figure 2: Scatter plot of Avena accessions with A-, AB-, AC- and ACD-genomes based on multidimensional scaling of cpSRR data. Symbols: violet rings—*A. agadiriana* accessions (AaBa-genome); green Xs—As-genome species accessions; blue stars—AB-genome species accessions; blue triangles—accessions of *A. longiglumis* (Al-genome); black dots—*A. damascene* accessions (Ad-genome); blue square—*A. prostrata* accession (Ap-genome); filled pink squares—accessions of *A. canariensis* (Ac-genome); light green diamonds—AC-genome species accessions; filled yellow triangles—accessions of ACD-genome species.

In the intergenic region *ndhF-rpl32*, an extended deletion was identified that was typical for all samples of the tetraploid species *A. magna* and *A. murphyi* with AC-genome and all samples of hexaploid species with ACD-genome (Fig. 3). In contrast, samples of another tetraploid species *A. insularis* with an AC-genome, did not possess this deletion.

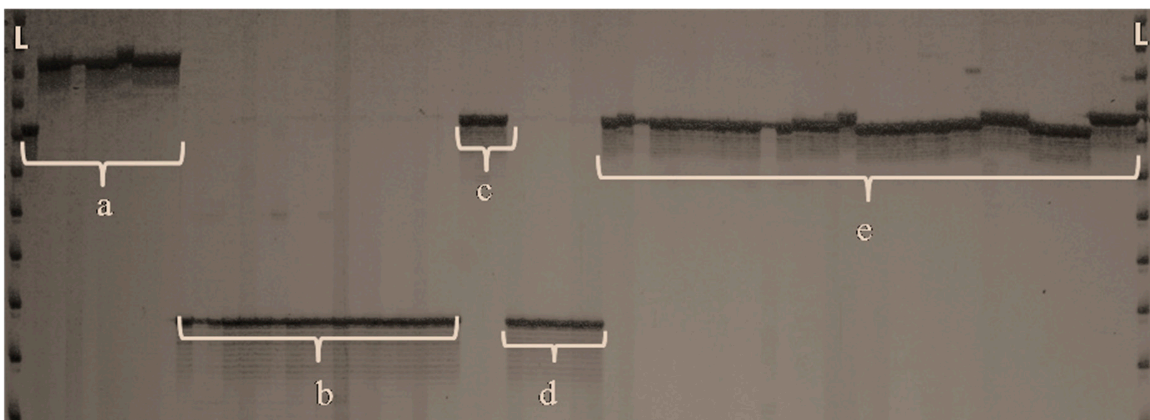

Figure 3: Length polymorphism of ndhF-rpl32 region in Avena accessions. The first and the last tracks (L)—10 bp DNA ladder. a—diploid Avena accessions with C-genome and *A. macrostachya* accession with CmCm-genome; b—hexaploid Avena accessions with ACD-genome; c—*A. insularis* accessions with AC-genome; d—*A. magna*, *A. murphyi* accessions with AC-genome; e—diploid Avena accessions with A-genome and tetraploid Avena accessions with AB- and AaBa-genome.

4 Discussion

Eighteen primer pairs developed by T. Ishii and colleagues [38] to study microsatellite variability in wheat were tested for their applicability to various *Avena* species. Although wheat and oats belong to different supertribes within the Pooideae subfamily of Poaceae, and their most recent common ancestor (MRCA) is estimated at 33.5 million years ago [43], twelve of the eighteen molecular markers proved suitable for use in *Avena*. Further analysis identified the most informative loci: *matK*−*5′trnK*, *petN*−*trnC*, and *ndhF*−*rpl32*, which can be employed to evaluate chloroplast genome polymorphism across large sets of *Avena* samples.

Considerable differences in gene diversity were observed among the studied oat species. The most polymorphic species were the C-genome diploids *A. eriantha* and *A. ventricosa*, the As-genome diploid *A. atlantica*, and the tetraploids *A. agadiriana* (AaBa-genome) and *A. insularis* (AC-genome).

Interestingly, *A. atlantica* has a relatively narrow distribution, being endemic to the Moroccan coast [44]. Despite its limited area, its gene diversity (H = 0.32) exceeded that of more widely distributed A-genome diploids such as *A. longiglumis*, *A. wiestii*, and *A. hirtula*. For *A. canariensis*, which is also narrowly distributed (Spain, Canary Islands, Fuenteventura), had a H value of only 0.07, while *A. damascena* (Syria, Damascus region and Morocco) exhibited no diversity (H = 0).

Among AB-genome tetraploids, *A. abyssinica* showed no variation among samples, reflecting its restricted distribution in Ethiopia. In contrast, *A. barbata*, which has a much wider range [44], exhibited the highest polymorphism among AB-genome species (H = 0.07). Another narrow-range AB-genome species, *A. vaviloviana*, had an intermediate diversity value (H = 0.04). Overall, AB-genome species displayed low polymorphism with very small differences among them. Earlier cytogenetic and molecular studies also reported distinct similarity among *A. abyssinica*, *A. barbata*, and *A. vaviloviana* [45,46].

The autotetraploid *A. macrostachya* appeared as the most isolated species on the scattergram, consistent with its pronounced morphophysiological differences from other oats. It is the only perennial and cross-pollinated *Avena* species and has been placed in a separate subgenus, *Avenastrum* [1,44]. Previous studies have also highlighted considerable genomic differences between *A. macrostachya* and other *Avena* species. On one hand, it possesses metacentric chromosomes similar to A-genome species [19,47,48]; on the other hand, meiosis in interspecific hybrids, banding patterns, and comparative analyses of ITS1 and ITS2 sequences indicate closer affinity to C-genome species [47,49–53].

Our results demonstrate marked differences between the chloroplast genomes of C-genome diploids and those of oat species with other genome types. Samples of diploid A-genome species, tetraploids with AB-, AaBa-, and AC-genomes, and hexaploids with the ACD-genome cluster together on the scattergram, whereas C-genome diploid species are distinctly separated. These findings are consistent with previous reports indicating different cytoplasm types in A- and C-genome diploids and support the hypothesis that the cytoplasmic genome of polyploid *Avena* species was derived from A-genome diploids [54–58].

Within the group of samples with A-type cytoplasm, those of *A. agadiriana* (AaBa-genome) were the most isolated. Notably, our findings do not support Fu's [54] observation of a closer relationship between the chloroplast genomes of *A. agadiriana* and *A. longiglumis* (AlAl). In the scattergram based on chloroplast microsatellite loci, these species form separate clusters. The origin of the *A. agadiriana* genome remains unclear. Previous comparisons of its chromosomes with the karyotypes of diploid *Avena* species bearing different A-genome variants suggested that one of its ancestors could be *A. damascena* [45]. However, in our study, the haplotypes of *A. agadiriana* differed markedly from those of *A. damascena* and other species. As noted above, *A. agadiriana* also exhibited high polymorphism. The observed remarkable intraspecific diversity in its chloroplast genome sequences is consistent with previous reports of high nuclear genome

polymorphism in this species [59–62]. Considering the relative conservatism of the chloroplast genome, the pronounced differences of *A. agadiriana* from other oat species, and its considerable intraspecific variation, it is possible that this species originated in ancient times, involving a currently unknown primitive A-genomic diploid as the cytoplasm donor.

Based on karyotype differences and analyses of chromosome pairing in F1 hybrids, genome A has been subdivided into several types, denoted by the letter "A" with the following indices: As, Ap, Al, Ac, and Ad [19,63–66]. According to our data, the chloroplast genomes of species with different A-genome types also differ considerably. Diploid A-genome species are divided into two groups. The first group includes diploid species *A. wiestii, A. hirtula, A. atlantica*, and *A. strigosa* (As-genome), together with tetraploid species *A. barbata, A. vaviloviana*, and *A. abyssinica* (AB-genome). The second group includes diploid species *A. prostrata* (Ap-genome), *A. damascena* (Ad-genome), *A. longiglumis* (Al-genome), and *A. canariensis* (Ac-genome), as well as tetraploid species with AC-genomes and hexaploid species with the ACD-genome.

The observed similarity of haplotypes among the AB-genome tetraploid species *A. abyssinica*, *A. barbata*, and *A. vaviloviana* with those of diploid As-genome species supports the hypothesis that the ancestor of these tetraploids was a diploid species from the As-genome group. Previous studies [19,67–70] also support the origin of *A. abyssinica*, *A. barbata*, and *A. vaviloviana* from As-genome diploids. Notably, seven of the nine analyzed AB-genome samples shared haplotype 8, which was also present in two of three *A. wiestii* samples and in the *A. hirtula* sample—both diploid As-genome species. Sample v522 of *A. vaviloviana* had haplotype 9, also detected in sample ws550 of *A. wiestii*, and sample br591 of *A. barbata* carried haplotype 11, also found in sample hr594 of *A. hirtula*. Consequently, no species-specific haplotypes were identified for AB-genome tetraploids; all analyzed samples possessed haplotypes shared with the diploid As-genome species *A. wiestii* and *A. hirtula*, which are likely the maternal progenitors of these tetraploids. The absence of species-specific haplotypes also suggests a relatively recent origin for this tetraploid group.

The similarity of haplotypes between tetraploid and hexaploid species with AC and ACDgenomes and diploid species with Ap-, Al-, Ac-, and Ad-genomes aligns with previous findings, supporting the derivation of the cytoplasm of ACgenome tetraploids from A-genome diploids and the cytoplasmic genome of hexaploid oats from ACgenome tetraploids [56–58,68,71,72]. However, the direct ancestor and exact cytoplasm donor of hexaploid oat species remain unknown. Following the description of *Avena magna* in 1968, it was considered the most likely ancestor of cultivated hexaploid oats [73]. After the discovery of *A. insularis* in 1998, chromosome pairing and hybridization studies with *A. sativa* as well as FISH-analysis suggested that *A. insularis* was closer to hexaploids than any other known tetraploid [74,75].

Our results, however, do not support this assumption. In the intergenic region *ndhF–rpl32*, an extended deletion of approximately 50 bp was detected, which is typical for all samples of the tetraploid species *A. magna* and *A. murphyi* (AC-genome) and all hexaploid ACDgenome species (Fig. 3). In contrast, *A. insularis* samples, as well as all diploid A-genome species, lacked this deletion. This deletion does not appear to be a microsatellite variant but rather another type of indel mutation. Because such a deletion represents a rare evolutionary event, it is highly unlikely to have occurred independently in different species. Therefore, *A. insularis* is likely the most primitive among AC-genome tetraploids and cannot be the direct maternal ancestor of hexaploid species. *A. magna* and *A. murphyi* likely share a common ancestor, and hexaploid oat species may have descended from one of these tetraploids or their shared ancestor. The antiquity of *A. insularis* among AC-genome tetraploids is further supported by its high genetic diversity.

It is also noteworthy that the chloroplast microsatellite markers used in this study were more informative than those used by Li and colleagues [76], with an average of 5.9 alleles per locus compared to 3.2 in their study. Our findings do not confirm Li et al.'s hypothesis that different diploid species could serve as cytoplasmic donors for various AC- and ACD-genome species.

5 Conclusion

This study demonstrates the possibility of transferring of chloroplast microsatellite markers between species belonging to different "core pooids" supertribes within the Pooideae subfamily (Poaceae). The most polymorphic species were the C-genome diploids *A. eriantha* and *A. ventricosa*, the As-genome diploid *A. atlantica*, and the tetraploids *A. insularis* (AC-genome) and *A. agadiriana* (AaBa-genome). Although *A. insularis* is often considered the species most closely related to hexaploid oats, it is likely the most primitive among tetraploid species with an AC genome and cannot be the direct maternal ancestor of hexaploid species. The absence of species-specific haplotypes in tetraploid species with an AB- genome suggest a recent origin for this group. The most possible maternal donors of these tetraploids are the the As-genome diploid species *A. wiestii* and *A. hirtula.* In contrast, *A. agadiriana* with the AaBa-genome, differed markedly from other oat species, this, together with the notably high level of intraspecific variation observed, may indicate the ancient origin for this species. Overall, this study identified new informative chloroplast markers for the genus *Avena* and provided further insights into the phylogenetic relationships among oat species.

Acknowledgement: We are grateful to Dr. Ekaterina Badaeva and Dr. Olga Shelukhina for the valuable discussion.

Funding Statement: The study was conducted under the state assignment of Vavilov Institute of general Genetics (125040704886-1) and Lomonosov Moscow State University (123063000008-9).

Author Contributions: The authors confirm contribution to the paper as follows: conceptualization, Svetlana Goryunova, Vitalii Pukhalskiy; resources, Igor Loskutov; investigation, Svetlana Goryunova and Aya Trifonova; data analysis, Svetlana Goryunova. and Margarita Lebedeva, visualization, Aya Trifonova and Aleksey Troitsky; writing—original draft, Svetlana Goryunova, Denis Goryunov and Anastasia Sivolapova, writing—review and editing, Vitalii Pukhalskiy, Igor Loskutov and Aleksey Troitsky. All authors reviewed and approved the final version of the manuscript.

Availability of Data and Materials: The authors confirm that the data supporting the findings of this study are available within the article.

Ethics Approval: Not applicable.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Loskutov IG, Rines HW. *Avena.* In: Kole C, editor. Wild crop relatives: genomic and breeding resources. Berlin/Heidelberg, Germany: Springer; 2011. p. 109–83. [CrossRef].
2. Song S, Lee YM, Lee YY, Yeum KJ. Oat (*Avena sativa*) extract against oxidative stress-induced apoptosis in human keratinocytes. Molecules. 2021;26(18):5564. [CrossRef].
3. Jibril AT, Arero AG, Kankam SB, Fuseini M. Effect of *Avena sativa* (Oats) on cognitive function: a systematic review of randomized controlled trials. Clin Nutr ESPEN. 2023;53:144–50. [CrossRef].
4. Li L, Zhang R, Hu Y, Deng H, Pei X, Liu F, et al. Impact of oat (*Avena sativa* L.) on metabolic syndrome and potential physiological mechanisms of action: a current review. J Agric Food Chem. 2023;71(41):14838–52. [CrossRef].

5. Amerizadeh A, Ghaheh HS, Vaseghi G, Farajzadegan Z, Asgary S. Effect of oat (*Avena sativa* L.) consumption on lipid profile with focus on triglycerides and high-density lipoprotein cholesterol (HDL-C): an updated systematic review. Curr Probl Cardiol. 2023;48(7):101153. [CrossRef].
6. Wankhede NL, Kale MB, Bawankule AK, Aglawe MM, Taksande BG, Trivedi RV, et al. Overview on the polyphenol avenanthramide in oats (*Avena sativa* Linn.) as regulators of PI3K signaling in the management of neurodegenerative diseases. Nutrients. 2023;15(17):3751. [CrossRef].
7. Alemayehu GF, Forsido SF, Tola YB, Amare E. Nutritional and phytochemical composition and associated health benefits of oat (*Avena sativa*) grains and oat-based fermented food products. Sci World J. 2023;2023:2730175. [CrossRef].
8. Kim HS, Hwang HJ, Seo WD, Do SH. Oat (*Avena sativa* L.) sprouts restore skin barrier function by modulating the expression of the epidermal differentiation complex in models of skin irritation. Int J Mol Sci. 2023;24(24):17274. [CrossRef].
9. Ling L, Li M, Chen N, Ren G, Qu L, Yue H, et al. Genome-wide analysis and expression of the GRAS transcription factor family in *Avena sativa*. Genes. 2023;14(1):164. [CrossRef].
10. Liu K, Ju Z, Jia Z, Liang G, Ma X, Liu W. Genome-wide identification and characterization of the oat (*Avena sativa* L.) WRKY transcription factor family. Genes. 2022;13(10):1918. [CrossRef].
11. Li D, Chen M, Meng X, Sun Y, Liu R, Sun T. Extraction, purification, structural characteristics, bioactivity and potential applications of polysaccharides from *Avena sativa* L.: a review. Int J Biol Macromol. 2024;265(Pt 2):130891. [CrossRef].
12. Guo Y, Tang K, Yao T, Zhang J, Liu Y, Meng J, et al. *Sphingosinicella rhizophila* sp. nov., isolated from oat (*Avena sativa* L.) rhizosphere soil. Curr Microbiol. 2025;82(4):163. [CrossRef].
13. Deng G, Nagy C, Yu P. Combined molecular spectroscopic techniques (SR-FTIR, XRF, ATR-FTIR) to study physiochemical and nutrient profiles of *Avena sativa* grain and nutrition and structure interactive association properties. Crit Rev Food Sci Nutr. 2023;63(25):7225–37. [CrossRef].
14. Oveisi M, Sikuljak D, Anđelković AA, Bozic D, Trkulja N, Piri R, et al. Application of artificial neural networks to classify *Avena fatua* and *Avena sterilis* based on seed traits: insights from European *Avena* populations primarily from the Balkan Region. BMC Plant Biol. 2024;24(1):537. [CrossRef].
15. Stefanello R, Puntel RT, da Silva Garcia WJ, Strazzabosco Dorneles L. Mitigating salt stress by conditioning seeds with ultraviolet light (UV-C) in white oats (*Avena sativa* L.). J Toxicol Environ Health Part A. 2024;87(13):533–40. [CrossRef].
16. Tinker NA, Wight CP, Bekele WA, Yan W, Jellen EN, Renhuldt NT, et al. Genome analysis in *Avena sativa* reveals hidden breeding barriers and opportunities for oat improvement. Commun Biol. 2022;5(1):474. [CrossRef].
17. Wen G, Ma BL, Shi Y, Liu K, Chen W. Selection of oat (*Avena sativa* L.) drought-tolerant genotypes based on multiple yield-associated traits. J Sci Food Agric. 2023;103(9):4380–91. [CrossRef].
18. Brzozowski LJ, Campbell MT, Hu H, Yao L, Caffe M, Gutiérrez L, et al. Genomic prediction of seed nutritional traits in biparental families of oat (*Avena sativa*). Plant Genome. 2023;16(4):e20370. [CrossRef].
19. Rajhathy T, Thomas H. Cytogenetics of oats (Avena L.). Ottawa, ON, Canada: Genetics Society of Canada; 1974.
20. Liu Q, Yuan H, Xu J, Cui D, Xiong G, Schwarzacher T, et al. The mitochondrial genome of the diploid oat *Avena longiglumis*. BMC Plant Biol. 2023;23(1):218. [CrossRef].
21. Zhang H, Liu N, Wang Y, Zheng X, Li W, Liu Z, et al. Super-pangenome analyses across 35 accessions of 23 *Avena* species highlight their complex evolutionary history and extensive genomic diversity. Nat Genet. 2025;57(9):2276–88. [CrossRef].
22. Liu Q, Xiong G, Wang Z, Wu Y, Tu T, Schwarzacher T, et al. Chromosome-level genome assembly of the diploid oat species *Avena longiglumis*. Sci Data. 2024;11(1):412. [CrossRef].
23. Daniell H, Lin CS, Yu M, Chang WJ. Chloroplast genomes: diversity, evolution, and applications in genetic engineering. Genome Biol. 2016;17(1):134. [CrossRef].
24. Provan J, Powell W, Hollingsworth PM. Chloroplast microsatellites: new tools for studies in plant ecology and evolution. Trends Ecol Evol. 2001;16(3):142–7. [CrossRef].
25. Schaal BA, Hayworth DA, Olsen KM, Rauscher JT, Smith WA. Phylogeographic studies in plants: problems and prospects. Mol Ecol. 1998;7(4):465–74. [CrossRef].

26. Shaw J, Lickey EB, Beck JT, Farmer SB, Liu W, Miller J, et al. The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. Am J Botany. 2005;92(1):142–66. [CrossRef].
27. Guo Q, Guo L, Li Y, Yang H, Hu X, Song C, et al. Development and characterization of microsatellite markers based on the chloroplast genome of tree peony. Genes. 2022;13(9):1543. [CrossRef].
28. Hoang TTT, Pham VD, Pham MP, Dang NH, Le XD, Vu DD. Development of chloroplast microsatellite markers for Bruguiera hainesii C.G. Rogers (Rhizophoraceae) in the Con Dao National Park, Ba Ria—Vung Tau Province, Vietnam. J Trop Sci Eng. 2025;37:21–34. [CrossRef].
29. Arroyo-García R, Lefort F, de Andrés MT, Ibáñez J, Borrego J, Jouve N, et al. Chloroplast microsatellite polymorphisms in *Vitis* species. Genome. 2002;45(6):1142–9. [CrossRef].
30. Desiderio F, Bitocchi E, Bellucci E, Rau D, Rodriguez M, Attene G, et al. Chloroplast microsatellite diversity in *Phaseolus* vulgaris. Front Plant Sci. 2013;3:312. [CrossRef].
31. Park H, Kim C, Lee YM, Kim JH. Development of chloroplast microsatellite markers for the endangered *Maianthemum bicolor* (Asparagaceae s.l.). Appl Plant Sci. 2016;4(8):1600032. [CrossRef].
32. Ebert D, Peakall R. Chloroplast simple sequence repeats (cpSSRs): technical resources and recommendations for expanding cpSSR discovery and applications to a wide array of plant species. Mol Ecol Resour. 2009;9(3):673–90. [CrossRef].
33. Xiong Y, Xiong Y, Shu X, Yu Q, Lei X, Li D, et al. Molecular phylogeography and intraspecific divergences in Siberian wildrye (*Elymus sibiricus* L.) wild populations in China, inferred from chloroplast DNA sequence and cpSSR markers. Front Plant Sci. 2022;13:862759. [CrossRef].
34. Deng Q, Zhang H, He Y, Wang T, Su Y. Chloroplast microsatellite markers for *Pseudotaxus chienii* developed from the whole chloroplast genome of *Taxus chinensis* var. *mairei* (Taxaceae). Appl Plant Sci. 2017;5(3):1600153. [CrossRef].
35. Pan L, Li Y, Guo R, Wu H, Hu Z, Chen C. Development of 12 chloroplast microsatellite markers in *Vigna unguiculata* (Fabaceae) and amplification in *Phaseolus vulgaris*. Appl Plant Sci. 2014;2(3):1300075. [CrossRef].
36. Saxena S, Kaila T, Chaduvula PK, Singh A, Singh NK, Gaikwad K. Novel chloroplast microsatellite markers in pigeonpea (*Cajanus* Cajan L. Millsp.) and their transferability to wild *Cajanus* species. Aust J Crop Sci. 2019;13(2):185–91. [CrossRef].
37. Diekmann K, Hodkinson TR, Barth S. New chloroplast microsatellite markers suitable for assessing genetic diversity of *Lolium perenne* and other related grass species. Ann Bot. 2012;110(6):1327–39. [CrossRef].
38. Ishii T, Mori N, Ogihara Y. Evaluation of allelic diversity at chloroplast microsatellite loci among common wheat and its ancestral species. Theor Appl Genet. 2001;103(6):896–904. [CrossRef].
39. Torres AM, Weeden NF, Martín A. Linkage among isozyme, RFLP and RAPD markers in *Vicia faba*. Theor Appl Genet. 1993;85(8):937–45. [CrossRef].
40. Bassam BJ, Gresshoff PM. Silver staining DNA in polyacrylamide gels. Nat Protoc. 2007;2(11):2649–54. [CrossRef].
41. Nei M. Molecular evolutionary genetics. New York, NY, USA: Columbia University Press; 1987. [CrossRef].
42. Hammer O, Harper DAT, Ryan PD. PAST: paleontological statistics software package for education and data analysis. Palaeontol Electron. 2001;4(1):9.
43. Pimentel M, Escudero M, Sahuquillo E, Minaya MÁ, Catalán P. Are diversification rates and chromosome evolution in the temperate grasses (Pooideae) associated with major environmental changes in the Oligocene-Miocene? PeerJ. 2017;5:e3815. [CrossRef].
44. Loskutov IG. Oat (Avena L.). Distribution, taxonomy, evolution and breeding value. Saint-Petersburg, Russia: SSC RF VIR; 2007.
45. Badaeva ED, Shelukhina OY, Goryunova SV, Loskutov IG, Pukhalskiy VA. Phylogenetic relationships of tetraploid AB-genome *Avena* species evaluated by means of cytogenetic (C-banding and FISH) and RAPD analyses. J Bot. 2010;2010:742307. [CrossRef].
46. Fominaya A, Vega C, Ferrer E. C-banding and nucleolar activity of tetraploid *Avena* species. Genome. 1988;30(5):633–8. [CrossRef].
47. Badaeva ED, Shelukhina OY, Diederichsen A, Loskutov IG, Pukhalskiy VA. Comparative cytogenetic analysis of *Avena macrostachya* and diploid C-genome *Avena* species. Genome. 2010;53(2):125–37. [CrossRef].
48. Loskutov IG. On evolutionary pathways of *Avena* species. Genet Resour Crop Evol. 2008;55(2):211–20. [CrossRef].

49. Leggett JM. A new triploid hybrid between Avena eriantha and *A. macrostachya*. Cereal Res Commun. 1990;18(1/2):97–101.
50. Leggett JM. Further hybrids involving the perennial autotetraploid oat *Avena macrostachya*. Genome. 1992;35(2):273–5. [CrossRef].
51. Leggett JM, Markhand GS. The genomic structure of Avena revealed by GISH. In: Brandham PE, Bennett MD, editors. Kew Chromosome Conference IV. London, UK: Royal Botanic Gardens; 1995. p. 133–9.
52. Pohler W, Hoppe HD. Homeology between the chromosomes of *Avena macrostachya* and the *Avena* C genome. Plant Breed. 1991;106(3):250–3. [CrossRef].
53. Rodionov AV, Tyupa NB, Kim ES, Machs EM, Loskutov IG. Genomic configuration of the autotetraploid oat species *Avena* macrostachya inferred from comparative analysis of ITS1 and ITS2 sequences: on the oat karyotype evolution during the early events of the *Avena* species divergence. Russ J Genet. 2005;41(5):518–28. [CrossRef].
54. Fu YB. Oat evolution revealed in the maternal lineages of 25 *Avena* species. Sci Rep. 2018;8:4252. [CrossRef].
55. Murai K, Tsunewaki K. Phylogenetic relationships between *Avena* Species revealed by the restriction endonuclease analysis of chloroplast and mitochondrial DNAs. In: Lawes DA, Thomas H, editors. Proceedings of the Second International Oats Conference; 1985 Jul 15–18; Aberystwyth, UK. Dordrecht, The Netherlands: Springer; 1986. p. 34–8. [CrossRef].
56. Murai K, Tsunewaki K. Chloroplast genome evolution in the genus *Avena*. Genetics. 1987;116(4):613–21. [CrossRef].
57. Peng YY, Wei YM, Baum BR, Jiang QT, Lan XJ, Dai SF, et al. Phylogenetic investigation of *Avena* diploid species and the maternal genome donor of *Avena* polyploids. Taxon. 2010;59(5):1472–82. [CrossRef].
58. Rines HW, Gengenbach BG, Boylan KL, Storey KK. Mitochondrial DNA diversity in oat cultivars and species. Crop Sci. 1988;28(1):171–6. [CrossRef].
59. Hayasaki M, Morikawa T, Leggett JM. Intraspecific variation of 18S-5.8S-26S rDNA sites revealed by FISH and RFLP in wild oat, *Avena agadiriana*. Genes Genet Syst. 2001;76(1):9–14. [CrossRef].
60. Jellen EN, Gill BS. C-banding variation in the Moroccan oat species *Avena agadiriana* (2n = 4x = 28). Theor Appl Genet. 1996;92(6):726–32. [CrossRef].
61. Leggett JM. Inter-and intra-specific hybrids involving the tetraploid species Avena agadiriana Baum et Fedak sp. nov. (2n = 4x = 28) [chromosome pairing]. In: Proceedings of the 3 International Oat Conference. Lund, Sweden: Svaloef AB; 1989.
62. Morikawa T, Leggett JM. Isozyme polymorphism and genetic differentiation in natural populations of a new tetraploid species *Avena agadiriana*, from Morocco. Genet Resour Crop Evol. 2005;52(4):363–70. [CrossRef].
63. Ladizinsky G, Zohary D. Notes on species delimination, species relationships and polyploidy in *Avena* L. Euphytica. 1971;20(3):380–95. [CrossRef].
64. Leggett JM. Interspecific diploid hybrids in *Avena*. Genome. 1989;32(2):346–8. [CrossRef].
65. Rajhathy T, Baum BR. *Avena damascena*: a new diploid oat species. Can J Genet Cytol. 1972;14(3):645–54. [CrossRef].
66. Thomas H. Cytogenetics of *Avena*. In: Marshall HG, Sorrells ME, editors. Agronomy monographs. 1st ed. Hoboken, NJ, USA: Wiley; 1992. p. 473–507. [cited 2025 Nov 16]. Available from: https://acsess.onlinelibrary.wiley.com/doi/10.2134/agronmonogr33.c14. [CrossRef].
67. Drossou A, Katsiotis A, Leggett JM, Loukas M, Tsakas S. Genome and species relationships in genus *Avena* based on RAPD and AFLP molecular markers. Theor Appl Genet. 2004;109(1):48–54. [CrossRef].
68. Fu YB, Williams DJ. AFLP variation in 25 *Avena* species. Theor Appl Genet. 2008;117(3):333–42. [CrossRef].
69. Rajhathy T, Morrison JW. Chromosome morphology in the genus *Avena*. Can J Bot. 1959;37(3):331–7. [CrossRef].
70. Sadasivaiah RS, Rajhathy T. Genome relationships in tetraploid *AVENA*. Can J Genet Cytol. 1968;10(3):655–69. [CrossRef].
71. Nikoloudakis N, Katsiotis A. The origin of the C-genome and cytoplasm of *Avena* polyploids. Theor Appl Genet. 2008;117(2):273–81. [CrossRef].
72. Yan HH, Baum BR, Zhou PP, Zhao J, Wei YM, Ren CZ, et al. Phylogenetic analysis of the genus *Avena* based on chloroplast intergenic spacer *psb*A–*trn*H and single-copy nuclear gene *Acc1*. Genome. 2014;57(5):267–77. [CrossRef].

73. Murphy HC, Sadanaga K, Zillinsky FJ, Terrell EE, Smith RT. *Avena magna*: an important new tetraploid species of oats. Science. 1968;159(3810):103–4. [CrossRef].
74. Fominaya A, Loarce Y, González JM, Ferrer E. Cytogenetic evidence supports *Avena insularis* being closely related to hexaploid oats. PLoS One. 2021;16(10):e0257100. [CrossRef].
75. Ladizinsky G. A new species of *Avena* from Sicily, possibly the tetraploid progenitor of hexaploid oats. Genet Resour Crop Evol. 1998;45(3):263–9. [CrossRef].
76. Li WT, Peng YY, Wei YM, Baum BR, Zheng YL. Relationships among *Avena* species as revealed by consensus chloroplast simple sequence repeat (ccSSR) markers. Genet Resour Crop Evol. 2009;56(4):465–80. [CrossRef].