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Genetic Diversity and Population Structure of Algerian Endemic Plant Species *Avena macrostachya* Bal. ex Cross. et Durieu

Paulina Bolc, Bogusław Łapiński, Wiesław Podyma and Maja Boczkowska *

National Centre for Plant Genetic Resources, Plant Breeding and Acclimatization Institute (IHAR)—National Research Institute, Radzików, 05-870 Błonie, Poland; p.bolc@ihar.edu.pl (P.B.); b.lapinski@ihar.edu.pl (B.Ł.); w.podyma@ihar.edu.pl (W.P.)

* Correspondence: m.boczkowska@ihar.edu.pl

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Abstract: *Avena macrostachya* is still a relatively unknown species. Using sequence-related amplified polymorphism (SRAP) markers, a simple and inexpensive technique, allowed us to conduct experiments on genetic differentiation and to study the population structure of this Algerian endemic oat. The results obtained showed lower than expected genetic diversity within the *A. macrostachya* species. The cause may be endemism of the species as well as genetic drift possible during collection, and maintenance of the accessions in gene bank and seed reproduction. No clear genetic structure was found in the examined collection, which indicates a close relationship between the populations collected in the Djurdjura National Park in Algeria. Considering the endemism of the species, its breeding potential and the small-scale ex situ collection, careful monitoring of natural sites and repeating of the collection mission are, therefore, absolutely crucial.

Keywords: *Avena macrostachya*; genetic diversity; germplasm; oat; SRAP

1. Introduction

Avena macrostachya Bal. ex Cross. et Durieu was discovered in 1853 by Benjamin Balans. The specimen no. 718 collected on 30 June 1853 at "Djebel Tougour prope Batna" at Bellezma Mountains is the holotype of the species [1]. It is a wild African oat species, classified as a relict endemic to the Atlas Mountains in north-east Algeria [1,2]. Its occurrence is limited to altitudes 1500–2200 m above sea level. It is typical of the alpine lawns of the Djurdjura mountains (*locus classicus*) [3]. It is the only perennial and allogamous species within the *Avena* genus [2]. *A. macrostachya* has 28 median or sub-median chromosomes, which are similar in size. Baum and Rajhathy [2] classified it as an autotetraploid, however, the most frequent meiotic configuration of four tetravalents and six bivalents indicates a level of homoeology developed in the course of the evolutionary process of diploidization. More recent studies have supported the hypothesis of an autopolyploid origin of this species [3]. According to Stebbins [4], the symmetry in karyotype, higher than in other *Avena* species, together with the perennial growth habit and cross-breeding system make the species most close to the ancestral forms of the genus. The morphological and karyological distinctness of *A. macrostachya* inspired even the question of assignment to another related genus *Helictotrichon* (as *H. macrostachyum*). However, cladistic analysis carried out by Baum has shown that *A. macrostachya* belongs to the genus *Avena* [2]. Further studies on hybridization of *A. macrostachya* with the diploid species of *Avena* indicated the C-genome group as the closest relatives [5,6]. Further molecular studies confirmed it [7–9] despite remarkable differences in chromosome symmetry level and localization of heterochromatic bands, and the lack of C-genome specific probe pattern [10]. Therefore, Rodionov et al. [8] assigned to *A. macrostachya* a new genome symbol C_m.

The general appearance of the *A. macrostachya* panicle is reminiscent of a laxed *Avena clauda* Dur. because of the long pedicels, the unequal glumes, and the florets which disarticulate at maturity (Figure 1). The epicuticular wax pattern of the glumes is a mixture of knobs and filaments rare in the genus. Sometimes, the uppermost florets may not disarticulate. Often, the lodicules vary in type even within the same floret, one may be of *A. sativa* type and the other of *A. fatua* type [11]. Spikelets comprising three to six fertile florets with diminished florets at the apex are 20–30 mm long. Florets disarticulate at maturity. *A. macrostachya* has short rhizomes, culms erect or geniculately ascending. Culms are 40–100 cm long, 2–3-noded. The ligule is 1–2 mm long, leaf-blades are 10–16 cm long and 3–6 mm wide and their surface is scaberulous, rough on both sides. *A. macrostachya* has apical sterile florets resembling those fertile though underdeveloped. Flowers have three anthers 8–9 mm long and ovary pubescent on apex. The fruit is a caryopsis with adherent pericarp, hairy at the apex [12].

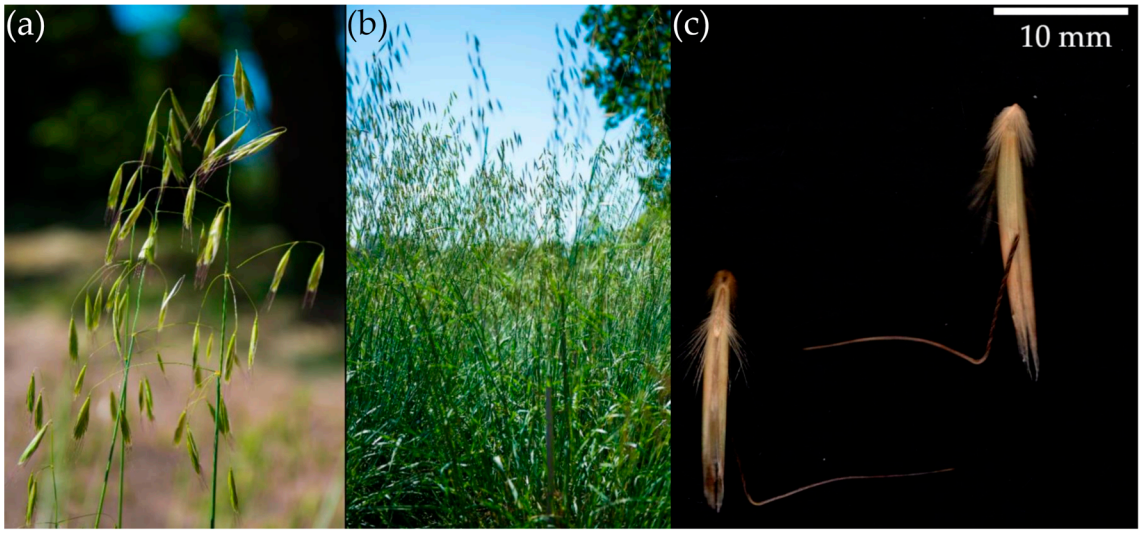

Figure 1. Morphology of *Avena macrostachya* Bal. ex Cross. et Durieu, (**a**) panicles (PL 52406), (**b**) the overall plant appearance (PL 52406), (**c**) grain morphology (PL 52409).

The Mediterranean basin is considered a global biodiversity hotspot primarily because of the size and diversity of its flora. About 10% of the world's vascular plants inhabit 1.6% of the world's terrestrial area [13]. In northern Algeria, 22 sites have been identified as Important Plant Areas (IPAs) for endemic plant species. The Atlas Mountains in Algeria include many valuable nature parks and reserves, the Bellezma National Park and the Djurdjura National Park contain the natural sites of *A. macrostachya* [14].

The Atlas Mountains are located in northern Africa extending over three countries: Morocco, Algeria, and Tunisia, and cover a total area of about 800,000 km^2. A characteristic feature of the Atlas Mountains are extensive cedar forests with unique species of trees and shrubs, including *Cedrus atlantica*, *Festuca algeriensis* Trab., *Lonicera kabylica* (Batt.) Rehder [15]. Besides, the ecoregion is rich in juniper *Juniperus africana* (Maire) H. del Villar and evergreen oak forests, *Quercus afares* Pomel [14]. The region of northern Algeria is rich in over 300 endemic taxa and the total number of taxa of elementary rank is 4000 [16]. A significant factor contributing to endemism is the climate in the Mediterranean basin: Altitude, large thermal amplitudes, west-east rainfall gradient. The geographical configuration, diverse topography and geology of this ecoregion enabled the creation of many microclimates and diverse habitats [17].

Plants of *A. macrostachya* were found only in two mountain ranges of the Atlas Mountains: The Aures and the Djurdjura Mountains (Table S1). The first region of the occurrence is the whole area of the Aures and Bellezma Mountains and the area of Djebel Chenntgouma, west of Kenchala. Eleven sites where *A. macrostachya* was found were located at an altitude of 1350–1800 m above sea level,

with a strongly moist climate, where grasses and spiny cushion shrubs (e.g., *Buplerum spinosum* Gouan, *Cytisus pungens* Spreng.) dominated. The second region was the Djurdjura Mountains, where this oat grew on the meadows of *Festuca* sp. 15 sites where this species occurred were found at an altitude of 1350–1650 m above sea level. The distribution of *A. macrostachya* covered a much larger area than in Aureus and Bellezma [18]. In the Djurdjura Mountains, there is a large population of *A. macrostachya* within the national park. It seems that this species prefers open spaces, such as screes and rocky places, with precipitation >500 mm of annual rainfall and mean minimum temperature of the coldest month of −0.8 °C or lower [1].

Our knowledge of the species, its distribution and variability is very limited. There are 150 records in the Global Biodiversity Information Facility (GBIF) database on the biological resources of *A. macrostachya* [19]. It includes 82 herbarium specimens and 37 living accessions. A critical analysis of these data narrows the knowledge about the presence of *A. macrostachya* to around 30 sites (Table S1). The information on *A. macrostachya* accessions in the other databases is very limited. The FAO database has in its collection only 13 accessions, the majority of which are the accessions preserved in the long-term storage of the Plant Breeding and Acclimatization Institute in Poland (acquisition year 2003). Apart from that, the database contains single accessions of *A. macrostachya* from Hungary (1995 acquisition year), Canada (1990 acquisition year), and Great Britain (1985 acquisition year) [20].

A. macrostachya is a species attracting special interest of oat breeders as a distinct source of new genes for building resistance to biotic and abiotic stresses [21]. It has been used in some oat breeding programs in UK, USA, and Poland. *A. macrostachya* accessions survived even severe winters in Canada [2]. The results of Santos et al. [22] confirmed the expected influence of the species variation on improvement of the cultivated oat resistance to low temperatures and the SBMV virus. Transfer of winterhardiness from *A. macrostachya* is the main objective of the Polish interspecific crossing program aimed at increasing the area of winter oat cultivation [23]. Yu and Herrmann [24] reported introgression of powdery mildew resistance to *A. sativa*. Loskutov [25] listed other resistances potentially useful for breeders: To crown and stem rust, septoria blotch, helminthosporium blotch, myrothecium blotch, frit fly. Weibull [26] announced resistance to the aphid *Rhopalosiphum padi* L. Only a small part of the species variation has been applied in oat improvement and the interest of breeders in this source of new genes is increasing. Therefore, better recognition in the level of individuality and population structure of the accessions kept in gene bank collections is important for the future of oat breeding.

The main aim of this work was to determine genetic diversity and population structure of a unique collection of *A. macrostachya* preserved in the Polish gene bank of the Plant Breeding and Acclimatization Institute (IHAR)—National Research Institute, Radzików.

2. Materials and Methods

2.1. Plant Material

The plant material used for the study consisted of nine accessions of *A. macrostachya* preserved by the National Centre for Plant Genetic Resources (NCPGR) in Radzików (Polish gene bank) (Table 1). Six accessions were acquired by germplasm-collecting expedition carried out by The International Board for Plant Genetic Resources (IBPGR) and Institut National de la Recherche Agronomique de l'Algerie (INRAA) in 1988. One accession (PL 52409) was collected through the NCPGR expedition to Algeria in the year 1989. Another accession (PL 52410) was obtained from the Plant Gene Resources of Canada. The last accession (PL 52412) resulted from open-pollination of PL 52411 and PL 52409. Locations of collection sites of the tested accessions are indicated in Figure 2.

Table 1. List of *A. macrostachya* accessions preserved by the National Centre for Plant Genetic Resources (NCPGR) in Radzików, i.e., Polish gene bank.

No.	Accession Number	Collection Site	Latitude	Longitude	Acquisition	Number of Plant
1	PL 52403	Tikjda, 6 km W (Djurdjura)	N 36°30′	E 4°5′	expedition [1]	10
2	PL 52404	Tikjda, 3 km E (Djurdjura)	N 36°30′	E 4°12′	expedition [1]	4
3	PL 52405	Tikjda, 8 km E (Djurdjura)	N 36°30′	E 4°15′	expedition [1]	10
4	PL 52406	Tala Guilef (Djurdjura)	N 36°30′	E 4°0′	expedition [1]	10
5	PL 52407	Tirourda (Djurdjura)	N 36°30′	E 4°20′	expedition [1]	10
6	PL 52408	Col de Tirouda (Djurdjura)	N 36°30′	E 4°20′	expedition	4
7	PL 52409	(Djurdjura)	N 36°30′	E 4°11′	Expedition NCPGR	4
8	PL 52410	Tikjda (Djurdjura)	N 36°27′	E 4°25′	exchange PGRC CAV 5264 [2]	2
9	PL 52412	n.a.	n.a.	n.a.	open-pollination PL 52411 and PL 52409	10

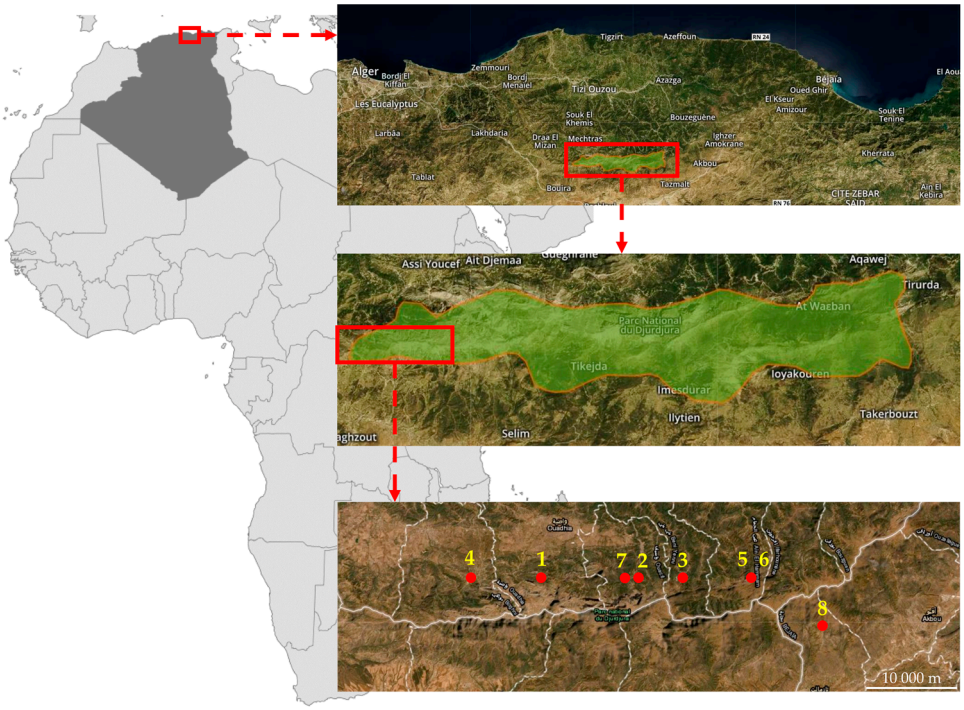

Figure 2. The *Avena macrostachya* Bal. ex Cross. et Durieu collection sites. Numbers indicating accessions according to Table 1. (UNEP-WCMC and IUCN (2020), Protected Planet: (protected area 4118; The World Database on Protected Areas (WDPA)) (On-line), (May 2020), Cambridge, UK: UNEP-WCMC and IUCN. Available at: www.protectedplanet.net.) Data available from the U.S. Geological Survey.

The history of maintenance of the accessions is well known, i.e., PL 52403–PL 52409 are first generation of originally collected seed material. PL 52410 is a descendant of seven clumps survived where 30 clones were established. Fifty seeds were received from Plant Gene Resources of Canada (PGRC). Other samples were small and a low number of seedlings were produced in the laboratory. All examined accessions have been preserved in long-term storage and multiplied on multiyear plots with spatial isolation. Morphologically and phenologically all accessions are very uniform.

According to Table 1 and geographical coordinates of collection sites distribution area of evaluated accessions covers 257 km^2 (5.55 km SN; 46.25 km WE) (Figure 2).

2.2. DNA Isolation

Genetic analysis was performed on 64 individuals representing nine accessions. The plant material was collected as young, healthy leaves picked separately from 64 plants. The tissue, immediately after collection, was frozen in liquid nitrogen and further stored in a deep freezer at −80 °C. Before isolation, the tissue was freeze-dried and ground in a bead mill. DNA isolation was carried out using the Genomic Mini Ax Plant kit (A&A Biotechnology, Gdynia, Poland) according to the manufacturer's protocol. A pellet was resuspended in 100 µL of 10 mM Tris-HCl. DNA quality was evaluated using electrophoresis on 1.5% (*w*/*v*) agarose gel. Concentration and purity were determined spectrometrically (NanoDrop Spectrophotometer ND-1000, NanoDrop Technologies, Willmington, DA, USA). DNA was diluted in 10 mM Tris-HCl to a working concentration of 25 ng/µL.

2.3. SRAP Analysis

A total of 64 combinations of sequence-related amplified polymorphism (SRAP) primer pairs published by Budak et al. [27] were tested in preliminary studies. The eight best combinations were selected for further analysis (Table 2). PCR amplification was carried out in 25 µL volume of a mixture containing 50 ng DNA, 0.2 u Walk DNA Polymerase (A&A Biotechnology, Gdynia, Poland), 1× reaction buffer (containing 100 mM KCl, 100 mM $(NH_4)_2SO_4$, 200 mM Tris-HCl pH 8.5, 20 mM $MgSO_4$, 1% Triton X-100), 120 µM of each dNTP and 0.5 µM of each primer (Table 3). Forward primers were labeled with fluorescent dyes on the 5′ end (Thermo Fisher, Waltham, MA, USA). The reaction was performed using Verity 96 Thermal Cycler (Applied Biosystems, Foster City, CA, USA under the following temperature profile: 3 min. at 94 °C of initial denaturation, followed by five cycles each one including 1 min at 94 °C, 1 min at 35 °C and 1 min at 72 °C, followed by 35 cycles each one including 1 min at 94 °C, 1 min at 55 °C and 2 min at 72 °C, and the final extension for 10 min at 72 °C. PCR products were separated using capillary sequencer Genetic Analyzer 3130XL (Applied Biosystems, Foster City, CA, USA). The 36 cm capillary array field with NanoPOP7 (Nimagen, Nijmegen, The Netherlands) was used. The length of fragments was assessed against the GeneScan 1200 LIZ Size Standard (Applied Biosystem, Foster City, CA, USA). Each PCR reaction and fragment analysis were tripled. Only repeatable fragments were scored.

Table 2. List of sequence-related amplified polymorphism (SRAP) primers used in the study.

	Primer	Sequence	Dye
Forward	Me1	TGA GTC CAA ACC GGA TA	6FAM
	Me2	TGA GTC CAA ACC GGA GC	VIC
	Me3	TGA GTC CAA ACC GGA AT	NED
	Me4	TGA GTC CAA ACC GGA CC	PET
Reverse	Em3	GAC TGC GTA CGA ATT GAC	-
	Em4	GAC TGC GTA CGA ATT TGA	-
	Em6	GAC TGC GTA CGA ATT GCA	-
	Em7	GAC TGC GTA CGA ATT CAA	-
	Em8	GAC TGC GTA CGA ATT CAC	-
	Em13	GAC TGC GTA CGA ATT CTG	-

Table 3. Primer combinations used in the study.

Set	Forward	Reverse
1	Me1	Em4
	Me2	Em3
	Me3	Em3
	Me4	Em8
2	Me1	Em13
	Me2	Em7
	Me3	Em6
	Me4	Em7

2.4. Data Analysis

Raw data were transformed into a binary matrix where 0 indicated absence and 1 presence of fragment using GeneMapper™ Software v4.1 (Applied Biosystem).

The polymorphism information content (PIC) coefficient for each marker was estimated using the following formula:

$$PIC = 1 - \sum_{i=1}^{n} p_i^2 \quad (1)$$

where *i* is the *i*th allele of the *j*th marker, *n* is the number of alleles of the *j*th marker and *p* is an allele frequency. Unbiased genetic diversity (uH_e) between accessions was estimated as follows:

$$uH_e = \frac{n}{n-1}\left(1 - \sum_{i=1}^{n} p_i^2\right) \quad (2)$$

where *n* is the sample size and *pi* is the frequency of the *i*th marker. The significance of differences was tested using ANOVA and post-hoc Tukey's HSD test.

XLSTAT Ecology (Addinsoft, Inc., Brooklyn, NY, USA) software was used to calculate the genetic distance based on the Jaccard coefficient and to perform the Principal Coordinate Analysis (PCoA). GenAlEx 6.501 software was used to perform a hierarchical analysis of molecular variance (AMOVA) [28,29]. STRUCTURE 2.3.4 software was used to determine population structure [30]. The number of group (K) values was set from 1 to 10, with ten independent runs for each K (50,000 burn-ins and 250,000 Markov Chain Monte Carlo generations) and an admixture model with correlated allele frequencies was used. The CLUMPAK software was used to identify the number of real groups in data (K). The optimal value of K was determined based on the *a posteriori* probability of data for a given K and ΔK using the full search algorithm to obtain the best alignment of the cluster analysis [31,32]. The raw supplementary data are available at https://osf.io/h3erq/.

3. Results

3.1. Marker Informativeness

The results of marker informativeness analysis showed an average number of obtained fragments about 100. The highest number, i.e., 113, was observed for the Me1/Em4 primers combination. The average polymorphism oscillated at the level of almost 70%, the highest, i.e., 81%, was observed for the pair of Me2/Em3 primers. PIC value oscillated on average at the level of 0.227, the highest value was also found for the pair of Me2/Em3 primers (0.270). More details are shown in Table 4.

Table 4. Marker statistics (PIC, Polymorphism Information Content).

Primers	No. of Fragments	% of Polymorphic Fragments	% of Rare Fragments	PIC
Me1/Em4	113	78%	17%	0.269
Me1/Em13	93	69%	20%	0.233
Me2/Em3	109	81%	17%	0.270
Me2/Em7	102	70%	22%	0.228
Me3/Em3	93	67%	31%	0.221
Me3/Em6	107	66%	33%	0.223
Me4/Em8	78	56%	35%	0.171
Me4/Em7	86	67%	26%	0.204
Mean	97.6	69.3%	27.8%	0.227

3.2. Genetic Diversity

The value of the genetic diversity coefficient (uH_e) within the studied collection was 0.227. The highest diversity appeared in PL 52405 (0.276) and the lowest in PL 52410 (0.158) (Figure 3).

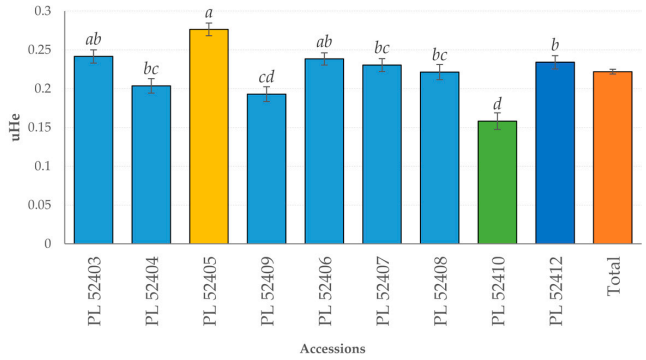

Figure 3. The unbiased genetic diversity coefficient (uH_e) within nine accessions of *Avena macrostachya* Bal. ex Cross. et Durieu based on sequence-related amplified polymorphism (SRAP) markers. Homogeneous groups were labeled with the letters. The significance of differences was tested using ANOVA. ($p < 0.05$) and post-hoc Tukey's HSD test.

3.3. Population Structure

To examine the relationships of *A. macrostachya* individuals, PCoA was performed. The first coordinate (Coord. 1), second coordinate (Coord. 2), and third coordinate (Coord. 3) explained 9.12%, 7.29%, and 6.64% of the observed variation, respectively. The gradual overlapping of gene pools of all accessions was clear (Figure 4). This result is highly consistent with the AMOVA for nine accessions, where 90% of the genetic variation was found among individuals, whereas 10% of the genetic variation was found among accessions (ΦPT = 0.104, $p = 0.0001$).

To infer the population structure from genotypic data of the nine accessions, the admixture model in the STRUCTURE software was implemented [30]. The largest ΔK was identified for K = 6, giving the highest *a posteriori* probability of K [32] (Figure 5). Individuals were assigned into six groups (numbered 1–6) based on a 70% membership threshold. According to this indicator, particular groups from one to nine individuals were assigned (9, 9, 2, 4, 1, and 4 individuals, respectively). The remaining individuals displayed a varying degree and pattern of admixture (Figure 6). The obtained results indicate that within the accession PL 52403 the 1st group was the most significantly represented. Its total contribution to the accession structure was estimated at 56.5% (Figure 7). In this accession, four of ten studied individuals were classified into the 1st group, one into the 3rd, whilst the others showed admixture. The 1st group also contributed the most to the accession PL 52406 and constituted 58.6% of its entire genetic background. Half of the individuals were classified as members of the 1st group, while the others showed an admixed genetic background. The 2nd group was mainly

present in the accessions PL 52404, PL 52410, and PL 52412, and its contribution to the genetic structure was 56.1%, 57.8%, and 50.4%, respectively. Among the 16 individuals studied in a total of these accessions, seven showed membership in the 2nd group. One plant originating from PL 52412 showed a genetic makeup typical for the 3rd group. The remaining individuals were not clearly assigned. The 3rd group appeared as an admixture in all the studied accessions and its contribution ranged from 2.8% (PL 52404) to 28.5% (PL 52410). Out of all 64 tested plants, only two were assigned to this group (from PL 52403 and PL 52412) according to the assumed criteria. The 4th group was the main contributor to the accession PL 52405 (45.4%) and 40% of the individuals have been assigned to it. In addition, one individual was assigned to the 2nd group, and the rest were admixed. The 5th group had the greatest impact on the genetic structure of the accession PL 52408 (39.4%), although all studied plants were considered as having a mixed origin. The last identified in the course of the analysis, the 6th group, prevailed in the genetic structure of the accession PL 52407. Four individuals showed homogeneous background, and the rest were admixed to varying degrees.

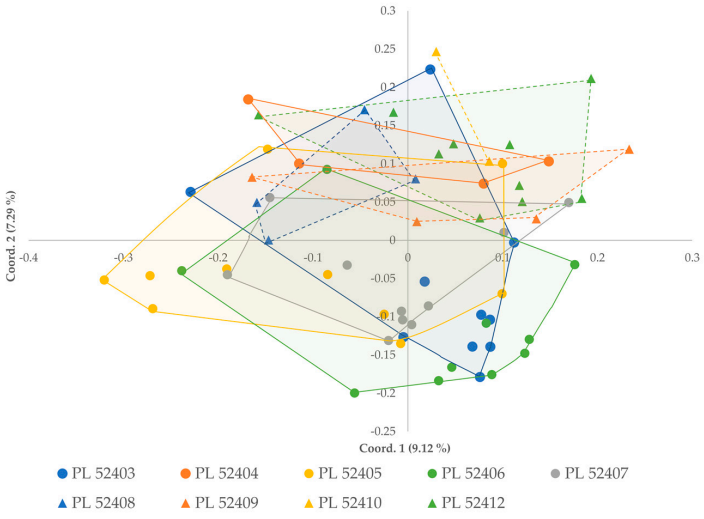

Figure 4. The results of the Principal Coordinates Analysis (PCoA) of nine accessions of *Avena macrostachya* Bal. ex Cross. et Durieu based on sequence-related amplified polymorphism (SRAP) markers.

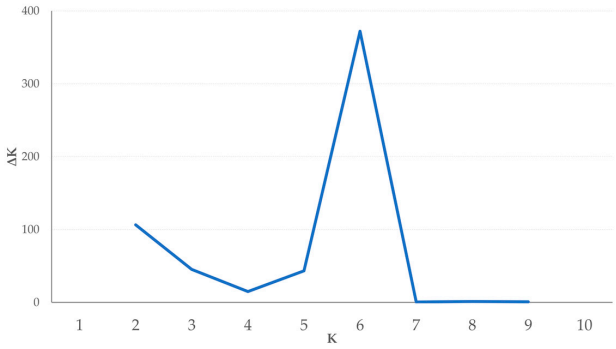

Figure 5. Results of the optimal population model investigation by plotting ad hoc measure ΔK of the data over ten runs generated by CLUMPAK software [31] The analysis performed for nine accessions of *Avena macrostachya* Bal. ex Cross. et Durieu based on sequence-related amplified polymorphism (SRAP) markers.

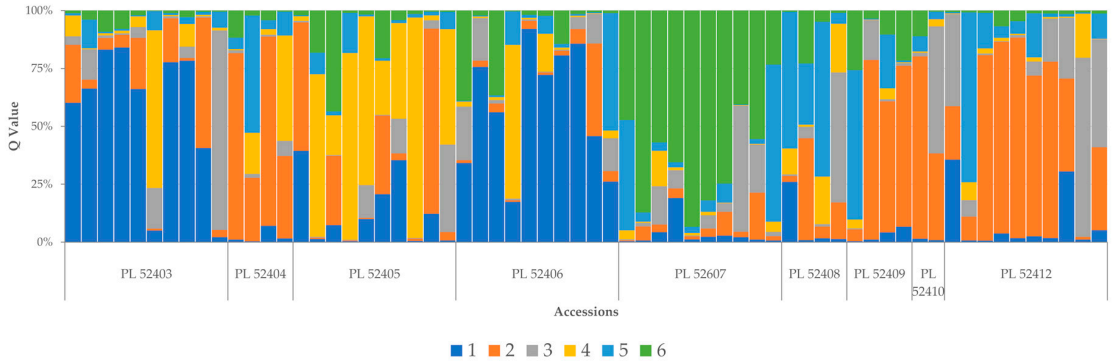

Figure 6. Q-plot of 50,000 burn-ins and 250,000 Markov Chain Monte Carlo generations of STRUCTURE software [33] with K = 6, where K is the number of ad hoc groups to be formed. Each vertical bar represents single plantlets. The length of the colored segment shows the estimated proportion of membership of that sample to each group. The analysis was performed for nine accessions of *Avena macrostachya* Bal. ex Cross. et Durieu based on sequence-related amplified polymorphism (SRAP) markers.

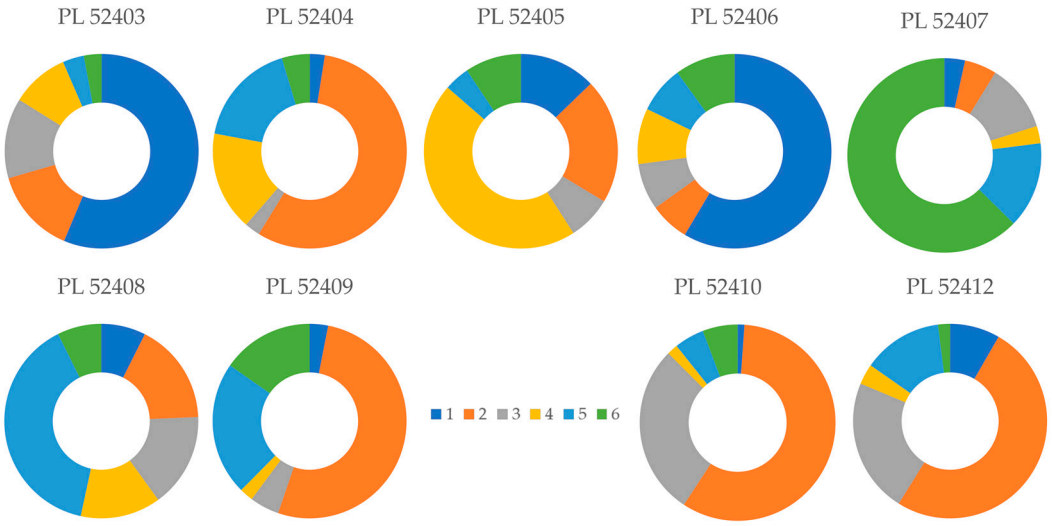

Figure 7. Proportion of six groups in nine accessions of *Avena macrostachya* Bal. ex Cross. et Durieu. Groups resulted from 50,000 burn-ins and 250,000 Markov Chain Monte Carlo generations of STRUCTURE software [33] with K = 6. The analysis based on sequence-related amplified polymorphism (SRAP) markers.

4. Discussion

4.1. Marker Effectivness

Here, the study of genetic diversity was performed using the sequence-related amplified polymorphism (SRAP) technique, which was developed by Li and Quiros in 2001 [20]. SRAP uses a combination of 17 or 18 nucleotides "forward" primers from GC-rich exons and "reverse" primers from AT-rich promoters or introns and spacers. SRAP technique is based on the amplification of open reading frames (ORF), which may strengthen the relationship between DNA polymorphisms and morphological characteristics [21]. The reproducibility of these markers is much better than that of the RAPD technique, and an additional advantage of the SRAP technique is the possibility to use fluorescent labeled primers and combine them with unlabeled primers to separate products in capillary sequencers [22]. Another advantage of SRAP is a small amount of starting DNA necessary for analysis (approx. 50 ng). The system does not require knowledge of the sequence or reference

genome information. The SRAP markers were used previously for assessing genetic relationships and diversity within and between *Saccharum* genera [23], *Brassica napus* L. [24], *Cynodon dactylon* L. [25], *Buchloë dactyloides* Nutt [26], *Cucurbita moschata* Duchesne [27], *Pinus kesiya* Royle ex Gordon var. *langbianensis* (A. Chev.) Gaussen [28]. SRAP markers amplify coding regions, which may result in their lower variability [34]. Research on the genetic diversity of the Egyptian pomegranate (*Punica granatum* L.) indicated SRAP markers had higher sensitivity, PIC and discrimination capacity comparing to ISSR and ISTR markers [35]. The effectiveness of SRAP markers for the genetic variation studies in the genus *Avena* was confirmed during the evaluation of 64 *Avena strigosa* Schreb. accessions [36]. The pattern of variation level within the groups was the same regardless of the method of analysis, i.e., morphological traits, isoenzymes, or SRAP markers. Based on the SRAP analysis results, it was also possible to identify the loss of genetic integrity of some accessions due to genetic drift [36]. In the presented study, eight combinations of SRAP primers pairs allowed the identification of 781 fragments of which 69% were polymorphic. Both of these values were higher than previously obtained for *A. strigosa* [36]. A higher level of polymorphism detected in the study of *A. macrostachya* comparing to *A. strigosa* may resulted both from a different flowering biology (cross- vs. self-pollination) and a different approach to the collection and analysis of samples (individuals vs. bulk samples). The mean value of PIC (0.227) in comparison to the analysis of *A. strigosa* (0.33) was lower [36]. This is a direct result of the high proportion of rare fragments with low frequency. In the studied accessions of *A. macrostachya,* their average contribution was 27.8%. According to Anderson et al. (1992) formula for dominant markers PIC range 0–0.5 [37]. Therefore, its value for the SRAP marker will be the average of the PIC values obtained for all fragments generated by a given pair of primers. Therefore, when a pair of primers detects a large number of unique alleles for which PIC is very low, the average value will be significantly decreased by them.

4.2. Genetic Diversity

The overall genetic diversity in the examined *A. macrostachya* collection at the level of 0.23 does not differ significantly from the values observed in previous studies of other *Avena* species. In the study of the eight accessions of *Avena strigosa* Schreb., the average value of the uHe coefficient was 0.21, whereas the genetic diversity of Polish landraces of *Avena sativa* L. was determined as 0.23 [38,39]. Significantly lower values of the differentiation were observed for common oat commercial cultivars, i.e., 0.07 [40]. However, the three oat species differ in terms of their biological status, ploidy level, and also breeding system and each of these factors substantially affects genetic diversity [41]. Generally, genetic diversity increases with ploidy [42]. This phenomenon was observed in the family *Poaceae* for *Cynodon* and *B. dactyloides* [43–45]. The three previously mentioned oat species represent three subsequent levels of ploidy, i.e., *A. strigosa* is a diploid species (2n = 14), *A. macrostachya* is a tetraploid (2n = 28) and *A. sativa* is a hexaploidy (2n = 42). The biological status, indicating whether the accessions under investigation represent wild species, landraces, or cultivars, is directly related to the expected degree of their genetic diversity. Along with the domestication of the species, a decrease in their genetic diversity is observed [46]. Accessions of *A. macrostachya* studied here reflect populations of the wild species, while both *A. strigosa* and *A. sativa* are domesticated species. Crops show a decreased diversity level caused by a serious population size reduction resulting from sub-sampling of wild ancestral species followed by targeted selection for the desired phenotypic characteristics during the breeding. Plant breeding systems have been identified as a major factor influencing genetic diversity. Cross-pollinated species are expected to have a higher level of genetic diversity compared to self-pollinating ones. In general, outcrossing reduces the relationship between uniting gametes, thus increasing the number of effective alleles and blurring population subdivision [47]. *A. macrostachya* is the only outcrossing species of the genus *Avena*. [40,46].

Considering all the above-mentioned facts, it was expected that the genetic diversity of the examined accessions of *A. macrostachya* would be higher than that observed in cultivated oats. Relatively low genetic differentiation may result both from the specificity of the species and the fact

that the investigated material was acquired from a gene bank. Thus, the loss of diversity may have resulted from a genetic drift that may have been caused by insufficient seed collection during the gene bank expedition, or the loss of alleles during the reproduction and regeneration of the gene bank accessions. Genetic diversity could also be lost due to the deterioration of seed viability during ex situ storage. In the absence of comparative material from natural sites, it is difficult to determine whether, and if so, to what extent, genetic diversity has been lost. According to Baum and Rajhathy [2], in genus *Avena*, a number of species seem to have a relict endemic distribution limited to isolated places in high elevations or on islands. As mentioned in the introduction *A. macrostachya* is an endemic species, occurring only in the Atlas Mountains. Populations of endemic species are more vulnerable to genetic drift and reduction of diversity [47]. Since the *A. macrostachya* sites occur in the mountains at an altitude exceeding 1300 m above sea level, natural selection related to the severe climate should also be considered as a diversity reducing factor. Furthermore, it seems to have a disjunct distribution (Aures and Djurdjura), which can be looked at as an intermediate stage of relict endemism in a process towards extinction. In the future, some of these small populations may become extinct, resulting in relict endemism in advanced stages of this reduction process. Thus, *A. macrostachya* appears to be one of the morphologically and geographically most isolated species in *Avena* and it probably originated from a very early branching off of the primitive *Avena* stock [2]. However, field studies in the Djurdjura showed that the partially isolated *A. macrostachya* populations were relatively numerous, so the low degree of variation was not caused by the bottleneck effect [1].

4.3. Population Structure

Knowledge about the population structure and diversity distribution, i.e., whether it is located within or between populations of the tested species, is important for preserving its genetic diversity [48]. In the study, AMOVA revealed no significant differentiation between the accessions ($\Phi PT = 0.094$). Uneven distribution of genetic variation towards the intra-population one is typical for wind-pollination outcrossing species because this type of breeding system and pollination mechanisms promotes heterozygosity of the individuals [49–53]. The analysis of the genetic structure showed that there is no clear distinction between the accessions representing natural populations. The gene pools permeated each other, indicating some gene flow between the populations. The gene flow between populations is most probably caused by the pollination mechanism. Pollen transported with the wind can reach long distances, eliminating the subdivisions [47]. As is generally known, even a small gene flow across distant populations leads to atrophy of their differentiation [54,55]. Another possible factor that increases gene flow is the transmission of grains by animals. Cattle pasturing was allowed in the Djurdjura National Park so that the seeds could be transported on cattle hair [1]. Descriptions of sites in the Djurdjura National Park made by Guarino et al. in 1988 [1] indicated the continuity of *A. macrostachya* in this region. The lack of a definite fragmentation of sites in a short time before seed collection to the Polish Gene Bank seems to have the greatest impact on the absence of a distinctive population structure.

5. Conclusions

To summarize, the research presented in this paper for the first time describes genetic diversity within the *A. macrostachya* species. Relatively low diversity value may be a consequence of both the endemic character of the species and the genetic drift during collection and preservation in the gene bank. No clear genetic structure has been found within the examined collection, which indicates that the gene flow between the populations in the Djurdjura National Park has been continuously maintained in the late 1980s.

The importance of this research is underlined by the applicability of *A. macrostachya* in common oat breeding and the uniqueness of the studied collection. It is extremely important to re-establish the expedition in order to identify the changes that have occurred at natural sites. The molecular study of *A. macrostachya* confirmed the genetic uniformity of the species as already evidenced by

morphological uniformity Genetic analysis of the currently occurring specimens is necessary to determine the effectiveness of in situ an ex situ conservation of this species. Considering the endemism of the species, its breeding potential, and the small-scale ex situ collection, careful monitoring of natural sites and repeating of the collection mission is therefore absolutely crucial.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/12/1984/s1, Table S1: The resources of accessions of *Avena macrostachya* in ex situ living collections and herbaria.

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