

Structural patterns and genetic diversity among oat (*Avena*) landraces assessed by microsatellite markers and morphological analysis

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Abstract As a counterweight to genetic erosion, landraces could become important germplasm resources for breeding. In the present study the genetic diversity of 62 Greek and Eastern European oat accessions (seven cultivars and 55 common and red oat landraces collected from 1904 to 1960) was studied using simple sequence repeats and 31 morphological descriptors. High levels of polymorphism were detected and 209 distinctive alleles were identified; on average 14.65 alleles per primer. Both, the principal component analysis derived from the morphological data, and the unweighted pair group method with arithmetic mean from the molecular analysis clustered the oat genotypes according to their type (common and red oats). In addition, the analysis of molecular variation showed that there were noteworthy differences within designated geographic regions. Moreover, a more discrete subpopulation structure was identified based on allele frequency

using a Bayesian clustering approach. A clear distinction was evident among the red and common oats suggesting that they should be considered as separate species.

Keywords *Avena. byzantina* · *A. sativa* · Morphological traits · Simple sequence repeats (SSR)

Introduction

Oat is a western Mediterranean cereal (Loskutov 2008) with a fairly recent agricultural history, since its cultivation began a few millennia later than the one of wheat and barley, and archaeological records documented oats—along with rye—as weed contaminants (Newton et al. 2010). In more recent years, this grain was mainly used as hay or silage; but its' main utilization has been as a livestock feed. Nowadays, oat is considered as one of the most important cereal crops in the world (being widely used for human consumption as a source of valuable nutrients; Boczkowska and Tarczyk 2013) and has received considerable attention with respect to collection and conservation.

More than 200,000 accessions of wild and cultivated *Avena* species are stored in seed gene banks worldwide (Diederichsen 2008). Landraces in particular, made a significant contribution to the current oat germplasm, since several modern varieties trace their origin back to a restricted number of them. Different

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definitions for landraces have been given through time, the most comprehensive being “landrace is a highly diverse population or mixture of genotypes naturally developed in a certain region under the influence of the regionally prevailing conditions of climate, soil and management, without or with only little mass selection” (Banga 1944). In contemporary breeding approaches, it is of great importance to preserve, characterize and utilize plant material that can provide useful genes in the genetic pool of the cultivated oats. A large number of oat cultivars was derived from individual selections from landraces, or crosses involving these selections. For instance, more than 100 cultivars were developed worldwide from ‘Red Rustproof’ oat landrace (Coffman 1977).

However, as a result to monoculture and pure lines plant breeding, landraces were widely replaced by modern cultivars in farming (Hammer et al. 2003). Furthermore, each modern cultivar presents a fraction of the genetic diversity comprised in the source population or the species (Wesenberg et al. 1992); hence the oat germplasm is suffering an ongoing genetic erosion (Loskutov 1998; Fu et al. 2003). Since the loss of genetic diversity has unpredictable consequences, a continuing effort is required to conserve, characterize and maintain wild populations, old landraces, and *Avena* populations in general.

The goal of the current study was to uniquely fingerprint and examine the genetic diversity existing among mostly Greek oat landraces using simple sequence repeats (SSR) molecular markers and morphological agronomic traits. The genetic structure of this germplasm collection was studied using a model-based Bayesian clustering method to assign genotypes to distinct gene pools. This study also offers vital baseline information for the association of Greece’s locally preserved germplasm with landraces of broader Eastern European origin, for the first time in literature.

Materials and methods

Plant material

A total of 55 *Avena* landraces originally collected from 1904 to 1960 (acquired from the USDA, Vavilov Institute of Plant Genetic Resources-VIR and the Agricultural Research Institute of Cyprus-ARI), plus

seven commercial cultivars were used in the current study (Table 1).

Morphology analysis

The entries were characterised at the experimental fields of NAGREF (Cereals Institute, Thermi-Thessaloniki, Greece) in unreplicated field plots. Seeding for the evaluation was performed in autumn (mid-November 2013) and harvesting in summer (mid-June 2014). Climate conditions are reported in Online Resource 1. Thirty-one morphological primary descriptors—according to the ECPGR *Avena* descriptors (IBPGR 1985)—were recorded from at least 30 plants within each entry (Online Resource 2). SPSS Statistics v 20 (IBM) was used to construct a principal component analysis (PCA) plot from the combined morphological descriptors.

DNA extraction and PCR amplification

Ten leaves from 15-day-old individual seedlings were harvested and DNA was extracted using the NucleoSpin[®] kit (Macherey–Nagel GmbH & Co, Düren, Germany) following the manufacturer’s instructions. DNA concentration was determined spectrometrically and its quality was established by agarose gel electrophoresis. After an initial screening, ten carboxyfluorescein (FAM) labeled SSR primer pairs derived from an oat genomic library (Li et al. 2000) were chosen because of their consistency in amplification and polymorphism in the oat panel (Table 2).

Amplification reactions were set up in a 10 μ L volume of a mixture containing 25 ng of genomic DNA, 1x Type-it[®] Multiplex PCR Master Mix (Qiagen, Venlo, Limburg, Netherlands) and 0.1 μ M of each primer. PCR amplification was performed in a Bio-Rad PTC-200 thermocycler (Applied Biosystems, Foster City, CA, USA) under the following temperature profile: 5 min at 95 °C followed by 35 cycles, each one included 30 s at 95 °C, 90 s at 57 °C, 30 s at 72 °C and a final extension for 30 min at 60 °C. SSR markers were analyzed on an ABI 3130 genetic analyzer. Size standard GeneScan 500 LIZ[™] (Applied Biosystems, Foster City, CA, USA) was included with each sample to define allele sizes. Data were analyzed using GeneMapper[®] (Applied Biosystems, Foster City, CA, USA).

Table 1 Accession number and origin of oat genotypes used in the current study

Number	Accession	Species ^a	Improvement status	Origin
1	Clav 357	<i>A. sativa</i> L.	Landrace	GR
2	PI 258566	<i>A. byzantina</i> C. Koch	Landrace	GR
3	PI 258579	<i>A. byzantina</i> C. Koch	Landrace	GR
4	PI 258580	<i>A. byzantina</i> C. Koch	Landrace	GR
5	PI 258584	<i>A. byzantina</i> C. Koch	Landrace	GR
6	PI 258585	<i>A. byzantina</i> C. Koch	Landrace	GR
7	PI 264838	<i>A. sativa</i> L.	Landrace	GR
8	PI 264839	<i>A. sativa</i> L.	Landrace	GR
9	PI 264840	<i>A. sativa</i> L.	Landrace	GR
10	PI 264841	<i>A. sativa</i> L.	Landrace	GR
11	PI 264842	<i>A. sativa</i> L.	Landrace	GR
12	PI 264843	<i>A. sativa</i> L.	Landrace	GR
13	PI 264844	<i>A. sativa</i> L.	Landrace	GR
14	PI 264845	<i>A. sativa</i> L.	Landrace	GR
15	PI 264846	<i>A. sativa</i> L.	Landrace	GR
16	PI 264847	<i>A. sativa</i> L.	Landrace	GR
17	PI 264848	<i>A. sativa</i> L.	Landrace	GR
18	PI 264849	<i>A. sativa</i> L.	Landrace	GR
19	PI 264850	<i>A. sativa</i> L.	Landrace	GR
20	PI 264851	<i>A. sativa</i> L.	Landrace	GR
21	PI 264854	<i>A. sativa</i> L.	Landrace	GR
22	PI 264862	<i>A. sativa</i> L.	Landrace	GR
23	PI 264863	<i>A. sativa</i> L.	Landrace	GR
24	NLD037 3693	<i>A. sativa</i> L.	Landrace	TR
25	NLD037 3694	<i>A. sativa</i> L.	Landrace	TR
26	NLD037 3695	<i>A. sativa</i> L.	Landrace	TR
27	CZE047 03C070203	<i>A. sativa</i> L.	Landrace	UKR
28	CZE047 03C070206	<i>A. sativa</i> L.	Landrace	UKR
29	CZE047 03C070359	<i>A. sativa</i> L.	Landrace	ROM
30	CZE047 03C070360	<i>A. sativa</i> L.	Landrace	ROM
31	CZE047 03C070417	<i>A. sativa</i> L.	Landrace	BLG
32	CZE047 03C070420	<i>A. sativa</i> L.	Landrace	BLG
33	RUS00110119	<i>A. sativa</i> L. var. <i>mutica</i> Alef.	Landrace	ALB
34	RUS00110003	<i>A. sativa</i> L. var. <i>aurea</i> Körn., <i>mutica</i> Alef.	Landrace	BLG
35	RUS00110109	<i>A. byzantina</i> C. Koch, <i>A. sativa</i> L.	Landrace	GR
36	RUS00110201	<i>A. byzantina</i> C. Koch	Landrace	GR
37	RUS00110211	<i>A. sativa</i> L. var. <i>mutica</i> Alef.	Landrace	ALB
38	RUS00110217	<i>A. sativa</i> L. var. <i>mutica</i> Alef.	Landrace	YUG
39	RUS00110218	<i>A. sativa</i> L. var. <i>mutica</i> Alef.	Landrace	GR
40	RUS00110220	<i>A. sativa</i> L. var. <i>mutica</i> Alef.	Landrace	GR
41	RUS00111516	<i>A. sativa</i> L. <i>A. byzantina</i> C. Koch	Landrace	GR
42	RUS00111655	<i>A. sativa</i> L. var. <i>inermis</i> Körn., <i>chinensis</i> Fisch.	Landrace	GR
43	RUS00110117	<i>A. byzantina</i> C. Koch	Landrace	GR
44	RUS00110528	<i>A. sativa</i> L. var. <i>aurea</i> Körn., <i>mutica</i> Alef.	Landrace	ROM

Table 1 continued

Number	Accession	Species ^a	Improvement status	Origin
45	RCAT011665	<i>A. sativa</i> L.	Landrace	ROM
46	RCAT012905	<i>A. sativa</i> L.	Landrace	USSR
47	RCAT013285	<i>A. sativa</i> L.	Landrace	ROM
48	RCAT011476	<i>A. sativa</i> L.	Landrace	ROM
49	RCAT011637	<i>A. sativa</i> L.	Landrace	BLG
50	RCAT011639	<i>A. sativa</i> L.	Landrace	BLG
51	Flega	<i>A. sativa</i> L.	Cultivar	GR
52	Kassandra	<i>A. byzantina</i> C. Koch	Cultivar	GR
53	Firth	<i>A. sativa</i> L.	Cultivar	DEU
54	Wistar	<i>A. sativa</i> L.	Cultivar	DEU
55	Lutz	<i>A. sativa</i> L.	Cultivar	DEU
56	Fuchs	<i>A. sativa</i> L.	Cultivar	DEU
57	Freddy	<i>A. sativa</i> L.	Cultivar	DEU
58	ARI 1-1	<i>A. sativa</i> L.	Landrace	CY
59	ARI 1-5	<i>A. sativa</i> L.	Landrace	CY
60	ARI 3-7	<i>A. sativa</i> L.	Landrace	CY
61	ARI 6-2	<i>A. sativa</i> L.	Landrace	CY
62	ARI 6-9	<i>A. sativa</i> L.	Landrace	CY

^a Species identification is according to donor genebank notation

Table 2 Characteristics of microsatellite markers selected for use in the study

	SSR marker	Repeat motif	Size (bp)	Number of alleles	<i>Dj</i>
1.	AM01	(AG) ₂₁ (CAGAG) ₆	156–219	25	0.97
2.	AM03	(AG) ₃₅	246–331	39	0.98
3.	AM04	(AG) ₃₄	113–172	37	0.99
4.	AM06	(AG) ₂₀	176–237	24	0.98
5.	AM14	(AC) ₂₁	109–183	15	0.90
6.	AM30	(GAA) ₁₄	178–231	25	0.98
7.	AM31	(GAA) ₂₃	132–198	21	0.95
8.	AM38	(GAA) ₉	153–157	3	0.29
9.	AM42	(GAA) ₁₆	143–205	16	0.88
10.	AM102	(AC) ₉	204–219	4	0.40
	Mean			20.9	0.83

Data analysis

Allele fragments were converted into a binary data matrix table. FreeTree (Pavlicek et al. 1999) was used to calculate the genetic similarities among taxa and for the construction of the bootstrapped dendrogram, which was depicted with the implementation of TreeView (<http://taxonomy.zoology.gla.ac.uk/rod/treeview.html>). The level of primer informativeness was estimated by means of the discriminating power (*Dj*), as described by Tessier et al. (1999). Genotypic

variations were assessed across various populations by means of analysis of molecular variance (AMOVA) using GenAlEx 6.4 (Peakall and Smouse 2006). The significance of the resulting variance components and the inter-population genetic distances were tested using 999 random permutations. Mantel test was conducted using the XLSTAT statistical software (Addinsoft), in order to test the goodness of fit between morphological and molecular markers.

Bayesian model-based clustering approach to identify the genetic structure in the oat germplasm was

performed using Structure 2.3.4 (Pritchard et al. 2000). The structure algorithm was run using the admixture model, with 10 independent replicate runs per K value (number of clusters) ranging from 1 to 10. Each run involved a burning period of 100,000 iterations and a post burning simulation length of 100,000. Validation of the most likely number of clusters K was performed with the Structure Harvester (<http://taylor0.biology.ucla.edu/structureHarvester>).

Results

Diversity and morphological characters

Differences were recorded between oat accessions for most of the descriptors (Online Resource 2). Among the agronomic descriptors, plant height was a variable trait that ranged from 105 (PI 264854) to 160 cm (RCAT013285), with an average of 131.33 cm. Variations were also recorded for leaf length (34.33 cm for PI 258580 vs 53.97 cm for RCAT012905, with an average of 44.12 cm) and leaf width (1.5 cm for PI 258580 vs 3.17 cm for NLD037 3694, with an average of 2.19 cm). Days to heading ranged from 161 days (PI 258580 and PI 264838) to 179 days (RUS00110528). Finally, the size of grain (weight of 1000 seeds) was also a variable character, since it fluctuated from 13 (RUS00111655) to 37 g (PI 264845).

Growth habit was rather a uniform trait since the majority of landraces (82 %) had a semi prostrate growth habit; seven Greek landraces had a prostrate growth habit and only two Greek landraces (CZE047 03C070360 and CZE047 03C070417) had an erect growth habit. Stem thickness was rather constantly thin and intermediate thin, since only five eastern European landraces (NLD037 3694, CZE047 03C070203, CZE047 03C070206, CZE047 03C070359 and RUS00110528) had a thick stem. Strikingly, only one genotype (RUS00111655) was found to have a unilateral panicle, naked grain and four fertile spikelets per floret. Finally, all landraces had low shedding at maturity (Online Resource 2).

Thirty-one scored attributes were combined in order to produce a PCA plot. The plot analysis classified the oat genotypes into two groups that mainly reflected their geographical origin (Greek and Eastern European) and their type (common—red oats;

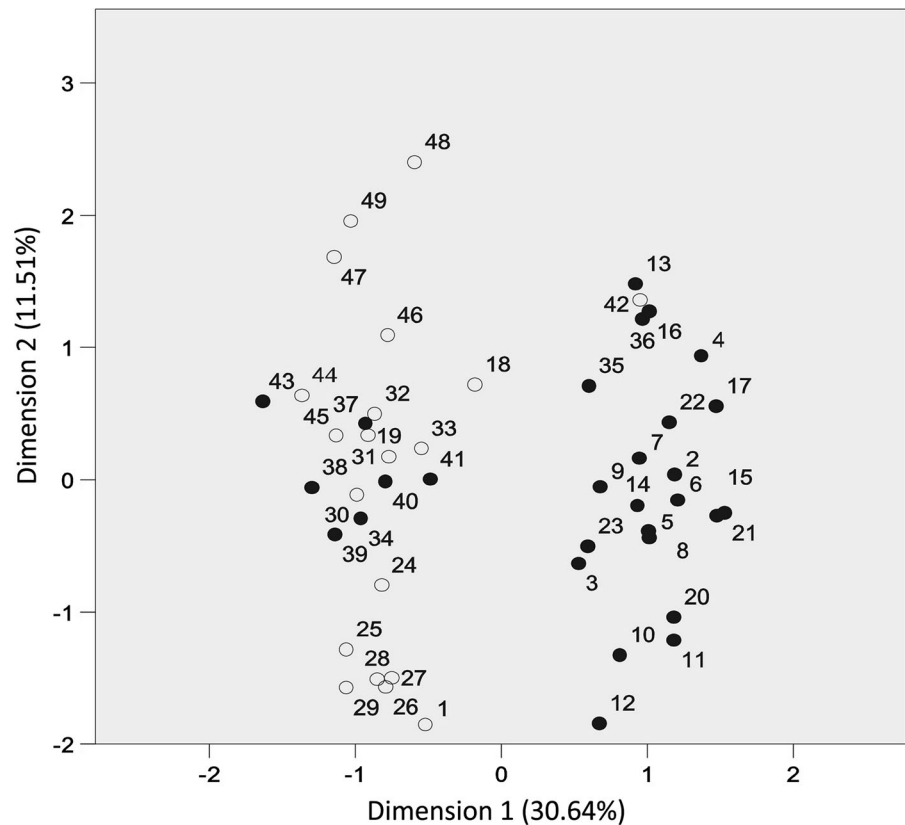
Fig. 1). Furthermore, it was detected that certain Greek landraces were more discrete from the core of the groups, having a greater heterogeneity.

Genetic diversity of oat landraces and cultivars

The SSR primers used to assess the genetic information in the oat collection, revealed a high level of diversity in landraces displaying a total of 209 alleles, with an average of 14.65 alleles per primer. The majority of alleles were polymorphic, since only five of them were common among all genotypes. The total number of alleles per marker varied from three (primer AM38) to 39 (primer AM3; Table 2), while 91 different electrophoretic patterns were detected. Furthermore, 49 alleles were unique, while 105 alleles had a frequency lower than 0.05. The discrimination power (D_j) varied from 0.29 (primer AM38) to 0.99 (primer AM04) with a mean of 0.83, illustrating that this primer set was very informative.

Genetic similarity calculated among the oat collection varied from 0.36 (landraces PI 258580 and NLD037 3695) to a maximum of 0.91 (landraces NLD037 3693 and NLD037 3695, as well as, landraces ARI 3-7 and ARI 6-2) with a mean similarity of 0.56. The UPGMA dendrogram classified the 62 *Avena* genotypes into two main clusters (100 % bootstrap value) according to their origin (Fig. 2). The first group which was the largest, contained all the oat landraces of Eastern European origin, a few Greek landraces and the majority of the commercial cultivars. Moderate to high bootstrapped values were detected among the oat landraces, while the highest genetic affinity was detected among two genotypes from Turkey (NLD037 3693 and NLD037 3695). Interestingly, the Greek landraces and variety ‘Flega’ that were clustered in this group, had low bootstrap supporting values. The second group included the majority of Greek, all the Cypriot landraces and the commercial cultivar ‘Kassandra’. The UPGMA clustering carried out for the SSR data showed a noteworthy within group difference among geographic regions of Greece and Cyprus, well supported by significant bootstrap values (Fig. 2). The highest affinity within the group was detected among the Greek landraces PI 264840 and PI 264843. Correlation coefficient among the morphological assessments and the molecular data was significant ($r = 0.51, p < 0.001$).

Fig. 1 Plot of PCA analysis of the combined 31 morphological data. *Black encircled*, are the Greek landraces. Numbers correspond to accessions shown in Table 1



Genetic structure of oat landraces and cultivars

The pattern of structure and the allocation of genetic diversity were additionally analyzed with a Bayesian-based approach implemented in the Structure 2.3.4 software (Pritchard et al. 2000). We investigated the range from $K = 1$ to $K = 10$ and calculated the posterior probability for each value of K using the estimated log likelihood of K . The obvious optimum for the ad hoc quantity based on the second order rate of change of the likelihood function with respect to ΔK was observed for $K = 2$ (Fig. 3). As a result, the entire oat accessions (four regional populations) were successfully assigned to two groups ($K = 2$, $\Delta K = 4.15$); inferred clusters were calculated with more than 90 % probability intervals. In addition, a minor peak at $K = 4$ ($\Delta K = 1.09$) was identified (data not shown). Structure analysis divided the oat genotypes into two groups.

The first population (Greek genotypes) was assigned to group I with a 0.764 proportion of membership. The 32 accessions of Greek origin

contained several fairly admixed genotypes. Even though the majority of these entries had a group I (red) membership value higher than 0.9, there were some landraces that had admixed genotypes and were assigned to group II (green; landraces PI 264849, PI 264850, RUS00110218, RUS00110220, RUS00111516, RUS00111655 and ‘Flega’). Cypriot accessions (five landraces) were also clustered in the first group, having a 0.968 proportion of membership. Furthermore, this population contained five rather genetically homogenous genotypes, since the only entry having a membership value lower than 0.9 (ARI 1–5). All Eastern European oats were assigned to group II with a 0.978 % of membership. Finally, the fourth population contained the remaining five commercial varieties (‘Firth’, ‘Wistar’, ‘Lutz’, ‘Fuchs’ and ‘Freddy’) that were assigned to group II having a 0.879 proportion of membership. Bayesian clustering and admixture analysis assisted the quantification of associations among oat landraces, according to their origin and revealed the presence of some sort of hierarchical structure in our entries. Furthermore,

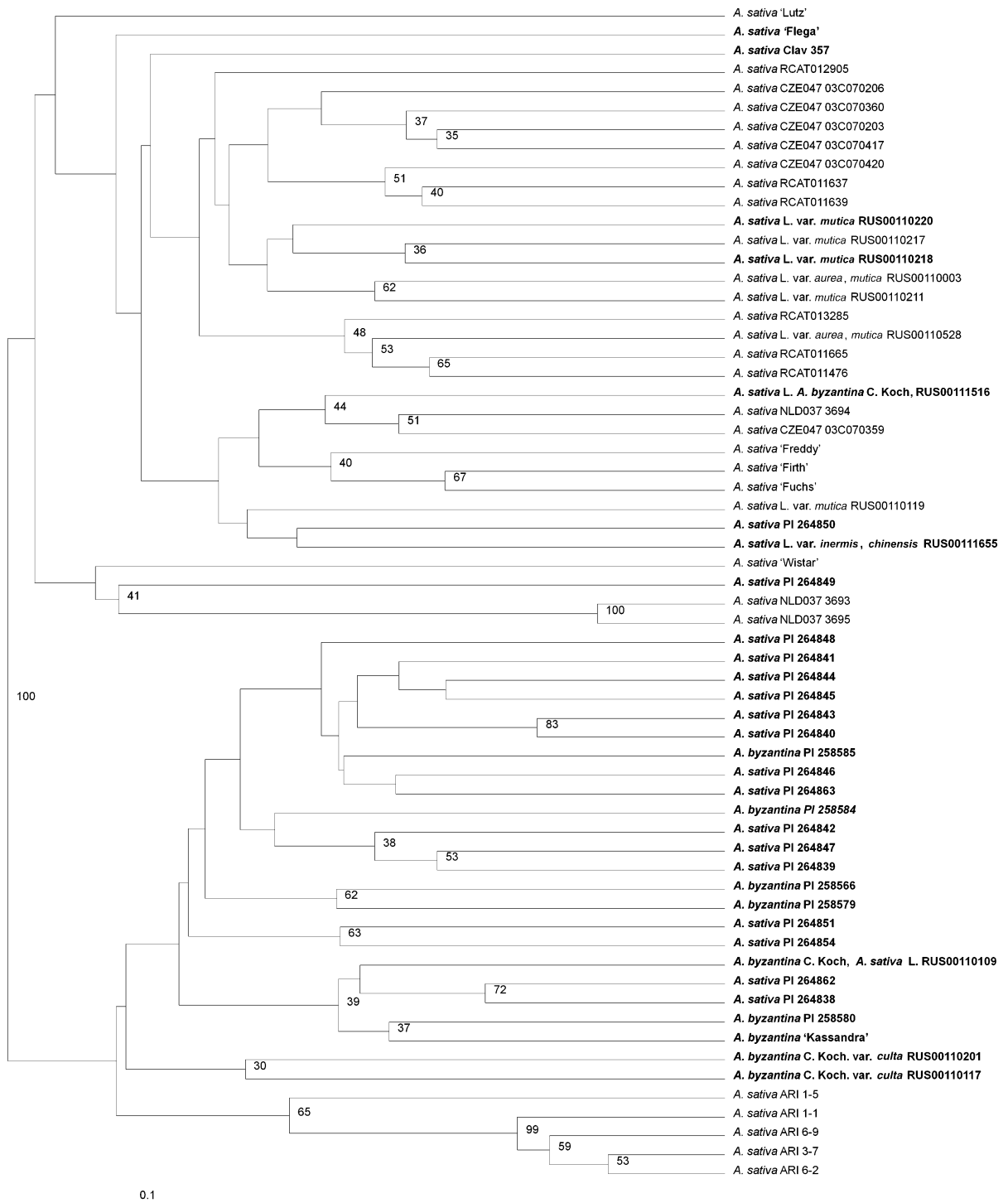


Fig. 2 Bootstrapped UPGMA dendrogram of 62 oat accessions based on Dice genetic similarity (calculated using SSR data). Values greater than 30 are presented. **Bold numbering** indicates the Greek landraces

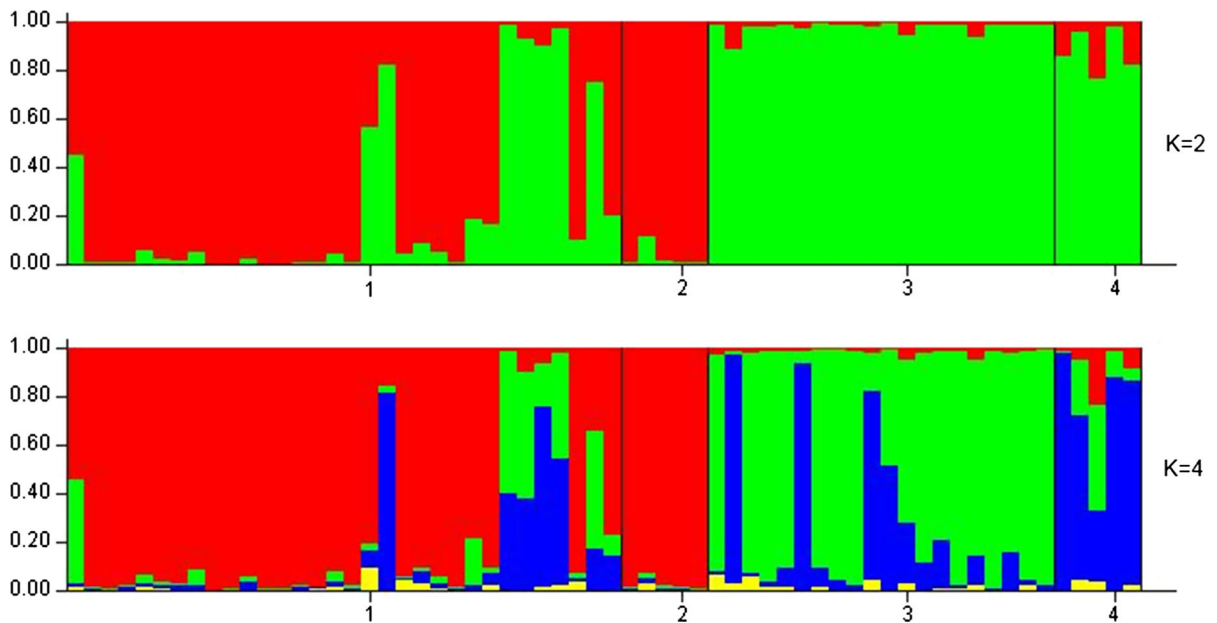


Fig. 3 Bayesian cluster analysis of the optimum K cluster. 1: Greek landraces, 2: Cypriot landraces, 3: Eastern European landraces and 4: commercial cultivars. Percentages of genotype

membership to clusters as inferred for $K = 2$ and $K = 4$. The color in each bar plot represents the probability of each individual belonging to a given group

structure analysis for $K = 4$ permitted us a better understanding of a more detailed genetic association, since it became evident that the commercial varieties were affiliated to specific Greek and Eastern European landraces (Fig. 3).

Analysis of molecular variance revealed that a high proportion of the total genetic diversity (78 %) was allocated within the four populations. The highest variability was recorded for the Greek genotypes ($SS = 439.125$), followed by Eastern European landraces ($SS = 266.750$) and commercial cultivars ($SS = 52.800$). Furthermore, it was also revealed that the genetic distance between clusters was significant ($F_{st} = 0.219$; $p = 0.010$). F_{st} values suggested the presence of divergence between populations. The closest genetic proximity was found among the Eastern European and commercial cultivars ($F_{st} = 0.138$; $p = 0.001$) and the more diverged populations were the Cypriot and the commercial cultivars ($F_{st} = 0.415$; $p = 0.005$).

Discussion

Agronomic traits and morphological analysis has been repeatedly used when characterizing large data sets of

Avena taxa in genebanks (Diederichsen 2008, 2009), or smaller sets of oat species, cultivars and landraces (Souza and Sorrells 1991; Sheikhepour et al. 2014; Boczkowska et al. 2014). As a result, morphological description has become a valuable source of information for breeding and agronomic research programs (Boczkowska et al. 2014). In the current study and in order to study the genetic diversity in our oat collection, 31 morphological traits were scored. It was detected that the resulting grouping reflected the principal area of origin; since Greek landraces were typically clustered separately from Eastern European oat landraces.

Eventhough morphological traits are generally employed in order to estimate genetic variation since their measurements are not laborious, still, diversity estimation based on morphology alone has limitations. Unfortunately, traits are heavily influenced by the environment, are limited in number and possibly unintentional selection for traits with agronomical value (i.e. selection for lightly colored seeds etc.) has further reduced them. As a result, oats germplasm has also been studied by a number of molecular markers types; for example AFLP (Fu et al. 2004, 2005), ISSR (Boczkowska and Tarczyk 2013) RAPD (Baohong et al. 2003), SSR (Li et al. 2000, 2007; Fu et al. 2003; Nersting

et al. 2006; Montilla-Bascón et al. 2013), or with an array of combined molecular techniques (He and Bjørnstad 2012; Boczkowska et al. 2014). In the current study, microsatellites were employed to estimate the genetic diversity in the oat collection, since they can be used to examine the allelic diversity changes and could be linked in the future to genomic regions that control diverse traits; thus enabling efficient and precise transfer of useful alleles from landraces to modern cultivars (Montilla-Bascón et al. 2013).

The molecular marker genetic analysis of the oat collection revealed a high level of variability among accessions. Moreover, this diversity was additionally apparent within the common oat (*A. sativa* L.) landrace germplasm that exhibited a total of 35 unique alleles, followed by the red oats (*A. byzantina* C. Koch), and the commercial cultivars, with eight and six unique alleles, respectively. Twenty three common oat landraces had unique alleles (ten of them were Greek), while three out of five Cypriot genotypes also contained unique alleles; something that underlines the distinctiveness of this germplasm. Finally, five Greek *A. byzantina* landraces (out of 15) had unique alleles. Montilla-Bascón et al. (2013) inquired the genetic diversity and the population structure among oat cultivars and reported that genetic variability was more evident in the common oat germplasm. Nonetheless, Diederichsen (2008) reported that even though red oat (*byzantina* type) accessions account for the 11.6 % of the PGRC oat collection still they covered the 34.7 % of all morphological groups. This means, that red oats are much richer in variety, since they comprise a great proportion of morphological groups. This is an indication that a significant conservation of genetic variation exists in landraces and thus offering opportunities for oat breeding.

Unfortunately, to date the vast genetic diversity of oats has not been fully utilized. Even though 564 *A. byzantina* landraces are catalogued in the EURISCO database (<http://eurisco.ipk-gatersleben.de/>), only 203 red oat cultivars have been developed. On the contrary, 1756 *A. sativa* landraces occur and 4464 common oat cultivars have been produced respectively. This has a major impact on the genetic erosion of the species. Fu et al. (2003) proved that modern plant breeding reduces genetic diversity by studying oat cultivars released from 1886 to 2001. They concluded that a significant decrease of alleles was evident in cultivars bred after 1970, with severe consequences to pest and disease resistance. In

that term, the genetic material characterized in the current study has a great significance; since several cultivars were bred from its genetic pool. For instance, the Greek landrace CIav 357 collected from Trace (Greece) in 1904 (<http://www.ars-grin.gov/>), was used for the selection of ‘Markton’, a globally milestone variety, that further produced numerous smut and rust resistant oats varieties.

In the present study it was detected from both morphological (Fig. 1) and molecular data (Fig. 2) that there is a clear disparity among common and red oat landraces, as well as, a clear geographical clustering, which is in agreement with previous studies (Odonoughue et al. 1994; Fu et al. 2005; Newell et al. 2011; Montilla-Bascón et al. 2013). *Avena sativa* cultivars differed substantially from the *A. sativa* landraces, and, for $K = 4$, they emerged as a separate group (Fig. 3). However, the red oat cultivar (‘Kassandra’) was clustered with the red oat landraces, signifying either lesser improvement of the red oat (*A. byzantina*) cultivars in contrast to common oats (*A. sativa*), or due to the more ‘active’ participation of red oat landraces in its lineage (Montilla-Bascón et al. 2013). Furthermore, it was established that the primary region of landraces, correlates to the degree of genetic affinity. This has also been reported from Diederichsen (2008) that concluded a strong positive correlation between the number of accessions originating from a given country and the number of morphological groups found.

Data support the assumption that common and red oats were domesticated independently of each other (Zohary and Hopf 2000). Loskutov (2008) proposed that *A. byzantina* had a different evolution history than *A. sativa*. It was suggested that the big-seeded *A. sterilis* L. endured modifications in the mode of floret dispersal and led to the establishment of the cultivated form of *A. byzantina*, while *A. sativa* was evolved from the smaller-seeded forms of *A. ludoviciana* Durieu (Loskutov and Rines 2011). Furthermore, the red oats are correlated to the Mediterranean climate and are winter annuals, opposed to common oats that are grown as spring annuals at temperate-climate geographical zones (Coffman 1961). However, the biological concept of oats contradicts to the taxonomy of the *Avena* species. Rodionova et al. (1994) identified 32 *A. sativa* and 17 *A. byzantina* botanical varieties and additionally distinct 19 botanical forms; summing up to 62 morphologically distinguishable taxonomical types within the cultivated

hexaploid oat (Diederichsen 2008). Danert (1972) categorized 19 botanical varieties of *A. sativa* and considered *A. byzantina* as a distinct species. On the contrary, Baum (1977) suggested that *A. byzantina* is not as a separate *Avena* species and it should be regarded as *A. sativa*. This has caused great nomenclature confusion, especially among different genebanks and thus it is realistic to accept that effective communication regarding oat genetic resources is far from accomplished when it comes to formal taxonomical names (Diederichsen 2004).

Furthermore, to make matters even more complicated, the mutual presence of both *Avena* types in the same regions over the centuries, has partially homogenized their genetic background. Especially in the northern region of Greece, where both the common hexaploid and red oat species overlap, such an admixture is expected. As a consequence, some Greek landraces were clustered among accessions of the Balkan region, while certain Southern Greek and Cypriot *A. sativa* accessions were grouped with the red oat landraces. This was also reported by Montilla-Bascón et al. (2013), that noted that some landraces described as common oats were grouped with red oat accessions and shared a specific *A. byzantina* allele. As a result, it was concluded that geographic proximity of these common oat accessions with their most related red oat landraces resulted from a potential cross among the landraces.

Interestingly, all Greek common oat landraces that were donated from VIR (RUS00110218, RUS00110220, RUS00111516 and RUS00111655) were clustered with the Eastern European *A. sativa* landraces. This may be attributed to the substantial germplasm exchange among different regions of origin that escalated during the last 150 years and has made it doubtlessly challenging to discriminate accessions between geographical origin and certain character states (Diederichsen 2008). Thus, the current diversity and genetic markup of *Avena* landraces maintained, may differ from the endemic landraces collected from Vavilov in the beginning of the twentieth century (Diederichsen 2008).

Conclusion

Overall, both morphological and molecular data support noteworthy divergence among designated

geographical areas. Furthermore, a more distinct subpopulation organization was identified grounded on allele frequency using a Bayesian assembling method. A strong discrepancy was apparent among the red and common oats suggesting that they ought to be considered as distinct species.

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Conflict of interest The authors declare that they have no conflict of interest.

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