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Comparative analysis of hexaploid *Avena* species using REMAP and ISSR methods

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Abstract: Taxonomic relationships in *Avena* genus are not evident. *Avena sativa* L. might have originated either from *Avena sterilis* L. or *Avena fatua* L. Alternatively, it may have evolved independently during formation of the hexaploid form. *A. fatua* may have a different A genome than the other hexaploids. Studies performed with *Ty1-copia* retrotransposon probes demonstrated that this retrotransposon is present at low copy number in the C genome, while it is abundant in A, B, and D. The observed differences provide an opportunity for analyzing the putative origin of *A. fatua* as well as its relationships with other hexaploids. Two marker systems were applied. Retrotransposon-microsatellite amplified polymorphism (REMAP) was used to obtain evidence that the A genome of *A. fatua* has a different origin than the other hexaploids, and inter-simple sequence repeat (ISSR) was applied to screen for whole genome polymorphisms. The results tend to favor the hypothesis that *A. sterilis* could be the progenitor of *A. sativa* and *A. fatua* and showed that *A. fatua* could not have originated via cross with a maternal species having a different A genome than *A. sterilis* and *A. sativa*.

Key words: *Avena* L., hexaploid oat species, genetic similarity, ISSR, REMAP

1. Introduction

The genus *Avena* L. (Poaceae) belongs to the tribe Aveneae (Leggett, 1992) and encompasses diploid, tetraploid, and hexaploid species (Rajhathy and Thomas, 1974). Diploid species have either an A or C genome; tetraploids can have AC, CD, AB, or CC; and all hexaploids have ACD. Zeller (1998) distinguished 31 *Avena* genus species within 7 sections. Under the rules of taxonomic classification the interfertile oat hexaploids are considered biological species (Ladizinsky and Zohary, 1971). However, according to Rajhathy (1991), 4 hexaploid species could be distinguished (*A. fatua* L., *A. sterilis* L., *A. byzantina* C. Koch, and *A. sativa* L.). Moreover, following the numerical taxonomy of Baum (1977), Leggett (1992) listed 8 hexaploids with 4 additional species (*A. trichophylla* C.Koch, *A. hybrida* Peterm., *A. occidentalis* Dur., and *A. atherantha* Presl.). Zeller (1998) distinguished 7 hexaploids, assuming that *A. byzantina* was a subspecies of *A. sativa*, while in Loskutov's (2008) opinion, the genus consisted of 6 hexaploid species (*A. fatua*, *A. sterilis*, *A. byzantina*, *A. sativa*, *A. occidentalis*, and *A. ludoviciana* Dur.). Nevertheless, independent of taxonomy, the most notable representatives having species status that are recognized in the genus are *A. sativa*, *A. fatua*, and *A. sterilis*.

The most common assumption is that the hexaploid species evolved from a single hexaploid ancestor followed by gain or loss of domestication genes (Leggett, 1992). Nevertheless, there are several concepts explaining the evolution of hexaploids. According to one, all hexaploid species moved from their Asian center predominantly westwards, and *A. fatua* occupied the northern and middle latitudes, while *A. sterilis* inhabited those to the south, reaching the western borders of the Mediterranean region (Malzev, 1930). Thus, *A. sterilis* might have originated from the same Asian center as *A. fatua*, but during its expansion differentiation took place. Nor is it obvious that *A. sterilis* L. or *A. fatua* L. is an ancestor of cultivated *A. sativa*. The former represents the oldest hexaploid. Study of oat chromosome translocations and correlation of that data with the geographic distribution of various forms demonstrated a high degree of genetic relationship among *A. sativa* L. and many forms of *A. sterilis* from eastern Anatolia (Zhou et al., 1999). *A. sterilis* is considered the ancestral form of the hexaploid oats (Jellen et al., 1993) due to the presence of the largest telomeric block in the long arm of 5C chromosome. Thomas (1992) claimed that *A. sterilis* generated *fatua*-type mutations that led to the emergence of *A. fatua*, from which weedy forms

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of *A. sativa* have evolved. Ladizinsky (1988) agreed that *A. fatua* was closer to *A. sativa*, suggesting the latter as putative ancestor of the former. C-banding chromosomal patterns and distribution of the rRNA genes support the hypothesis that *A. fatua* diverged from *A. sterilis*, and the cultivated hexaploid forms, in turn, derived from *A. fatua* (Badaeva et al., 2011). Alternatively, *A. fatua* might have evolved independently of *A. sterilis* and *A. sativa* (Peng et al., 2010a). Relationship studies among *Avena* accessions representing diploid, tetraploid, and hexaploid species based on sequences of the chloroplast genome fragments (Peng et al., 2010a) allowed analysis of the evolutionary pathways. According to the authors, diploid species with A genomes could have been maternal parents of different polyploid oats. The most probable ancestor of *A. fatua* A genome was *A. damascena* and for the other hexaploids, *A. wiestii*.

Despite numerous cytological (Rajhathy and Thomas, 1974; Thomas, 1992; Jellen et al., 1993; Zhou et al., 1999) and molecular evidence (Drossou et al., 2004; Fu and Williams, 2008; Nikoloudakis et al., 2008; Peng et al., 2008, 2010a, 2010b) evolution of the genus *Avena* and species distinctiveness remain unclear. Moreover, suggestions regarding a different origin of the A genome of *A. fatua* conflict with the common assumption that the hexaploid species evolved from a single hexaploid ancestor followed by gain or loss of domestication genes (Leggett, 1992).

Analysis of *Ty1-copia* retrotransposons distribution in *Avena* demonstrated that those mobile elements were present in A, B, C, and D genomes; however, they were detected at low copy number in C genome. This presents a unique opportunity to verify whether the A genome of *A. fatua* is distinct from its *A. sativa* and *A. sterilis* counterparts. This could be accomplished if an appropriate molecular marker system directed towards retrotransposon sequence is used for taxonomic purposes. Alternatively, a marker system directed towards the simple sequence repeats that used to be randomly distributed among chromosomes (Zietkiewicz et al., 1994) could be applied due to its usability for taxonomic purposes (Achrem et al., 2014; Choudhary et al., 2014.

The primary aim of this study was to investigate the genetic relationships among hexaploid species of oat (*Avena* L.). For the analysis, out of 27 *A. sterilis* studied with ISSRs (Paczos-Grzeda et al., 2009a) and 12 genotypes of *A. fatua* of different origin evaluated with ISSRs and REMAPs (Paczos-Grzeda et al., 2009b), the most diverse were chosen. We were also interested in verifying whether *A. fatua* could have a different A genome than *A. sterilis* and *A. sativa*. To accomplish this, 2 marker platforms (REMAP, directed towards BARE-1 retrotransposon sequence, and ISSR markers reflecting the whole genome) were used.

2. Materials and methods

2.1. Plant materials

Ten *Avena* accessions representing 3 hexaploid species (*A. sativa* L., *A. fatua* L., and *A. sterilis* L.) were included in this study (Table 1). The *A. fatua* group was represented by *A.*

Table 1. Origin of *Avena* species accessions analyzed in this study.

Species	Cultivar name/ accession number	Source	Origin/producer
A. sativa L.	Bandicoot PI 573721	United States Department of Agriculture, Aberdeen, Idaho, USA	SARDI, Australia
	Mostyn CN 57959	Plant Gene Resources of Canada, Saskatoon, Canada	Welsh Plant Breeding Station, Aberystwyth, UK
	Sprinter	Plant Breeding, Strzelce, Poland	Plant Breeding, Strzelce, Poland
	Rajtar	DANKO Plant Breeding, Choryn, Poland	DANKO Plant Breeding, Choryn, Poland
A. sterilis L.	AVE 531	Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany	Italy
	CN 20321	Plant Gene Resources of Canada, Saskatoon, Canada	Syria
	PI 287211	United States Department of Agriculture, Aberdeen, Idaho, USA	Israel
A. fatua L.	AVE 2407	Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany	Libya
	PI 52190	Plant Breeding and Acclimatization Institute, Radzikow, Poland	Poland
	1963 VIR	NI Vavilov All-Russian Scientific Institute of Plant Industry, St Petersburg, Russia	Japan

fatua AVE 2407, *A. fatua* PI 52190, and *A. fatua* 1963 VIR; *A. sterilis* encompassed *A. sterilis* AVE 531, *A. sterilis* CN 20321, and *A. sterilis* PI 287211; and the *A. sativa* group was formed with cultivars 'Bandicot', 'Mostyn', 'Sprinter', and 'Rajtar' from different breeding programs.

2.2. DNA extraction

Genomic DNA was extracted from 15–20 coleoptiles from seedlings that were several days old following the CTAB procedure (Doyle and Doyle, 1987) in 2 replications.

2.3. REMAP analysis

A polymerase chain reaction was carried out according to the REMAP method described by Kalendar et al. (1999) with modifications. The PCR mixture of 15 µL contained 30 ng of template DNA, 1 U of Taq DNA polymerase in 1X PCR buffer (10 mM Tris, pH 8.8; 50 mM KCl; 0.08% Nonidet P40), 200 µM of each dNTP, 0.35 pmol of each primer, 2.2 mM $MgCl_2$, and 0.4 mM spermidine. Two types of primer were used. Primer REMAP-LTR (5' CTA GGG CAT AAT TCC AAC A 3'), directed towards 5' terminal LTR sequence Bare-1 retrotransposon and designed by Yu and Wise (2000), was combined with 20 ISSR random primers (Table 2). Amplifications were performed in a T1 Biometra thermal cycler. The cycling profile consisted of an initial denaturation step of 4 min at 94 °C followed by 35 cycles of 45 s at 94 °C, 45 s at 54 °C, and 2 min at 72 °C with a final extension of 10 min at 72 °C. Amplification products were separated on 2.5% agarose gels containing 0.1% EtBr in 1X TBE buffer (89 mM Tris-borate, 2.5 mM EDTA). DNA marker GeneRuler 100 bp Plus DNA ladder was used.

2.4. ISSR analysis

PCR amplification was performed according to the ISSR method described earlier (Zietkiewicz et al., 1994), with minor modifications. A reaction mixture of 15 µL contained 1X PCR buffer (10 mM Tris, pH 8.8; 50 mM KCl; 0.08% Nonidet P40), 160 µM *of each* dNTP, 0.35 pmol of primer (Table 2), 1.3 mM $MgCl_2$, 0.4 mM spermidine, 0.5 U of Taq DNA polymerase, and 30 ng of template DNA. Amplifications were carried out in a T1 Biometra thermal cycler with an initial denaturation step at 95 °C for 4 min, followed by 3 cycles of 30 s at 95 °C, 45 s at 54 °C, and 2 min at 72 °C; 3 cycles of 30 s at 95 °C, 45 s at 53 °C, and 2 min at 72 °C; 32 cycles of 30 s at 95 °C, 45 s at 52 °C, and 2 min at 72 °C; and a final extension step of 7 min at 72 °C. The amplification products were separated and visualized as in REMAP.

2.5. Data analysis

The amplified ISSR and REMAP fragments were scored as present (1) or absent (0) and assembled in 0/1 data matrices. Using GenAlEx software Nei's genetic distance (Nei and Li, 1979), the Shannon's information index (*I*), polymorphic information content index (*PIC*), as well as a percentage of polymorphic markers were calculated. Analysis of molecular variance (AMOVA) was evaluated by GenAlEx Excel add-in software (Peakall and Smouse, 2012). AMOVA calculations were performed using 999 permutations. The Mantel 2-tailed test was performed using Spearman correlation. P-value was estimated using 1000 permutations.

The unweighted pair group method of analysis (UPGMA) was performed in PAST software (Hammer et al., 2001) using Dice coefficients with 1000 bootstraps. Principal coordinate analysis (PCoA) was calculated by XlStat v. 2014.1.01 Excel add-in software.

3. Results

The level of DNA polymorphism among oat species (*A. sativa*, wild *A. fatua*, and *A. sterilis*) was studied using REMAP and ISSR. The most diverse genotypes were chosen for the study based on previous analyses (Paczos-Grzeda et al., 2009a, 2009b). Twenty REMAP primer pair combinations and 19 ISSR primers were used for DNA profiling, and they amplified, respectively, 535 and 280 fragments (Figure 1) (Table 2); 73.5% of REMAP and 66.8% of ISSR products were polymorphic. REMAP primer combinations produced 68 (12.7%) rare bands specific for single genotypes. ISSR primers amplified 39 (13.9%) accession-specific products. Polymorphic information content (*PIC*) values ranged from 0.28 to 0.40 for REMAP and from 0.28 to 0.44 for ISSR. Mean values of *PIC* for REMAP and ISSR were the same (0.35).

For further analyses only polymorphic markers were used. Independent of the marker system the number of unique bands was relatively low, with the lowest values for *A. fatua* group and the highest for *A. sativa* cultivars (Table 3). Similarly, percentage of polymorphic markers (*P%*), as well as Shannon polymorphic index (*I*) and *PIC* increased from *A. fatua* via *A. sterilis* towards *A. sativa*. Independent of marker system the values for Shannon index, *PIC*, and percentage of polymorphic bands were nearly identical, with slightly higher values for the ISSR marker system, and comparable to the characteristics evaluated for combined REMAP and ISSR data.

Analysis of Nei's genetic distance showed that the populations were separated by a great genetic distance. REMAP demonstrated that *A. fatua* group was 0.30 apart from *A. sterilis*. Nearly the same distance was evaluated between *A. sterilis* and *A. sativa*, while *A. fatua* was a little closer to *A. sativa* (0.26). ISSR markers also separated populations; however, in the given case genetic distance values for *A. fatua* and *A. sterilis* were the highest (0.22), and the lowest (0.18) were between *A. sativa* and *A. sterilis*. The Mantel test of the genetic distance matrices evaluated by REMAP and ISSR markers, respectively, demonstrated that they were highly correlated ($r = 0.86$, $P = 0.001$).

Table 2. Characteristics of ISSR and REMAP primers and products.

ISSR primer	Sequence 5'-3'	Range of product size (bp)	Total product number	Polymorphic products number	Accession-specific products number	*PIC*	REMAP * primers	Range of product size (bp)	Total product number	Polymorphic products number	Accession-specific products number	*PIC*
sr1	$(AG)_8G$	220–1100	12	6	0	0.39	REMAP-sr1	120–960	23	12	1	0.33
sr6	$(GT)_8C$	350–2300	17	12	2	0.33	REMAP-sr6	80–1170	18	9	2	0.37
sr11	$(AC)_8G$	340–1400	16	9	2	0.35	REMAP-sr11	140–1190	32	27	2	0.36
sr14	$(GA)_7YG$	160–1400	11	3	1	0.33	REMAP-sr14	160–1500	28	20	5	0.36
sr16	$(GA)_8C$	320–1700	16	14	5	0.28	REMAP-sr16	150–1500	33	25	4	0.35
sr17	$(GA)_8YC$	190–1500	15	7	0	0.35	REMAP-sr17	100–1500	36	24	4	0.32
sr22	$(CA)_8G$	370–1600	14	9	3	0.32	REMAP-sr22	130–1500	32	28	9	0.34
sr23	$(CA)_8GC$	250–1350	11	7	2	0.33	REMAP-sr23	95–1600	33	25	4	0.37
sr27	$(TC)_8G$	410–1600	16	14	3	0.37	REMAP-sr27	170–1500	32	29	1	0.33
sr28	$(TG)_8G$	350–1250	18	14	2	0.35	REMAP-sr28	100–1250	27	20	4	0.36
sr31	$(AG)_8YC$	380–1280	8	4	0	0.44	REMAP-sr31	200–1450	32	28	3	0.36
sr32	$(AG)_8YT$	200–1600	17	7	2	0.33	REMAP-sr32	200–1400	21	15	3	0.39
sr33	$(AG)_8T$	360–2200	19	11	3	0.39	REMAP-sr33	100–1480	22	16	2	0.36
sr34	$(TC)_8CC$	290–1650	30	27	3	0.41	REMAP-sr34	250–1070	29	23	2	0.32
sr35	$(TC)_8CG$	500–1800	6	5	2	0.30	REMAP-sr35	160–1150	21	15	8	0.31
sr36	$(AC)_8CG$	650–1650	11	7	0	0.29	REMAP-sr36	100–1180	29	22	3	0.38
sr37	$(AC)_8C$	350–2500	14	13	7	0.31	REMAP-sr37	80–1100	26	16	2	0.37
sr38	$(CT)_8G$	na	na	na	na	na	REMAP-sr38	405–1650	23	22	3	0.37
sr39	$(GA)_8GG$	230–1500	15	9	0	0.42	REMAP-sr39	250–980	12	9	1	0.28
sr40	$(AC)_8T$	360–1350	14	9	2	0.31	REMAP-sr40	100–1000	26	8	5	0.40
Total			280	187	39	-	-	-	535	393	68	-
Average per primer			14.0	9.4	1.9	0.35	-	-	26.7	19.7	3.4	0.35

* REMAP-primer sequence: 5' CTA GGG CAT AAT TCC AAC A 3'

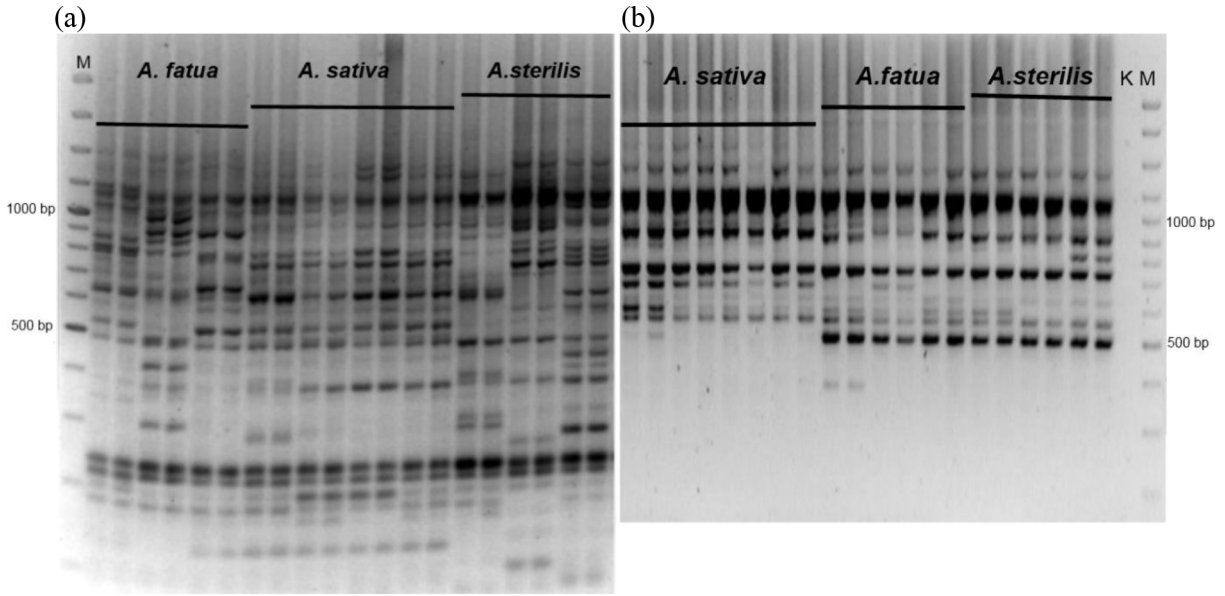

Figure 1. DNA fragments amplified with primers REMAP-LTR. SR22 (a) and ISSR primer SR22 (b).
(a) M – 100 bp Gene Ruler; 1,2 - *A. sterilis* AVE 531; 3,4 - *A. sterilis* CN 20321; 5,6 - *A. sterilis* PI 287211; 7,8 - *A. sativa* 'Bandicoot'; 9,10 - *A. sativa* 'Mostyn'; 11,12 - *A. sativa* 'Sprinter'; 13,14 - *A. sativa* 'Rajtar'; 15,16 - *A. fatua* AVE 2407; 17,18 - *A. fatua* PI 52190; 19,20 - *A. fatua* 1963 VIR.
(b) 1,2 - *A. fatua* AVE 2407; 3,4 - *A. fatua* PI 52190; 5,6 - *A. fatua* 1963 VIR; 7,8 - *A. sterilis* AVE 531; 9,10 - *A. sterilis* CN 20321; 11,12 - *A. sterilis* PI 287211; 13,14 - *A. sativa* 'Bandicoot'; 15,16 - *A. sativa* 'Mostyn'; 17,18 - *A. sativa* 'Sprinter'; 19,20 - *A. sativa* 'Rajtar'; K – negative control; M – 100 bp Gene Ruler.

Table 3. Arrangement of marker system characteristics. *I*: Shannon index; *He*: heterozygosity; *P%*: percentage of polymorphic markers; SE: standard error.

Marker system	Population	Bands	Unique bands	I (SE)	He	*P%* (SE)
REMAP	*A. fatua*	418	28	0.108 (0.01)	0.073	19.25
	A. sterilis	413	51	0.188 (0.012)	0.127	33.08
	A. sativa	419	36	0.199 (0.012)	0.135	35.14
	Total/(mean)	535	38.3	0.165 (0.007)	0.112	29.16 (4.99)
ISSR	*A. fatua*	210	13	0.097 (0.013)	0.065	19.43
	A. sterilis	219	24	0.182 (0.016)	0.122	37.65
	A. sativa	231	25	0.205 (0.017)	0.139	41.29
	Total/(mean)	280	20.7	0.161 (0.09)	0.109	32.79 (6.76)
REMAP and ISSR	*A. fatua*	628	41	0.104 (0.008)	0.070	18.5
	A. sterilis	632	75	0.186 (0.009)	0.125	33.13
	A. sativa	650	61	0.201 (0.01)	0.136	35.58
	Total/(mean)	815	59.0	0.164 (0.005)	0.11	29.08 (5.3)

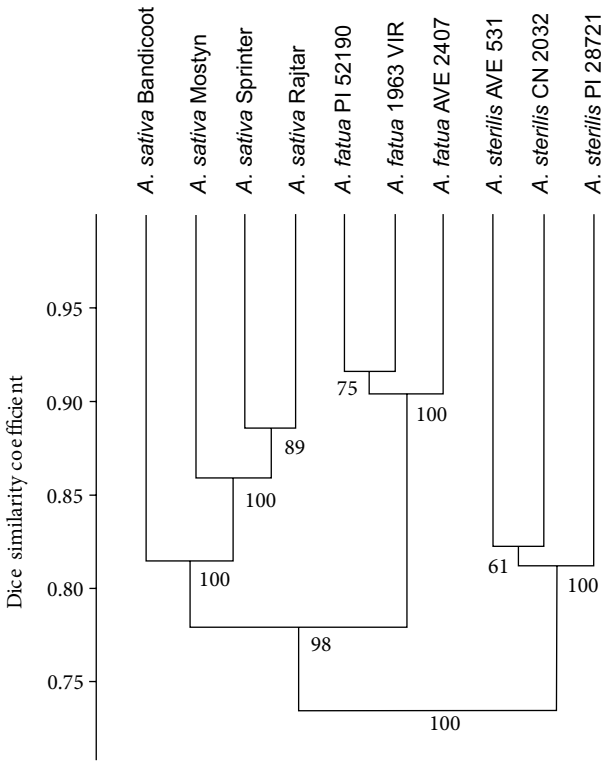

Figure 2. Cluster analysis using UPGMA and Dice genetic coefficients. Evaluation based on REMAP and ISSR markers. Bootstrap value is indicated on clods.

Analysis of molecular variance Φ_{PT} indices that could be considered analogues of the genetic distances evaluated by REMAP demonstrated that *A. fatua* samples were 0.48 apart from *A. sterilis*; however, the explained variance between *A. fatua* and *A. sativa* samples suggested they should be related (0.44). The Φ_{PT} value estimated between *A. sterilis* and *A. sativa* was the lowest (0.43). Similar studies based on ISSR markers gave comparable results with slightly lower values of Φ_{PT} indices. When markers amplified by REMAP and ISSR techniques were combined it became evident that the highest Φ_{PT} value was between *A. fatua* and *A. sterilis* (0.46) and the lowest was between *A. sterilis* and *A. sativa* (0.39). Most of the explained variance was due to intra-group variation.

UPGMA analyses based on all marker types grouped oats into 2 main clusters (Figure 2). The first encompasses *A. sterilis* species and the second, all remaining materials. The latter cluster is subdivided into *A. sativa* and *A. fatua* species. Based on bootstrap values, such differentiation is significant. Moreover, the relations among species within each cluster, as indicated on the dendrogram, are also significant. Grouping based on REMAPs and ISSRs taken separately (not shown) resulted in similar clusters.

Principal coordinate analysis based on both REMAPs and ISSRs (Figure 3) as well as either REMAPs or ISSRs (not shown) gave congruent results, confirming the cluster analyses. The 3 coordinates explained nearly 76% of variance; the *A. sterilis*, *A. fatua*, and *A. sativa* groups formed separated clouds. *A. fatua* was closer to *A. sativa* than to *A. sterilis*, and *A. sativa* group was represented by the most distinct samples.

4. Discussion

REMAPs and ISSRs have been widely used for the assessment of intraspecific and interspecific genetic relationships but rarely to analyze *Avena* L. species. For instance, ISSRs were employed to assess genetic diversity in *A. sativa* mutants (De Suoza et al., 2005) or to estimate genetic similarity of common oat cultivars (Paczos-Grzeda, 2007; Boczkowska et al., 2014) and landraces

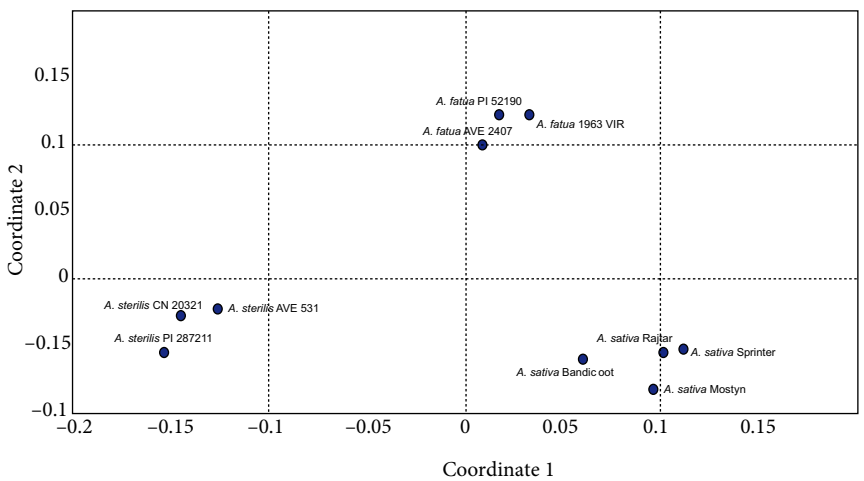

Figure 3. Principal coordinate analysis based on REMAPs and ISSRs. The first 2 coordinates explained 66.7% of variance.

(Boczkowska and Tarczyk, 2013). REMAP has been applied for the identification of oat dwarfing gene *Dw6* markers (Tanhuanpää et al., 2006) and markers associated with a gene affecting grain cadmium accumulation in oat (Tanhuanpää et al., 2007). However, the REMAP method has not been used to estimate the relationships between *Avena* L. species.

Comparing ISSR and REMAP approaches, both techniques generated reliable molecular markers for diversity studies in *Avena* species. The REMAPs and ISSRs were efficient in detecting polymorphisms in the genus *Avena*. Moreover, based on Shannon's index and *PIC* values they were informative enough to differentiate between the *A. sterilis*, *A. fatua*, and *A. sativa* species used in the current study. The percentage of polymorphisms value was higher for ISSRs than for REMAPs. Similarly, the efficiency of REMAP was a little bit lower than ISSR. This may be explained by the fact that species representing *A. fatua* are highly similar in the current experiment and in data published earlier (Paczos-Grzeda et al., 2009b). This notion is confirmed by the smaller number of unique bands amplified among *A. fatua* species independently, whether using REMAPs or ISSRs. Alternatively, the lower efficiency of REMAP compared to the ISSR marker system could be due to the BARE-1 sequence directed primer used in the approach. Utilization of such a primer should have resulted in the amplification of the *Bare*-1 oat homologue (*OARE*-1) retrotransposons. However, as it was designed based on the 5' terminal LTR sequences of barley rather than oats, fewer polymorphisms could be evaluated under stringent PCR conditions. On the other hand, the higher efficiency of the ISSR markers could be due to inter-simple sequence repeat sequences that are more variable than REMAPs and seem to be randomly distributed along the genome (Agarwal et al., 2008). Whatever the reason for these slight differences between the 2 marker systems, the results are highly correlated, demonstrating that both platforms could be used for studies in oat.

The studies of other authors (Fu and Williams, 2008; Peng et al., 2010b) demonstrated that *A. sativa*, *A. fatua*, and *A. sterilis* species are easily differentiated. Based on RAPD marker polymorphism (Chrząstek et al., 2004), genetic similarity was lowest between *A. fatua* and *A. sativa* (0.66) and highest between *A. sativa* and *A. sterilis* (0.77). Comparable data were evaluated based on AMOVA, and Φ_{PT} indexes confirmed that the differences among *A. sativa*, *A. sterilis*, and *A. fatua* groups were large and significant. Moreover, UPGMA and PCoA data demonstrate that *A. fatua* and *A. sterilis* are distinct from *A. sativa*. Dendrograms based on REMAP and ISSR markers were topologically similar and consistent with *Avena* L. genus taxonomy (Leggett, 1992). All accessions were clustered together according to their species membership. No major clustering differences were present between phenograms. Very similar clustering was present using SSR markers (Paczos-Grzeda et al., 2007) where genetic relationships between the hexaploids *A. sativa*, *A. fatua*, and *A. sterilis* were studied. Those results seem to be in agreement with the hypothesis (Loskutov, 2008) that *A. sterilis* is the progenitor of *A. sativa* and *A. fatua*. On the other hand, *A. sativa* is a cultivated form, while *A. fatua* is wild. It may be expected that wild forms exhibit higher variation than cultivated forms. However, the observed differences in *A. fatua* illustrated on the dendrograms were lower than those for *A. sativa*. This seems to contradict studies based on C-banding patterns of chromosomes and distribution of the rRNA gene families in which *A. fatua* displayed the highest intraspecific variation of the karyotype (Badaeva et al., 2011). The results also demonstrate that due to the higher diversity of *A. sterilis* it could be used as a putative source of valuable traits for *A. sativa* improvement in breeding programs, while *A. fatua* seems to be less promising for such purposes.

The reasoning behind using REMAP markers in the current study was that there is a growing body of evidence indicating that retrotransposable elements were involved in the evolution of plant genomes (Flavell et al., 1992; Yu and Wise, 2000). Moreover, *Bare-1* retrotransposons (a member of the *Ty1-copia* family), found in at least 10,000 copies in hexaploid oats (Kimura et al., 2001), prevailed in the telomeric and centromeric regions of A and B genomes but were not abundant in C genome (Katsiotis et al., 1996). Thus, utilization of the REMAP in combination with *Bare-1*–directed primer may help to differentiate *A. fatua* and other hexaploids if the species have different A genome donors. Under this assumption, REMAP markers may have helped in solving the problem of *A. fatua* evolution. Based on hierarchical analysis performed on REMAPs, the results do not to confirm the hypothesis of a different origin for A genome of *A. fatua* and the other hexaploids; however, they are congruent with the hypothesis that *A. sterilis* was the progenitor of *A. fatua* and *A. sativa*. Such a conclusion was also drawn based on ISSRs. Nevertheless, both REMAP and ISSR marker systems were not capable of identifying putative differences between A genomes in *A. fatua* and the other analyzed species.

Summing up, the results demonstrate that both ISSR and REMAP marker systems are capable of differentiating between the most recognized representatives of *Avena* genus hexaploids and indicate that *A. fatua* is closer to *A. sativa* than to *A. sterilis*. We tend to think that *A. sterilis* is a progenitor of *A. fatua*. Analysis performed based on REMAPs utilizing BARE-directed primer failed to support the hypothesis of a different origin for A genomes in *A. fatua* compared to the other hexaploids.

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