

Diversity of avenanthramide content in wild and cultivated oats

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Разнообразие содержания
авенантрамидов у культурного
и дикого овса

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Background. Oat grains accumulate substantial amounts of various phenolic compounds that possess biological activity and have a potential to considerably increase health benefits of oats as a food. Avenanthramides (AVA) is an important group of these compounds due to their antioxidant, anti-itching, anti-inflammatory, antiproliferative activities. **Materials and methods.** Using combined HPLC and LC-MS analyses, we provide the first comprehensive review of the total avenanthramide content and composition in cultivated and wild oats. The AVA content was measured in 32 wild and 120 cultivated oat accessions obtained from the global collection of the N.I. Vavilov Institute of Plant Genetic Resources (VIR), St. Petersburg, Russia. **Results and conclusion.** The wild hexaploid *A. sterilis* L. had the highest total AVA content, reaching 1825 mg kg⁻¹. Among cultivated accessions, naked oat cv. 'Numbat' (Australia) had the highest AVA content, 586 mg kg⁻¹. The AVA composition exhibited a wide diversity among the analyzed samples. Accessions were identified where AVAs A, B and C, which are generally considered as major AVA, had a low percentage, and instead other AVAs prevailed. The AVA content in eight oat cultivars revealed significant annual changes in both the total AVA content and the proportions of individual AVAs. Using HPLC analyses, 22 distinguishable peaks in AVA extracts of oat seeds were detected and quantified. Several of these peaks, which have not been previously documented, presumably represent different AVAs. Further analyses are needed to detail these findings and to determine the specific AVA structures in oat grains.

Key words: oat, cultivated and wild *Avena* spp., avenanthramides, genetic resources.

Актуальность. Овес накапливает значительные количества различных фенольных соединений, которые обладают биологической активностью и способны значительно повышать питательную значимость продуктов из овса. Авенантрамиды (AVA) являются важной группой этих соединений благодаря их антиоксидантной, противовоспалительной и других видов активности. **Материал и методика.** Используя комбинированный анализ HPLC и LC-MS, впервые были получены комплексные данные по общему содержанию и составу авенантрамидов у образцов культурных и диких видов овса. Было определено содержание AVA у 32 образцов дикого и 120 образцов культурного овса, полученных из мировой коллекции Всероссийского института генетических ресурсов растений им. Н.И. Вавилова (ВИР, Санкт-Петербург, Россия). **Результаты и выводы.** Образец дикого гексаплоидного вида *A. sterilis* L. имел самое высокое общее содержание AVA в зерновке (1825 мг/кг), а среди образцов культурного овса самое высокое содержание AVA (586 мг/кг) имел голозерный сорт 'Numbat' (Австралия). Данные по компонентному составу AVA показали большое разнообразие среди анализируемых образцов. Были идентифицированы образцы, у которых основные компоненты А, В и С данного вещества имели низкое содержание, а вместо них преобладали другие, менее значимые компоненты. Содержание AVA у восьми сортов овса показало значительные изменения по годам как общего содержания AVA, так и соотношения отдельных его компонентов. Используя анализ HPLC, установили и количественно определили 22 различных пика компонентов AVA в экстрактах зерновок овса. Некоторые из этих пиков, которые ранее не были документированы, предположительно представляют разные компоненты AVA. В дальнейшем необходимо детализировать полученные результаты и определить конкретные компоненты AVA в зерне овса.

Ключевые слова: овес, культурные и дикие виды *Avena*, авенантрамиды, генетические ресурсы.

Introduction

Oat grains accumulate substantial amounts of various phenolic compounds that possess biological activity and have a potential to considerably increase health benefits of oats as a food. Avenanthramides (AVA) is an important group of these compounds due to their antioxidant, anti-itching, anti-inflammatory, antiproliferative activities (Hitayezu et al., 2015; Koenig et al., 2014; Meydani 2009; Ren et al. 2011; Yang et al., 2014), and preventing effects in cancer and heart diseases (Guo et al., 2010). In a recent study, AVA and its metabolites have exhibited bioactivity against human colon cancer cells (Wang et al., 2014). In addition, due to their antioxidative activity, AVA can help to prevent the rancidity of food products and thus improve their storage properties (Peterson, 2001).

AVAs are phytoalexins, i.e. compounds produced in response to pathogenic attack. However, they are constitutively expressed in both oat seeds and leaves (Peterson, Dimberg 2008). Chemically, AVA are derivatives of anthranilic (2-aminobenzoic) acid, connected either with hydroxycinnamic (type I AVA) or with avenalamic (5-(4-hydroxyphenyl)-2,4-pentadienoic) acid (type II AVA). Anthranilic, hydroxycinnamic and avenalamic acids can have substitutions with hydroxyl and/or methoxyl functions, and thus over 30 different compounds with molecular mass from 283 to 387 are built up (Collins 1989; Wise 2014).

Most of the reports on the AVA structure and composition have confined to cultivated oat and describe the presence and antioxidant activity of only few AVAs; in most cases these are major three A, B and C (Boz, 2015; Collins, 2011). Several publications showed levels of AVA A, B and C produced in different fractions and products of oat seeds. In the study of Hitayezu et al. (2015), fine bran had the highest AVA content, while the whole groat oat flour had the lowest. This is in contrast with the data of Mattila et al. (2005), where oat flakes had double AVA content than that in oat bran. Oat hulls had comparable amounts of three major AVAs in the studies of Bryngelsson et al. (2002) and Ortiz-Robledo et al. (2013). Several researchers have documented the influence of environmental conditions, i.e. year and location, on AVA accumulation (Dimberg et al. 2005; Emmons, Peterson 2001; Peterson 2005; Wise et al., 2008). The amount of AVA in oat grains increases significantly during imbibition (Matsukawa et al., 2000), plant development (Peterson, Dimberg, 2008), stimulation by elicitors (Mayama, 1995a; Ishihara et al., 1996; Matsukawa et al., 2000; Ren, Wise, 2012; Wise, 2011), steeping (Bryngelsson et al., 2003), storing (Dimberg et al., 1996), and fungal infection (Mayama et al., 1995a, b; Miyagawa et al., 1995).

Regarding AVA antioxidant activity, Hitayezu et al. (2015) found correlation between AVA and radical scavenging data, while there was no such correlation for five free phenolic acids. Studies on antioxidant activity showed differences between AVA A, B and C extracted from seeds of cultivated oat (Bratt et al., 2003; Hitayezu et al., 2015; Ren et al., 2011; Yang et al., 2014). This indicates that other AVAs may also possess properties different from those reported for AVA A, B and C, and further studies are needed to discover their potential as bioactive compounds.

To the best of our knowledge, there is only one publication on AVA content and composition in wild oat, where up to 13 wild and 80 cultivated oat accessions were analyzed (Redaelli et al., 2016). In that study again only the major three AVAs, i.e. A, B and C, were taken into consideration. Wild oats present a crucial source of variation for breeding

programs, which dictates the need to further investigate the variability pattern in AVA content and composition across the genus *Avena* L. Wild oat species may have a unique composition of AVA that potentially can be used in various applications.

The aim of this study was to analyze the AVA content and composition in 32 wild and 128 cultivated (including commercial cultivars and landraces) oat accessions from the collection of N.I. Vavilov Institute of Plant Genetic Resources (VIR). We employed HPLC and LC-MS analyses and focused specifically on identification and quantitative analyses of several minor AVA in wild and cultivated oats.

Materials and methods

Plant material

Thirty two accessions of wild species of different ploidy level and one hundred and twenty accessions of cultivated hexaploid species of different geographical origin (Table 1) were selected from the VIR collection for comprehensive field trials which were conducted at VIR's field station in 2010–2014. Taxonomical definition was done according to Rodionova et al. (1994), Loskutov (2007), and Loskutov and Rines (2011). All accessions were sown manually on 1.0 m² plots in six 1 m rows with the 15 cm spacing between the rows and 30 cm between the plots. Harvesting was done manually and followed by the manual threshing of panicles using VIR's standard guidelines (Loskutov et al., 2012).

Extraction and analyses of avenanthramides on HPLC and LC-MS

Grains of oat were dehulled manually and milled (Pulverisette). Avenanthramides were extracted with 80% ethanol according to the method described elsewhere (REF). Two to three replications were made for each accession.

HPLC

HPLC model Agilent 1100 with a diode array detector was used to analyze the extracted AVA with detection at 340 nm. We used a C18 column, Kromasil 100-3.5C18, dimensions 4.6 × 150 mm. We used H₂O with 0.1% formic acid as buffer A and acetonitrile as buffer B. Flow rate was 0.8 ml/min with a gradient as follows: during first 24 min 20–45% buffer B; from 24th to 25th min, 80% buffer B; stayed so till 30 min; till 30min 10 sec, 20%; stayed there till 40 min. AVA reference standards A, B, C, AA, BB, CC, D, G and L, kindly provided by Prof. A. Ishimata (Kyoto University, Japan), were used for building calibration curves to quantify corresponding peaks. All other peaks were quantified using calibration curve of AVA A.

Total avenanthramide level was calculated from the HPLC chromatogram by summing up the peaks that appear in the region between AVA CC and L. Quantification was based on basis of the fresh weight of the samples.

LC-MS

Several representative samples (with the highest amount of avenanthramides other than those for which standards were available) were analyzed by LC-MS. An Agilent Technologies 1260 Infinity HPLC system (CA, USA) equipped with a diode array detector (DAD, G4212) was used under the conditions described above. A mass spectrometer (Agilent Technologies 6120 Quadrupole, Germany) equipped with an API-ES was connected with the HPLC-DAD system. The ionization source parameters were as follows: API-ES negative ion mode; nebulizer pressure 25 psig; dry gas temperature 300°C;

Table 1. List of analyzed oat species accessions**Таблица 1. Список проанализированных образцов видов овса**

	Species	Ploidy 2n	Genome	Number of accessions	Geographical origin
1	<i>A. sativa</i> L.	3	ACD	110	Russian Federation, Ukraine, Belarus, Norway, UK, Sweden, Finland, France, Netherland, Germany, Austria, Czech Republic, Slovakia, Kyrgyzstan, Mongolia, China, Japan, Canada, Australia
2	<i>A. byzantina</i> C. Koch	3	ACD	10	Russian Federation, China, Portugal
3	<i>A. atlantica</i> Baum	1	As	2	Morocco
4	<i>A. canariensis</i> Baum et Fedak.	1	Ac	2	Spain, Canary Islands
5	<i>A. clauda</i> Durieu	1	Cp	2	Turkey, Iran
6	<i>A. damascena</i> Rajh. et Baum	1	Ad	2	Syria
7	<i>A. hirtula</i> Lagas.	1	As	1	Tunisia
8	<i>A. longiglumis</i> Durieu	1	Al	1	from the U.S. genebank
9	<i>A. wiestii</i> Steud.	1	As	2	Israel, Iran
10	<i>A. agadiriana</i> Baum et Fedak	2	AB?	2	Morocco
11	<i>A. barbata</i> Pott	2	AB	2	Azerbaijan, Lebanon
12	<i>A. insularis</i> Ladiz.	2	CD?	2	Italy, Sicily
13	<i>A. magna</i> Murphy et Terr.	2	AC	2	Morocco
14	<i>A. murphyi</i> Ladiz.	2	AC	2	Spain
15	<i>A. vaviloviana</i> (Malz.) Mordv.	2	AB	2	Ethiopia
16	<i>A. fatua</i> L.	3	ACD	2	Ethiopia, China
17	<i>A. ludoviciana</i> Durieu	3	ACD	2	Iran, Ethiopia
18	<i>A. occidentalis</i> Durieu	3	ACD	2	Spain, Canary islands
19	<i>A. sterilis</i> L.	3	ACD	2	Israel, Algeria

dry gas flow 10 L/min; capillary voltage +3.0 kV; VCap 3000V; Quad temp 100°C. The mass signal dimension was 100–1500 *m/z*, fragmentor set to 70 V.

Statistics

Individual AVAs were calculated as percentage of the total AVA. All statistical analyses were done using R programming.

Results

1. Total AVA content

To check the effect of year-long AVA accumulation, eight accessions were grown at one place during two years: 2014 and either 2011 or 2012 or 2013. We checked the difference in the total AVA content in these accessions and discovered that for four accessions 2014 was much more favorable for AVA accumulation; for three accessions 2014 was less favorable than other years; and for cv. 'Premjer' the total AVA content was the same in two years (Table 1). The lack of a clear tendency for each analyzed year led to the absence of statistical difference when the analysis was run for the summarized data of 2014 and the other year (supplementary material, Table 2 3).

Then we looked at the total AVA in accessions belonging to different species and with different ploidy levels. Tables 2 and 3 give the overview of the analyzed accessions. Total AVA in cultivated oat varied from 12.35 (Mongolian landrace, naked) to 586.63 mg kg⁻¹ (cv. 'Numbat', naked) (Fig. 1; supplementary material, Table 1). In wild oats, both analyzed accessions of the diploid species *A. atlantica* had the lowest values (4–9 mg kg⁻¹), while tetraploid *A. insularis*, *A. longiglumis* and hexaploid *A. sterilis* (the accession with the origin from Algeria) showed the highest levels of AVA (613, 662 and 1825 mg kg⁻¹, respectively). Comparison of accessions of different ploidy (only for wild species) revealed no significant variation in the total AVA. Therefore, we ran further statistical analyses of different species, ignoring their ploidy. That analysis, i.e. comparison of species, also showed no significant difference, as accessions within one species differed more in their AVA content than accessions belonging to different species.

We compared total AVA between cultivated (hexaploid species *A. sativa* and *A. byzantina*) and hexaploid wild oat accessions; within *A. sativa*, hulled and naked oats were compared. Again, no significant difference was observed in either of those two analyzes (data not shown).

Table 2. Total AVA content (triplicate average) in cultivars replicated in different years
(mean ± standard deviation in mg kg⁻¹ fresh weight; *p < 0.05, **p < 0.01, ***p < 0.001)

Таблица 2. Общее содержание AVA (среднее значение трех повторностей) у сортов, выращенных в разные годы (среднее ± стандартное отклонение, мг кг⁻¹ сырого веса; *p < 0.05, **p < 0.01, ***p < 0.001)

Cultivar, variety	Total AVA, mg kg ⁻¹ , Year 1	Total AVA, mg kg ⁻¹ , Year 2 (2014)	p*
Argamak, <i>mutica</i> 14648	18.15 ± 3.3 (2011)	84.40 ± 18.4	***
Numbat, <i>inermis</i> 14851	551.35 ± 31.5 (2011)	60.08 ± 5.2	***
Persheron, <i>inermis</i> 15275	82.26 ± 9.6 (2012)	43.85 ± 5.4	***
Eklips, <i>aurea</i> 15187	171.01 ± 34.5 (2013)	64.34 ± 6.4	***
Premjer, <i>A. byzantina</i> 15238	60.57 ± 3.1 (2013)	59.46 ± 6.9	ns
Krechet, <i>mutica</i> 14857	37.57 ± 6.0 (2012)	127.08 ± 16.4	***
Konkur, <i>mutica</i> 15068	70.31 ± 3.0 (2010)	133.03 ± 36.3	**
Bulanyi, <i>mutica</i> 15277	22.04 ± 5.5 (2013)	46.14 ± 7.0	**

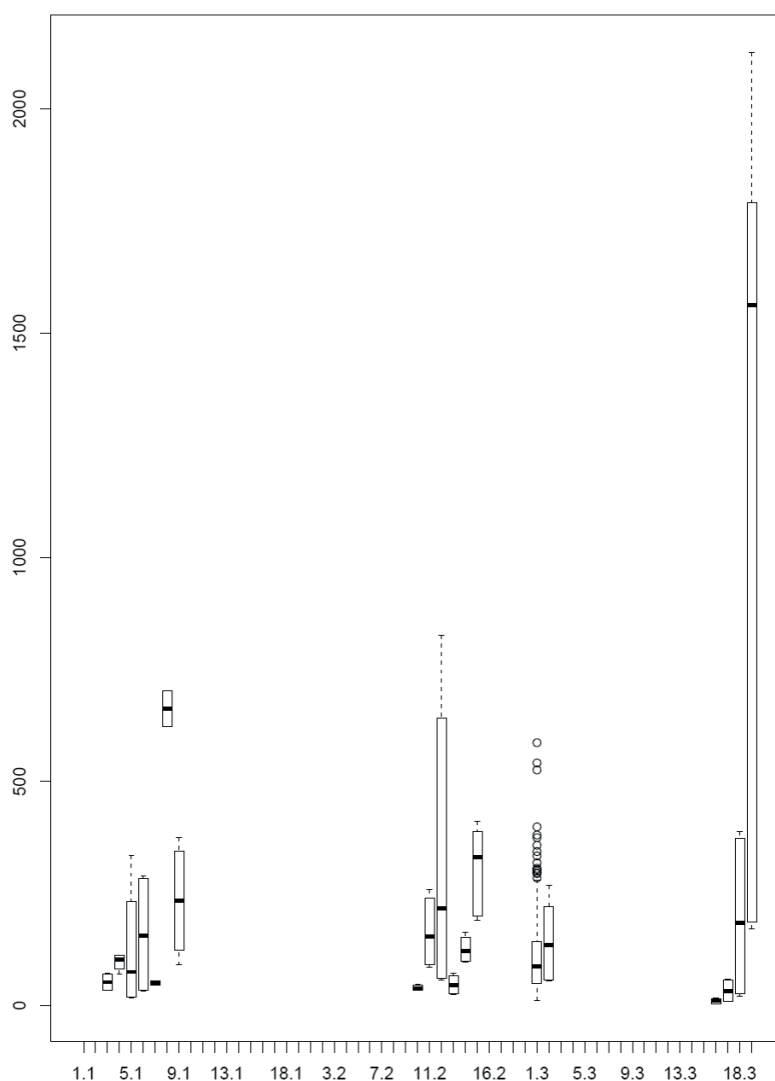


Fig. 1. Total AVA content (mg kg⁻¹) in diploid ('1' after dot), tetraploid ('2' after dot) and hexaploid ('3' after dot) wild and cultivated oat species. The digit(s) before dot designate species according to Table 1 (1 - *A. sativa*, 2 - *A. byzantine*, etc.)

Рис. 1. Общее содержание AVA (мг кг⁻¹) у диплоидных («1» после точки), тетраплоидных («2» после точки) и гексаплоидных («3» после точки) диких и культурных видов овса. Цифры перед точкой указывают виды согласно таблице 1 (1 - *A. sativa*, 2 - *A. byzantina* и т. д.)

2. AVA composition

HPLC analyses suggested the presence of over 20 AVAs in oat grains (Fig. 2, A), i.e. peaks appearing between CC and L - 'AVA region' (Fig. 2, B). We identified them by comparing the retention times with those of the available nine standards (Fig. 2, B) and by checking the molecular weight by LC-MS. LC-MS analyses revealed several AVAs that were eluting at the same time and thus appearing as one peak on the HPLC chromatogram. For example, AVA B co-eluted with minor AVA QQ, and thus we could not calculate the exact

amount of these two compounds. Thus, we identified about 25 AVAs. Of them, 17 were the AVAs known from before, and 8 compounds were unknown. Major AVAs in most accessions were A, B and C (Fig. 2, A). However, there were accessions that had unusual AVA composition. Several HPLC chromatograms on most interesting examples of unusual AVA composition are presented in Fig. 3. For instance, they had relatively low levels of major AVAs, compensating it by high levels of other compounds, which sometimes appeared on chromatograms in the areas outside the AVA region (Fig. 3, I).

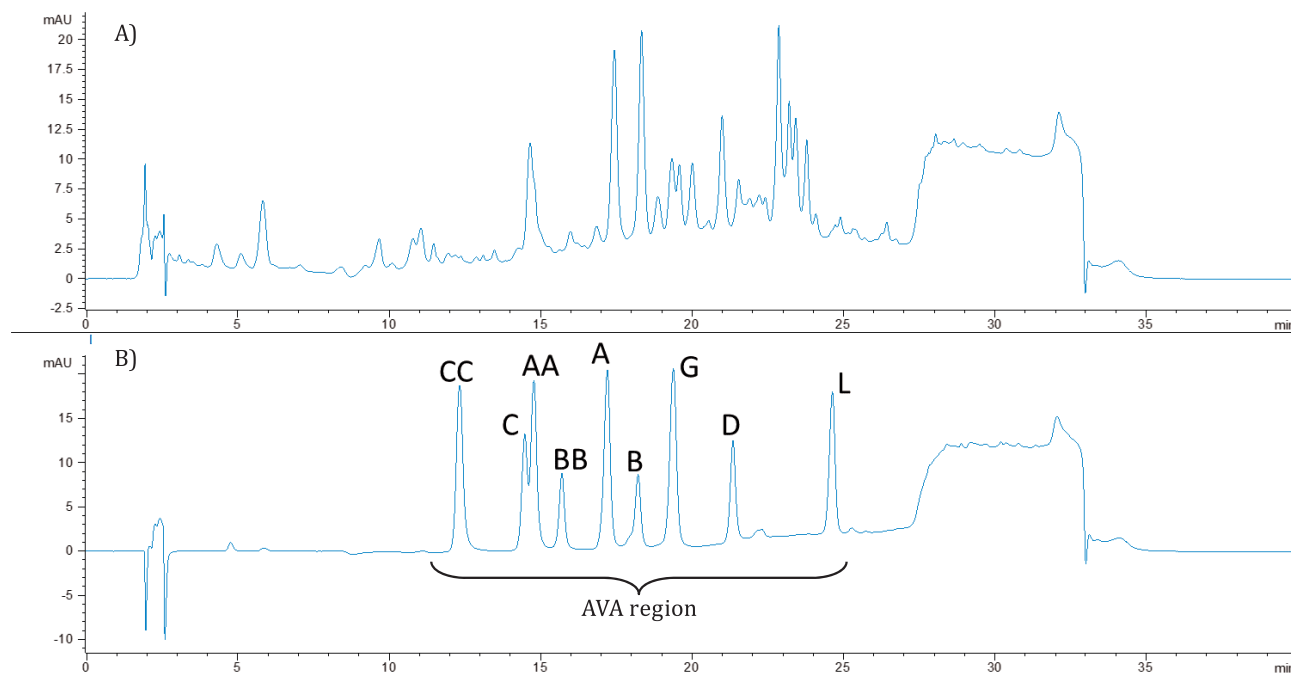


Fig. 2. HPLC chromatogram of AVA content extracted from the grains of oat cv. Sprint 3 (A) and of nine AVA standards (B)

Рис. 2. HPLC-хроматограмма содержания AVA, экстрагированных из зерен овса сорта Спринт 3 (A) и девять стандартов AVA (B)

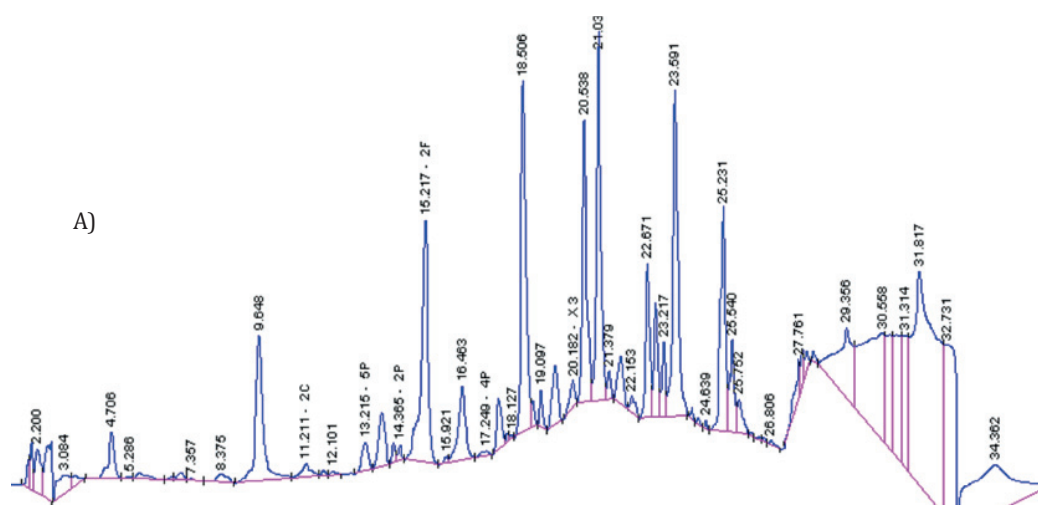


Fig. 3. HPLC chromatograms of cultivated (A-C) and wild (D-I) accessions of oat species with unusual AVA composition

Рис. 3. HPLC-хроматограммы образцов культурных (A-C) и диких (D-I) видов овса с необычным составом AVA

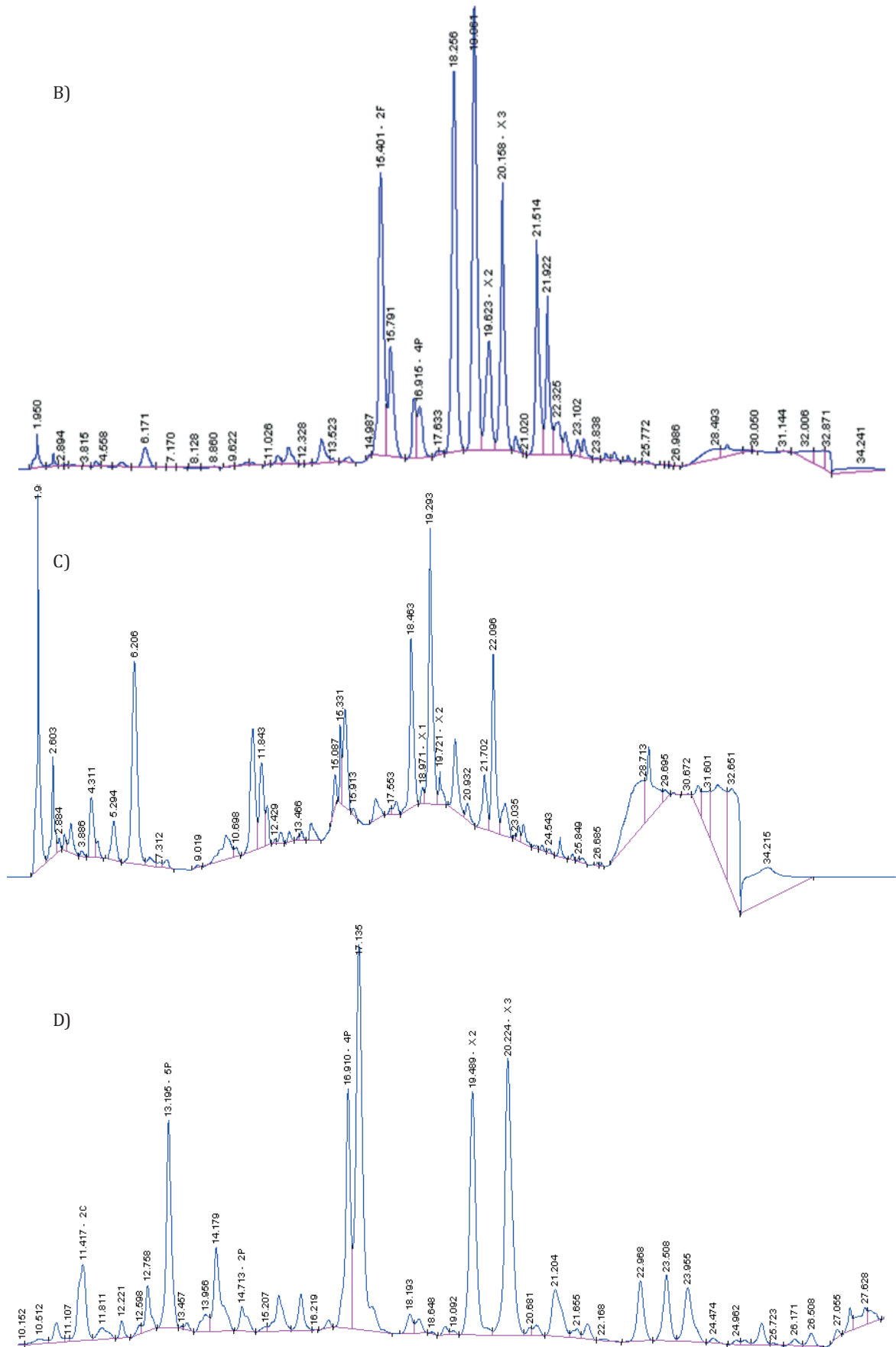


Fig. 3. HPLC chromatograms of cultivated (A-C) and wild (D-I) accessions of oat species with unusual AVA composition

Рис. 3. HPLC-хроматограммы образцов культурных (А-С) и диких (D-I) видов овса с необычным составом AVA

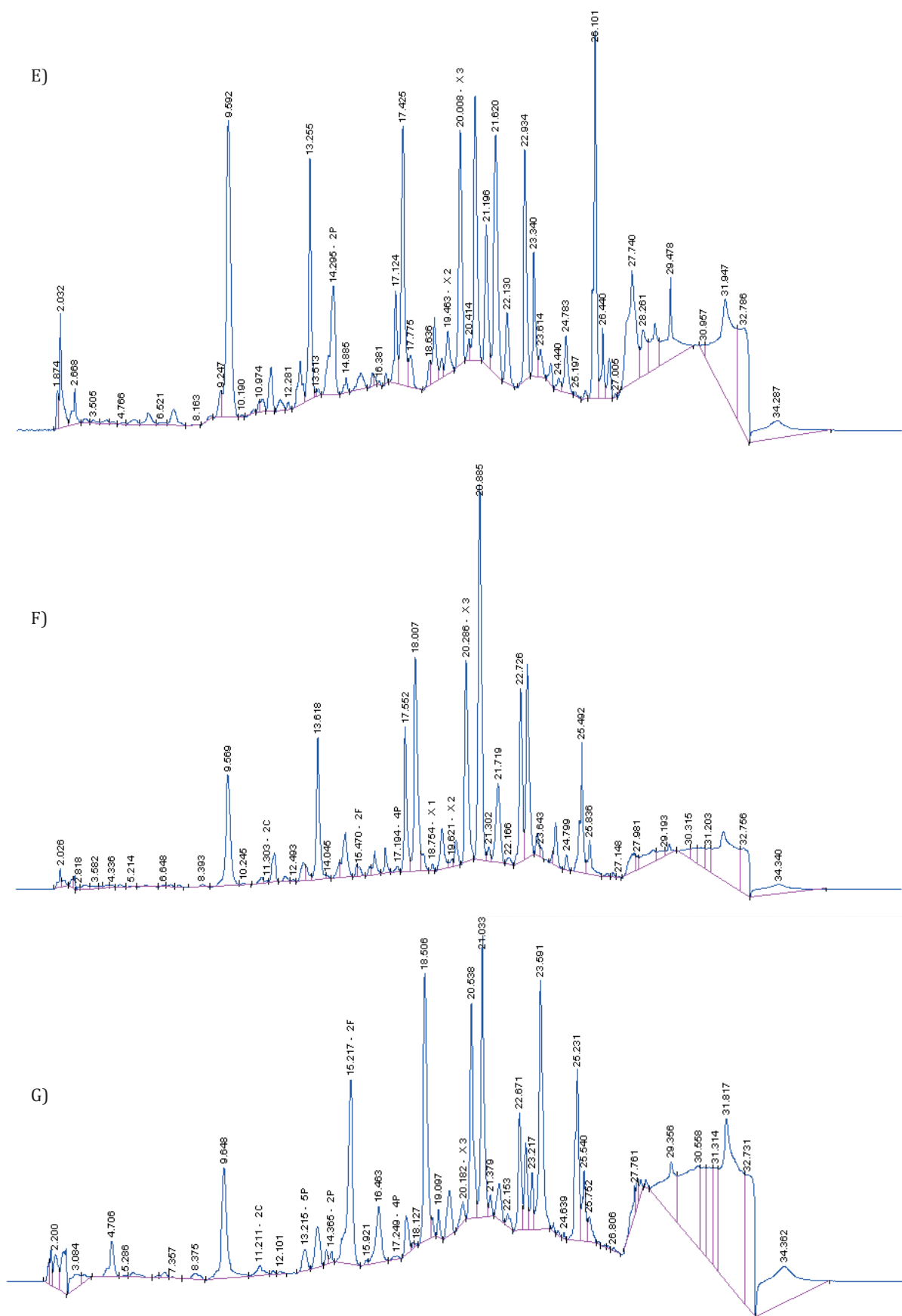


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Рис. 3. HPLC-хроматограммы образцов культурных (А–С) и диких (D–I) видов овса с необычным составом AVA

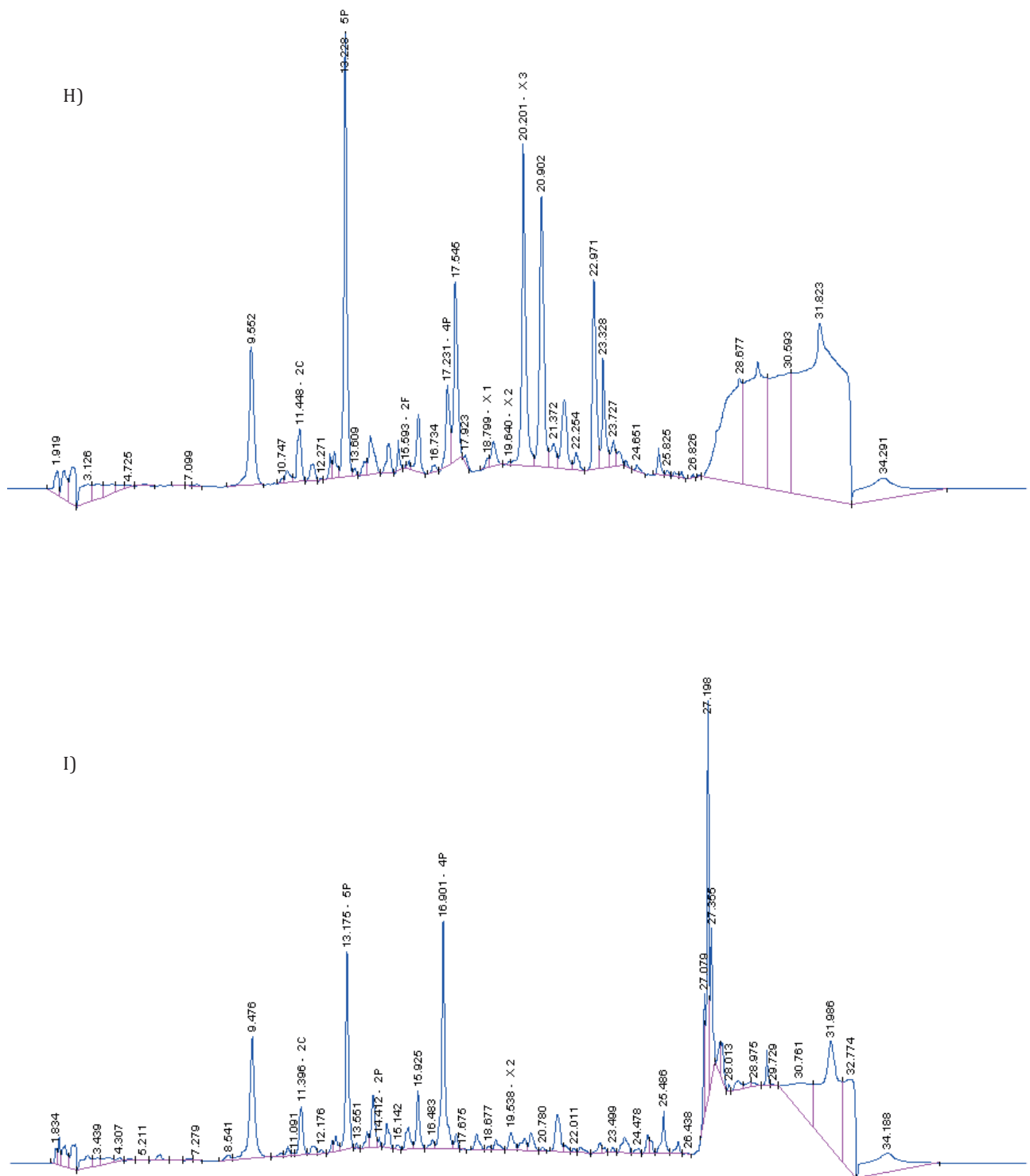


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Рис. 3. HPLC-хроматограммы образцов культурных (А-С) и диких (D-I) видов овса с необычным составом AVA

LC-MS was used to analyze molecular weight (MW) of AVA and unknowns. Fig. 4 illustrates the HPLC chromatogram and MW of unknown 2 and AVA C extracted from wild oat *A. damascena*. Judging by the HPLC chromatogram only, a perception would be that the largest peak is AVA C. However, LC-MS analyses revealed that the largest peak had MW 326 instead of 316, as MW of

AVA C, and thus we identified the largest compound as an unknown.

In case of other unknowns, the levels of unknowns 1, 3 and 4 were too low for being detectable by LC-MS. There were accessions with high levels of unknowns 5, 6, 7 and 8; all of them had more or less the same ion composition; MW of the major ion was 316 (Fig. 4, B).

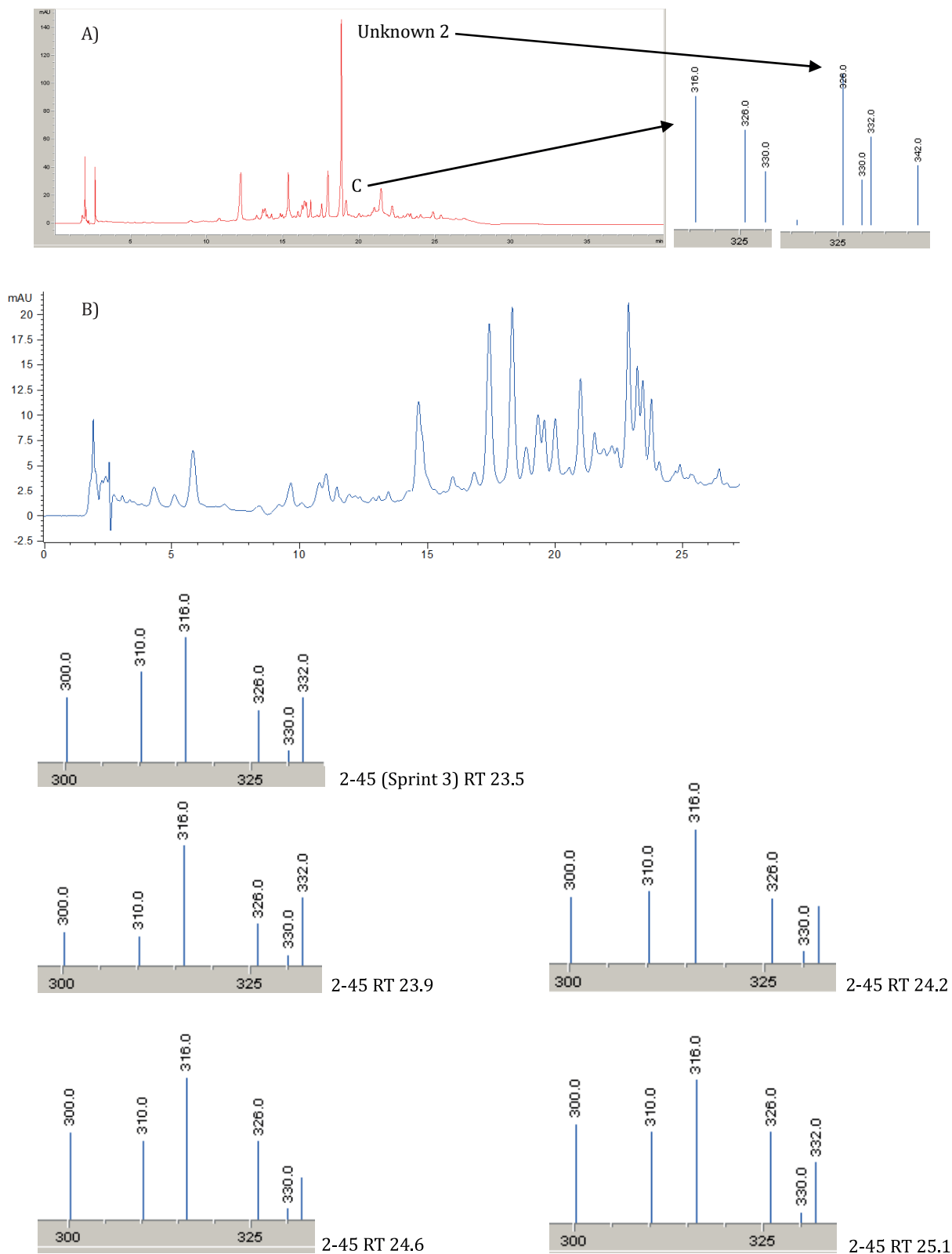


Fig. 4. LC-MS analysis of the AVA content extracted from oat with depicted MW of unknowns 5-8
Рис. 4. LC-MS анализ содержания AVA, выделенных из овса с неизвестными пиками МВ 5-8

Tables 3 and 4 give the lowest and the highest percentage of different AVAs in wild and cultivated oats, respectively. For the majority of the accessions, AVA A, B and C comprised the largest portion of AVAs, but there was one exception. One accession of *A. damascena* had 48.6% of unknown 2 and 9.1% of unknown 1, while levels of AVA A, BQ and C were much lower: together they comprised only 18% (Fig. 4, A; Table 3). In cultivated oat, the highest level of unknown 2

and unknown 1 was 11.9 and 5.8% respectively (Table 4). The other interesting results were higher portion of R/N in wild oat with maximum 18.27% in *A. magna* vs. maximum 7.76% in cultivated oat (cv. Podgorny), 16.97% of unknown 7 in wild oat (*A. hirtula*) vs. 4.67 in cultivated oat, and large difference in the highest level of B+Q between wild and cultivated oats: maximum 24.71% in wild vs. 39.5% in cultivated oat.

Table 3. Lowest and highest percentage of different AVA in wild oat

Таблица 3. Самые низкие и самые высокие значения (%) различных компонентов AVA у образцов диких видов овса

Compound	Lowest value, % (species)	Highest value, % (species)
AVA_CC	0.41 (<i>A. canariensis</i> -1)	6.28 (<i>A. atlantica</i> -2)
AVA_UN1	0.05 (<i>A. occidentalis</i> -2)	9.1 (<i>A. damascena</i> -1)
AVA_UN2	0.02 (<i>A. insularis</i> -2)	48.6 (<i>A. damascena</i> -1)
AVA_C	4.78 (<i>A. canariensis</i> -1)	41.7 (<i>A. longiglumis</i>)
AVA_AA	0.31 (<i>A. wiestii</i> -1)	11.9 (<i>A. sterilis</i> -1)
AVA_BB	0.12 (<i>A. agaridiana</i> -2)	4.39 (<i>A. agaridiana</i> -1)
AVA_QQ	0.00 (<i>A. vaviloviana</i> -1)	5.03 (<i>A. sterilis</i> -2)
AVA_UN3	0.05 (<i>A. ludoviciana</i> -2)	2.26 (<i>A. clauda</i> -2)
AVA_A	3.88 (<i>A. clauda</i> -2)	33.02 (<i>A. canariensis</i> -1)
AVA_B+Q	3.34 (<i>A. clauda</i> -2)	24.71 (<i>A. insularis</i> -2)
AVA_X+OO	0.43 (<i>A. occidentalis</i> -1)	6.69 (<i>A. canariensis</i> -1)
AVA_G+Y	1.26 (<i>A. barbata</i> -1)	12.49 (<i>A. hirtula</i>)
AVA_H	0.07 (<i>A. vaviloviana</i> -2)	5.63 (<i>A. sterilis</i> -2)
AVA_O	1.92 (<i>A. ludoviciana</i> -1)	16.67 (<i>A. canariensis</i> -1)
AVA_P/S	0.31 (<i>A. longiglumis</i>)	9.69 (<i>A. sterilis</i> -1)
AVA_R/N	0.78 (<i>A. damascena</i> -1)	18.27 (<i>A. magna</i> -1)
AVA_UN4	0.00 (<i>A. insularis</i> -2)	2.80 (<i>A. sterilis</i> -2)
AVA_UN5	0.00 (<i>A. murphyi</i> -1)	4.09 (<i>A. clauda</i> -2)
AVA_UN6	0.00 (<i>A. insularis</i> -2)	2.49 (<i>A. hirtula</i>)
AVA_UN7	0.06 (<i>A. occidentalis</i> -2)	16.97 (<i>A. hirtula</i>)
AVA_UN8	0.01 (<i>A. longiglumis</i>)	5.70 (<i>A. fatua</i> -2)
AVA_L	0.02 (<i>A. longiglumis</i> , <i>A. murphyi</i> -1, <i>A. sterilis</i> -1)	3.11 (<i>A. damascena</i> -1)

Table 4. Lowest and highest percentage of different AVA in cultivated oat
Таблица 4. Самые низкие и самые высокие значения (%) различных компонентов AVA у образцов культурного овса

Compound	Lowest value, % (cultivar)	Highest value, % (cultivar)
AVA_CC	0.01 (Borot, <i>mutica</i>)	4,71 (Effectiv)
AVA_UN1	0.06 (Borot, <i>mutica</i>)	5,8 (Mongolian landrace, <i>inermis</i>)
AVA_UN2	0.04 (Chinese local, <i>A. byzantina</i>)	11,9 (Faust, <i>mutica</i>)
AVA_C	6.25 (Faust, <i>mutica</i>)	29.1 (Guzeripl, <i>A. byzantina</i>)
AVA_AA	0.67 (Gere)	13.74 (Soku, <i>mutica</i>)
AVA_BB	0.51 (Premjer, <i>A sativa</i> , <i>A. byzantina</i>)	5.75 (Borot, <i>mutica</i>)
AVA_QQ	0.04 (Drug, <i>mutica</i>)	6.48 (Gorisont, <i>mutica</i>)
AVA_UN3	0.04 (Metis, <i>aurea</i>)	2.19 (Dans, <i>mutica</i>)
AVA_A	9.84 (Bulanyi, <i>mutica</i>)	30.77 (Kirovetc, <i>aurea</i>)
AVA_B+Q	8.32 (Argamak, <i>mutica</i>)	39.50 (Universal-1, <i>mutica</i>)
AVA_X+OO	0.34 (Pisarevskiy, <i>mutica</i>)	8.26 (Gorisont, <i>mutica</i>)
AVA_G+Y	1.43 (Narymskii 943, <i>mutica</i>)	14.99 (Dans, <i>mutica</i>)
AVA_H	0.00 (Kirovetc, <i>aurea</i>)	11.77 (Argamak, <i>mutica</i>)
AVA_O	0.02 (Persheron, <i>inermis</i>)	12.79 (Drug, <i>mutica</i>)
AVA_P/S	0.24 (Pomor, <i>inermis</i>)	14.25 (Bulanyi, <i>mutica</i>)
AVA_R/N	0.21 (Sprint 2, <i>aurea</i>)	7.76 (Podgorniy, <i>mutica, grisea</i>)
AVA_UN4	0.00 (local <i>A. byzantina</i> ; Murom, <i>inermis</i>)	5,27 (Argamak, <i>mutica</i>)
AVA_UN5	0.00 (several accessions)	9,71 (Sprint 3, <i>aurea</i>)
AVA_UN6	0.01 (Canyon)	8,8 (Sprint 3, <i>aurea</i>)
AVA_UN7	0.00 (Skakun, <i>mutica</i>)	4,67 (Taezhnik, <i>aurea</i>)
AVA_UN8	0.01 (Numbat, <i>inermis</i>)	5.01 (Hurdal)
AVA_L	0.01 (Numbat, <i>inermis</i> ; Soku, <i>mutica</i>)	4.19 (Tumenskii golozerniy, <i>inermis</i>)

3. Cluster analyses

Analyzing the variation of all 22 detected peaks in different species, it was possible to organize them into 5 clusters for wild oat (Fig. 5) and into 10 clusters for cultivars (Fig. 6). The main common tendencies for both wild and cultivated oats were that O was in one cluster with X+OO; L with un1 and un2; un3, un4, in 5, un6 and H (but for wild

ones, there was also G+Y in this cluster). The most striking difference between wild and cultivated oat was AVA C and R/N: in wild oat, they were the two the most distant AVA while in cultivated oat they appeared in one cluster. A similar situation was observed for AVA P/S and QQ: sitting next to each other in cultivars, they belonged to the two most remote clusters in wild oats.

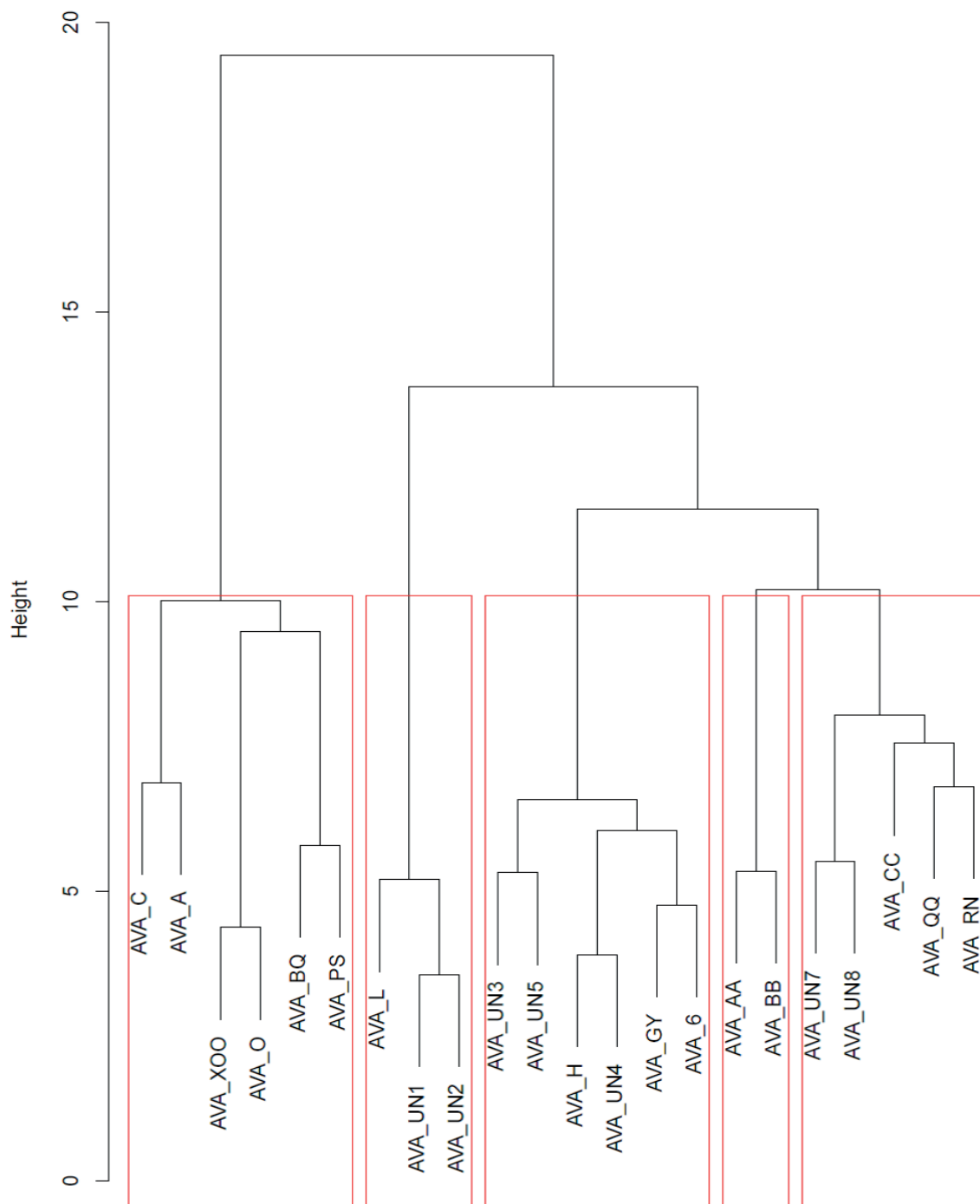


Fig. 5. Cluster analysis in wild oat

Рис. 5. Результаты кластерного анализа данных у образцов диких видов овса

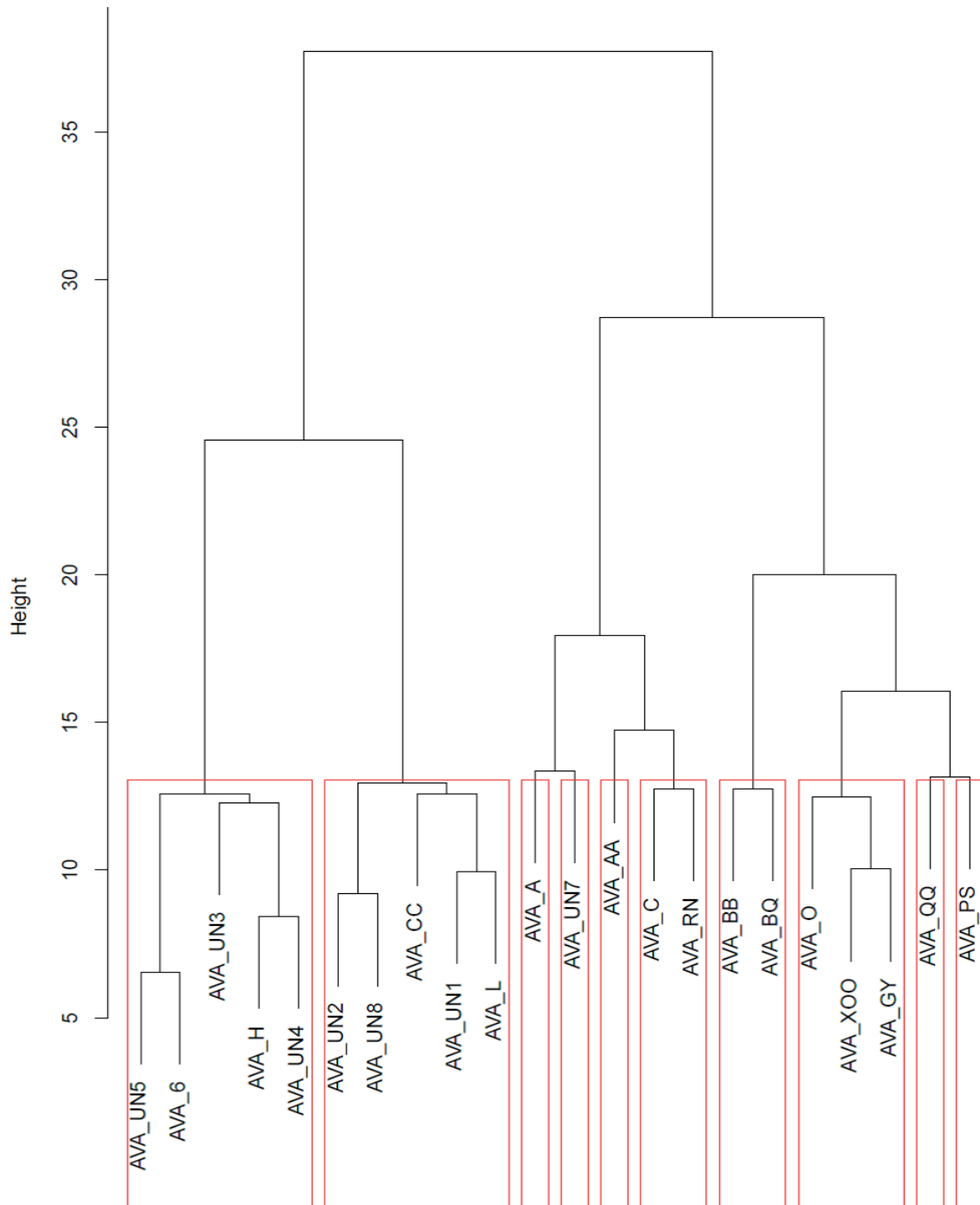


Fig. 6. Cluster analysis in cultivars

Рис. 6. Результаты кластерного анализа данных у образцов культурного овса

Further statistical analyses were done on clusters instead of individual AVAs, separately for wild and cultivated oat. For wild oat, ploidy and species effects on clusters were analyzed. Ploidy was significant for cluster 2 (AVA L, un1,

and un2), with diploid oats having higher levels of these AVAs (Fig. 7); the species identity had a significant effect on cluster 5 (un7, 8, CC, QQ, R/N), with *A. magna* and *A. fatua* as the species with the highest levels (Fig. 8).

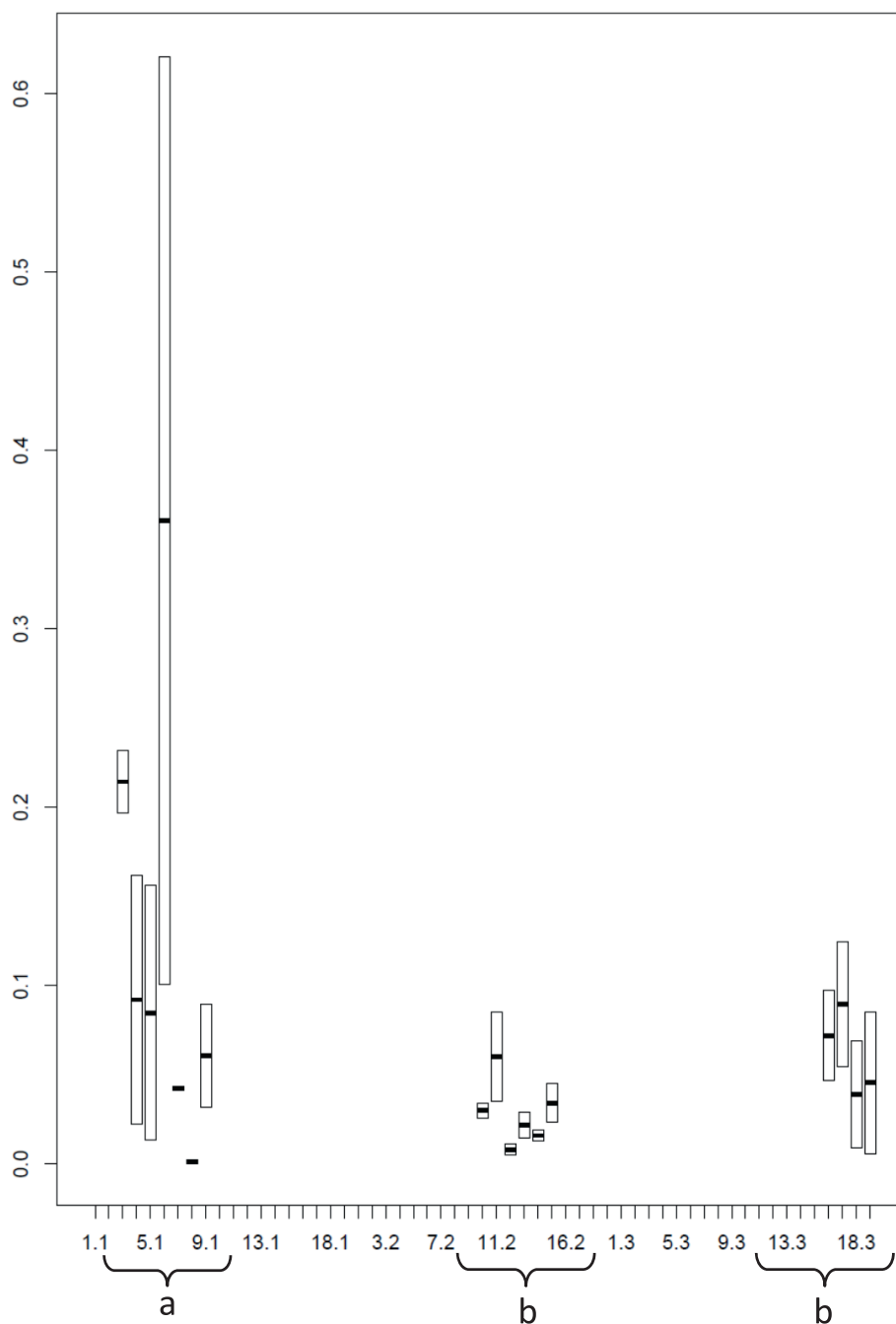


Fig. 7. Analysis of cluster 2 in diploid ('1' after dot), tetraploid ('2' after dot), and hexaploid ('3' after dot) wild oat species.

The digit(s) before dot is a code for species ID according to Table 1 (1 – *A. sativa*, 2 – *A. byzantina*, etc.). Different letters designate the statistical difference

Рис. 7. Анализ кластера 2 у диплоидных («1» после точки), тетраплоидных («2» после точки) и гексаплоидных («3» после точки) образцов видов дикого овса.

Цифры перед точкой – это код для идентификации вида согласно таблице 1 (1 – *A. sativa*, 2 – *A. byzantina* и т. д.). Разные буквы обозначают статистическую разницу

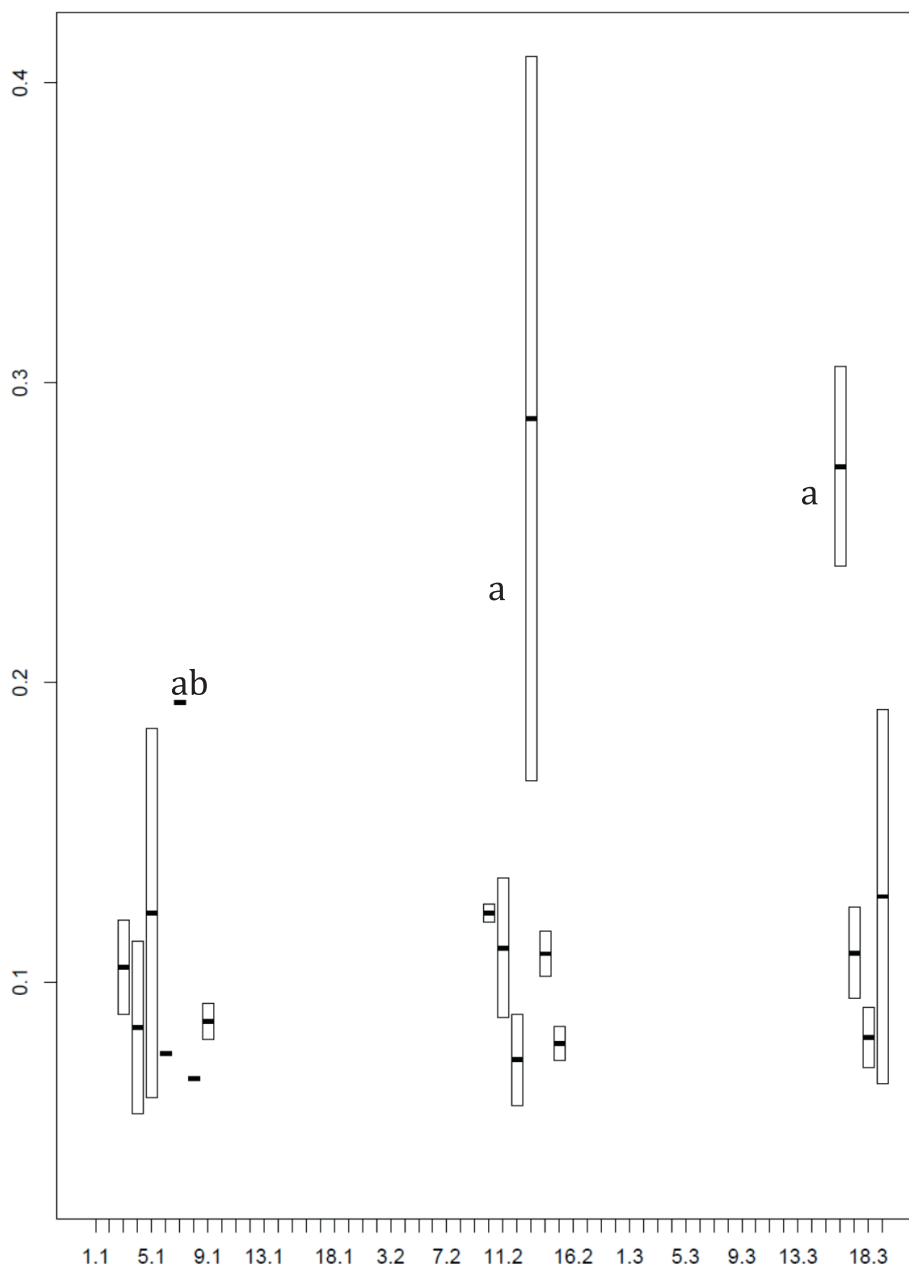


Fig. 8. Cluster 5 in different species of wild oat.

Species with higher AVA levels are marked by 'a'; species with lower levels are not marked with any letter; 'ab' designates the species belonging to both groups

Рис. 8. Кластер 5 у разных образцов диких видов овса.

Виды с более высокими уровнями AVA отмечены «а»; виды с более низкими уровнями не обозначены ни одной буквой; «ab» обозначает вид, который принадлежит к обеим группам

Discussion

1. Total AVA.

In this study, we analyzed the AVA content and composition in 32 wild and 120 cultivated oat accessions and found the AVA levels within the range from 4 to 1825 mg kg⁻¹. In hexaploid cultivars, the lowest AVA content was reported to be 7.7 mg kg⁻¹ (Bryngelsson et al., 2002) and the highest 3.0 g kg⁻¹ (Redaelli et al., 2016). In a marginally cultivated diploid accession of *A. strigose* Schreb., the total AVA reached 4.1 g kg⁻¹ (Redaelli et al., 2016). For wild oats, levels from 240 to 1585 mg kg⁻¹ were reported (Redaelli et al., 2016), and this was the only publication on the AVA content in wild oats. In our study, the highest AVA content among wild oats was registered for one accession of hexaploid *A. sterilis* (almost 2 g kg⁻¹), and tetraploid *A. insularis* and diploid *A. longiglumis* (about 600–700 mg kg⁻¹). However, other accessions of *A. insularis* and *A. sterilis* had much lower AVA contents. Thus, we cannot conclude that these species are generally richer in AVAs than other species. Among cultivars, both the lowest (12 mg kg⁻¹) and the highest (up to almost 600 mg kg⁻¹) AVA level were found in naked oat. Thus, once again we cannot conclude that the presence or absence of hulls alone makes an effect on AVA accumulation. Total AVA levels varied drastically between accessions of the same species, which led to the absence of a statistically significant difference between accessions with different ploidy level, hulled and naked accessions, or between species.

Analyses of eight accessions that were reproduced during two years revealed a large difference between the two replications. Remarkably, the conditions in 2014 favored AVA accumulation for four out of eight accessions, while for the three accessions that year was unfavorable, and one accession had the same AVA content in both years. Thus, our data indicate that the conditions promoting AVA accumulation for one cultivar might not have a similar effect on, or can even be highly unfavorable for another cultivar. This is generally in line with other data that showed strong influence of environmental conditions on AVA accumulation (Emmons, Peterson, 2001; Peterson et al., 2005; Redaelli et al., 2016).

2. AVA composition.

Chemical structure and nomenclature of 36 AVAs have been described by M. L. Wise (Wise, 2014). In our study, we were able to detect 25 compounds based on their MW analyzed by LC-MS; of those, 15 compounds were identified, two compounds (R/N and P/S) could not be separated due to the same eluting time and fragmentation pattern, and 8 compounds appeared unknown. Since unknowns eluted at the retention times corresponding to avenanthramides, they were accounted for as AVAs. However, further LC-MS studies are needed to confirm their belonging to the AVA class. There are just a few reports that mention AVAs other than A, B, and C in oat grains; all of them were done on cultivated oat. Those are the works done by Okazaki et al. (2004), where AVAs D, G, and L were reported in addition to the major A, B, and C; Skoglund et al. (2008), who depicted 21 peaks on the HPLC chromatogram; Elene Karlberg with coauthors, who quantified 11 AVAs; M. Wise (2011), who provided MS data of 10 AVA compounds; and A. Ishihara et al. (2014), when AVAs CC, AA, and BB were studied in detail. Our study is the first where analyses of AVAs other than A, B and C were done on wild oats.

Studies on biochemical activity, such as antioxidant and phytoalexin capacity, have only been done for A, B and C. For example, in the study of oat resistance to crown rust, levels

of AVA A and B were higher in the resistant cultivars and lower in the susceptible and highly correlated with retardation of hyphae growth (Mayama et al., 1982). On the other hand, in the study of antioxidant capacity, AVA C was shown to outperform A and B (Yang et al., 2014). These two examples demonstrate that different AVAs have different properties. Thus, it is important to further study the properties of other AVAs as they might be more efficient than A, B and C in phytoalexin or antioxidant capacity and/or may possess valuable capacities other than those reported for A, B and C. Our study provides important information on cultivars and wild oats that possess high levels of unusual unstudied AVAs and may serve as a source for production of these AVAs for further investigations. A method called false malting can be used to increase dramatically the levels of AVAs in oat grains, if needed (Collins et al., 2012).

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