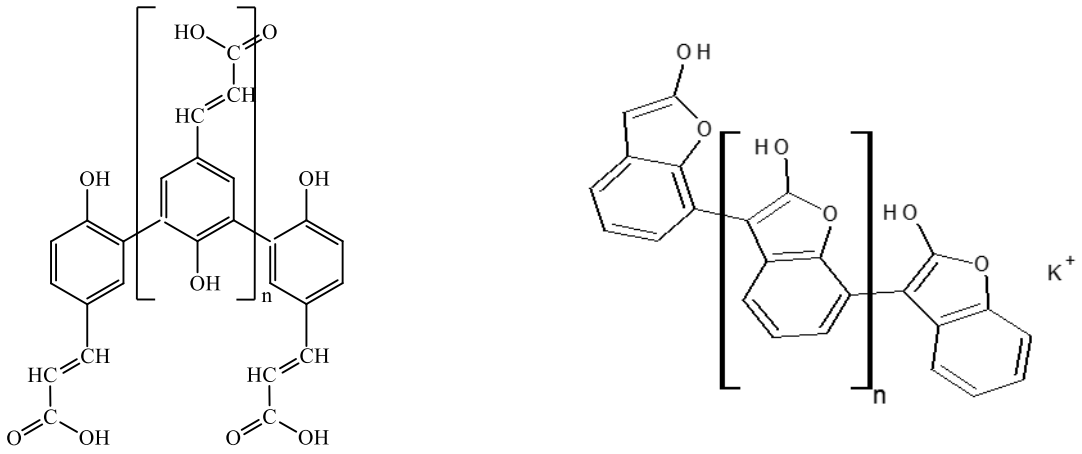
Closing report NKFI-ID: 129516

Title: Zab genetikai források jellemzése
PI: Dr. Mónika Varga

The objective of the project was to characterize the chemical composition of spring- and winter-type oat varieties with different hull color (white, yellow, grey, red and black) with the main question: Is there any correlation between the hull color and the groat/hull composition? Additionally, identification of the alleopatic compounds including L- DOPA in oat residues during plant growth was also planned.
Forty oat genotypes, mainly cultivated hexaploid *Avena sativa* species, a wild hexaploid *Avena fatua*, a tetraploid *Avena murphyi* and a diploid *Avena strigosa* were examined in this project. Ultraviolete-visible (UV-Vis) spectroscopy, high-performance liquid chromatography (HPLC) with different detection methods (diode array detection, DAD; fluorescent detection, FLD and mass spectrometry, MS) and enzymatic methods were applied for the characterization of the genotypes.
The following compounds/compound groups and properties were investigated to achieve the main objective:
1. black pigment,
2. phenolic aldehydes and acids,
3. avenanthramides,
4. mono-, and diglycerides,
5. antioxidant capacity,
6. oat fat soluble vitaminers – carotenoids and tocopherols, tocotrienols,
7. carbohydrates – β-glucan and mono-, di-, and trisaccharides,
8. allelopatic compounds.

The results are follows:
1. <u>Black pigment</u>
The quantification of the black pigment was essential for the correlation between the hull color and the chemical composition. However, the structure of the black coloration in plants has not yet been elucidated. As a first step, the black pigment was isolated from the hull of an *A. sativa*, an *A. strigosa* and an *A. fatua* specieses and purificated via a multi-step process. By means of UV-Vis spectroscopy and EPR spectroscopy, the pigment was identified as melanin, a biopolymer formed from phenolic compounds. The matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) experiments showed that there are different types of oat melanin depending on the species. All of them consist mainly of low molecular weight (527-1499 Da) oligomers built up from 3 to 9 monomer units, the tetramer oligomers being dominant. Large polymeric structures were not observed. These plant melanins proved to be homogeneous, in contrast with the heterogeneous animal and bacterial melanins. The MALDI-TOF MS and FourierTtransform Infrared (FT-IR) spectroscopy analysis proved that the melanin obtained from *A. sativa* species was built up from *p*-coumaric acid. As the plant melanins are formed by the action of polyphenol oxidases, we have attempted the *in vitro* enzymatic synthesis of melanin from *p*-coumaric acid for comparison. The resulting polymer consisted of oligomers of 4-9 monomer units similarly to those in oat melanin. However, the building blocks proved to be connected to each other via C-O-C linkages in contrast with the C-C linkages in oat melanin. An another type of melanin was identified in *A. strigosa* and *A.*

fatua specieses by MALDI-TOF and FT-IR measurements. According to the MALDI-TOF analysis the molecular mass of the monomer was 134 Da. We could surmise that the *A. strigosa* /*A. fatua* melanin was built up from 2-hydroxybenzofurane formed from 2-hydroxyphenylacetic acid via intramolecular lacton formation (Fig. 1.).



A **B**

Fig.1. The proposed structure of oat melanin extracted from *A. sativa* (A) and *A. strigosa/ A. fatua* (B).

FT-IR measurements supported the structure depicted in Fig.1. The oligomers involved an aromatic system containing trisubstituted aromatic rings in the 1,2,7 positions, a phenolic OH group and a C-O-C bond. This type of melanin was purified from the black hull of a durum wheat either. A MSc diploma work has been defended from this topic in 2015 (A. Cseh: „Structural elucidation of a plant melanin", University of Szeged).

To gain more information about the color appearance in the *A. sativa*, the concentration of the *p*-coumaric acid and the total phenol content during the development of the spike were determined. In parallel, the polyphenol-oxidase enzyme activity was measured either. The monomer concentration was the highest in the milk-ripening phenological stage, when the black color started to appear. In parallel the polyphenol-oxidase enzyme activity was the highest at this stage.

As melanins are insoluble in water and common organic solvents and can be dissolved only in alkaline solutions, the range of methods available for their quantitative analysis is limited. Therefore, the color of the oat samples was determined by a physical method, a colorimeter. Measuring the L, a^* and b^* color space coordinates the samples can be divided into three color groups: there are yellow, redness and black oat accessions. This classification is harmonized with the visual partition. L represents lightness (100 as white and 0 as black). Yellow samples had the highest mean L, while the black samples were the darkest and had the lowest L.

A journal paper has been published from the structural determination of the *A. sativa* melanin: *Varga, M., Berkesi, O., Darula, Zs., Nagy, N. V., Palágyi, A. (2016). Structural characterization of allomelanin from black oat. Phytochemistry, 130, 313-320.*

2. Phenolic aldehydes and acids

An extraction and HPLC-UV-MS/MS method were developed for the determination of bound and free phenolic aldehydes and acids both of the groats and the hulls. The groat and hull of the whole seeds of all genotypes were separated by hand. Contrary to mechanical dehulling, manual separation of groats and hulls provid an intact groat containing undamaged bran the main source

of phenolic acids. Therefore, the manually dehulled groat was composed of the bran, the endosperm and the germ and covered with trichomes. Due to the ball milling procedure there were no milling residues.

Analysing the methanolic extract of the groats and hulls 3 phenolic aldehydes and 7 phenolic acids were identified by comparison of their retention times and MS2 or MS3 data with authentic references and previously reported data in the literature (Table 1.)

Table 1. Average concentration (mg/kg) of soluble and bound phenolic compounds detected in groat and hull of yellow, reddish and black oat samples.

Compound	Soluble Phenolic Extract		Bound Phenolic Extract	
	Groat	Hull	Groat	Hull
PROald	n.d.*	9.0 ± 6.5	n.d.	n.d.
pHBald	0.5 ± 0.2	11.5 ± 5.0	9.9 ± 1.7	134.7 ± 32.3
Vald	2.1 ± 1.0	41.6 ± 20.69	15.2 ± 4.5	282.5 ± 127.4
PROA	n.d.	4.2 ± 3.9	83.0 ± 53.1	302.4 ± 102.1
pHBA	n.d.	11.0 ± 4.4	5.4 ± 1.3	22.5 ± 24.9
VA	2.1 ± 1.1	27.0 ± 12.2	8.1 ± 2.3	206.0 ± 171.6
CA	8.6 ± 3.5	4.2 ± 3.6	n.d.	n.d.
SYRA	1.3 ± 0.5	5.5 ± 2.3	4.9 ± 1.6	38.8 ± 12.4
pCA	0.7 ± 0.5	26.7 ± 7.2	62.2 ± 30.6	3689.0 ± 1126.8
FA	3.3 ± 1.2	14.5 ± 7.4	327.4 ± 108.5	8095.1 ± 3967.7
iFA	n.d.	n.d.	16.4 ± 4.4	415.2 ± 237.2
diFA	n.d.	n.d.	17.2 ± 13.6	152.4 ± 131.6
C-GLYC	6.9 ± 4.9	39.4 ± 49.6	n.d.	n.d.
F-GLYC	2.6 ± 2.2	5.1 ± 7.4	n.d.	n.d.
pC-GLYC	0.4 ± 0.4	3.6 ± 4.4	n.d.	n.d.
5p	27.9 ± 21.3	7.6 ± 6.6	n.d.	n.d.
diC-GLYC+2c	6.6 ± 6.2	63.7 ± 90.7	n.d.	n.d.
5f	3.6 ± 4.2	21.3 ± 34.1	n.d.	n.d.
2p/4p	25.6 ± 19.1	24.8 ± 19.1	n.d.	n.d.
F-C-GLYC+2f	27.5 ± 21.5	109.2 ± 175.6	n.d.	n.d.
$5p_d$	17.1 ± 17.7	23.6 ± 32.7	n.d.	n.d.
2p/4p	n.d.	47.5 ± 28.5	n.d.	n.d.
3f	9.9 ± 8.2	22.3 ± 23.1	n.d.	n.d.
2f/4f	21.8 ± 20.1	30.3 ± 26.3	n.d.	n.d.
diF-GLYC	n.d.	39.6 ± 51.8	n.d.	n.d.
$2p_d/4p_d$	1.8 ± 1.3	28.3 ± 17.3	n.d.	n.d.
$2f_d/4f_d$	11.5 ± 12.9	7.2 ± 7.6	n.d.	n.d.
$2p_d/4p_d$	n.d.	26.8 ± 15.7	n.d.	n.d.

*n.d. not detected

PROald: protocatechuic aldehyde; pHBald: *p*-hydroxybenzaldehyde; Vald: vanillin; PROA: protocatechuic acid; pHBA: p-hydroxybenzoic acid; VA: vanillic acid; CA: caffeic acid; SYRA: syringic acid; pCA: *p*-coumaric acid; FA: ferulic acid; iFA: isoferulic acid; diFA: diferulic acid; C-GLYC: caffeoylglycerol; F-GLYC: feruloylglycerol; pC-GLYC: *p*-coumaroylglycerol; 5p: avenanthramide; diC-GLYC+2c: dicaffeoylglycerol and avenanthramide 2c; 5f: avenanthramide; 2p/4p: avenanthramide; F-C-GLYC+2f: feruoyl-caffeoylglycerol and avenanthramide 2f; $5p_d$: avenanthramide; 2p/4p: avenathramide; 3f: avenanthramide; 2f/4f: avenanthramide; diF-GLYC: diferuloylglycerol; $2p_d/4p_d$: avenanthramide; $2f_d/4f_d$: avenanthramide; $2p_d/4p_d$: avenanthramide.

Beside the typical phenolic aldehydes and acids previously observed in oat (i.e. protocatechuic acid, *p*-hydroxybenzaldehyde, vanillin, *p*-hydroxybenzoic acid, vanillic acid, caffeic acid, *p*-coumaric acid, and ferulic acid), protocatechuic aldehyde and syringic acid were also identified in the samples. Vanillin proved to be clearly the most abundant phenolic aldehyde in both the hull and the groat. Vanillic acid and *p*-coumaric acid were found to be the major phenolic acids in hull, and caffeic acid was the predominant in groat.
After liberation of bound phenolics a total of 10 phenolic compounds, namely, 2 phenolic aldehydes and 8 phenolic acids were identified. The phenolic aldehydes included *p*-hydroxybenzaldehyde and vanillin. Protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, syringic acid, *p*-coumaric acid, ferulic acid and isoferulic acid were observed as simple phenolic acids. Besides the monomeric phenolic acids, a ferulic acid dehydrodimer was also detected referred to as diferulic acid. Ferulic acid and *p*-coumaric acid were the most abundant bound phenolics. To the best of our knowledge no data has so far been published about the bound phenolic composition of oat hull.
The soluble and bound phenolic content of the hulls was higher than that of the groats. In addition, our results showed that the amount of bound phenolic compounds was higher than that of the soluble phenolics both in the hulls and the groats.

3. Avenanthramides

An extraction method was developed for the determination of the avenanthramides, solely present in oats among cereals. The effect of extraction solvents (methanol, ethanol, propanol), extraction methods (heating, ultrasound, horizontal shaker) and mass/volume ratio on the yield of avenanthramides was investigated. The final method was applied for the determination of aventhramides in the hull and the groat of the genotypes.
Several avenanthramides, 5p, 2c, 5f, 2p/4p, 2f, 5pd, 3f, 2f/4f, 2pd/4pd, 2fd/4fd using the Dimberg's nomenclature were detected in the groats and hulls (Table 1.). Their identification was based on the characteristic MS2 fragmentation pattern. Some of the avenanthramides could not be identified unambiguously, because the compounds differed from each other only in the position of the hydroxyl groups could not be distinguished by MS. Avenanthramide 2p and 5p proved to be dominant in groats, while 2p/4p, 2f/4f and 2pd/4pd were the major forms in hulls. The avenanthramide content of the hulls was two times higher than that of the groats in all color groups.

4. Mono-, and diglycerides

Glycerol derivatives of caffeic and ferulic acid and a lesser amount glycerol derivative of p-coumaric acid were detected in the methanolic extracts of the hulls and groats. The MS2 and MS3 experiments revealed that caffeoylglycerol, feruloylglycerol, *p*-coumaroylglycerol as monoglycerides and dicaffeoylglycerol, feruloyl-caffeoylglycerol, diferuloylglycerol as diglycerides were identified in the extracts (Table 1.). So far there were no mass spectrometric evidences about the presence of mono-, and dicinnamoylglycerols in oat. The amount of the glycerols was the highest in the hulls (especially in the black hull) and exceeded the amount of the phenolic acids.

5. Antioxidant activity

The antioxidant activity (AOA) of the genotypes was characterized using the DPPH method, which assesses the scavenging capacity of hydrogen donating antioxidants toward the stable free radical. Mean AOA of the hull soluble phenolic fraction ranged among genotypes between 1.52 and 8.78 μmol/g and between 0.55 and 2.06 μmol/g in the case of the groat soluble fraction. Therefore, soluble phenolic fraction of hulls had higher AOA than that of soluble phenolic

fraction of groats in all color groups. Similar differences were observed in the case of the bound phenolic fraction of the samples. Namely, hull extracts had higher AOA than groat extracts. Mean AOA of the hull bound phenolic fraction ranged among genotypes between 1.58 and 9.32 μmol/g and between 0.02 and 0.06 μmol/g in the case of the groat-bound phenolic fraction. It is important to note that the scavenging capacity of the hull soluble fraction was comparable to that of the hull bound fraction. Furthermore, the antioxidant activity of the groat soluble fractions proved to be 20-50-fold higher than those of bound fractions. Comparing the average AOA of the different color groups, it can be concluded that the black hull extracts exhibited the highest AOA (5.99 μmol/g for the soluble, 8.50 μmol/g for the bound) almost twice as high the AOA of yellow extracts (3.80 μmol/g for the soluble, 3.61 μmol/g for the bound). AOA of both the hull and groat extracts for the yellow and red colored groups were not different from one other, except the hull bound fraction. To identify the origin of the higher AOA of the black hull extracts and explain the difference between the AOA of the groat and hull, correlation analysis was performed. The results supported the observed relationship between the hull color and hull AOA. A strong negative correlation ($r = -0.685$, $p < 0.05$) was obtained for L color space coordinate with AOA of the hull soluble phenolic fraction. However, there was no correlation between L and AOA of the groat soluble phenolic fraction. According to the correlation analysis, color dependence of the AOA of the hull soluble fraction may be related to the phenolic aldehydes ($r = -0.742$, $p < 0.05$), monoglycerides ($r = -0.817$, $p < 0.05$), and to the mixed compound (avenanthramides and diglycerides) content of the hull ($r = -0.717$, $p < 0.05$). To explain the higher AOA of hull compared to those of groats, we have compared the soluble phenolic content of the hulls and groats. Avenanthramides and diglycerides were the predominant phenolics determined in both hulls and groats. Every compound group detected min. 3 times more quantities in hulls than groats. Moreover, the phenolic aldehyde content of the hulls was 25 times higher than that of groats.

Similar to the soluble phenolic fraction, a strong negative correlation ($r = -0.763$, $p < 0.05$) was obtained for L color space coordinate with AOA of the hull bound phenolic fraction. However, a weak correlation ($r = -0.481$, $p < 0.05$) was observed between L and AOA of the groat bound phenolic fraction. On the basis of the correlation analysis we could surmise that the higher AOA of the hull bound phenolic fraction is due to the high amount of vanillic acid, vanillin, diferulic acid, and especially ferulic acid, in the hull. Interestingly, the correlation between hull AOA and *p*-coumaric acid was opposite to the relationship between hull AOA and the other phenolic acids. Specifically, hull AOA was negatively correlated with the *p*-coumaric acid content. This observation is in agreement with the results about the pigmentation of *A. sativa* species. The black color arises from melanin which is proved to be built from *p*-coumaric acid monomers. The higher AOA of hull bound phenolic extracts compared with groat bound phenolic extracts can be explained by bound phenolic compounds in the hulls present in a 20-fold higher amount. Principal component analysis was performed on the determined variables of the hulls and the groats in order to confirm any relationships between the variables and assess similarities and differences among the genotypes. Because soluble and bound phenolic compositions of the samples were completely different, PCA was applied to these fraction separately. Color space coordinates, antioxidant activity, and phenolic compounds presented as sum values according to their compound type: total phenolic aldehydes, total phenolic acids, total avenanthramides, total monoglycerides and total mixed compounds were subjected to PCA in the case of the soluble phenolic fraction of the groats and hulls. The statistical analysis retained two principal components of which the first explained 46.75% of the variance, while the second added 21.74% (Fig.2.).

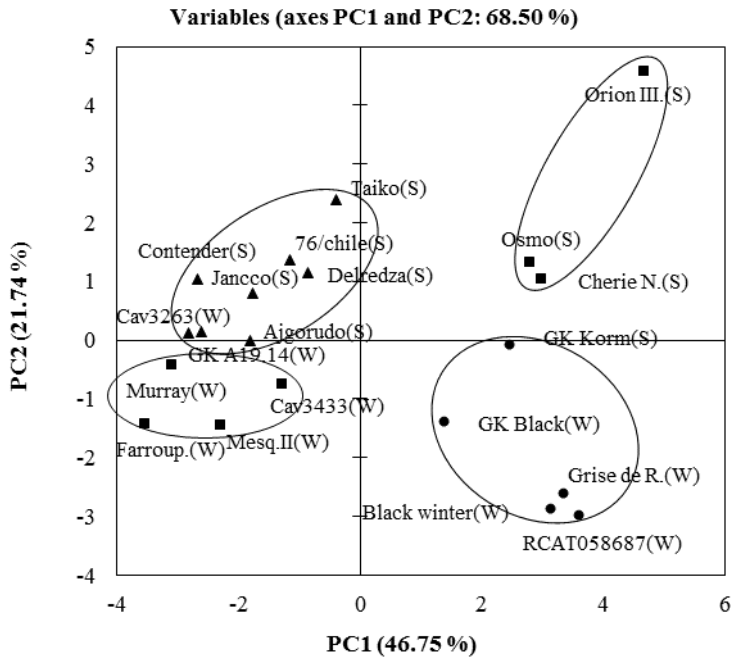


Fig.2. Results of the PCA analysis performed on the determined variables of the soluble extracts of the hulls and the groats. White-hulled (▲), reddish-hulled (■), black-hulled (●) genotypes. Spring-type (S), winter-type (W) genotypes.

The AOA of the hull, the mixed compound content of the hull, the total aldehyde, total acid, and total monoglyceride content both of the hull and the groat were the dominant contributors for PC1. Regarding the color space coordinates, the L, b* parameters represented negative loadings in PC1. Investigating the contributors to the PC2, the AOA of the groat, mixed compound content of the groat and avenanthramides found both in the hull and the groat were the main loadings. The scores plot (Fig. 2) indicates that there were differences between the genotypes. According to this figure, the first principal component resolved the varieties according to their hull color and a differentiation of spring cultivars from winter ones was observed by PC2. Black-hulled varieties showed a tendency to higher hull AOA, higher total content of phenolic acids and aldehydes and glycerides in comparison to the white-hulled varieties. However the reddish-hulled samples were not separated from the accessions of the other color groups. While the winter varieties were grouping with the white-hulled samples, the spring cultivars positioned with the black-hulled samples. The spring cultivars from each color group were characterized by higher groat AOA and greater amounts of avenanthramides in contrast to the winter ones.

With respect to the bound phenolic fraction of the samples, the color space coordinates, antioxidant activity of the groats and hulls, and the insoluble phenolic acids recovered through alkaline hydrolysis from the groats and hulls were subjected to PCA. The statistical analysis retained two principal components of which the first accounted 40.33% of the variance, while the second explained 18.26% (Fig.3.). AOA of the hull, most of the bound phenolic compounds detected in the hull, and p-hydroxybenzaldehyde, protocatechuic acid, and syringic acid quantified in the groat bound fraction were the main contributors for PC1.

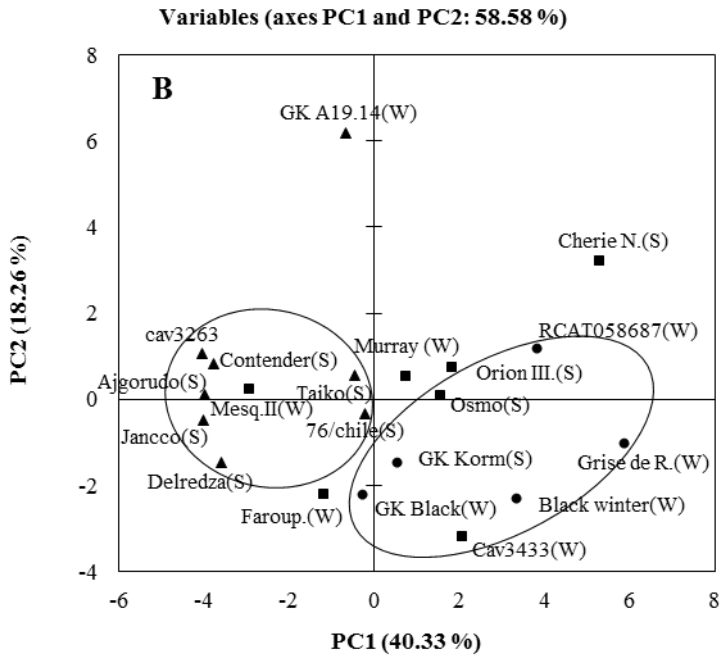


Fig.3. Results of the PCA analysis performed on the determined variables of the bound extracts of the hulls and the groats. White-hulled (▲), reddish-hulled (■), black-hulled (●) genotypes. Spring-type (S), winter-type (W) genotypes.

L and b* color space coordinates presented negative loadings in PC1. AOA of the groat, most of the bound phenolics found in the groat and bound pCA identified in the hull were the main loadings in PC2. The scores plot (Fig.3.) indicates a clear separation of the samples along the first principal component. In contrast, the genotypes were found over a narrower range along the second principal component. Therefore, black-hulled varieties showed a tendency to higher hull AOA and higher concentration of bound phenolics in the hull in comparison to the white-hulled varieties. However the reddish-hulled samples were not separated from the accessions of the other color groups.

A journal paper has been submitted and accepted with minor revision from the results about the phenolic composition and antioxidant activity of oats with different hull color.

Varga, M., Jójárt, R., Fónad, P., Mihály, R., Palágyi, A.. Phenolic composition and antioxidant activity of colored oats. Food Chemistry.

6. Oat fat soluble vitaminers – carotenoids and tocopherols, tocotrienols

Oats contain a significantly higher level of oil than other cereal grains with a high proportion of unsaturated fatty acids. Beside their health-preventing role, phenolics, avenanthramides and vitamin E as endogenous antioxidants contribute to the stability of oat food products by preventing the oxidation of free fatty acids and affect the flavor of processed oat products.

A method was developed and validated for the simultaneous extraction of E vitaminers (tocotrienols, tocopherols) and carotenoids (lutein, zeaxanthin, cryptoxanthin and beta-carotene) from the oat genotypes. It involves sample saponification (70°C, 50 min, in potassium hydroxide, ethanol, sodium chloride, pyrogallol mixture) and extraction (n-hexane/ethyl acetate, 9:1 v/v) followed by reversed phase HPLC separation with UV (carotenoids, λ=450nm,) and FLD (tocopherols, λ_{Ex}=290nm, λ_{Em}=330nm,) detection using a linear gradient of tetrahydrofuran, acetonitrile and water mixtures.

The genotypes were found to exhibit simple carotenoid profiles consisting mainly of lutein (0.94 mg/kg) and zeaxanthin (0.40 mg/kg) with small concentrations of β-carotene (0.06 mg/kg). Regarding the E vitaminers, α-tocopherol (8.86 mg/kg), and α-tocotrienol (17.78 mg/kg) were the major formes. The mean summa concentration of the β- and γ-tocotrienols was 6.62 mg/kg, while 0.87 mg/kg was measured for the β-tocopherol. No σ -tocotrienol or σ-, and γ-tocopherols were found in the genotypes. PCA analysis was performed on the eight variables including all measured carotenoids, tocopherols and tocotrienols. The first principal component (PC1) explained 43.79% of the total variance. The second principal component (PC2) accounted for an additional 26.53% of the total variance (Fig.4.). The first component positively correlated with the concentration of β-carotene and α-tocopherol. Lutein, zeaxanthin, β-tocopherol, α-tocotrienol, β- and γ-tocotrienols were the main contributors to the PC2.

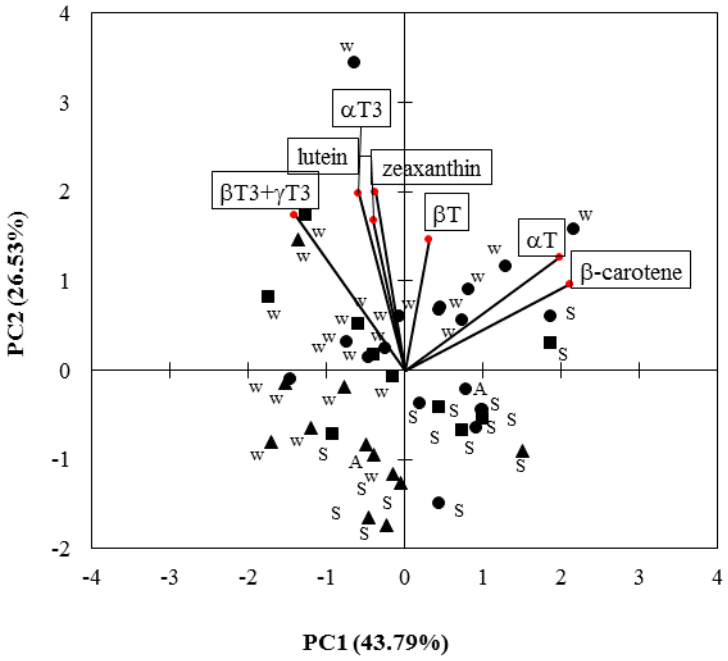


Fig.4. Results of the PCA analysis performed on the determined variables of the fat soluble vitamin extract of groats. White-hulled (▲), reddish-hulled (■), black-hulled (●) genotypes. Spring-type (S), winter-type (W) genotypes. αT: α-tocopherol; βT: β-tocopherol; αT3: α-tocotrienol; βT3+γT3: β- and γ-tocotrienols.

PCA resolved winter genotypes from the spring ones. Winter-type genotypes were characterized by a higher content of lutein, zeaxanthin and E vitaminers than spring accessions. Spring genotypes had higher β-carotene and α-tocopherol content. The black-hulled samples were noticeably separated from the other accessions due to their higher carotenoids and vitamin E content.

7. Carbohydrates – mono-, di-, and trisaccharides

A novel extraction and LC-MS method have been developed for the determination of mono-, di-, tri-, and tetrasaccharides from cereal grains. The extraction capacity of methanol and water at 25 and 85 °C using ultrasound and vertical shaker were tested. In order to obtain maximum sensitivity, different MS parameters were optimized. The effects of the drying gas flow and pressure, the nebulizer gas flow and pressure, the fragmentor voltage, and the the capillary

voltage on the ionization efficiency were investigated. Ionization of the carbohydrates was tested by addition of H^+, Li^+, Na^+ and Cs^+ post-column to the effluent. The highest signal intensity was achieved on the use of Na^+. The influence of the mobile phase composition on the intensity of the carbohydrates signal was optimized by using acetonitrile, methanol and water. The combination of acetonitrile and water with a linear gradient was found to be optimum for the separation of carbohydrates. The method developed was applied to the analysis of wide array of cereal grains (Fig.5.). In general, the samples contained manly disaccharides except sorghum and buckwheat varieties, which proved to be a rich source of monosaccharides.

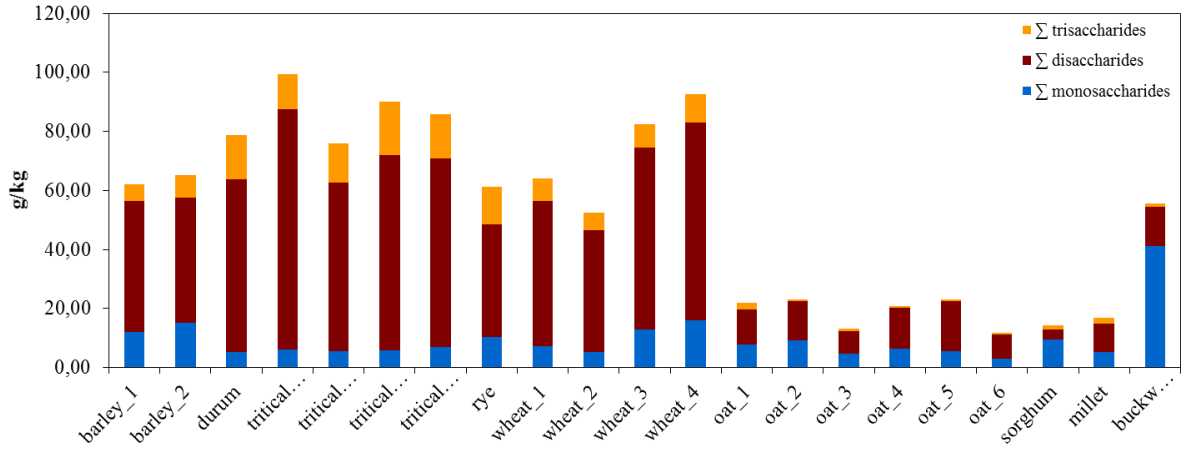


Fig.5. Mono-, di-, and trisaccharid content of cereal grains.

Oat, sorghum and millet had the lowest level of carbohydrates whereas triticale, barley and wheat had the highest. Fructose and especially glucose as free monosaccharides, saccharose and mainly maltose as free disaccharides and 6G-kestose, 1-kestose and raffinose in equal quantity as trisaccharides were detected in all cereal grains.
A MSc diploma work has been defended from these results in 2016 (T. Tasi, Determination of mono-, di-, and oligosaccharides in cereals by LC-MS, University of Szeged).

8. Allelopatic compounds

It is known that oats has an inhibitory effect to seed germination and early seedling growth of weed plants. This allelopathic potential might be due to phenolic compounds, for example L-DOPA. For identification and quantitative determination of of these compounds an extraction method and a HPLC-MS/MS method were developed. Various LC and MS parameters were optimized from the aspect of sensitivity. The effects of the nebulizer gas pressure and temperature and the drying gas pressure were studied. The results revealed that the investigated parameters did not have significant effects on the ionization efficiency of L-DOPA. However, an outstanding signal intensity was observed at a nebulizer gas pressure of 60 psi and a drying gas pressure of 25 psi at a nebulizing gas temperature of 350 °C. After the establishment of the MS parameters, the influence of the mobile phase composition on the efficiency of L-DOPA ionization was investigated using acetonitrile, methanol, formic acid, acetic acid and ammonium acetate. It was found that formic acid and acetic acid were more suitable than ammonium acetate as mobile phase additives. No matter which individual solvent was used, there was no marked effect on the signal intensity. The validated method was applied for the determination of the L-DOPA content of different oat varieties. Correlation analysis between *L* color space coordinate and L-DOPA content of the seeds revealed a week negative correlation

(r = -0.421) between these parameters. L-DOPA content of root, stem, leaf and spike of a white and a black hulled oat at booting, heading and milk-ripening phenological stages in greenhouse were determined either. In the case of the black hulled genotype the highest amount of L-DOPA was detected in the root at all developmental stages (Fig.6.).

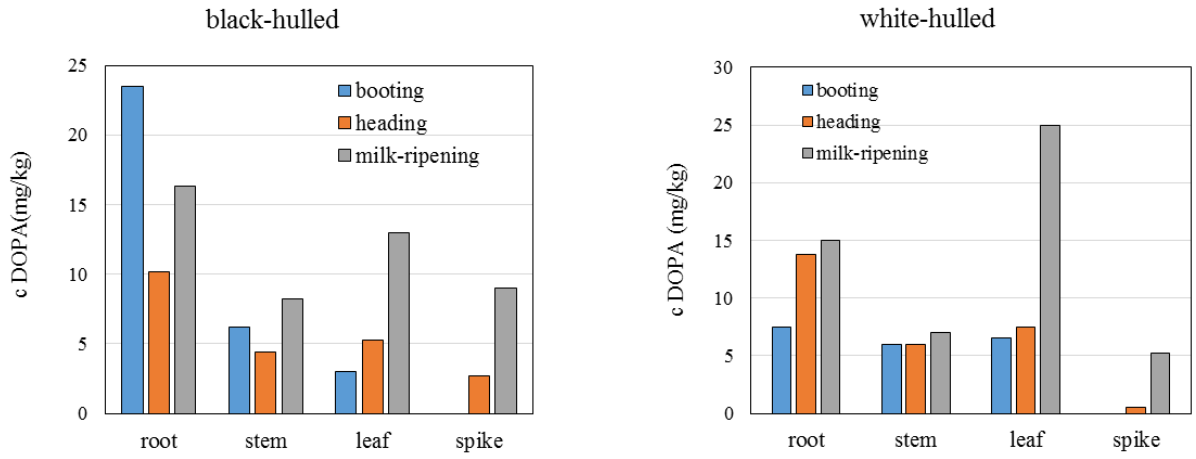


Fig.6. L-DOPA content of the different parts of a black-hulled and white-hulled oat genotypes.

The root extracts of *A. fatua* and some *A. sativa* genotypes with different hull color at different developmental stages were analysed in order to estimate the allelopathic potential of the samples. Thirteen phenolic compounds were identified, namely ferulic acid, isoferulic acid, caffeic acid, vanillic acid, 5-hydroxyferulic acid, gentisic acid, *p*-coumaric acid, hydroxyphenylacetic acid, dimethoxyphenylacetic acid, DOPA, methyl-DOPA, DIBOA and scopoletin. To make an appropriate interpretation of root composition, related to the effect of genetic origin and growth stage on oat genotypes, principal component analysis was performed on the all compounds detected in the root (Fig.7.).

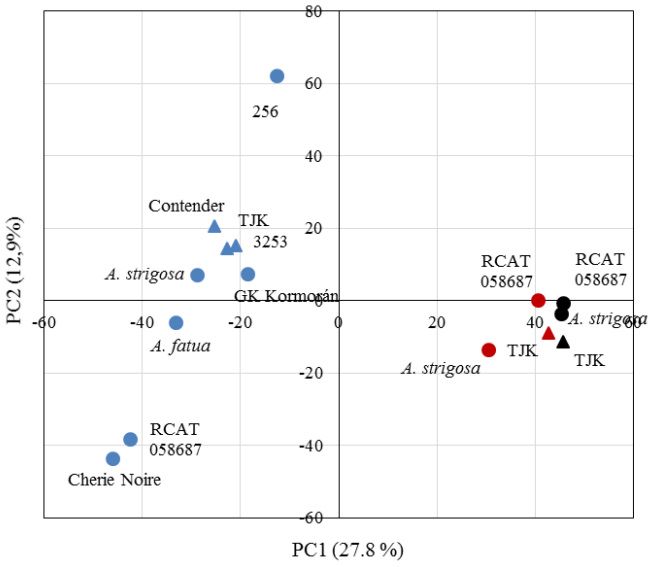


Fig.7. Results of the PCA analysis performed on all compounds detected in the root extract of the genotypes at different developmental stages. White-hulled (▲), black-hulled (●) genotypes. Black: heading, red: milk-ripening, blue: grain.

The loading plot showed that the genotypes were separated according to their developmental stage. Therefore, the concentration of the individual compounds varied considerably in relation to the plant growth stage. Furthermore, a differentiation of the black-hulled genotypes from the white-hulled ones was observed at all developmental stage. Consequently, the root composition of the black-hulled genotypes was different from the white-hulled ones.
A journal paper has been published from the results about the method development of L-DOPA analysis.
Varga, E., Varga, M.. (2014). Development and validation of an LC-MS/MS method for the analysis of L-DOPA in oat, Acta Biologica Szegediensis, 130, 313-320.

The main results of the project:
Determination of the structure of an allomelanin.
Detailed description of the free and bound phenolic composition (including the first described oat mono-and diglyceride esters of phenolic compounds and several avenanthramides) of 20 oat genotypes.
Correlation between the hull color and the antioxidant activity.
Correlation between the hull color and the carotenoid and vitamin E content.
Demonstration of the allelopatic potential of black-hulled genotypes.
Comparison of the mono-, di-, and trisaccharide content of cereal grains.
Development of numerous analytical methods for routin analysis.