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CHARACTERISTICS OF THE DARK GRAIN CORN COLLECTION BY ANTHOCYANIN COMPLEX

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Abstract

Corn (*Zea mays* L.) with dark-colored grains accumulate anthocyanins - pigments from the class of flavonoids, which are products of secondary metabolism and have antioxidant properties. Numerous epidemiological studies have shown that consuming foods rich in anthocyanins leads to a significant reduction in diabetes, obesity, cardiovascular and oncological diseases. Participating in metabolism, anthocyanins have a beneficial effect on maintaining health. Based on this, the present study was evaluated the total content and composition of anthocyanins in the grains of 21 local dark corn samples. For this purpose, reversed-phase high-performance liquid chromatography (HPLC) with spectrophotometric and mass-spectrometric detection was used. In the grains of the studied forms, mainly cyanidin-3-glucoside and pelargonidin-3-glucoside, as well as isomeric products of their mono- and diacylation with malonic acid, were identified. Pelargonidin-3-glucoside derivatives predominated in the grain extracts of some studied forms. Promising corn samples were selected as a source material for further breeding work to create local forms with improved nutritional and therapeutic properties.

Key words: corn, anthocyanin, cyanidin-3-glucoside, pelargonidin-3-glucoside, HPLC.

1. Introduction

Corn (*Zea mays* L.) is an important cereal plant, widely grown in the world, with high nutritional value and industrial significance. Corn is important for the biosynthesis of many micronutrients and biologically active substances, the most important of which are anthocyanins. Anthocyanins are water-soluble pigments belonging to the flavonoid group that are synthesized in complex metabolic pathways and, accumulating in cell vacuoles, give plant parts a wide range of colors from pink to purple. Having arisen evolutionarily during the adaptation of plants to a terrestrial lifestyle, their main function is to attract animals as pollinators and seed distributors [1]. Their photoprotective effect is manifested in protecting the photosynthetic apparatus from oxidative damage by absorbing excess solar radiation [2].

Anthocyanins as natural dyes are actively introduced into the food and cosmetic industries, replacing

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undesirable or prohibited synthetic dyes in food products, for which their connection with degenerative diseases of people has been discovered [3–5].

Possessing high antioxidant properties, these pigments may provide various health benefits. Numerous epidemiological studies have shown that in food they exhibit, in addition to antioxidant properties, also antimicrobial, anticarcinogenic, anti-inflammatory and other health benefits [6–11].

The total content of anthocyanins have been established for many fruits and vegetables. But, compared with an equivalent amount of soft fruits or commonly eaten vegetables, cereal grains contain more of these substances in bound form. This determines their absorption and inclusion in metabolism in the large intestine, where their positive effect is manifested [12, 13]. Thus, the task of increasing anthocyanins in the daily diet, including through the consumption of cereal seeds, becomes relevant.

Almost all parts of the corn accumulate anthocyanins. The color shade of corn grain depends on the content and localization of different types of anthocyanins.

The combination of nutritional value and beneficial properties of anthocyanin-rich corn grains is the basis for their use as a functional product or one of its components. A high content of anthocyanins is characteristic mainly of Mexican and American varieties of corn with pigmented grains. They are traditionally grown in Mexico, Guatemala, Bolivia, and in the South American states of Colorado, Arizona, New Mexico and Texas - regions of the center of origin of corn [9, 14, 15]. During their spread across Europe, these forms of corn were unable to grow under the conditions of the long photoperiod and colder European climate. In addition, their spread was hampered by a cultural factor in the form of preference for yellow- and white-grained forms of corn for flour production [9].

Based on the above, the use of the genetic potential of local pigmented forms of corn, the selection of promising genotypes with respect to the composition and content of beneficial anthocyanins is the basis for breeding to improve and increase the preventive and nutritional properties of grain.

Local forms of dark grain corn represent a potential source of genetic variation adapted to environmental conditions. For the collection of corn with anthocyanin grain coloration that we created, a high level of genetic polymorphism was identified [16]. The purpose of this work was to study the anthocyanin complex in the grains of these samples to identify promising forms of corn as a source material for further selection to increase the anthocyanin content in the grain.

2. Materials and Methods

A corn collection with dark-colored grain, including inbred lines, simple and complex hybrids, obtained by us as a result of several successive years of inbreeding, hybridization and selection of forms with the most homogeneous and intensely colored grain served as research material. The color of the grain of the studied forms in this work, during a visual assessment, was determined as red (r) and blue (b) shades (corn forms of similar grain color by other authors were called purple and blue, respectively). Quantitative determination of the total content of anthocyanins and analysis of the composition of the anthocyanin complex in the grains of the studied corn samples was carried out using reverse-phase HPLC with spectrophotometric and mass spectrometric detection at the Institute of Pharmacy, Chemistry and Biology of Belgorod State National Research University.

Extraction of grain anthocyanins was carried out by infusing crushed plant material in a 0.1M HCl solution at room temperature and periodically stirring, leaving the mixture overnight at room temperature. The resulting solution after filtration was used for spectrophotometric determination of the total anthocyanin content. To perform HPLC, the extracts were purified by solid-phase extraction using DIAPAK C18 concentrating cartridges (BioChemMakST, Moscow) [17].

Spectrophotometric determination was carried out as follows: an aliquot portion of the extract (*Va*) was diluted by adjusting the pH to 1 (1M HCl in water) in a flask of volume *Vk* and the spectrum was recorded relative to ethanol. Another portion of a solution of the same volume was brought to pH 4.5 with a 1M aqueous solution of NaOH, diluted with acetate buffer pH 4.5, and a new spectrum was recorded.

Based on the obtained optical density valuesat the wavelength with maximum absorption at pH 1, the following was calculated:

a) optical density – according to the equation:

$$A=[A_{\lambda max}(\text{pH } 1)-A_{700nm}(\text{pH } 1)]-[A_{\lambda max}(\text{pH } 4.5)-A_{700nm}(\text{pH } 4.5)];$$

b) the content of anthocyanins in the original solution - according to the formula:

$$\alpha = (A/26900)\bullet(Vk/Va)\bullet Ve\bullet 484.8\bullet 100/m,$$

where 26900 - is the molar absorption coefficient, l/(mol•cm); *Ve* – initial volume of extract, l; *m* – mass of a sample of plant material, g; 484.8 – molar mass of cyanidin-3-glucoside chloride, g/mol; 100 – coefficient for conversion per 100 g of raw materials.

To determine the composition of anthocyanins, an Agilent 1260 Infinity Chromatographic System with diode array and mass spectrometric (Agilent 6130 Quadrupole LS/MS) detectors was used. Mass spectra were recorded in ESI mode (electric sputter ionization) with scanning mode for positively charged ions; voltage on the fragmenter: 150V was used to record the initial flavylium ions, and 250V was used to determine aglycones during fragmentation of the initial ions. The separation was carried out on a 150×2.1 mm Kromasil-100 5C18 column (column thermostat temperature – 40°C) with a mobile phase: 10vol.% CH_3CN, 10vol.% HCOOH, 80vol.% water (0.150 ml/min); the chromatogram was recorded at 515 nm, processed and stored using the ChemStation 32 program [18].

3. Results and discussion

The chromatographic profile of anthocyanins in the grains of all studied samples was similar, but with different ratios of their types. Cyanidin-3-glucoside - Cy3G and pelargonidin-3-glucoside - Pg3G, as well as their mono- and diacylated derivatives were identified (Fig.1; Tab. 1, 2).

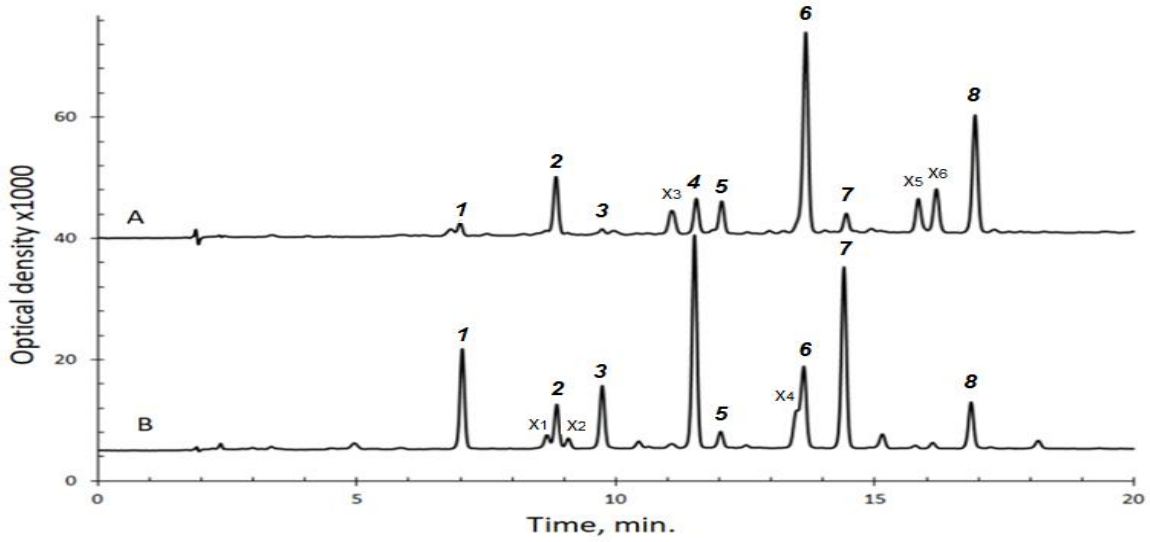

Fig.1. Chromatograms of pelargonidin (A) and cyanidin (B) types of anthocyanins in corn grains.

Substances: 1- Cy3G; 2 - Pg3G; 3 - Cy3(3″MG); 4 - Cy3(6″MG); 5 - Pg3(3″MG); 6 - Pg3(6″MG);

7-Cy3(3",6″diMG); 8 - Pg3(3",6″diMG); x_1, x_2 - minor isomers of malonated Cy3G

Of the derivatives of these anthocyanins, isomeric cyanidin-3-malonylglucoside with an unknown (marked with ?) and a known acylation position was identified: cyanidin-3-(?-malonylglucoside) - Cy3?MG, cyanidin-3-(3"-malonylglucoside) - Cy3(3″MG), cyanidin-3-(6"-malonylglucoside) - Cy3(6″MG), cyanidin-3- (?, 6′-dimalonylglucoside) - Cy3(?,6″diMG), cyanidin-3-(3″6"-dimalonylglucoside) - Cy3(3",6″diMG) (Tab. 1), pelargonidin-3-(3″-malonylglucoside) - Pg3(3″MG), pelargonidin-3-(6″-malonylglucoside)-Pg3(6″MG), pelargonidin-3-(?,6"-dimalonylglucoside) - Pg3(?,6″diMG), pelargonidin-3-(3",6"-dimalonylglucoside) - Pg3 (3″,6″diMG) (Tab. 2).

Table 1. Content of cyanidin-3-glucoside and its acylated derivatives in corn grains, mol.%

Samples	Cy3G	Cy3?MG	Cy3?MG	Cy3(3″MG)	Cy3(6″MG)	Cy3(?6″diMG)	Cy3(3″6″diMG)

UgSh70(b)	11.6	3.4	2.6	7.4	25.1	6.4	21.7
UgSh145	12.3	3.2	2.1	8.3	32	16.5	23.3
EHM221	12.5	2.5	2.1	8.4	35.7	11.4	25.3
EHM250	9.7	3.2	2.3	7.9	30.7	14	29
CSp-100(b)	15.1	3.3	2.2	7.7	35	15.1	20.3
CSp-100(r)	11.6	2.4	1.9	8.9	31.2	13.2	27.1
KF104	11.1	3.4	2.3	5.8	37.7	16.2	9.5
KF94(b)	15.8	4.7	2.5	7.2	34.8	11.4	16.6
KF94(r)	2.5	0	0	0	5.2	0	3.1
Julfa(b)	16.6	3.4	2	7.6	32.9	14	18.7
KF60(b)	11.2	1.8	2.1	7.9	27	12.1	31.3
KF5	11.9	1.8	1.8	10.4	25.7	9	31.7
KF88	12.9	3	2	7.6	31.4	14.6	22.2
EHM247	13.9	2.6	2.6	7.2	39	7.9	16.2
EHM250×Julfa	20	2.8	2	6.3	32.7	11.3	13.9
KF94(r)×EHM269	12.4	3.3	4.1	2.5	7.1	11.6	18
EHM269×KF94(b)	14	3.9	2.8	7.4	33.1	11.2	16.7
EHM250×KF60(b)	17.9	2.4	2.1	7.5	34.9	13.8	19.1
UgSh176×KF94(r)	11.2	1.9	1	7.6	24.7	5.4	22
EHM269×KF104	16.2	2.6	2	6.5	35.8	11.6	18.4
EHM250×KF94(r)	12	1.8	1.8	10.3	24.2	9.8	31.4

Not: ? – unknown localization of the malonyl radical in the glucoside substituent.

Acylated anthocyanin derivatives have been shown to have greater solubility and resistance to changes in pH compared to their glucosides, which is an important prerequisite for protecting glucosides from enzymatic degradation and for stabilizing the structure of anthocyanins during their transport to the vacuole [19, 20].

Table 2. Content of pelargonidin-3-glucoside and its acylated derivatives in corn grains, mol.%

Samples	**Pg3G**	**Pg3(3″MG)**	**Pg3(6″MG)**	**Pg3(?,6″diMG)**	**Pg3(3″,6″diMG)**
UgSh70(b)	3.7	2.7	6.3	0	4
UgSh145	0.6	0	0	0	1.6
EHM221	0.5	0	0	0	1.6
EHM250	0.3	0	0	0	3
CSp-100(b)	0.7	0	0	0	0.7
CSp-100(r)	0.8	0	0	0	2.5
KF104	1.3	0	0	0.9	0.6
KF94(b)	3.3	0	0	9.3	3.8
KF94(r)	7.9	8.8	32.7	8.3	19
Julfa(b)	1	0	0	0	1
KF60(b)	0.8	0	0	0	3.3
KF5	0.9	0	0	0	2.5
KF88	0.5	0	0	0	1.4
EHM247	4	0	9.4	0	3.8
EHM250×Julfa	1.7	2.8	4.7	0	1.8
KF94(r)×EHM269	4.1	2.5	17.3	0	7.9
EHM269×KF94(b)	3.9	0	0	11.6	7
EHM250×KF60(b)	0.7	0	0	0	1.6
UgSh176×KF94(r)	5	2	10.2	0	5.9
EHM269×KF104	0.9	0	0	0	1
EHM250×KF94(r)	0.9	0	0	0	1.7

Not: ? – unknown localization of the malonyl radical in the glucoside substituent.

Anthocyanin biosynthesis is a branch of the complex phenylpropanoid metabolic pathway of plant cells and occurs with the obligatory participation of the key enzymes dihydroflavonol reductase (DFR) and flavanone-3′-hydroxylase (F3′H) [9]. Depending on the activity of these enzymes, different types of anthocyanins accumulate in different organs of corn.

In most of the studied forms of corn, the content of cyanidin-3-glucoside and its derivatives in grain extracts exceeded the content of pelargonidin-3-glucoside and its derivatives, which were represented by minor components of the mixture.

The results obtained on the composition of anthocyanins in corn grains were, in general, consistent with the literature data that the main component of the anthocyanin complex in corn grains is cyanidin-3-glucoside and its derivatives [4, 5]. At the same time, the indicators of four samples of our collection did not correspond to the above-mentioned ratio of anthocyanin types (about the predominance of cyanidins over pelargonidins) (Fig. 2): in the grains of the inbred line UgSh70 and the hybrid UgSh176×KF94(r), the Pg3G content was 1/4 and 1/3 part of the anthocyanins, respectively, in another hybrid KF94(r)×EHM269, the C3G**:**Pg3G ratio was close to 4**:**3. In the grains of the inbred line KF94(r), a multiple excess of Pg3G over the C3G content was found.

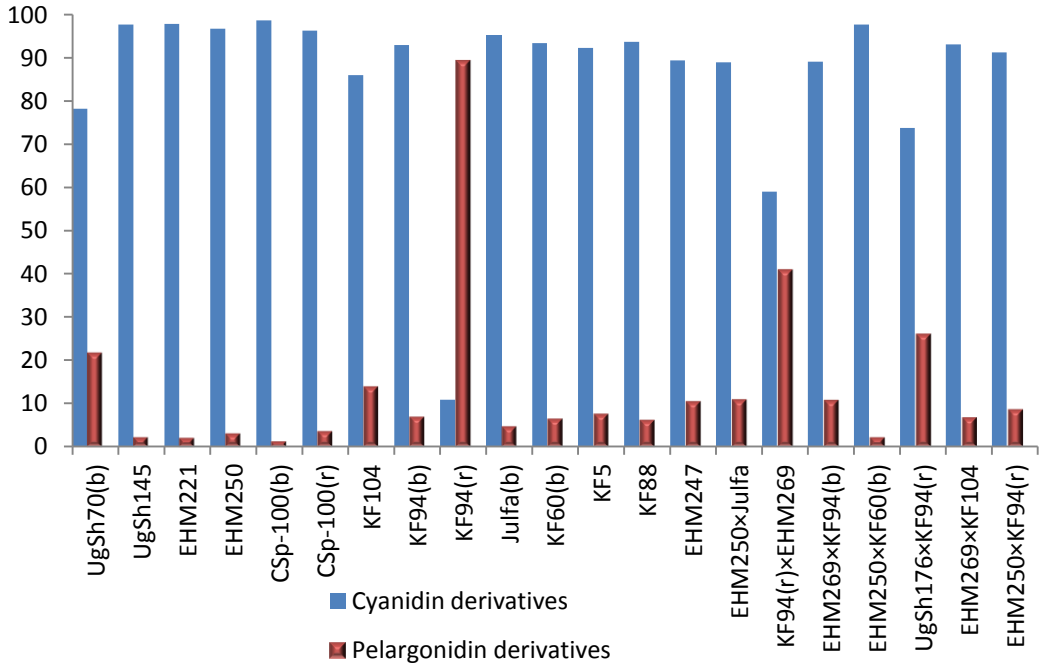

Fig 2. Relative content of anthocyanins in corn grain extracts

The data obtained on the content of these two types of anthocyanins suggest that in the cells of these forms there is a high activity of DFR and/or low activity of F3′H - key enzymes that direct metabolism along the path of pelargonidin biosynthesis. The presence of such "deviations" among samples enriches the genetic variability of the studied collection.

In all samples, with the exception of CSp-100b, Julfa(b) and the hybrid EHM269×KF104, the content of acylated anthocyanin derivatives exceeded the content of their glucosides. In the grain extracts of the noted samples, the ratio of Pg3G and its derivatives was equal. The content of monoacylated anthocyanin derivatives in the grains of all studied forms of corn was approximately two times higher than the content of diacylated ones.

Determination of the total accumulation of anthocyanins showed that their content in the grains of the studied sample was in the range of 0.0073–0.0270g per 100g of dry weight. The highest content of anthocyanins (0.0270g) was found in the hybrid UgSh176×KF94(r) (Fig. 3a), the lowest amount (0.0073g) was found in another hybrid – KF94(r)×EHM269.

It is noteworthy that one of the parental forms of these hybrids is the inbred line - KF94(r) (Fig. 3b), in the grain extracts of which a "non-standard" ratio of anthocyanins was revealed. Taking into account that

pelargonidin-3-glucoside is more resistant to breakdown in digestive processes in the intestine than cyanidin-3-glucoside [21], it can be said of the high practical value of the mentioned corn form.

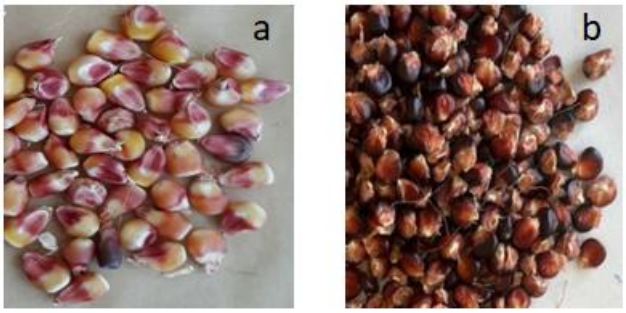

Fig. 3. Grains: a) corn hybrid UgSh176×KF94(r); b) inbred line KF94(r).

In 8 samples, the total accumulation of anthocyanins in the grain exceeded 0.0150g per 100g of dry weight (Fig. 4, highlighted in black).

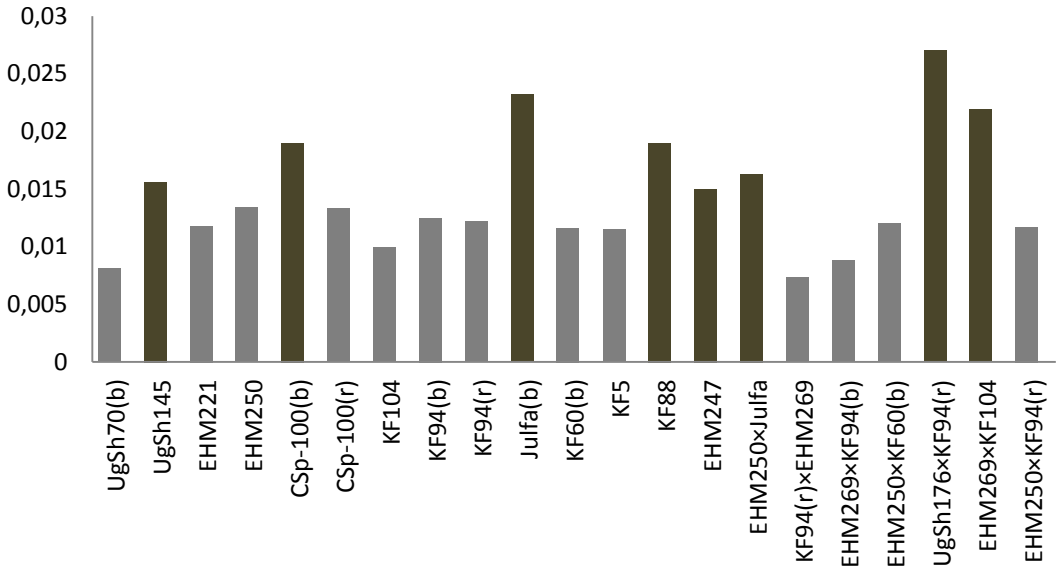

Fig. 4. The level of total accumulation of anthocyanins in the grains of the corn forms.

It should also be noted that the level of anthocyanin accumulation in the samples we studied was low compared to Mexican varieties of purple and blue corn. These varieties carry dominant alleles of regulatory genes that activate structural genes for anthocyanin biosynthesis [5, 9]. In addition, differences in the environmental and agronomic conditions of plant growing, the physicochemical properties of grain, and methods of anthocyanin extraction also affect the results obtained [5, 12].

4. Conclusions

Thus, in studying the anthocyanin complex of a local corn collection with dark-colored grain, mainly cyanidin-3-glucoside and pelargonidin-3-glucoside, as well as their mono- and diacylated derivatives with malonic acid, were identified. The content of cyanidin-3-glucoside and its derivatives dominated in most corn samples. At the same time, samples were identified in whose grain extracts the content of pelargonidin-3-glucoside derivatives was equal to or prevailed over cyanidin-3-glucoside derivatives. The samples selected as a result of this study - inbred lines - KF94(r), UgSh145, CSp-100(b), Julfa(b), EHM247, as well as hybrid forms - KF88, EHM250×Julfa(b), UgSh176×KF94(r) and EHM269×KF104 - are used as a source

material in breeding work on creating local corn forms with improved nutritional, therapeutic and prophylactic properties.

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