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Basic Characteristics of the Maize Grain Processing Products with *wx*, *ae* and *su* Mutations in The Genome of Endosperm (*Zea mays L.*) on Starch, as well as Morphological and Thermodynamic Features of The Isolated Starcheses

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Abstract: To understand the relationship between the genotype of maize plants, differing in their origin and the ploidy of the genome, which carry gene alleles programming the biosynthesis of various starch modifications, the thermodynamic and morphological features of starches from the grains of such plants have been studied. The peculiarities of the isolation of starch extracted from subspecies of maize (the dry matter (DM) mass fraction, starch content in DM grain, ash content in DM grain, amylose content in starch) belonging to different genotypes have been studied. Among the starch types of maize studied, four groups were earmarked to comprise the *wx, ae, su* and transitional starch types. The starches of the *su* genotype were shown to have the smallest starch granules compared to the *wx* and *ae* genotype starches. An increase in amylose content in the investigated starches was found to be accompanied by a decrease in their thermodynamic melting parameters, thus inducing the accumulation of defective structures in the studied starches. At the same time, the starches of *ae* and *su* genotypes possessed a more ordered structure than those of *wx* genotype. In the *ae*- and *wx*-type starches, thermodynamic parameters were evaluated for amylose–lipid complex dissociation, i. e. temperature (T_{aml}) and enthalpy (H_{aml}); in the case of the *su* genotype, temperature and enthalpy values of amylose–lipid complex dissociation were higher than those in the starches from the *ae* and *wx* genotypes. The thickness of the crystalline lamellae (L_{crl}) for all studied genotypes was virtually independent of the amylose content or the starch genotype. It was shown that the thermodynamic melting parameters of studied starches are determined both by amylose content in starch and by the individual features of the maize genotype.

Keywords: *Zea mays* L.; diploid and tetraploid genotypes; grain characteristics; starch; amylose; morphological and thermodynamic properties

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1. Introduction

Cereal crops are the main sources of starch. Besides, starches are contained in tuber crops (e. g. potato, manioc, etc.) and legumes as well as in some unripe fruits, such as bananas and mangoes [1]. It is well known that starches consist of two polysaccharides – linear amylose and branched amylopectin; there are also starches consisting of amylopectin molecules only. Both polysaccharides are homopolymers of α-D-glucopyranose (glucose), differing from one to each other in molecular mass and physicochemical properties. A linear molecule of amylose consists of α-glucose residues linked with

α-(1→4)-glycoside bonds. An amylopectin macromolecule is formed by branched chains of α-glucose residues connected with α-(1→4) and, in the chain branching points, with α-(1→6) glycosidic linkages. The structure of amylopectin is three-dimensional: its branches point in all directions, thus contributing to the spherical shape of its molecule [2,3]. The ratio of the two main polysaccharides in starches determines their functional and physicochemical properties. The ratio of amylose to amylopectin in starch is responsible for its rheological properties, whereas starch swelling power and solubility depend on the interaction among polymer chains, incorporating amorphous and crystalline fractions of starch granules [4]. The degree of this interaction is determined by the ratio of amylose to amylopectin and depends on the degree of polymerization, chain length and degree of branching, molecular mass, and molecular configuration [5,6]. Starch swelling power is directly linked to amylopectin content, since amylose acts as a diluent and swelling inhibitor [7,8]. In a large-scale production of native starches, the ratio of amylose to amylopectin averages 1:3. The amylose content in starches produced from the most widespread maize cultivars is usually 24–28%. An exception is maize with the amylose content higher than 50%. The rheological properties of maize starches with increased amylose content were shown to differ significantly from the conventional maize starches or those produced from waxy maize [2]. The content of amylose-lipid complexes in high-amylose starches in many aspects determines their functional properties [9, 10]. Amylopectin maize starches are characterized by an amylose content less than 10%, and by their rheological properties comes close to those of potato starch. Amylopectin maize starches are characterized by high melting temperatures of crystalline lamellae [11], high swelling power and viscosity, the durability of their colloids during the pasting process, and ability to impede amylose retrogradation, compared to conventional and high-amylose starches.

Structural and thermodynamic properties of starch were shown to be affected by the conditions of starch-yielding plant cultivation and by the presence of certain genes involved in the biosynthesis of starch polysaccharides [12]. The ratio of the main polysaccharides in starch and its physicochemical properties are determined by the activity of various enzymes involved in the biosynthesis of starch polysaccharides. The synthases responsible for the synthesis of starch and glycogen polysaccharides belong to the glycosyltransferase-B (GT-B) superfamily (CL0113) and are represented by: starch synthase (SS), glycogen synthase (GS), and starch phosphorylase (SP) [13]. Five independent conservative SS (GBSSI, SSI, SSII, SSIII и SSIV) have been found in plants [14,15]. The granule-bound starch synthase (GBSS) is crucial for amylose synthesis [16], while mutations in SSIIb and SSIII alter the levels of amylopectin content [17,18]. Three forms of expressed enzymes are known that promote branching of starch molecules in the endosperm of cereals with varying degrees: enzyme I (SBEI), enzyme IIa (SBEIIa), and enzyme IIb (SBEIIb) [19,20]. The genotypes with the waxy type of grain endosperm (*wx*) are characterized by the absence of GBSS, accompanied by the accumulation of only amylopectin in the complete absence of amylose, and the amylose type of grain endosperm (*ae*) characterized by a violation of SBEIIb, which is accompanied by the effect of accumulation of weakly branched polysaccharides and the accumulation of amylose in starch [21–24]. The maize with a very high level of amylose (>90%) was obtained when the enzymatic activity of SBEI was considerably reduced and the activity of SBEII was almost completely suppressed [25,26]. Mutant SBEIIb genotypes form starch granules in the endosperm with abnormal structure morphology due to the increased content of amylose in them, which is accompanied by the effect of increasing the starch melting temperature. Besides, this genotype was found to contain a considerable amount of a substance of the intermediate type between amylose and amylopectin [8,27]. The *sugary* (*su*) genotype with sucrose synthases (SS), generating phytoglycogen as a precursor of amylopectin, is characterized by a deficit of such enzymes as isoamylases and pullulanases, disturbing branching in starches. The high sugar content and low starch content in sweet sorghum and sweet corn indicate a high activity of this enzyme. Grains of these plants, as a rule,

are wrinkled, with a low DM concentration [28–30]. The results of studies on the effect of different maize genome ploidy on their starch were not found.

It is possible to regulate the synthesis of starch with pre-set structure and properties by arranging certain gene combinations. The objective of this study was to determine the thermodynamic and structural features of starches extracted from maize genotypes differing in their origin and genome ploidy, which carry gene alleles programming the biosynthesis of various starch modifications. It is also possible to correct the granule morphology and crystallinity in the extracted starches at the genetic level. As a rule, morphological traits in starch, including its physicochemical properties, are regulated not by one or two genes but by the gene network. Therefore, genotypes containing a certain portion of genes determining the structure and physicochemical properties of starch will differ in all or any of their parameters, thus representing an optimal raw material for various sectors of industry.

2. Experimental Section

2.1. Materials

The maize material was reproduced for the research in 2017 in a foothill area at the Kabardino-Balkarian Research Institute of Agriculture. The breeding site was located within the piedmont zone of the North Caucasus, at the watershed dividing the rivers Urvan and Nalchik. The soils were mostly represented by meadow chernozems. Cultivars and hybrids were reproduced under paper cages using the technique of cross-pollination within a population on a plot. Harvesting was manual, scheduled as soon as the ears reached full ripeness. The maize seed material was represented by 11 accessions of non-transgenic flint, dent and sweet maize subspecies from the global collection held by the N.I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR), selected for their high starch content in grain. Among the selected accessions there were cultivars (*Kabardinskaya belaya zubovidnaya, White Flint, Gornaya chechenskaya, Mais agestano, Populyatsiya MRPP22, Baksanskaya sakharnaya and Luch, Alina, Rannya Lacomka, Mestnaya*) of Russian and foreign origin, and a hybrid (*Otbornyy 150SV*) developed domestically. The accession *Baksanskaya sakharnaya* was isolated from Populyatsiya МРППП20 as a mutant for the su2 gene.

2.2. Methods

2.2.1. Cytological Analysis

In the morning hours, specimens of maize seedling rootlets prepared for chromosome analysis were fixed in acetalcohol (Carney's fixative solution) in the phase of three-day-old plantlets. Chromosome staining was performed according to the Feulgen protocol, using the Schiff reagent after hot hydrolysis in 1N HCL at 60 °C for 10 min, beyond that point chromosomes were stained at room temperature for one hour with the subsequent triple washing of specimens in sulphur water [31]. To facilitate crashing of the specimens, maceration with cellulase from *Aspergillus niger* (Sigma-Aldrich, USA) was employed.

2.2.2. Extraction of Starch and By-Products

Starch was isolated from maize grain using the modified technique offered by Adkins and Greenwood [33]. Maize grain (100 g) was immersed in a 0.4% solution of sodium metabisulphite (initial bulk concentration of SO_2 was ~0.20%) and infused for 48 h at 48–50°C. The maize extract was then separated from the grain, and the grain was coarsely crushed in a PLU-1 laboratory crusher (Research Institute for Starch Products, Russia). The ground grain was thoroughly washed from the crusher with 100 mL of distilled water. The maize germ was isolated from the grain matter and rinsed to remove free starch through a 0.5 mm metal sieve mesh with 100 mL of water; then, it was dried in a drying cabinet at 50–52°C. After that, the grain matter separated from the germ un-

derwent fine grinding in a Waring LB20ES laboratory blender (Conair Group, USA) at 40°C for 5 min with the rotational speed 3000 rpm. The resulting fine substance was sifted through a 70 μm nylon 6 sieve mesh. The grain matter left on the sieve surface underwent multiple was washed with distilled water (9 times, using 100 mL of water for each washing) until the rinsing water had become starch-free, which was verified by the iodine test. The resulting cellulose contained different mass fractions of the 'bound' starch. This cellulose was dried at 55–58°C until the constant weight was fixed. The starch/protein suspension was separated by centrifugation (Hermle Labortechnik, Z300K, Wehingen, Germany) for 30 min at 6000 rpm. The centrifuged starch and protein (maize gluten) sediment was further separated by washing the gluten layer out with distilled water. The starch was thoroughly mixed with 150 mL of distilled water, and gluten was separated from starch by a repeated centrifugation. The gluten suspension separated from starch was centrifuged (Hermle Labortechnik, Z300K, Wehingen, Germany) for 20 min at 6000 rpm. The obtained starch and gluten were dried separately at 50–55 °C for 24 h. The product yield and the mass fraction of starch in by-products were measured, including the same in the rinsing water. The resulting starch was studied using the described physicochemical methods.

2.2.3. Assessment of Grain Characteristics

Grain characteristics (moisture, ash and starch content) were assessed in compliance with the standard European techniques (ISO 6540:1980: Maize – Determination of moisture content [on milled grains and on whole grains]; ISO 2171:2007: Cereals, pulses and by-products – Determination of ash yield by incineration; ISO 10520:1997: Native starch — Determination of starch content — Ewers polarimetric method).

2.2.4. Measuring the Amylose Content

Amylose content was measured colourimetrically according to the described technique [34]. The result was compared with the Megazyme amylose/amylopectin assay procedure, utilizing the commercial kit (Megazyme Ireland International, Ltd., Bray, Ireland), followed in compliance with the manufacturer's recommendations.

2.2.5. Light Microscopy

Chromosome numbers of the specimens were calculated using the transmitted light microscopy. For each maize accession, the number of chromosomes in a somatic cell from the rootlet was counted in 15 metaphase plates of the crushed specimen under an Olympus CX43 microscope (Olympus Optical Co., Ltd, Japan) using 1600× zoom with immersion.

Light microscopy was used to analyse the morphology of the studied starch granules. Starch was placed on a microscope slide and stained with one drop of Lugol's iodine (a solution of potassium iodide with iodine in water) [32]. Then, the preparation was covered with a cover glass to ensure that starch granules were evenly spread under the glass. Filter paper was used to remove the excess of the dye. Starch granules were analysed using 400× zoom and a blue filter under the Micromed 3 lm optical microscope in transmitted light with an attached Oplenic psc 600 – 15c (B51) imaging camera (Oplenic Corp., USA).

2.2.6. Scanning electron microscopy (SEM)

Morphological changes of the starch granule were investigated with scanning electron microscopy (SEM). Photos of starch granules were obtained with the use of scanning electron microscope Mira3 LMU (Tescan, Brno, Czech Republic) at room temperature in the conditions of high vacuum with accelerating voltage 500 V.

Size and morphology of the maize starch granules were measured or estimated using an extended suite of morphometric algorithms under the "Altami Studio" licensed

software GUI. Besides the standard granulometric characteristics, a number of dimensionless shape descriptors (such as ellipticity factor) was analyzed and compared. Ellipticity factor was calculated by the above-mentioned software as the ratio between the starch granule squares and squares of the ellipses with equivalent moments of inertia. In the case of elliptic approximation the ellipticity coefficient was taken to be unity.

2.2.7. Differential Scanning Calorimetry

Thermodynamic melting parameters for 0.3% (w/w) water dispersions of the investigated maize starches were measured using the method of highly sensitive differential scanning calorimetry (DSC) on a DASM-4 microcalorimeter (Pushchino, Russia). The volume of the studied sample was 0.5 cm^3 in a closed capsule. Measurements were conducted at the temperature range of 20–120 °C, at the heating rate of 2°C/min and under the constant pressure of 2.5 mPa. DSC measurements for each sample were repeated three times. The excess heat capacity scale was calibrated using the Joule–Lenz effect for each experiment. Earlier it was shown that under the given conditions there was no need to take into account thermal lags or the sample treatment duration in the calorimeter capsule [35]. Deionized water (Millipore Direct-Q3) with the special resistance of 18.2 MΩ at 25 °C was used for comparison as the standard solution.

Mean values of thermodynamic melting parameters for the crystalline lamellae were identified in starch out of no less than three concurrent measurements. The value of melting temperature matched the maximum of the heat capacity peak on the thermogram. The value of experimental enthalpy corresponded to the area under the peak of the excess heat capacity curve as a function of temperature. Molar enthalpy of melting (ΔH_m) was calculated for the anhydroglucose residue (162 g/mol). As a rough approximation, the starch melting process may be regarded as a quasi-equilibrium one [36,37], which opens an opportunity to apply a one-stage melting model where the starch melting process is described as an equilibrium reaction between the native and melted states.

The van't Hoff enthalpy values (ΔH^{vH}) were calculated as described earlier [38], using the following equation:

$$\Delta H^{vH} = 2\,R^{1/2}\,T_m\,(C_p\text{-}0.5\Delta C_p\,\exp)\,½ \quad (1)$$

where R is the universal gas constant; T_m is the melting temperature for a crystalline lamella in starch; C_p is the maximum on the ordinate of the heat capacity peak on the thermogram; $\Delta C_{p\ exp}$ is the difference in heat capacity values between the in the heat capacities of phase native and molten state. The cooperative melting unit (ν) and thickness of the crystalline lamella (Lcrl) were calculated as described in the published source [39]

$$\nu = (\Delta H^{vH})/(\Delta H_m) \quad (2)$$

where ΔH_m is the experimental enthalpy of melting for the crystalline lamella and ΔH^{vH} is the van't Hoff enthalpy of melting.

$$Lcrl=0.35\nu \quad (3)$$

considering that 0.35 nm is the projection of the anhydroglucose residue on the axis of the amylopectin double helix [40,41].

2.2.8. Statistical analysis

The data was reported as an average of triplicate observations. This data was subjected to statistical analysis by Origin.

3. Results and Discussion

3.1. Ranking Maize Accessions According to the Type of Starch

Studying the ranking of the accessions according to their grain starch genotypes and analysing the results helped to identify the accessions belonging to the *wx* (*Mais agestano*,

Mestnaya), *ae* (*White Flint and Gornaya chechenskaya*) and *su* types (*Baksanskaya sakharnaya K-23426, Alina, Rannyaya lakomka*) (Table 1). The remaining accessions (*Populyatsiya MRPP22, Kabardinskaya belaya zubovidnaya, Luch and Otbornyy 150SV*) were attributed to mixed types. Cytological analysis resulted in finding out that accessions *Baksanskaya sakharnaya* and *Populyatsiya MRPP22* are characterized by the tetraploid chromosome number (4n), whereas all other accessions have the diploid (2n) number of chromosomes in their genome (Table 2). Tetraploid accessions differ from diploid ones in that the latter have a twofold set of all genes in their genome, and tetraploid ones a fourfold *sugary2* gene in the homozygous recessive *su2su2su2su2* (*Baksanskaya sakharnaya*) and dominant *Su2Su2Su2Su2* (*Populyatsiya MRPP22*) states. Grains carrying alleles of the su2, wx, ae gene in the genome show slight phenotypic differences, which are most pronounced in sweet corn (Fig. 1 (I)).

3.2. Maize Grain Characteristics

Table 1 presents genetic characteristics of the studied maize grains, their starch content, and amylose content in starch. The presented data make it clear that the maize accessions with the amylopectin genotype (*wx*) and those with the amylose one (*ae*) demonstrated close values of the DM mass fraction; with this, the amylopectin genotype possesses higher amounts of minerals compared to the amylose genotype, which is reflected in the mass fraction level of ash content in grain. Both genotypes have close to similar levels of starch content in grain: from 69.3 to 73.7%. At the same time, the starches from the amylose genotype contain 32.0–38.8% of amylose, and those from the amylopectin genotype 0–15 % of amylose. The sugary genotype (*su*), represented by tetraploid accession, showed the highest value of the DM mass fraction (from 92.8% up to 93.3%), the lowest starch mass fraction (58.8–65.9%) in grain DM, and the ash mass fraction of 1.5-1.7% in grains DM. Concurrently, the minimum mass fraction of starch from grains of *su* genotypes were accompanied by the amylose fraction in starch equal to 25.5% – 27.7% of grains DM. These data confirm the assumption that the amounts of the main polysaccharides in starch (amylose and amylopectin) and their ratios are determined by the plant genotype. Nevertheless, there are some differences in the studied parameters within- each of the four groups with the sugary, amylose, amylopectin and mixed endosperm types.

Table 1. Values of the main components in the grain's endosperm of different maize genotypes, and effectiveness indicators of their processing into starch.

Cultivar	Ploidy, n	Geno-type	Dry matter mass fraction in grain, %	Starch content in grain (S1), %	Mass fraction of ash content in grain, %	Amylose content in starch, %	Starch yield after maize grain processing under laboratory conditions (S2), % of grain DM	Starch extraction ratio (S2/S1·100), %
Mais agestano	2	*wx*	92.2±0.3	70.5±0.1	1.7±0.2	0.0 ± 0.5	58.6±0.7	83.1
Mestnaya	2	*wx*	92.1±0.4	70.8±0.5	1.65 ±0.2	0.0 ± 0.5	59.9±1.4	84.6
Populyatsiya MRPP22	4	-	91.9±0.4	73.7±0.3	1.7±0.1	15.0 ± 0.7	62.9 ±0.9	85.3
Luch	2	-	92.1±0,2	75.3±0.4	1.34±0.1	17.5 ± 1.0	64.7±0.7	85.9
Kabardinskaya belaya zubovidnaya	2	-	91.4±0.3	73.0±0.1	1.5±0.1	21.0 ±0.9	59.9±0.6	82.1
Otbornyy 150SV	2	-	92.1±0.2	70.9±0.1	1.4±0.1	26.4 ± 0.9	56.8±0.4	80.1
Gornaya che-	2	*ae*	92.0±0.3	71.3±0.3	1.5 ±0.2	32.0 ± 0.9	62.0±0.8	86.9

chenskaya								
White Flint	2	*ae*	91.2±0.5	69.3±0.3	1.5±0.1	38. 0 ± 0.1	62.6±0.7	90.3
Baksanskaya sakharnaya	4	*su*	93.3±0.3	59.3±0.2	1.7±0.1	25.5 ± 1.1	20.8±1.4	35.1
Alina	2	*su*	92.8±0.4	65.9±0.6	1.4 ±0.2	26.4 ± 1.2	10.5±2.3	15.9
Ranyaa lacomka	2	*su*	93.3±0.2	58.8±0.4	1.5 ±0.1	27.7± 0.8	17.8±2.1	30.3

Maize accessions with the *wx*, *ae* and mixed *wx* and *ae* genotypes showed high starch content after profound grain processing (from 69.3 to 75.3% of dry matter). The content of the *su*-type starches in the grains with corresponding genotypes were high enough for sweet maize reaching 59.3-65.9% of the grain dry matter on dependently from variety.

The results of starch extraction from the grain with different maize genotypes during laboratory-based processing (Table 1) demonstrated the effectiveness of starch extraction from the grain of the *ae* maize genotype. Starch extraction from the tested maize cultivars *White Flint* and *Gornaya chechenskaya* showed the ratio of 87–90%, i.e. more than the other studied accessions with different genotypes. However, grain samples of the *wx* type had the starch extraction ratio of 83.1–84.6%, which is high enough for the processed grain of waxy maize. The research results, presented in Table 1, as certain that a high mass fraction of starch in grains is not always a criterion of high starch extraction. It should be noted that starch yield from grains of *su* genotype is the smallest among investigated maize cultivars. A low ratio of starch extraction from the *su*-type grains (*Baksanskaya sakharnaya, Alina, Rannya lacomka*) is explained by very small starch granules, non-typical for maize grains, so in this case the losses in by-products tend to increase significantly.

The mass fraction of ash in the studied grain samples with the *wx* genotype did not change significantly and amounted to 1.4 - 17% of dry matter. The mass fraction of amylopectin in starch from maize with *wx* genotype was close to 100%.

The highest mass fraction of amylose in starch among the investigated maize starches was obtained for starch of the ae genotype (varieties *Gornaya Chechenskaya* and *White Flint*, 32% and 38%, respectively). Among the cultivars with the *su* genotype, it should be noted that in variety *Rannya Lakomka* mass fraction of amylose in starch is 27.7% that is highest in comparison with other investigated varieties with *su* genotype.

Thus, the lowest starch content in the grains of the *su* genotype is observed, the highest starch content was in the grains of the transitional genotype from *wx* to *ae*, while at the same time, the following set can be drawn up according to the increase of the amylose content in starches depending on the plant genotype: wx ≤ transitional genotype from wx to ae ≤ su ≤ ae.

3.3. Analysis and Characteristics of Starch Granule Size

The results of a microscopic examination performed on the starch granules from different genotypes in the presence of Lugol's solution are presented in Figure 1 (II). Amylopectin and amylose molecules are known to be stained with Lugol's solution in a different manner. Amylopectin macromolecules turn brown, and amylose macromolecules turn blue [32]. The microscopic images show that brown amylopectin molecules are mostly present in the *wx*-type starch, but the images of the *ae* and *su* genotypes contain both blue macromolecules of amylose and brown ones of amylopectin. These micrograph images also demonstrate that the starches isolated from different maize genotypes have different granule sizes. It is shown that the granules of the large and small starch fractions contain different quantities of amylose and amylopectin molecules. According to the published data, the intensity of crystalline reflexes is higher in starch samples with larger granules than in those with smaller granules. Therefore, these factors may effect on the structural arrangement of starch granules and on thermodynamic parameters of starch melting [45].

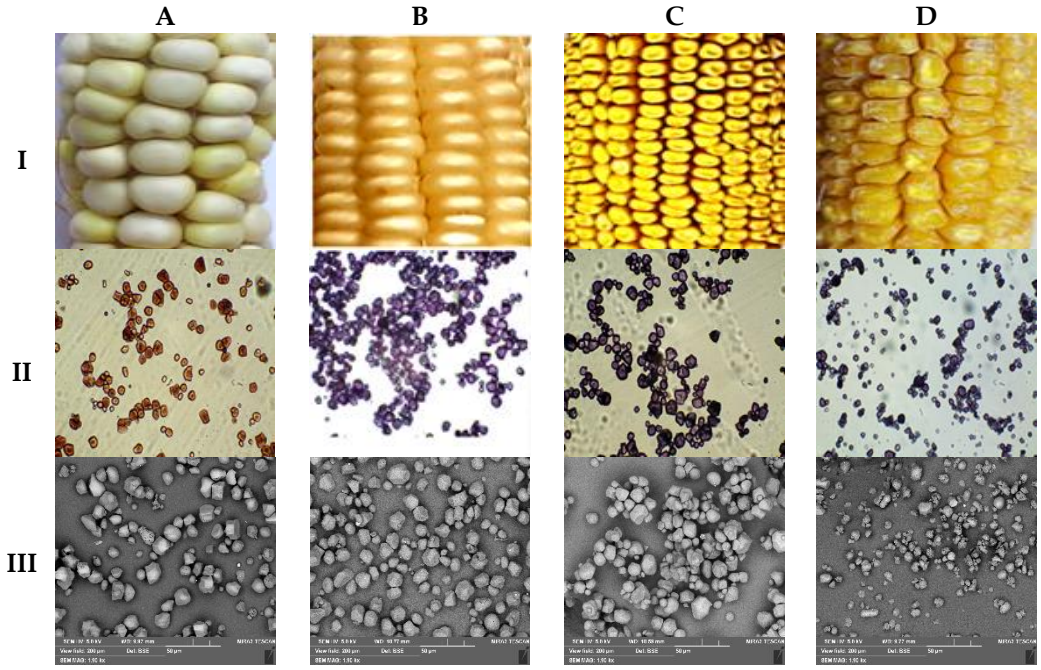

Figure 1. Examples of **i**mages of phenotypic trains in maize grain (I), as well as light (II) and electron scanning (III) microscopies images of starch granules with different genotypes (A-*wx*, B-*ae*, C-transitional *f*rom *wx* to *ae* , D-*su*). Ligth microscopy images of starch granules stained with Lugol's solution with a 400x zoom.

The morphology of the starch in the granules was analyzed by scanning electron microscopy (SEM). The size and shape of maize starch granules were evaluated both using direct morphometric parameters and relative values (the ellipticity coefficient (calculated as the ratio of the area of the object to the area of the ellipse with the same moments of inertia). Examples of micrographs of maize starch granules of different genotypes are presented on Fig. 1, III. It can be seen from the microphotographs that all studied starches contain both irregular or cubic granules and elliptical granules with clear edges, the ratio of which differs in different genotypes.

It should be noted that the size of maize starch granules from plants with different genotypes are various (Fig.2). Thus, from the above morphological data, it can be seen that the maize starch granules of the *ae* genotype are characterized by large geometric dimensions compared to the starch granules of the *wx* genotype, while the proportion of irregular or cubic granules in the starch of the *ae* genotype is higher than in the starch of the *wx* genotype. It should be emphasized that starch granules of the *su* genotype are characterized by significantly smaller sizes compared to granules of the *ae* and *wx* genotypes, which may be due to the individual genotypic features of this genotype. It should be noted that our results on the granule size of the *su* maize genotype correlate with the literature data [46]. It is possible that the size of maize starch granules can serve as a phenotypic marker for distinguishing starches of the *su* type from those isolated from the *wx* and *ae* genotypes.

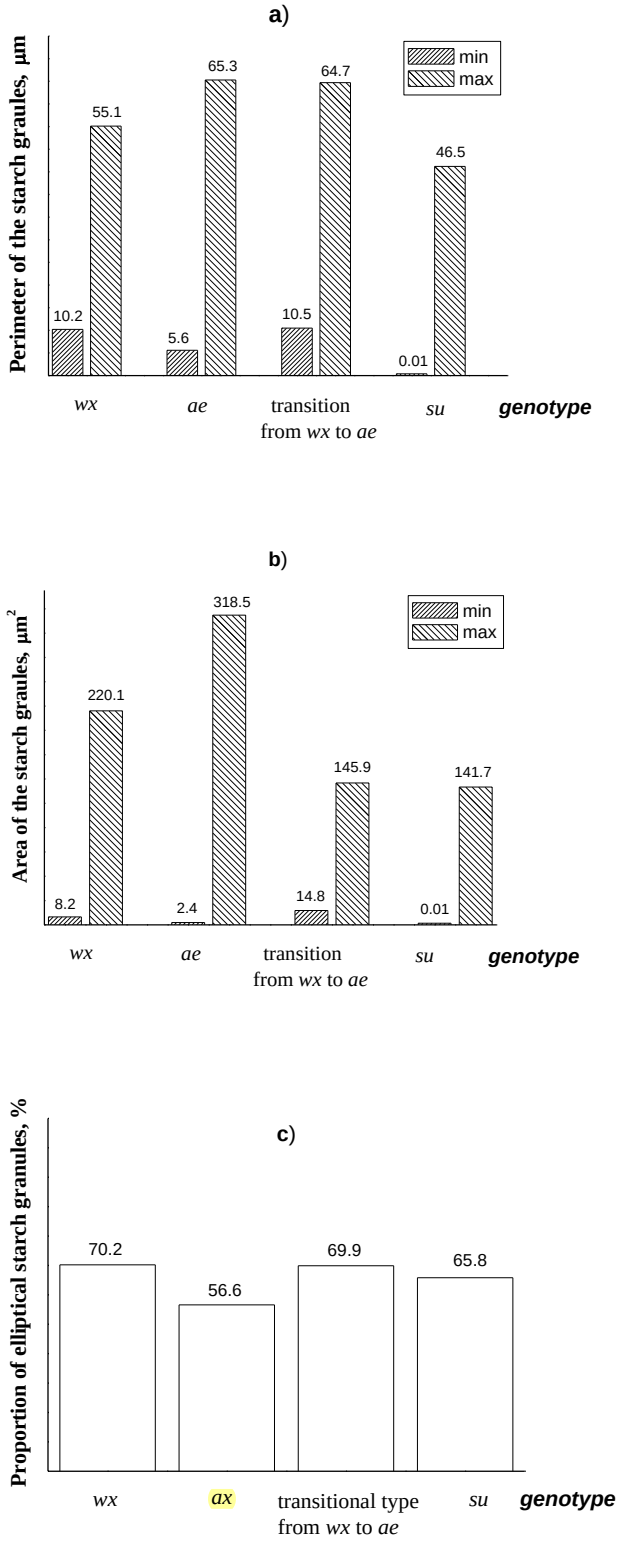

Figure 2. Examples of granulometric data (perimeter (a), area (b) and proportion of elliptical (c) starch granules) in dependently on genotype of maize plants from which extracted starch.

3.4. Thermal Properties of Starches in Different Maize Genotypes

Examples of the DSC thermograms describing the melting of aqueous starch dispersions for various maize genotypes with different amylose content are presented on Figure 3.

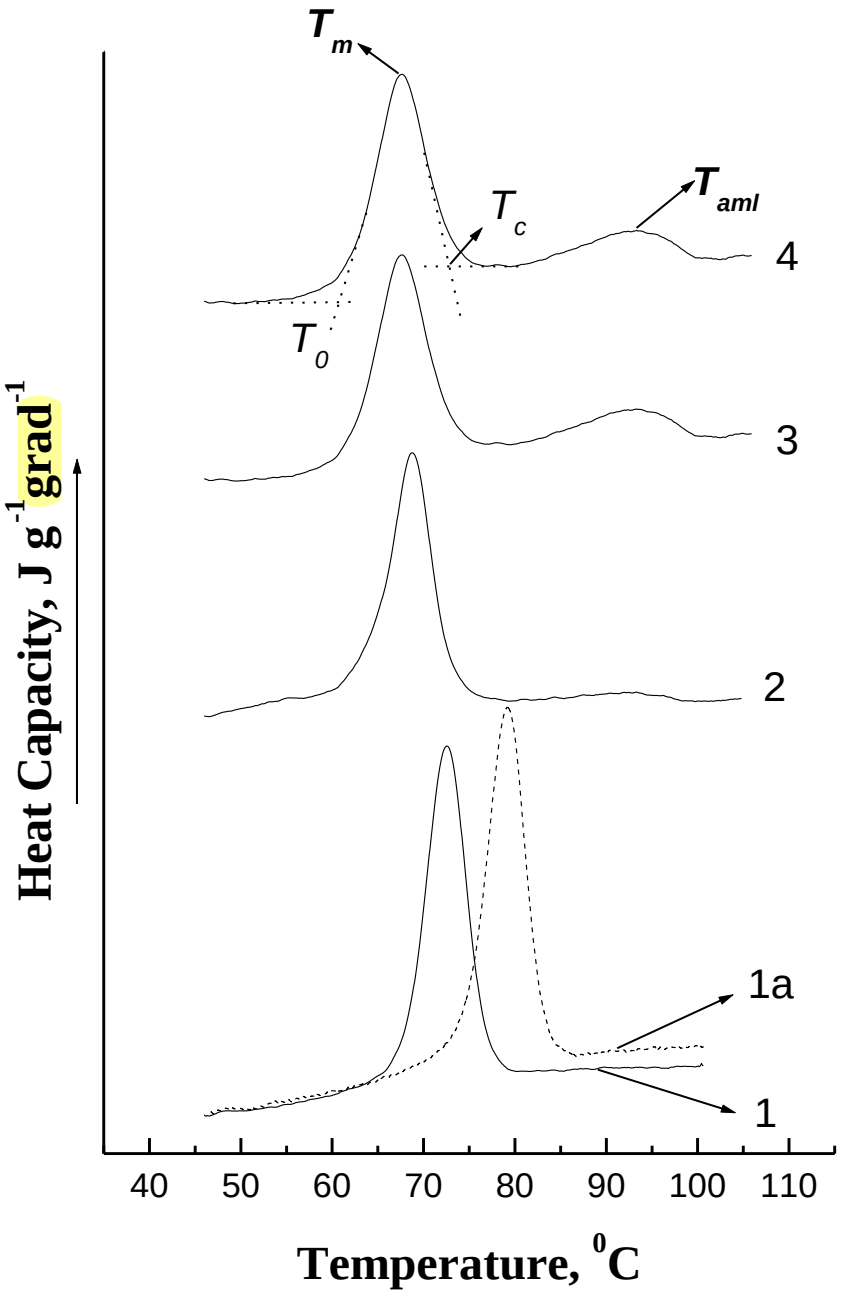

Figure 3. The examples of DSC thermograms of aqueous starch dispersion (0.3%) for different maize genotypes and amylose content (1 - *wx* genotype (0% the amylose content), 2- transitional genotype from *wx* to *ae* (15 % amylose content), 3 – *su* genotype (25.5.% amylose content), 4 –*ae* genotype (38.0 % amylose content), as well as for starch dispersion of *wx* genotype with 0 % amylose content in 0.6 M KCl (1 a). T_m is the melting temperature for amylopectin crystalline lamellae; T_0 and T_c - onset and conclusion temperature of the melting of amylopectin crystalline lamellae; T_{aml} is the dissociation temperature for the amylose–lipid complex.

The observed DSC thermograms are typical for the melting of aqueous dispersions of maize starches with different amylose content [35, 39]. It is obvious from the DSC thermograms that there are two peaks observed in the melting process of maize starch when starch contains a certain amount of amylose. The first peak corresponds to the melting of amylopectin crystalline lamellae or destruction (untwisting) of the double helices in amylopectin, while the second one marked the dissociation (melting) of amylose–lipid complexes [35, 36, 37,39, 47, 48]. Naturally, only one peak was observed while melting the amylopectin (*wx*-genotype) maize starch extracted from varieties *Mais agestano and Mestnaya*, because this starch was amylose-free. Melting temperature values for amylopectin crystalline lamellae in the investigated starches decreased from 345.8 K (variety *Mais agestano*) to 340.8 K (variety *Baksanskaya Sakharnaya*), which is probably due to an intensified accumulation of defects in partially crystalline starch granules with an increase in their amylose content. The values of melting enthalpy decreased with an increase of amylose content, which may be explained by the reduction in the degree of crystallinity with an increase in the amylose content. The highest values of melting temperature and enthalpy for amylopectin crystalline lamellae were observed in amylopectin starches as compared to normal maize starches (15–30% of amylose content), suggesting that amylopectin starches require more energy to disrupt their structure and that these starches have a more ordered crystalline structure compared to normal maize starches [37, 39, 47] . With this, starch melting enthalpy values in the transitional from *wx* to *ae* genotype decreased with an increase in amylose content, while in the *ae*-type starches with the amylose content of 32.0 and 38.0% the amylopectin melting enthalpy values practically match the respective values in amylopectin starches from the *wx* genotype. Higher transition temperature associated with higher degree of cristallinity, with provided structural stability [47,48]. The starches of *wx* genotype (varieties *Mais agestano and Mestnaya*) shows the highest value of the melting temperature among the investigated maize starches (Table 2) what may be connected with structural rigidity and more resistant to gelatinization, while the lowest was shown by starches of *su*-genotype (varieties *Baksanskaya sahharnaya*, *Alina*, *Rannya Lacomka*). The differences (ΔT) between temperature conclusion (Tc) and temperature onset (T_0) of amylopectin crystalline lamellar melting for investigated maize starches may be due to the changes in the crystalline regions in starch granules [47]. Higher value of ΔT for *su*-genotype (varieties *Baksanskaya sahharnaya*, *Alina*, *Rannya Lacomka*) among investigated maize starches (Table 2) shown that these starches are characterized by lower crystallinity and the presence of crysttaline regions of different strengths in starch granules that is correlated with literature data [48, 49].

It is known that maize starches are characterized by A-polymorphous structure [47]. It was shown earlier that during heating in an excess of salt solution the temperature transition of starches shifts to a higher value, at least the differences for A-type of polymorphous structure correspond to 6–12 degree [50]. The melting temperature of the investigated aqueous starch dispersions and starch dispersions in the presence of 0.6 M KCl solution shows that the melting temperatures of starches suspended among KCl are up to 6–7 K higher (Figs. 2, 1a). This is typical for A-type polymorph, so the type of polymorphic structure doesn't depend on the plant's genotype.

The value of the melting enthalpy of amylose-lipid complexes is determined by the crystalline state of amylose-lipid complexes and is proportional to the content of lipids in them [51]. It should be mentioned that the temperature and melting enthalpy values for amylose–lipid complexes in the investigated maize starches were practically unaffected by an increase in amylose content, and remained invariable. Therefore it can be assumed that the lipid content and the crystalline state of amylose–lipid complexes in the investigated maize starches are practically the same.

Thus, the analysis of the studied thermodynamic parameters in the maize starch melting patterns of different genotypes with varying amylose content showed that in increase of amylose content in starch was accompanied by a decrease in amylopectin

melting temperature values, and was also determined by the genotypic features of the plant whose grain was used for starch extraction (Table 2), which is in line with published data [39, 52]. It is shown that an increase in amylose content in starches is accompanied by the formation of more defective (less ordered) crystalline structures melting at lower melting temperatures. The development of more defective (less ordered) structures occurs as a result of accumulation of non-ordered amylose chains in amorphous lamellae and amylose terminal chains in crystalline lamellae with increasing of amylose content in starches [39, 52]. The same pattern was observed in the studied maize starches as well. It was shown earlier that DSC data can be used to estimate the thickness of crystalline lamellae, and these data correlate well with data of X-ray [for example, 47, 53]. Crystalline lamella thickness values in all tested starches were similar enough, their mean value being 5.9 ± 0.9 nm. Therefore, the thickness of crystalline lamellae in the tested maize starches remains practically constant regardless of any increase in amylose content and does not depend on the starch genotype.

Table 2. Thermodynamic melting parameters of the studied maize starches (T_m is the melting temperature of crystalline lamellae; ΔH_m is the melting enthalpy of crystalline lamellae; ΔH^{vHof} is the van't Hoff enthalpy; v is the value of the cooperative melting unit; L_{crl} is the thickness of a crystalline lamella; T_{aml} and ΔH_{aml} are the temperature and enthalpy of amylose–lipid complex dissociation).

Cultivar	T_m, K	$\Delta T=Tc-T_0$	ΔH_m, kJ/mol	ΔH^{vH}, kJ/mol	v, anhydroglucose residue	L_{crl}, nm	T_{aml}, K	ΔH_{aml}, kJ/mol
Mais agestano	345.8±0.1	9.7	3.7±0.2	48.6±1.5	13.4±0.7	4.7±0.2	–	–
Mestnaya	345.2±0.1	10.0	2.7±0.2	38.1±1.5	14.2±0.6	5.0±0.2	–	–
Populyatsiya MRPP22	342.1±0.1	10.7	2.4±0.1	38.2±0.1	16.0±0.5	5.6±0.2	366.5±0.1	0.2±0.05
Luch	343.7±0.1	8.4	1.9±0.1	34.9±1.8	17.9±0.1	6.3±0.0	365.6±0.0	0.30±0.05
Kabardinskaya belaya zubovidnaya	343.1±0.0	8.6	1.9±0.3	36.2±4.0	20.0±0.8	7.0±0.3	365.7±0.1	0.10±0.0
Otbornyy 150SV	343.2±0.1	7.3	2.1±0.3	42.5±2.4	21.0±3.0	7.4±0.7	366.1±0.0	0.30±0.05
Gornaya chechenskaya	343.5±0.0	10.2	2.8±0.3	43.2±2.5	15.5±0.8	5.5±0.2	366.0±0.0	0.30±0.03
White Flint	342.9±0.0	10.4	2.7±0.1	42.1±1.2	16.0±0.4	5.6±0.1	365.8±0.0	0.40±0.0
Baksanskaya sakharnaya	340.8±0.1	12.3	2.4±0.1	35.1±0.4	14.7±0.8	5.2±0.2	367.3±0.0	0.7±0.1
Alina	342.1±0.1	12.1	1.6±0.1	27.9±1.2	16.9±0.4	6.0±0.1	367.5±0.1	0.3±0.05
Ranyaa Lacomka	341.8±0.1	14.5	1.8±0.1	28.5±0.8	15.8±0.4	5.5±0.2	366.6±0.1	0.5±0.1
Mean value						5.9±0.8	365.9±0.7	0.4±0.1

It is worth mentioning that the thermodynamic melting parameters of the *su*-type maize starch extracted from *Baksanskaya sakharnaya, Alina, Rannya lacomka* to some extent fall out of the line of the tested starches. Melting of crystalline lamellae is associated with the lowest values of the melting temperature and enthalpy among the investigated starches, suggesting the presence of less ordered structures in this starch than in the starches with (Table 2). At the same time, these starches demonstrate a large amount and the highest thermostability of its amylose–lipid complex. It was shown that maize starches from plants of *su*-genotype are characterized by the presence of V_h-crystalline patterns that are attribute of amylose-lipid complexes [47, 53]. Specific features of the starches extracted from the maize of *su*-genotype seem to be associated with the genotypic peculiarities of the sugary maize grain endosperm, characteristic of such cultivars, i. e. the effects of the *su2* gene on starch granule development in the cultivar's grain (the *su* genotype) are expressed in the form of disturbances in the starch polysaccharide branching process, as opposed to the other tested starches from the *wx* and *ae* genotypes. This fact, however, requires further and more profound researching.

The thermodynamic melting parameters of crystalline lamellae, namely the surface entropy (qi) and enthalpy (si) for the faces of crystalline lamellae it is possible to obtain from the Gibbs-Thomson equation for semicrystalline polymers [54, 55].

$$T_m = T_{0m} (1- 2\gamma_i /(\Delta H0m \rho_{crl} L_{crl})) \quad (4)$$

where T_{0m} and ΔH_{0m} are the melting temperature and enthalpy, respectively, of a hypothetic crystal of an unlimited size (ideal crystallite) or such crystal whose the role of free surface energy may be ignored in favour of bulk energy; γ_i is the free surface energy for the faces of crystalline lamellae of crystalline lamellae; p_{crl} and L_{crl} are the density and thickness of a crystalline lamella, respectively.

$$q_i = (\Delta H_{0m} - \Delta H_m) \rho_{crl} L_{crl} /2.5 \quad (5)$$

$$s_i = (q_i- \gamma_i) / T_m \quad (6)$$

It is well known that melting of polymer crystals begins with the melting of defects in an amorphous lamella. Disordered B-chains of amylopectin and amylose pass-through chains localized in clusters of amylopectin and passing through several crystalline and amorphous lamellae may be played the role of defects in starch granules [37, 54, 56]. The quantity of defects in the structural arrangement of starch granules is proportional of the value of surface energy on the terminal faces of a crystalline lamella of starch granule [54,57]. Qualitative evaluation of defects is possible using the method of differential scanning microcalorimetry.

Table 3. The values of free surface energy (γi), enthalpy (qi) and entropy (si) on the face side of crystalline lamellae in the studied maize starches from different genotypes.

Cultivar	$\gamma_i \times 10^7$ **J/cm²**	$q_i \times 10^7$ **J/cm²**	$s_i \times 10^7$ **J/cm²**
Mais agestano	6.89	35.93	0.08
Mestnaya	7.66	55.75	0.13
Populyatsiya MRPP22	9.80	68.74	0.17
Luch	10.27	87.74	0.23
Kabardinskaya belaya zubovidnaya	11.74	100.73	0.20
Otbornyy 150SV	12.30	100.63	0.26
Gornaya chechenskaya	8.98	59.05	0.15
White Flint	9.39	62.97	0.16
Baksanskaya sakharnaya	9.47	63.34	0.16
Alina	10.49	91.01	0.23
Rannaya Lacomka	9.74	79.41	0.20

The accumulation of defects is accompanied by the development of crystallites with a more 'mellow' surface. Thermodynamic parameters were assessed for the surface of the faces of crystalline lamellae in the tested starches (Table 3). The calculations involved the values of T_{0m} (366.5 K) and ΔH (35.5 J/g) as well as p_{crl} (1.48 g/cm^3) for the A-type crystalline spherulites to which maize starches are attributed [58, 59]. The values of melting entropy, melting temperature, and crystalline lamella thickness were taken from the experimental data. It is evident from the presented data (Table 3) that the surface entropy value for the faces of crystalline lamellae slightly increases for starches extracted from the *ae*-genotype maize with an increase of their amylose content. This phenomenon suggests the conclusion that, with a rise of amylose content in the transitional from *wx* to *ae*-genotype maize starches, accumulation of defects occurs in the starch structure. It can be seen from the presented data (Table 3) that the highest values of the surface entropy of the ends of crystalline lamellae are characterised by the transitional genotype starches, as well as the starches isolated from su-genotypes (*Alina* and *Rannaya Lacomka* varieties), probably due to their less ordered structure compared to other starches studied. It should be noted that for starches of the *ae* genotype with an amylose content of 32.0 and 38.0%, the value of the entropy of the end faces is close to the corresponding value for starches of the transitional type from *wx* to *ae* genotype with an amylose content of 15% and the

su-genotype of the *Baksanskaya sakharnaya* variety, which probably indicates about the similarity in the degree of their order.

Thus, a rise in the amylose content in the tested maize starches was accompanied by a decrease in their thermodynamic melting parameters; at the same time, the thickness of crystalline lamellae and the amounts of amylose–lipid complexes remained practically unchanged for starches from the transitional and *ae* genotypes. Changes of the thermodynamic parameters of investigated maize starches are determined both by the amylose content in them and by the genotype of plants from which the starches were extracted.

4. Conclusions

The studied high-starch maize accessions were found to contain different genotypes of starch with variable amylose/amylopectin ratios. Among the starch types of maize studied, four groups were earmarked to comprise the *wx, ae, su* and transitional starch types. The samples of maize varieties *Baksanskaya sugar (su-genotype)* and *Population MRPP22 (*transitional from *wx* to *ae* genotype*)* had a tetraploid genotype, did not show signs of a pronounced specific effect of genome ploidy on the composition or ratio of amylose/amylopectin.

It was found that an increase in the content of amylose in maize starches *wx* and *ae* is accompanied by a decrease in their thermodynamic melting parameters and the accumulation of defective structures in the studied starches, regardless of their ploidy. It should be pointed out that the starches from the *wx*, *ae* and *su* genotypes possess a more ordered structure than those of transitional from *wx* to *ae* genotype. The starches extracted from *su*-genotype differed in its thermodynamic melting parameters from other accessions and are characterized by lower crystallinity among maize starches investigated genotypes. Thus, the thermodynamic melting parameters of studied starches are determined both by amylose content in starch and by the individual features of the maize genotype.

The studied high-starch waxy maize accessions with the *wx* genotype (varieties *Mais agestano, Mestnaya*), with transitional genotype maize accessions (varieties *Luch and Kabardinskaya belaya zubovidnaya*), and an accession with the *su* genotype (varieties *Baksanskaya sakharnaya, Alina,Rannaya Lacomka*) are of practical importance: they may serve as valuable raw materials for starch and treacle production industries and as sources for the development of new high-starch cultivars of waxy maize and traditional grain cultivars with a high mass fraction of starch.

A comparative analysis of tetraploid samples differing only in the presence of one allele of the sugar content gene in the dominant and recessive states clearly showed a significant effect of the su gene on the phenotypic, morphological and thermodynamic characteristics of native maize starch. This study shows that the genes involved in the biosynthesis of maize starch have a multifactorial effect, which manifests itself both in the form of parameter regulation on the thermodynamic and structural features of maize starch, and on the biochemical trait of grain, which can change under the influence of several genes, but not their quantity. Methods of breeding and genetic engineering make it possible to obtain starch with a wide range of physicochemical properties and phenotype of starch granules due to various combinations of a few genes (wx, ae, su) that affect starch synthesis. The study of the characteristics of various maize genotypes and the deciphering of their genetic components that affect the synthesis of starch will significantly accelerate the technology for obtaining starches with desired physical and chemical properties as raw materials for the relevant industries.

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