

Conference Paper

Creating Tetraploid Lines of Sweet Corn (*Zea Mays Saccharata*) and Studying Biochemical Content of Their Grain

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Abstract

A collection of tetraploid sweet corn has been created on the basis of Baksanskaya Sugary var. (VIR catalog no. k-23426) obtained from dent corn tetraploid population MRPP-20 characterized with high grain yield of its ear. The variety has been created by inbreeding separate caryopses homozygotic for *su1* and *su2* genes (*sugary endosperm*); the caryopses were selected from MRPP-20 population with subsequent selection work for valuable traits during a number of generations. The collection includes over 300 specimens, among them there are genotypes promising for selection of foodcrop sweet corn. The research was conducted into principal phenotypic traits of the plant and chemical components of its caryopsis (protein, starch, oil). Analysis has shown that the chemical composition of grain varies widely. Structural phenotypic traits of vegetative organs differ mainly in the number of ears per plant, ear size, number of seeds per ear and the number of seed rows, caryopsis length, color of corncob and the caryopsis itself. Specimens with high (17.0–18.0 %) and low (11.0–12.0 %) protein content, high (9.0–10.0 %) and low (5.0–6.0 %) oil content, high (66.0–67.0 %) and low (60.0–61.0 %) starch content in caryopses were produced. Specimens have been obtained that combine high starch content with low oil content (C-1042) and vice versa (C-1114, C-1116), with low protein and oil content in a combination with high starch content (C-1180, C-1370). The research results allowed choosing starting material for selection work with hybrids of sweet corn to get predefined flavor properties.

Keywords: tetraploid maize, sugary endosperm, protein, oil, starch, VIR collection.

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1. Introduction

Maize (*Zea mays* L.) is the third most important crop among cereal crops, taking up over 30 % of the global cereal balance 30 % [1] (Shpaar, 2008). Global market in maize reached 1112 million tonnes in 2017, while in Russia 13,235.7 thousand tonnes of grain maize were produced, [2, 3]. The most popular type of maize is sweet corn that is used as foodstuff after it reaches milky-wax ripeness. High nutritional properties of sweet corn determines its significant advantages over green peas and kidney beans that

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are used for conservation and consumed fresh. Biochemical content indicators of corn grain takes an important place in selection work. Methods of selection and evaluation of selection materials of sweet corn differ from those used with other types of maize. The main difference is that commercial ears of sweet corn are harvested during their milky ripeness phase, when water content in the seeds is 70-80%, thus, there are additional requirements to evaluation of its nutritional, processing and commercial value.

Among current problems in sweet corn selection, a number of authors note a significant narrowing of genetic polymorphism of source material [4]. There are several ways to resolve it: creating synthetic populations involving genetic plasma of exotic varieties and wild relatives, different sub-species and forms of maize. There are promising results in all these directions [5--9]. Researchers also note viability of much wider use of high-carotene and supersugary synthetics and self-pollinating lines in selection [10, 11]. A way to effectively expand genetic polymorphism is using mutagenesis. G.-H. Zan, J.L. Brewbaker [12] in their studies of recombinant capabilities of mutant forms showed promising nature of this research direction. Genetic parameters of mutagenesis-induced mutant forms of sweet corn were studied by M.N. Sitnikov [13, 14]. Additional notion should be given to selection of sweet corn for prolificacy with simultaneous flowering of ears and reaching technical ripeness, as well as evenness of ears for main phenotypic traits. A new direction in selection of sweet corn that is capable of accumulating a large part of traits in a single genome is creation of tetraploid populations and hybrids of sweet corn with high grain productivity. The tetraploid genotype has a number of advantages over its diploid analog in yield of leaf and stalk mass, size of commercial ears and caryopses, wider polymorphism in biochemical composition of seeds [15]. That is why creation and studies of selective value of new tetraploid sweet corn lines allows expanding diversity of source material for selection of high-yield hybrids.

2. Methods and Materials

The target of this research were tetraploid sweet corn lines obtained as a result of studying progeny of caryopses carrying the *su2* gene in homozygotic state, selected from the tetraploid sweet corn variety Baksanskaya Sugary (VIR catalog no. k-23426). The variety was created from the sweet corn lines obtained by selection from MRPP-20 population (VIR catalog no. k-23425). Sweet corn varieties Nika-353 (produced by Kabardino-Balkarian Agricultural Research Institute) and Ranniaia Lakomka (Early Gourmet, produced by IPA OTBOR) were used as a diploid standard. Phenotypic tests of the collection were carried out in the territory of IPA OTBOR during 2017-2018. The

selection plot is located within the boundaries of North Caucasus piedmont zone, at a watershed between Urvan and Nalchik rivers. The soil is largely meadow chernozem. The climate in the area is characterized as moderately hot, with the sum of active temperatures of 3000--3200 °C and moderate humidity (the humidity factor is 0.5--0.9), the hydrothermic factor is 0.9--1.2. Altogether, during the period of research, growth and development of maize underwent under conditions of excess heat and deficient moisture.

All tests of sweet corn specimens were performed twice. Working plots were two-row, with an area of 4.9 m². Inter-row distance was 0.7 m, plant population was 60 thousand plant per 1 ha. Measurements and counts were conducted on 10 plants and 10 ears in two-fold repeatability. Protein, starch and oil content in caryopses were determined by infrared spectroscopy with the Infratec 1241 Grain Analyzer instrument (made in Sweden) using 100 g of dry seeds. Analysis of seed metabolites was conducted by gas-liquid chromatography with mass spectrometric study at a Agilent 6850 chromatographer with a quadrupole mass-selective detector Agilent 5975B VL MSD manufactured by Agilent Technologies, USA. The obtained results processed in UniChrom software [16]. Cytological analysis followed the procedure established by Z.P. Pausheva [17].

3. Results

The VIR's collection of tetraploid sweet corn that includes 300 specimens was created on the basis of lines obtained from progeny of individual caryopses with sugary endosperm found among tetraploid population of dent corn MRPP-20. The source for MRPP-20 population was created in Krasnodar Agricultural Research Institute named after P.P. Lukianenko by V.S. Shcherbak on the basis of Synthetic B population imported from the USA, Kubanskaya Sugary variety and 1/16 of genome from perennial tetraploid teosinte *Zea perennis* Hitchc ($2n=40$) [14]. As a result of 10 cycles of positive selection for high seed productivity of ear for traits of preferred bivalent conjugation of chromosomes in meiocytes in the diakinesis stage, as well as minimal difference between actual and expected seed production (grain/ear ratio) of ear, several genotypes with high grain productivity were established. Selection criteria were the traits of high frequency of bivalent conjugation of chromosomes in meiosis against quadrivalent with subsequent re-pollination of individual selected genotypes of plants (Fig. 1).

After propagation, analysis and selection for seed production was conducted. The analysis involved counting seeds in the ear; this count was recorded as actual seed production of the ear. After that the ear was milled and the corncob was scorched with

a blowtorch. After removal of flower scales in such a way, the corncob was scraped with a knife and the number of seed cells on the corncob was counted (Fig. 2). The doubled sum of the seed cell count was recorded as expected (potential) seed production of the ear. Only seeds from the ears with minimal difference between the expected and actual seed production were selected for subsequent participation in the next cycle of selection.

With each selection cycle the value of diploidization of the tetraploid genome increased, the same as seed production with a shift of multivalent associations of chromosomes in meiocytes from quadrivalent to bivalent [14]. In the end of the 10th cycle, a population MRPP-20 was created, which served as a source material for establishing the lines of tetraploid sweet corn (Fig. 3).

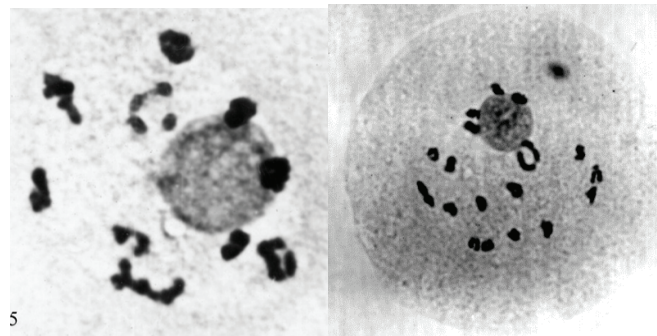


Figure 1: Meiocytes of tetraploid corn with quadrivalent (left) and bivalent (right) preferred conjugation of chromosomes. [14].

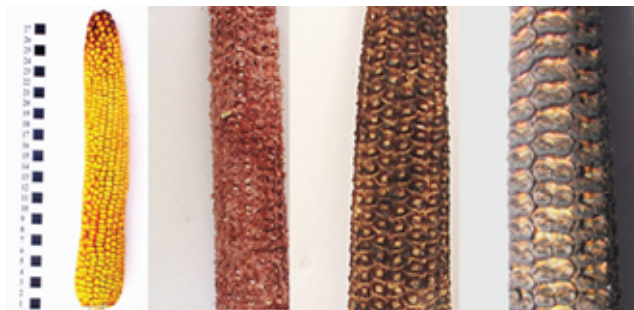


Figure 2: Sequence of actions to prepare corncob to seed cells count (left to right).

During the selection improvement of the MRPP-20 population, selection was also conducted for other traits of selective value, such as early ripening, multiple-row ears, moisture-yielding capacity, propensity for prolificacy, resistance against biotic and abiotic environmental factors. Formation of caryopses homozygotic for *su2* gene was largely facilitated by inclusion of the Kubanskaya Sugary variety into the initial breeding background of the MRPP-20 population. Individual caryopses were propagated and subjected to multiple rounds of inbreeding to increase the frequency of homozygotes



Figure 3: Ears of tetraploid MRPP-20 corn after 10 cycles of selection (left) and its source material from the first cycle of selection (right) [14].

of *su2* gene alleles and to improve evenness of sweet corn phenotypic traits. From the best lines of tetraploid sweet corn selected for their seed production, a new variety of sweet corn had been created by the method of polyhybrid crossing. It was registered as Baksanskaia Sugary with the Russian State Register of Selection Achievements in 2011 (patent no. 6335 dated 01.02.2012) (Fig. 4). The Baksanskaia Sugary var. of tetraploid sweet corn has a number of advantages over the diploid standard to to a larger multiple-row ear with a good seed production and longer and larger caryopses on the ear (Table 1).

The research has shown that trait values vary wider in tetraploid lines of sweet corn than in diploid lines. During the selection of tetraploid lines of sweet corn, genotypes were selected with high and low content of protein, oil and starch, at that, the variation range of the values was broader than for diploid genotypes grain (Table 3).

TABLE 1: Characteristics of Baksanskaia Sugary variety of tetraploid sweet corn (2010, 2011, HCP_{0,5}=3.08 centner/ha)[18].

no.	Traits	Nika 353 (St)	Baksanskaia Sugary	Deviations from the standard
1	Milky ripeness ear yield, centner/ha	82.0	146.6	+64.6
2	Commercial grain output from an ear, %	64.0	85.5	+21.5
3	Total sugar content, mg/100 g of flour	47.1	47.7	+0.6
4	Protein content, %	10.06	12.75	+2.69
5	Oil content, %	4.55	2.9	-1.65
6	Number of seed rows per ear	12.7	22.4	+9.7
7	Number of seeds per row	29.3	35.5	+6.2
8	Caryopsis height, mm	7.0	11.0	+0.4



Figure 4: Ears of tetraploid sweet corn, variety Baksanskaia Sugary in the phase of milky (left) and full (right) ripeness. [18].

TABLE 2: Distribution of 4n sweet corn specimens for content of protein, starch and oil in grain.

Grain content	Sample	St, 2n	4n lines established
Protein, %		13.1±2.2	
high	17.1--18.0		17
Average	12.0--17.0		287
low	11.0--11.9		6
Starch, %		64.4±3.5	
high	66.1--67.0		10
Average	61.0--66.0		284
low	59.0--60.9		6
Oil, %		6.1±2.1	
high	9.1--10.0		7
Average	6.0--9.0		285
low	4.0--5.9		8

It is fair to assume that the wider variability in biochemical composition of tetraploid maize caryopses is due to presence of more chromosomes and gene alleles, or by means of increased size of both caryopsis and corcule (Fig. 4). However, it does not cause only increase or only reduction in the values of phenotypic traits, including biochemical ones.

In order to determine chemical composition diversity by fatty acids content in oil, 5 lines with high oil content (9.1--10.0 %) in their grain were selected. The results of gas-liquid chromatography showed that the oil largely consists of 9 fatty acids (Table 3). All

the essential for human fatty acids (oleic, arachidonic, linoleic and linolenic) were found in tetraploid sweet corn grain, however their content in different specimens varied. The maximum total content of fatty acids, including essential ones, was found in specimen C-1576.



Figure 5: Caryopses of tetraploid sweet corn, Baksanskaia Sugary (left) and Nika-353 (right). [18]

TABLE 3: Fatty acid content in the grain of high-oil specimens of 4n sweet corn.

Fatty acids	C-1576	C-1577	C-1320	C-1121	C-1119	Average
tridecanoic	3.34	0.71	0.13	0.13	4.17	1.70±1.92
undecylic	2.12	1.69	0.05	0.69	1.09	1.13±0.81
palmitic acid	169.24	103.79	87.26	72.24	70.59	100.62±40.63
linoleic acid	369.64	280.96	362.90	239.69	177.30	286.10±82.00
oleic acid	235.94	156.13	166.32	142.02	85.76	157.23±53.90
linolenic	27.56	16.15	25.77	27.50	11.38	21.67±7.45
stearic	51.03	25.81	14.71	16.09	10.73	23.67±16.27
arachic	2.74	0.74	0.51	0.63	0.25	0.97±1.00
behenic	6.24	0.90	0.90	0.39	4.03	2.49±2.54
Total fatty acids	867.85	586.87	658.55	499.38	365.30	595.59±187.39

Content of amino acids that cannot be synthesized in animal tissue (valine, isoleucine, leucine, methionine, threonine, tryptophane and phenylalanine) are of critical importance for humans and animals alike. Analysis of free amino acids content in high-oil specimens showed that of the essential amino acids, valine and tryptophane are present in the grain. High valine content was found in specimen C-1121, high tryptophane content was found in specimen C-1576. Specimen C 1121 was characterized with high total free amino acids content. Conclusion

TABLE 4: Free amino acids content in the grain of high-oil specimens of 4n sweet corn.

Free amino acids	C-1576	C-1577	C-1320	C-1121	C-1119	Average
a-alanine	4.71	0.86	1.59	4.21	2.15	2.71±1.68
glycine	0.98	1.04	2.25	1.51	0.76	1.31±0.59
proline	74.86	30.86	17.30	159.92	18.79	60.35±60.36
valine-norvaline	1.82	0.47	1.22	2.87	2.27	1.73±0.93
pipecolic acid	0.49	0.21	0.02	0.10	0.03	0.17±0.19
serine	4.67	0.70	0.14	0.43	0.47	1.28±1.90
asparagine	14.17	3.34	2.48	2.17	1.60	4.75±5.30
glutamine	2.67	1.37	1.02	3.75	2.87	2.33±1.12
aspartic acid, asparagine	5.74	2.68	3.34	2.47	2.98	3.44±1.33
oxyproline	0.91	0.96	0.06	1.54	0.86	0.87±0.53
tryptophane	0.49	0.04	0.16	0.13	0.11	0.19±0.18
tyrosine	3.21	2.51	2.28	4.00	2.64	2.93±0.69
Total free amino acids	114.72	45.04	31.86	183.10	35.53	82.05±65.82

Research into selection of caryopses with sugary genotypes on the basis of high-productivity tetraploid population MRPP-20 has proven its efficiency in expanding polymorphism of phenotypic traits of biochemical composition of tetraploid sweet corn grain. Selection in the initial tetraploid population MRPP-20 for preferred bivalent conjugation of chromosomes in meiocytes allowed overcoming low seed production typical of autotetraploid sweet corn. Creation of synthetic population is possible from the best lines with a long selection work; such populations will serve as a foundation for variation of biochemical content in grain from new lines of sweet corn in a broader range than that of diploid analogs. Lines were created that are characterized with high (17.1--18.0 %) and low (11.0--11.9 %) protein content, high (9.1--10.0 %) and low (4.0--5.9 %) oil content, high (66.1--67.0 %) and low (59.0--60.9 %) starch content in caryopses, as well as specimens combining high starch content with low oil content (C-1042) and vice versa (C-1114, C-1116), with low protein an oil content combined with high starch content (C-1180, C-1370). High total content of fatty acids, including essential acids, was found in the specimen C-1576, while high content of essential amino acid valine was found in the specimen C-1121 and high content of essential amino acid tryptophane was found in the specimen C-1576. Specimen C-1121 was characterized with high total free amino acids content. The research results allowed choosing starting material for selection work with hybrids of sweet corn to get predefined nutritional and flavor properties.

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