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NAS RK is pleased to announce that Bulletin of NAS RK scientific journal has been accepted for indexing in the Emerging Sources Citation Index, a new edition of Web of Science. Content in this index is under consideration by Clarivate Analytics to be accepted in the Science Citation Index Expanded, the Social Sciences Citation Index, and the Arts & Humanities Citation Index. The quality and depth of content Web of Science offers to researchers, authors, publishers, and institutions sets it apart from other research databases. The inclusion of Bulletin of NAS RK in the Emerging Sources Citation Index demonstrates our dedication to providing the most relevant and influential multidiscipline content to our community.

Қазақстан Республикасы Ұлттық ғылым академиясы "ҚР ҰҒА Хабаршысы" ғылыми журналының Web of Science-тің жаңаланған нұсқасы Emerging Sources Citation Index-те индекстелуге қабылданғанын хабарлайды. Бұл индекстелу барысында Clarivate Analytics компаниясы журналды одан әрі the Science Citation Index Expanded, the Social Sciences Citation Index және the Arts & Humanities Citation Index-ке қабылдау мәселесін қарастыруда. Web of Science зерттеушілер, авторлар, баспашылар мен мекемелерге контент тереңдігі мен сапасын ұсынады. ҚР ҰҒА Хабаршысының Emerging Sources Citation Index-ке енуі біздің қоғамдастық үшін ең өзекті және беделді мультидисциплинарлы контентке адалдығымызды білдіреді.

НАН РК сообщает, что научный журнал «Вестник НАН РК» был принят для индексирования в Emerging Sources CitationIndex, обновленной версии Web of Science. Содержание в этом индексировании находится в стадии рассмотрения компанией Clarivate Analytics для дальнейшего принятия журнала в the Science Citation Index Expanded, the Social Sciences Citation Index и the Arts & Humanities Citation Index. Web of Science предлагает качество и глубину контента для исследователей, авторов, издателей и учреждений. Включение Вестника НАН РК в Emerging Sources Citation Index демонстрирует нашу приверженность к наиболее актуальному и влиятельному мультидисциплинарному контенту для нашего сообщества.

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ASSESSMENT OF MAIZE GRAIN QUALITY BY DNA MARKERS

Abstract. Research goal. To evaluate maize lines on the basis of the high content of lysine and tryptophan and waxiness using SSR markers.

Methods. Laboratory, statistical.

Results. The article presents the results of maize lines examination using SSR markers carried out in order to select genotypes with a high content of lysine and tryptophan and waxiness. For the selection of lines based on the high content of lysine and tryptophan, a complex of two specific SSR markers was used, namely a dominant phi1212 marker and a codominant phi057 one. With the aid of these markers, three alleles of 141, 153, 165 bp and 141, 150, 160 bp were detected at the polymorphism information content (PIC) values of 0.51 and 0.61, respectively. The homozygous state of the recessive allele *o2* associated with a high content of lysine and tryptophan in grain was detected in 2 out of 77 maize genotypes under examination. The W4 microsatellite marker was used to detect genotypes for the trait of waxiness, i.e. those having zero mutation of the *wx* gene. Resulted from PCR of 77 maize lines, 5 alleles of 176 to 200 bp were detected at the PIC value of 0.73. Amplicons of size 194 and 200 bp were detected in 24 examined maize lines that allegedly contain a zero mutation of the *wx* gene in a recessive homozygous state. Among the promising genotypes with double recessive homozygote for the alleles *o2* and *wx*, two genotypes which are homozygous by *opaque-2- waxy* genes have been selected to be used in further breeding work aimed at improving protein quality of maize grain.

Keywords: SSR markers, lysine, tryptophan, waxiness, *wx* gene.

Introduction. At present, breeding of grain crop is aimed not only at high yield but also at improved biochemical characteristics of the grain. In particular, maize grain contains 8 to 10% protein and a small amount of amino acids. The maize seed protein contains 1.5 to 2% lysine, which is less than a half the human nutrition requirement [1, 2]. In addition, typical maize contains leucine-isoleucine-rich protein which has low biological value.

There are favourable mutations of maize that lead to an improved quality of grain protein. Such mutations are found in genes *opaque2 (o2), opaque6 (o6), opaque7 (o7), opaque11 (o11), floury2 (fl2), floury3 (fl3), Mucronate (Mc) and Defective endosperm (Dc-b30).* They provide for significantly higher concentrations of lysine and tryptophan in maize grain and to a decrease in the accumulation of zein as compared with conventional maize varieties [3, 4].

In particular, the recessive mutation *opaque-2 (o2)* induces a specific decrease in the accumulation of 22-kDa α-zein. It was found that *O2* encodes the main transcriptional regulatory protein bZIP that is specifically expressed in the endosperm and directly or indirectly regulates a number of genes associated with protein accumulation and the level of lysine-ketoglutarate reductase and aspartate kinase. This allows assuming that *O2* plays an important role in the development of grain as a gene expression coordinator which controls protein accumulation, metabolism of nitrogen and carbon [5-9].

Among the well-known starch-modifying maize mutations, mutation *wx* (bearing zero mutation of the *wx* gene, which is a trait of waxiness) deserves special attention. It causes a significant decrease in the activity of the granular-bound starch synthase, suppresses the synthesis of amylose and causes the formation of starch that almost completely consists of amylopectin. Amylopectin is better digested with amylase compared with amylose; therefore, it is more technological feedstock for the industry. The results

of the latest research on plant genome allow effective selection of genotypes and creation of new high-productive genotypes with improved grain quality [10, 11]. Sinkangam et al. (2011) documented the effective combination of traits of high lysine and tryptophan content and waxiness for an individual genotype [10].

For the examination of such maize lines and hybrids, it is necessary to exploit DNA markers, because as a result of the selection for improved quality characteristics, such lines and hybrids (i.e. with increased content of lysine and tryptophan or waxiness) may be inferior to other genotypes in terms of valuable economic and agronomic properties. To evaluate the polymorphism of maize lines in terms of lysine and tryptophan content and waxiness we used of a set of three SSR markers *phi057, phi112* and *W4* [12, 13]. Thus, **the purpose of the work** was to evaluate maize lines on the basis of the high content of lysine and tryptophan and waxiness using SSR markers.

Methods and materials. In the research, 77 maize lines of the Research Institute of Agrarian Business (Dnipro, Ukraine) were used. The work was carried out at the Ukrainian Institute for Plant Variety Examination (Kyiv, Ukraine) during the 2017−2018 period.

Isolation of DNA and PCR. DNA was isolated from 5-day maize seedlings using CTAB (1%) as a lysing buffer. Purification from proteins and polysaccharides was carried out using chloroform; DNA sedimentation was carried out under isopropyl alcohol. The extracted DNA was washed with 95% ethanol solution and dissolved in TE buffer (1mM EDTA pH 8.0, 10 mM Tris-HCl pH 8.0). In the experiment, three SSR markers related to the trait of high lysine and tryptophan content and the trait of waxy seed were used [14, 15] (table 1).

Table 1 - Characteristics of SSR markers

Gene	Marker	Position (bp)	The nucleotide sequence of primers 5'…3'	Hybridization temperature (°C)	Expected size of amplicones (bp)
O2	Phi112	1218-1368	F* - gccctgcaggttcacattgagt	57.0	150
			R** - aggagtacgcttggatgctcttc		
	Phi057	3616-3769	F - ctcatcagtgccgtcgtccat	57.0	165
			R - cagtcgcaagaaaccgttgcc		
Wx	W4	4597-4791	F - aataatccctgctgttcggt	60.0	194
			R - cagcttttggtggccaga		

Note: *Forward primer; **Reverse primer

10 μl of reaction mixture contained: 1 × DreamTaqTMGreen buffer, 0.75 u DreamTaqTM polymerase (ThermoScientific, USA), 100 μM of each dNTP, 25 ng of DNA sample, 0.1 μM of each primer according to the marker. Polymerase chain reaction (PCR) was performed on the TC-Y CreaCon (USA) amplifier. For each primer, the following parameters of PCR were set: step 1 − initial denaturation: (94°C) 5 min; step 2 − development of specific reaction products: denaturation: (94°C) 1 min, hybridization of primers (57-60°C) 1 min, elongation (72°C) 1 min, number of cycles 30; step 3 − final elongation: (72°C) 5 min.

The amplicons were visualized by electrophoresis in a 2% agarose gel in 0.5 × TBE (Tris-borate buffer solution) with bromine ethidium [16]. DNA electrophoresis was performed for 1.5 h. at an electric field intensity of 5 V/cm.

Statistical data analysis. Size of identified alleles was determined using TotalLab TL120 (Trial version) software. In order to evaluate the characteristic of the discriminatory force of the locus not only in terms of the number of detected alleles but also relative frequencies of their encounter, polymorphism information content (PIC) was calculated according to the formula: $PIC = 1 - \sum_i p_{li}^2$, where p_{li} is the frequency of the *i* allele of *l* locus [17].

Results and discussion. To identify maize genotypes based on the trait of high lysine and tryptophan content, a set of two specific SSR markers was used: dominant marker phi1212 that identifies the *O2* allele in the homozygous and heterozygous state [13] and codominant marker phi057 that identifies and isolates three forms of *o2o2, o2O2* and *O2O2*. The results of the electrophoretic separation of the obtained DNA amplicons of maize lines with primers to the markers phi057 and phi112 are shown in figure 1 and 2, respectively.

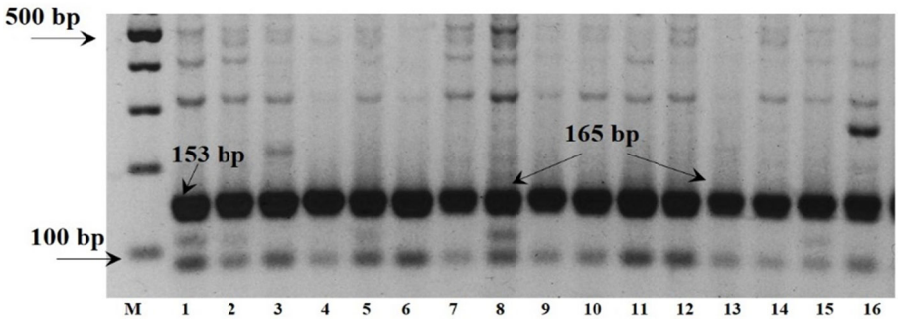

Figure 1 - Electrophoresis of amplicons by the phi057 marker: 1−12 correspond to maize lines RAM 1-RAM 12; 13−16 to RAM 14−RAM 17; M − molecular weight marker Thermo Scientific O'RangeRuler 100 bp DNA Ladder.

As it can be seen from Fig. 1, RAM 1, RAM 2, RAM 3, RAM 4, RAM 5, RAM 6 and RAM 7 lines on tracks 1−7 contain a 153 bp allele. Maize lines RAM 11, RAM 12, RAM 14, RAM 15 and RAM 16 on tracks 11, 12, 14−16 by the marker phi057 also have an allele of 153 bp. Therefore, the indicated lines, in the case of detection of alleles of any size by marker phi112, may have genotype *O2O2*. Amplicons of 165 bp were found in lines RAM 8, RAM 9 and RAM 14 that are shown on tracks 8−10 and 13. This means that in the absence of any amplicon by the phi112 marker, these lines may be homozygous for the recessive allele *o2* and, accordingly, be promising in terms of lysine and tryptophan synthesis.

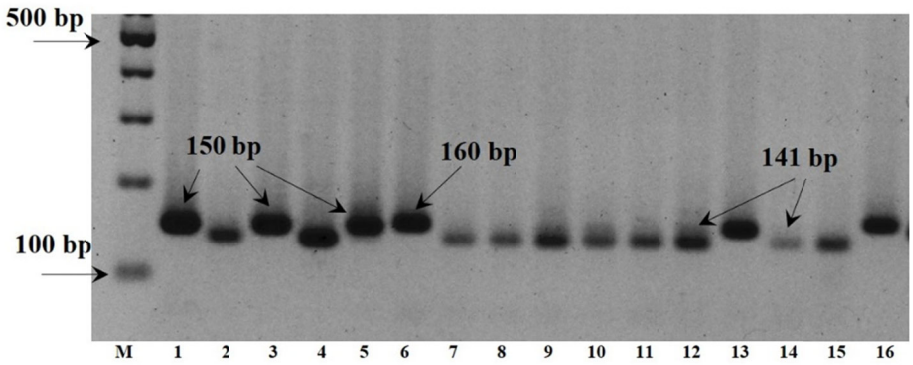

Figure 2 - Electrophoresis of amplicons by the phi112 marker: 1−12 correspond to maize lines RAM 1-RAM 12; 13−16 to RAM 14−RAM 17; M − molecular weight marker Thermo Scientific O'RangeRuler 100 bp DNA Ladder

Figure 2 shows the electrophoresis of DNA amplicons of maize lines by the phi1212 marker. According to the obtained data, alleles of 150 bp were detected in lines RAM 1, RAM 3 and RAM 5 corresponding to tracks 1, 3 and 5, respectively. On tracks 2, 4, 7−12 and 14−15 that represent lines RAM 2, RAM 4, RAM 7, RAM 8, RAM 9, RAM 10, RAM 11, RAM 12, RAM 15 and RAM 16, 141 bp alleles were detected. Presented on tracks 6, 13 and 16 are 160 bp alleles that are characteristic for RAM 6, RAM 14 and RAM 17 lines. The lines described above, in which the allele size detected by the marker phi057 was 153 and 165 bp, may have two genotypes at the heterozygous or homozygous state of the *O2* allele: *O2O2 or O2o2*. However, given the identified alleles by the phi112marker, maize line shown in the figure cannot be homozygous for the allele *o2* that is connected to the increased synthesis of lysine and tryptophan.

According to the obtained data, the size and frequency of the alleles by the markers under study and PIC valued were determined. The markers phi057 and phi112, which identify genotypes with a high content of lysine and tryptophan, detected three alleles: 141, 153, 165 bp and 141, 150, 160 bp, respectively (table 2).

The frequency of the alleles detected by the phi057marker ranged from 0.03 to 0.58 and by the phi112 marker from 0.05 to 0.47. According to the obtained data, the frequency of the 141 bp allele detected by the phi057 marker was 0.03 and the frequency of the 160 bp allele detected by the phi112 marker was 0.05. The frequency of favourable 153 bp allele by the phi057 marker was 0.39. The estimated PIC values for each marker (0.51 and 0.61), indicate a sufficient degree of identified variability among the genotypes under examination. The obtained amplicons allow determining the allelic state of maize lines (table 3).

Table 2 - Alleles, detected in maize lines by markers of high lysine and tryptophan content

SSR marker	The number of alleles	Allele size (bp)	Allele frequency	PIC
phi057	3	141	0.03	0.51
		153	0.39	
		165	0.58	
phi112	3	141	0.47	0.61
		150	0.40	
		160	0.05	

Table 3 - Maize genotypes identified by markers of high lysine and tryptophan content

Genotype	Alleles by the phi057 marker (bp)	Alleles by the phi112 marker (bp)	The share of genotype (%)
O2O2	153	141 or 150 or 160	35
o2O2	165		23
o2o2	165	–	3

Given that phi057 is a codominant marker, the genotypes in which an allele of 153 bp or any of the expected alleles was detected by dominant phi112 marker may be homozygous for the dominant *O2* allele. Of all maize lines under examination, 27 may have *O2O2* genotype. Accordingly, these lines can be considered not promising in terms of lysine and tryptophan content. The largest number of genotypes, according to a combination of dominant and codominant markers, may have a heterozygote for the investigated allele. It was found that 18 genotypes may contain heterozygote *O2o2*. As a result of the amplification of their DNA with the corresponding primers, the presence of 165 bp alleles by the phi057 marker and amplicons of 141, 150 and 160 bp by the phi112 marker was proved. To determine the homozygous state by the recessive allele *o2*, the absence of any amplicons by the phi112 gene marker and the presence of alleles of about 165 bp by the phi057 marker is important. Only two lines (3% of the total number of studied maize genotypes) which conform to the described above conditions were selected.

Volkova et al. (2015) carried out studies of populations of lines GK26 and Mo17 using DNA markers nc030, phi061, phi064, phi083, phi031, phi044, phi057, phi084, phi080 and phi112, which demonstrated clear amplification products for the determination of polymorphism in eight chromosomes. The authors found a link between the phi112 and phi057 marker loci with quantitative trait loci (QTL). The analysed 58 maize lines and detected four alleles for marker locus phi 057 and five for phi112 at PIC values of 0.60 and 0.57, respectively. The obtained results are suggested to be used for the individual genotype forecasting in terms of development of certain agronomic traits, which allows to significantly accelerate the selection of the necessary material within a year, starting from F2, and to model the selection of genotypes with a high level of the trait manifestation in subpopulations by marker alleles, which allows to improve basic maize populations and use them as a source material for heterozygous breeding [18].

Singh et al. (2018) studied the genetic diversity of maize lines and hybrids. The authors analyzed 30 maize genotypes by 23 markers, including the phi112 marker. In the studied genotypes, four alleles in the range from 140 to 165 bp were detected. In our research, this marker also detected the alleles of the specified range with the corresponding frequency [19].

Similar studies were carried out by Smith et al. (1997). The authors evaluated maize lines using RFLP and SSR markers to determine genetic distances between genotypes. The phi057 marker identified four alleles, followed by the phi112 marker with five alleles. The PIC values were 0.60 and 0.57, respectively [20].

The employment of the phi057 and phi112 markers to evaluate maize genotypes with a high content of lysine and tryptophan is documented by Magulama and Sales (2009) and Yang et al. (2008). The authors prove the use of the codominant marker phi057 for the selection of maize lines with a homozygous state of alleles *o2o2* [12, 21]. Danson et al. (2006) recommend using the phi112 marker in marker-associated breeding. The authors studied parent lines of maize by the markers umc1066, umc1216, phi057 and phi112. According to the results of their research, it was found that the presence of zero allele by the phi112 markers is related to the state of a mutant allele of the gene *opaque-2 (o2)* [22].

Consequently, the studies carried out by other authors were aimed at the use of SSR markers not only for the evaluation of genetic diversity but also for the selection of maize genotypes with a high content of lysine and tryptophan in grain. In our studies, the markers phi057 and phi112 were also used to select

promising genotypes with a high content of lysine and tryptophan. However, the obtained data require further investigation to confirm the effectiveness of the approach of using DNA markers.

Since the nutritional value of grain depends not only on the protein content but also on the type of starch, we studied a trait of waxiness by the *wx* gene. Microsatellite W4 marker was used to detect the maize genotypes containing a zero mutation of the *wx* gene (a trait of waxiness). As a result of the amplification of the lines under study, the fragments promising for the presence of a zero mutation (homozygous recessive state of *wx*) were obtained (figure 3).

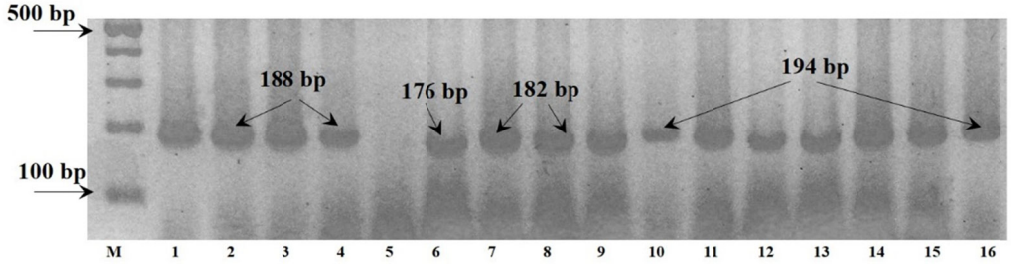

Figure 3 - Electrophoresis of amplicons by the W4 marker: 1−4 correspond to RAM 87−RAM 90; 5 to RAM 94; 6−14 to RAM 99 − RAM 107; 15 to RAM 109; 16 to RAM 111;
M – molecular weight marker Thermo Scientific O'RangeRuler 100 bp DNA Ladder

Shown in figure 3 are the results of the electrophoretic separation of amplicons by the marker of waxiness. The alleles of 188 bp were detected in lines RAM 88, RAM 89, RAM 90, RAM 104, RAM 105 and RAM 109, alleles of 182 bp in lines RAM 100, RAM 101, RAM 102, RAM 106 and RAM 107 and alleles of 176 bp in RAM 99. Given that the presence of alleles of the specified size is not related to zero mutation of the *wx* gene, the lines are not considered promising for further breeding work in terms of the trait of waxiness. Favourable alleles of 194 bp were detected on tracks 1, 10 and 16 in RAM 87, RAM 103, RAM 111 lines, respectively.

According to the PCR results of 77 maize lines, five alleles with a size range between 176 and 200 bp were obtained by the W4 marker (table 4).

Table 3 - Maize genotypes identified by a marker of waxiness

SSR marker	The number of alleles	Allele size (bp)	Allele frequency	PIC
W4	5	176	0.14	0.73
		182	0.12	
		188	0.42	
		194*	0.23	
		200*	0.09	

Note: *favourable alleles on the basis of waxiness.

For the detected alleles, their frequencies were calculated; their values ranged from 0.09 to 0.42. The PIC value was 0.73. According to the obtained data, amplicons of 194 and 200 bp were detected in 24 maize lines. This suggests that these genotypes may contain a zero mutation of the *wx* gene in a recessive homozygous state.

Dang (2010) describes the use of four markers for assessing Chinese maize lines on the basis of waxiness. For this purpose, the author used four pairs of primers W1, W2, W3 and W4. According to the genetic sequences of the various alleles associated with the waxiness, the pair of primers W1, W2 and W3 should amplify fragments 202, 555 and 364 bp, respectively, in the genotypes that are not related to zero mutation of the *wx* gene. Since in practice, similar fragments were observed in both waxy and conventional genotypes, these three pairs of primers did not allow selecting waxy genotypes [13]. Therefore, only one W4 primer was effective, and therefore we used it in our research too.

Sinkangam et al. (2011) investigated the transfer of *opaque-2* genes from inbred maize lines with high-quality protein to elite waxy inbred lines by crossing and selecting homozygous recessive lines using MAS methods as well as their biochemical parameters. The researchers found that lines that have the *opaque-2-waxy* genotype have a high content of sugar and amylopectin. As a result of crosses and selections, the authors obtained six lines homozygous for *opaque-2-waxy* genes [10].

In our study, among the promising genotypes with the trait of waxiness and high content of lysine and tryptophan, i.e. with double recessive homozygote for the alleles *o2* and *wx*, two genotypes can be selected according to the detected by the phi057 marker alleles and with no amplicons by the phi112 marker, as well as by the presence of favourable alleles by the W4 marker.

Considering the fact that the traits of high lysine and tryptophan content and waxiness allow obtaining high-quality processed grain products, there is a need to continue the works on the evaluation of protein composition and the type of starch in promising maize lines selected by SSR markers [10]. The maize lines we selected will be used in further breeding work aimed at improving the quality of protein of maize grain.

Conclusions. Using a combination of two SSR markers phi057 and phi112 we evaluated 77 maize lines on the basis of the high content of lysine and tryptophan in grain. According to the obtained data, two of the studied lines were selected. They were identified as possible genotypes with a homozygous recessive mutation *o2*.

In order to select waxy-seeded breeding materials, an estimation of the genotypes by the W4 marker was carried out. According to the presence of two alleles of 194 and 200 bp, 24 maize lines were found promising. Two promising genotypes have been identified by double recessive homozygote for the alleles *o2* and *wx*. They will be used in further breeding work.

The proposed set of SSR marker is useful for identifying both double and single recessive homozygotes for valuable economic and agronomica traits. As these two traits can be clearly detected only at the later stages of plant development, specific molecular markers for *wx* and *o2* genes may be appropriate to facilitate the early selection of specific genotypes in the respective breeding programs.

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ДНҚ-МАРКЕРЛЕРІН ҚОЛДАНУ АРҚЫЛЫ ЖҮГЕРІ ДӘНІНІҢ САПАСЫН БАҒАЛАУ

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ОЦЕНКА КАЧЕСТВА ЗЕРНА КУКУРУЗЫ С ПОМОЩЬЮ ДНК-МАРКЕРОВ

Аннотация. Цель исследования – ценить линии кукурузы по признакам повышенного содержания лизина и триптофана, а также восковидности с помощью SSR-маркеров**.**

Методы. Лабораторные, статистические.

Результаты. В статье представлены результаты исследований линий кукурузы с помощью SSR-маркеров с целью отбора генотипов с повышенным содержанием лизина и триптофана в зерне, а также восковидным типом зерна. Для отбора линий по признаку повышенного содержания лизина и триптофана применяли комплекс из двух специфических SSR-маркеров: доминантный маркер phi112 и кодоминантных phi057. Определено, что по маркерам phi057 и phi112 получено по три аллели с размерами 141, 153 и 165 пн и 141, 150 и 160 пн соответственно, значение PIC составили 0,51 и 0,61. Гомозиготное состояние по рецессивной аллели *o2*, которая связана с повышенным содержанием лизина и триптофана в зерне была обнаружена в двух из 77 исследуемых генотипов кукурузы.

Для выявления генотипов по признаку восковидности, содержащих ноль-мутацию гена *wx*, применяли микросателлитный маркер W4. По результатам ПЦР анализа 77 линий кукурузы получено пять аллелей размером от 176 до 200 пн, значение PIC составило 0,73. Ампликоны размером 194 и 200 пн были идентифицированы в 24 исследуемых линиях кукурузы, которые могут содержать ноль-мутацию гена *wx* в рецессивной гомозиготной форме.

Среди перспективных генотипов с двойной рецессивной гомозиготой по аллелям *o2* и *wx* отобрано 2 генотипа (гомозиготные по *opaque-2-waxy* генами), которые будут использованы в дальнейшей работе в селекционных программах на улучшение качества зерна кукурузы.

Ключевые слова: SSR маркеры, лизин, триптофан, восковидность, ген w*x.*

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