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GRAIN PROPERTIES OF NEW INBRED LINES IN COMPARISON WITH MAIZE HYBRIDS

KARAKTERISTIKE ZRNA NOVIH SAMOOPLODNIH LINIJA U POREĐENJU SA HIBRIDIMA KUKURUZA

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ABSTRACT

Grain quality parameters of five new maize inbred lines were investigated and compared with the corresponding properties of two commercial hybrids used in flour production. Kernel of the line L2 had the highest 1000-kernel mass (267.54 g), and line L2 had the highest test mass (844.68 kg m^{-3}*). The milling response ranged from 9.80 (L2) to 14.03 s (L1). The largest share of the soft fraction of endosperm was determined in L3 (37.13 %), which is slightly lower than in hybrid ZP 633. The highest protein (12.37 %), as well as crude fibre content (2.59 %) was determined in inbred line L4, while the lowest protein was found in the line L2 (9.36 %), and the lowest crude fibre content (1.81 %) was detected in line L3. All tested maize inbred lines were found to be highly prospective for breeding of new hybrids with improved grain quality parameters.*

Key words*: *inbred lines, maize hybrids, chemical composition, physical traits, whole-grain maize flour.

REZIME

*Kukuruz (*Zea mays *L.) predstavlja jednu od najviše gajenih i najznačajnijih žitarica koje se vekovima koriste u ishrani ljudi i životinja. Cilj ovog istraživanja bio je da se ispitaju novi genotipovi kukuruza sa povećanim potencijalom za stvaranje komercijalne kategorije semena visoke nutritivne, funkcionalne i tehnološke vrednosti koje bi imalo povoljne karakteristike mlevenja za proizvodnju integralnog kukuruznog brašna.*

Ispitivani su parametri kvaliteta zrna pet novih samooplodnih linija kukuruza i upoređivani sa odgovarajućim svojstvima dva komercijalna hibrida koja se koriste u proizvodnji brašna. Zrno linije L2 imalo je najvišu apsolutnu masu zrna (267,54 g), a zrno linije L2 najvišu nasipnu masu (844,68 kg m^{-3}*). Otpornost na mlevenje kretala se od 9,80 (L2) do 14,03 s (L1). Najveći udeo meke frakcije endosperma utvrđen je u liniji L3 (37,13%), što je nešto niže nego u standardnom hibridu zubanu ZP 633 (41,97%). Najviši sadržaj proteina (12,37%), kao i sirove celuloze (2,59%) određen je u liniji L4, dok je linija L2 imala najniži sadržaj proteina (9,36%), a linija L3 najniži sadržaj sirove celuloze (1,81%). Utvrđeno je da sve ispitane nove samooplodne linije kukuruza poseduju visok potencijal za selekciju novih hibrida sa poboljšanim parametrima kvaliteta zrna.*

Ključne reči: *samooplodne linije, hibridi kukuruza, hemijski sastav, fizičke osobine, integralno kukuruzno brašno.*

INTRODUCTION

Maize (*Zea mays* L.), also known as corn, is one of the most important cereal food and feed crops worldwide. Production of maize ranks as the third after wheat (*Triticum aestivum* L.) and rice (*Oryza sativa* L.) with estimated world production of 1,186 million metric tons in 2020. This cereal grain was first domesticated from ancient grass teosinte between 5000 and 7000 years ago by indigenous peoples of southern Mexico. After Columbus arrived in the New World and brought maize to Europe, cultivated maize spread worldwide (*FAO, 2020; Liu et al. 2020; Terzić et al., 2020*).

The first double-cross hybrid was created by Donald F. Jones in 1918. It was later improved and introduced on a trial basis in 1924 by Henry Agard Wallace, and in 1933 during a devastating drought in rural parts of the USA, farmers started using these novelty seeds (*Sutch, 2011*). As a result of long-term breeding processes, modern single-cross hybrids provide significantly higher yields than previous double-cross hybrids and open pollinated varieties (*Milenković et al., 2014*). The modern directions of maize breeding have been particularly focused on creating new specialty hybrids with altered and improved nutritional properties (*Pollak and Scott, 2005*).

In general, main chemical components of maize grain are, in average: 71.3 % starch, 9.91 % protein, 4.45 % oil, 1.42 % ash, and 2.66 % crude fibre (*Eckhoff and Watson, 2009*). Maize hybrids differ in endosperm hardness or vitreousness, i.e., breakage susceptibility, which is greatly dependent on genetic background. The ratio of vitreous (hard) to floury (soft) endosperm is an important agronomic trait that may influence grain hardness, post-harvest resistance to pests, and microorganisms, as well as the rate of starch digestibility. Even though endosperm hardness is generally genetically predisposed, other factors such as environment and post-harvest handling, i.e., transportation, drying, storage, and processing, may also affect grain hardness (*Córdova-Noboa et al., 2020; Kljak et al., 2011*). Furthermore, maize grain hardness is extremely important in food processing and grain trading because it influences end-use processing performance by large, including dry-milling yield, and power usage, as well as dust formation during processing. Dent maize hybrids, which are predominant nowadays, originate from flint-flour genotypes and differ in their ratio of vitreous to floury endosperm (*Kljak et al., 2018*). The interactions between starch granules ant the protein matrix that surrounds them provide the variations in texture and strength of vitreous and floury endosperm of the grain (*Kljak et al., 2011; Philippeau et al., 2000*).

There are two main maize grain processing technologies: 1) wet milling, and 2) dry milling. Wet milling of maize grain is a process developed to obtain high yields of starch (mostly used for the production of sweeteners, food thickeners, bioethanol, etc.) from the kernels. The objective of this process is to separate germ, fibre, protein, and starch from the maize kernel by steeping the maize kernels in solutions containing SO_2 first, which softens the kernel and helps in the separation of the kernel constituents. Dry milling was developed to obtain food grade grits, hominy, and other food fractions by removing the germ and bran to produce products with a longer shelf life and lower oil content. Whole-grain maize flour, on the other hand, obtained by grinding kernels without removing the germ first, is naturally gluten free, and therefore suitable for persons suffering from celiac disease (*Semenčenko et al., 2013; Parris et al., 2006*).

Research within the field of technological value and grain quality contributes to better valorisation of maize in the industrial processing, especially in the production of high-quality functional food, which has the objective to increase the economic value of this, for our country the most important, carbohydrate feedstock (*Milašinović-Šeremešić et al., 2018; Radosavljević et al., 2001*).

The quality of maize is determined by the joined effects of the cellular structure, physical and biochemical properties of the components in the grain (*Paulsen et al., 2003*). Great number of factors, including environment, genetics, growing and post-harvesting conditions, kernel physical properties, chemical composition, etc., may influence variations in maize quality. In comparison to softer maize kernels that are more suitable for wet-milling, harder maize kernels exhibit better performance during storage, handling, transportation, alkaline cooking, and dry-milling (*Lee et al., 2007*).

The aim of this study was to examine the physical properties and chemical composition of five new inbred lines and to compare them with two commercial yellow kernel hybrids, one dent and one popping maize hybrid created in the Maize Research Institute, Zemun Polje. Furthermore, the objective was to identify the best inbred maize lines for further breeding of hybrids with increased potential for high nutritional, functional and technological value that would have favourable milling characteristics, primarily for food production, i.e., whole grain maize flour.

MATERIAL AND METHODS

Five new inbred maize lines and two hybrids, one yellow kernel dent (ZP 633), and one yellow kernel popcorn (ZP 611k) developed at the Maize Research Institute, Zemun Polje, were investigated in this study. In spring of 2019, at the experimental field located at the Maize Research Institute, Zemun Polje, the two-replicate trial was set up according to the randomized complete-block design. The plot size was 21 m^2, while the sowing density was 60,000 plants ha^{-1}. Maize ears of each replicate were harvested in the full physiological maturity stage from the area of 7 m^2 (two inner rows). Twenty average ears per replicate were selected for further analysis. Whole grain maize flour was obtained by a dry grind process on a laboratory mill (Perten Instruments, Hägersten, Sweden) for fine samples preparation (mesh 0.5 mm).

Methods applied for determining physical properties (1000-kernel mass, test mass, milling response, absolute density, soft and hard endosperm portion, and water absorption index), were described in detail in a previously published paper by *Radosavljević et al., 2001*. Absolute density or specific gravity is determined in a specially designed glass column based on the difference in 96% ethanol levels before and after immersion of 100 grains of previously determined mass. The water absorption index is determined by measuring the amount of water that is absorbed by a certain amount of maize grain under precisely defined conditions. The milling response (i.e., time-to grind), determined by a Stenvert hardness test (*Pomeranz et al., 1985*) is a measure of kernel hardness which presents the time (s) necessary for kernel grinding until the top level of the material collected in a glass cylinder (125×25 mm) reaches the level of 17 ml. The samples of cornmeal obtained after grinding maize kernels during the Stenvert hardness test are merged and sieved through 0.5 mm diameter nylon mesh. The fraction that is sifted through the sieve is denoted as the soft endosperm, and the remaining one as hard endosperm. The fractions are measured afterwards, and the hard to soft endosperm ratio is calculated.

Dry matter content in the maize flour was determined by the standard drying method in an oven at 105 °C to constant mass. The protein content was determined by the Kjeldahl method as the total nitrogen multiplied by 6.25 (AOAC, 1990). Crude fibre content was determined by Weende method adjusted for Fibretec™ Systems, Foss, Denmark (Agricultural food products, 1993). The results are expressed in the percentages per dry matter (d.m.). All analyses were performed in two replicates, and the results are presented as means.

Statistical analysis was performed in Minitab19 Statistical Software using one-way ANOVA analysis of variance with Fisher's LSD (Least Significance Difference) test. Differences between the means with probability $p<0.05$ were accepted as statistically significant.

RESULTS AND DISCUSSION

In order to identify genotypes with increased potential for creating a commercial category of seeds with high nutritional, functional and technological value that would have favourable milling characteristics for the production of whole grain maize flour, grain quality parameters of five new maize inbred lines were investigated.

Table 1. Kernel physical properties of the investigated inbred lines and maize hybrids

Genotype	1000-kernel mass (g)	Test mass (kg m^{-3})	Absolute density (g cm^{-3})	Water absorption index
Inbred lines				
L1	243.24 ± 1.87^{d}	844.67 ± 6.44^{b}	1.32 ± 0.01^{b}	0.231 ± 0.00^{c}
L2	267.54 ± 5.67^{b}	$829.22 \pm 3.15^{b,c}$	$1.30 \pm 0.00^{b,c}$	0.277 ± 0.01^{b}
L3	265.75 ± 3.30^{b}	$831.28 \pm 6.19^{b,c}$	$1.30 \pm 0.01^{b,c}$	0.272 ± 0.00^{b}
L4	198.94 ± 2.15^{e}	808.61 ± 3.54^{d}	1.29 ± 0.00^{c}	0.341 ± 0.00^{a}
L5	252.00 ± 2.27^{c}	$815.14 \pm 9.07^{c,d}$	$1.31 \pm 0.01^{b,c}$	0.271 ± 0.02^{b}
Hybrids				
ZP 611k	133.15 ± 4.02^{f}	886.40 ± 6.28^{a}	1.37 ± 0.01^{a}	0.221 ± 0.01^{c}
ZP 633	303.81 ± 2.79^{a}	$831.60 \pm 10.95^{b,c}$	1.26 ± 0.00^{d}	0.233 ± 0.00^{c}

Results are given as mean ± standard deviation. Means that do not share a letter are significantly different.

The data shown in in this article represent the analyses results of the selected inbred lines in comparison with the corresponding physicochemical characteristics and chemical properties of two commercial hybrids used in flour production: ZP 611k and ZP 633.

The kernel physical properties of the investigated inbred lines and ZP maize hybrids are shown in Table 1.

Regarding physical properties of the inbred maize lines, kernel of the line L2 had the highest 1000-kernel mass (267.54±5.67 g), and kernel of the line L2 had the highest test mass (844.67±6.44 kg m^{-3}). The 1000-kernel mass is considered as one of the most important physical indicators of grain quality essential for maize grain processing in dry and wet milling technologies. Higher 1000-kernel mass is a preferred wet-milling characteristic because it is associated with greater starch and protein yield and lower yields of fibre (*Milašinović et al., 2007; Milašinović, 2005*). In a study conducted by *Milenković et al. (2014)*, 1000-kernel mass of the investigated lines ranged from 311.3 to 352.65 g. *Somavat et al. (2016)* found that 1000-kernel mass of some differently coloured dent maize kernels, such as blue maize, is lower than the same parameter of yellow and red kernel maize. The test mass of popcorn hybrid ZP 611k kernel was higher than in all tested lines (886.40±6.28 kg m^{-3}), as well as the absolute mass of the standard hybrid ZP 633 (303.81±2.79 g), which is in accordance with studies of ZP maize hybrids previously published (*Milašinović-Šeremešić et al. 2019; Milašinović-Šeremešić et al. 2018; Semenčenko, 2013*). Test mass is also a valuable indicator of maize grain quality, it is the oldest and easily measurable standard. This parameter is used in determining maize grade and significantly influences its selling price in the market, although it is a poor indicator of maize quality for processing and milled products (*Paulsen et al. 2003; Lee et al. 2007*). All maize inbred lines and hybrids used in this study had test mass greater than 650.0 kg m^{-3}, which is a requirement for animal feed according to Serbian regulations (*Pravilnik o kvalitetu hrane za životinje, 2016*), and 69.50 kg h l^{-1} (695.0 kg m^{-3}) required for US Grade No. 2 maize (*Somavat et al., 2016*). In Serbian regulations there is no minimal request for this parameter for maize grain quality for human food consumption. Water absorption index is a crucial parameter for the wet milling processing of the maize grain that tends to separate the maize kernels into their basic chemical components (starch, protein, oil, and fibres). During the steeping (soaking or hydration) step of the process, the morphological and biochemical changes that occur are responsible for all subsequent stages of the process and, thus, for the end quality of the finished product (*Botelho et al., 2013*). Water absorption index in maize inbred lines ranged from 0.231±0.00 (L1) to 0.341±0.00 (L4), while hybrid ZP 633 had the value of water absorption index of 0.233±0.00, and ZP 611k result was 0.233±0.00 (Table 1).

The milling response results obtained in these analyses ranged from 9.80±0.40 (L2) to 14.03±0.15 s (L1) for inbred lines, while dent hybrid ZP 633 had milling response of 11.97±0.97 s and popcorn hybrid ZP 611k had the highest milling response of all samples - 15.83±0.42 s (Figure 1). *Milašinović Šeremešić et al. (2019)* reported that milling responses of ten differently coloured maize hybrids from Serbia ranged from 12.10 to 25.40 s.

Kernel hardness is closely related to the ratio of hard (glassy) and soft (floury) endosperm. Observed from the industrial point of view, maize starch processing, in particular, milling response and the share of hard and soft fractions of the endosperm are parameters of grain hardness, which represent its most important physical properties (*Milašinović, 2005*). Maize hybrids that contain larger portion of soft endosperm enable easier extraction of starch because of weaker protein matrix that surrounds starch granules and are, therefore, more suitable for wet-milling. On the other hand, maize hybrids with higher share of hard endosperm are more appropriate for dry-milling as they yield grits with larger sizes (*Lee et al., 2007*).

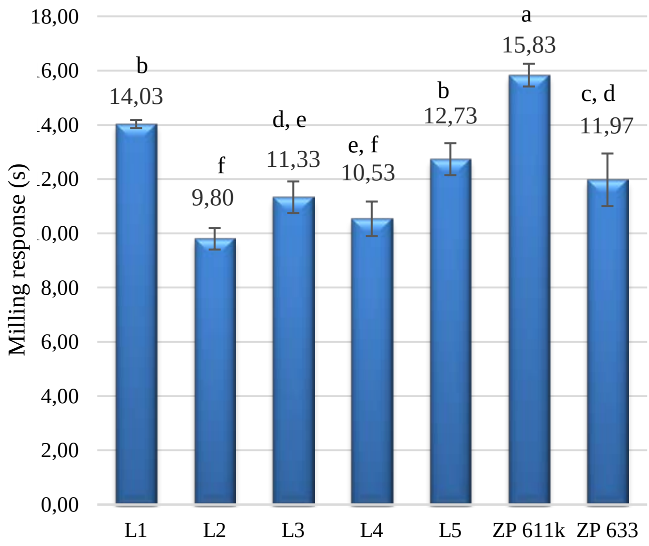

Fig 1. Milling response of the investigated inbred lines and maize hybrids

The ratio of hard and soft endosperm fractions in the kernel depends on different factors such as the genetic background and the environmental conditions. The hard endosperm fraction content in inbred line kernels ranged from 62.87 % (L3) to 68.52 % (L1), and soft endosperm share from 31.48 % (L1) to 37.13 % (L3). The corresponding values in maize hybrids were 58.03 % of hard endosperm in ZP 633 and 71.47 % in ZP 611k, i.e., the detected soft endosperm share in ZP 633 was 41.97 %, and 28.58 % in popcorn hybrid ZP 611k (Figure 2).

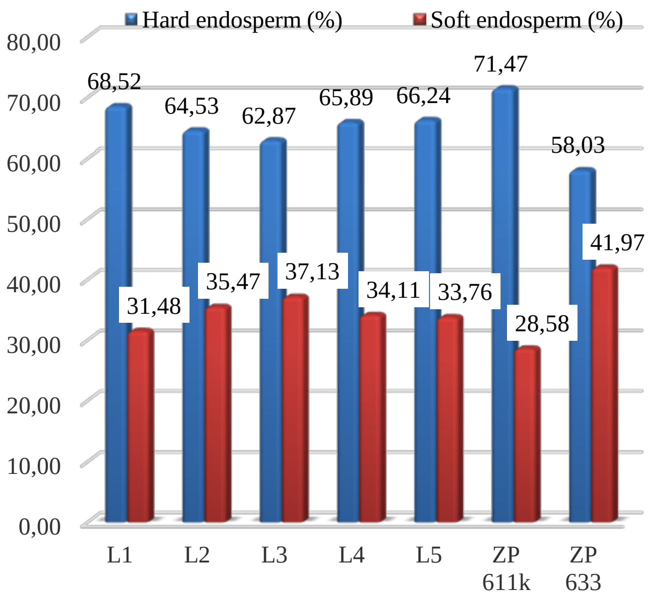

Fig 2. Hard and soft endosperm fractions of the investigated inbred lines and maize hybrids

Line L1 showed high milling response (14.03±0.15 s), slightly lower than the popcorn hybrid ZP 611k (15.83±0.42 s), as well as a very high proportion of hard (glassy) fraction of endosperm in the kernel (68.52 %). The largest share of the soft fraction of endosperm (floury endosperm) was determined in the line L3 (37.13 %), which is slightly lower than in the standard dent hybrid ZP 633 (41.97 %, Figure 2).

Dry matter content of the investigated samples of whole-grain maize flour ranged from 88.61±0.06 % (L1) to 91.41±0.03 % (ZP 633) (Table 2). Moisture content of the kernel, and therefore dry matter content are very important because they influence most grain quality parameters and milling properties such as kernel mass and volume, absolute density, stress crack, breakage susceptibility, as well as chemical composition (*Lee et al., 2007*). Kernel moisture content of the inbred maize lines reported by *Milenković et al. (2014)* was in the same range as in our study.

Table 2. Kernel chemical composition of the investigated inbred lines and maize hybrids

Genotype	Dry matter (%)	Protein (%)	Crude fibre (%)
Inbred lines			
L1	88.61±0.06^{c}	12.00±0.02^{b}	2.28±0.21^{b}
L2	89.09±0.04^{b,c}	9.36±0.06^{d}	2.04±0.14^{b,c,d}
L3	89.69±0.04^{b}	10.48±0.23^{c}	1.81±0.17^{d}
L4	89.37±0.07^{b}	12.37±0.02^{a}	2.59±0.09^{a}
L5	89.08±0.07^{b,c}	12.22±0.14^{a,b}	1.90±0.03^{c,d}
Hybrids			
ZP 611k	88.88±0.75^{b}	12.19±0.12^{a,b}	2.66±0.06^{a}
ZP 633	91.41±0.03^{a}	10.80±0.22^{c}	2.11±0.06^{b,c}

Results are given as mean ± standard deviation. Means that do not share a letter are significantly different.

When it comes to the content of protein, maize is accounted for one of the poor cereal staple foods. Even though starch is the main source of calories consumed, protein provides the essential nutrients for our food supply (*Milenković et al., 2014*). The highest protein content (12.37±0.02 %), as well as crude fibre content (2.59±0.09 %) was determined in inbred line L4, while the lowest protein content was found in the line L2, and the lowest crude fibre content (1.81±0.17 %) was detected in line L3 (Table 2). Protein content results are in accordance with findings of *Milenković et al. (2014)*. Results of kernel the chemical composition are in accordance with results of the studies previously conducted in the Maize Research Institute (*Milašinović Šeremešić et al., 2019, Semenčenko et al., 2015a, 2015b, 2014*).

CONCLUSION

All samples of new inbred lines and commercial maize hybrids investigated in this study showed good quality parameters regarding physical properties and chemical composition. The results obtained in this research indicate various possibilities of application of the examined maize inbred lines in the following stages of breeding, which represent a starting point for further research of possibilities for their industrial utilization. These findings are implying that genetic variability of these new inbred lines opens up various possibilities for their technological processing and use, primarily for obtaining the gluten-free whole-grain maize flour for the production of functional food. However, further, more detailed studies regarding physical traits, chemical composition and biochemical properties are needed in order to assess the complete nutritive potentials of these new maize inbred lines.

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