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A GENETIC BASE OF UTILISATION OF MAIZE GRAIN AS A VALUABLE RENEWABLE RAW MATERIAL FOR BIOETHANOL PRODUCTION

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Maize (*Zea mays L.*) is one of the most important cereal crops, and as such, one of the most significant naturally renewable carbohydrate raw materials for the production of energy and multitude of different products. Many studies have shown that the kernel composition and starch structure of maize are highly influenced by genetic background of the maize. Maize grain consists of approximately 70% of starch, which makes it a very suitable feedstock for the bioethanol production. This study was conducted with aim to understand how different genetic background affects bioethanol yield and other fermentation properties of the selected maize genotypes in the process of maize grain-based bioethanol production. Twenty seven maize hybrids, including genotypes of standard chemical composition as well as specialty maize hybrids such as popping, waxy, white kernel and red kernel hybrids, developed at the Maize Research Institute, Zemun Polje, were investigated in this study. The lowest bioethanol yield of 7.25% w/w obtained for hybrid ZP 611k after 48 h of fermentation and the highest by genotype ZP 434 (8.96% w/w). A very significant positive correlation was determined between kernel starch content and the bioethanol yield after 48h of fermentation, as well as volumetric productivity (48h) (r=0.67). Between bioethanol yield after 48h of fermentation and soft endosperm content in kernel of the investigated ZP maize hybrids a very significant positive correlation was assessed (r=0.66). Higher overall bioethanol yields have been obtained from genotypes containing higher starch and lower protein and lipid contents.

Key words: bioethanol, genetic background, maize, simultaneous saccharification and fermentation (SSF), starch

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INTRODUCTION

Trends of producing bioethanol have been rising over the past several decades due to the increasing demand for renewable alternative fuels driven by depletion of fossil resources and increasing attention to climate change. Bioethanol produced from renewable biomass is believed to be one of the dominating biofuels (MOJOVIĆ *et al.,* 2013). It is a liquid alternative fuel which can be produced from different starch-containing (e.g. maize, wheat, triticale, rice, potatoes, cassava, Jerusalem artichoke, sweet potatoes and barley), sugar-based feedstocks (e.g. sugar cane, sugar beet, sweet sorghum and fruits), lignocellulosic materials (e.g. wood, straw and grasses) or algae (MOJOVIĆ *et al.,* 2012). Nearly all bioethanol is produced by fermentation of maize glucose in the United States, or sugar cane sucrose in Brazil. Maize grain is currently considered one of the best renewable raw materials for the production of this alternative fuel due to the high content of starch in the grain which provides the highest bioethanol yields. According to the Renewable Fuels Association (RFA, 2014), global bioethanol production has reached the level of 85 billion litres in 2013.

Maize (*Zea mays* L.) is one of the most important cereal crops, and as such, one of the most significant naturally renewable carbohydrate raw materials for the production of energy and multitude of different products (RADOSAVLJEVIĆ *et al.*, 2012). The actual value of maize is determined by the mode of its use; each new application results in the increase of its value. A great diversity of maize grain properties provides the alteration of the grain composition in relation to quantity and quality of certain components. This is achieved by the breeding process (PAJIĆ *et al.,* 2010). Maize grain consists of approximately 70% of starch on the average (MILAŠINOVIC *et al*., 2007). Furthermore, some researchers point out that maize is a valuable model for the study of genetics, evolution and domestication (WEI *et al.,* 2007). Organisation for Economic Co-operation and Development (OECD) in collaboration with Food and Agriculture Organization of the United Nations (FAO) claims that in the near future maize will continue to expand and diversify as a research model, as an industrial resource and as a crop for feed and fuel (OECD/FAO, 2013).

Serbia is one of very important maize producers in the world and the surpluses of this cereal grain, which are not used for food and animal feed, should be carefully redirected to the production of bioethanol.

Bioethanol is mainly produced from a starchy part of the maize grain leaving significant amounts of valuable by-products such as distillers' dried grains with soluble (DDGS) which can be used as animal feed (SEMENČENKO *et al*., 2013). The kernel composition and starch structure of maize are to a significant degree determined by genetic background of the maize (MEDIĆ, 2011). Starch is a carbohydrate component that has the greatest influence on maize grain yields, as well as the significant effect on bioethanol yield (RADOSAVLJEVIĆ *et al.*, 2012, SEMENČENKO, 2013).

Traditionally, the main goals of maize breeding are production of high yielding hybrids tolerant to drought and pests (RADOSAVLJEVIĆ *et al.*, 2012). However, in recent years the demands for utilisation of maize for ethanol, biodegradable polymers and nutritional products imposed a new direction of breeding programmes towards modifying and increasing the kernel composition of starch, protein and oils, as well as their efficient extractability and fermentability. Early efforts are in selective breeding versus transgenic approach (BOTHAST and SCHLICHER, 2005). Choosing proper crop management practices, such as selection of desirable hybrids, dates of planting, kernel drying and storage conditions, can lead to further improvement in the ethanol production and ethanol yield maximisation (MEDIĆ, 2011). Wet milling and dry milling are two methods of maize grain processing. Both methods can be applied in bioethanol production, although dry grind

technology is predominant (SEMENČENKO *et al.,* 2013a). Maize hybrids for ethanol production are being developed either with higher extractable or with higher fermentable sugars content, for wet milling or dry grind ethanol production, respectively. Numerous studies have shown that the kernel composition and starch structure of maize grain are determined by genetic background of the maize, but can also be influenced by environmental conditions (e.g. growing temperature and soil moisture). Hybrid variability has been reported by several authors to affect final bioethanol concentration in a conventional dry grind corn process (SINGH *et al.,* 2005, MURTHY *et al.,* 2009).

The main goal of the present study was to investigate the effects of genetic background on suitability of twenty seven maize hybrids developed at the Maize Research Institute, Zemun Polje, Belgrade, Serbia, for bioethanol production.

MATERIALS AND METHODS

Twenty seven maize hybrids of the FAO maturity groups 100–800 developed at the Maize Research Institute, Zemun Polje, were investigated in this study. The two-replicate trial was set up according to the randomized complete-block design in the experimental field of the Maize Research Institute. 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 cobs 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 (mash 0.5 mm).

Termamyl SC, a heat-stable α-amylase (EC 3.2.1.1) from *Bacillus licheniformis* was used for whole grain maize flour starch liquefaction. The enzyme activity was 133 KNU g^{-1} (KNU, kilo novo units of α-amylases: the amount of enzyme which breaks down 5.26 g of starch per hour according to Novozyme's standard method for the determination of α-amylase). SAN Extra L, *Aspergillus niger* glucoamylase (EC 3.2.1.3), activity 437 AGU g^{-1} (AGU is the amount of enzyme which hydrolyses 1 μmol of maltose per minute under specified conditions) was used for maize flour starch saccharification. The enzymes were a gift from Novozymes (Bagsvaerd, Denmark). *Saccharomyces cerevisiae* var. *ellipsoideus* yeast was used for the fermentation of hydrolyzed maize flour starch. The culture originated from the collection of the Department of Biochemical Engineering and Biotechnology, Faculty of Technology and Metallurgy, Belgrade, and was maintained on a malt agar slant. The agar slant consisted of malt extract (3 g l^{-1}), yeast extract (3 g l^{-1}), peptone (5 g l^{-1}), agar (20 g l^{-1}) and distilled water (up to 1 l). Before use as an inoculum for the fermentation, the culture was aerobically propagated in 500 ml flasks in a shaking bath at 30˚C for 48 h. The liquid media consisted of yeast extract (3 g l^{-1}), peptone (3.5 g l^{-1}), KH_2PO_4 (2.0 g l^{-1}), $MgSO_4 \cdot 7H_2O$ (1.0 g l^{-1}), $(NH_2)_2SO_4$ (1.0 g l^{-1}), glucose (10 g l^{-1}) and distilled water.

Whole grain maize flour (100 g) was mixed with water in 1 : 3 weight ratio and 60 ppm of Ca^{2+} (as $CaCl_2$) ions was added. The mixture was treated with enzymes in two steps. The first step, liquefaction, was performed at 85˚C and pH 6.0 with 0.02% v/w concentration of Termamyl SC in 1 h. The liquefaction was performed in flasks in a thermostated water bath with shaking (150 rpm). Starch hydrolysates obtained by liquefaction of the whole grain maize flour starch were then subjected to simultaneous saccharification and fermentation (SSF) by *S. cerevisiae* var. *ellipsoideus* under semi-anaerobic conditions (concentration of inoculum 2% w/w) with 0.12% v/w concentration of SAN Extra L (pH 5.0; 30˚C), up to 48h. It was considered that the pasteurization of the substrate achieved during the enzymatic liquefaction (85˚C for 1 h) was

sufficient thermal treatment, and thus no additional sterilization prior to SSF process was performed. The simultaneous saccharification and fermentation was performed in flasks in a thermostated water bath. Experiments were performed in triplicates.

The starch content was determined by Ewers polarimetric method (ISO 10520, 1997). Dry matter content in the maize flour was determined by the standard drying method in an oven at 105˚C to constant mass. Lipid content was determined according to the Soxhlet method (AOAC, 2000). Protein content was estimated as the total nitrogen by the Kjeldahl method multiplied by 6.25, and the ash content was determined by slow combustion of the sample at 650˚C for 2h (AOAC, 2000). Crude fibre content was determined by Weende method adjusted for Fibretec^{TM} Systems, Foss, Denmark. The bioethanol concentration was determined based on the density of alcohol distillate at 20˚C. At least three measurements were made for each sample analysis and the data given were averages.

The experimental data were statistically processed by the analysis of variance (ANOVA) and the LSD multiple test was used for any significant differences at the $P<0.05$ level between the means. All the analyses were conducted using statistical software package STATISTICA 8.1. (StatSoft Inc. USA).

RESULTS AND DISCUSSION

Maize grain quality traits, such as chemical composition and physical properties increase the value of end-use products (OSORNO and CARENA, 2008). Grain quality traits can also be used as genetic diversity indicators in the same way that agronomic traits have been used, providing valuable information about the genetic relationships among genotypes (MUNAMAVA *et al.,* 2004). Differences in kernel characteristics caused by genetic inheritance, environment or handling can influence the processing and utilisation of maize.

Physical properties of the investigated hybrids varied significantly between genotypes, as presented in Table 1.

Ear physical property of great importance is 1000-kernel weight because it indicates total yield potential of the hybrid crop (Table 1). The highest value of 1000-kernel weight was determined in hybrid ZP 877 (423.02 g) followed by ZP 600 (418.92 g) and ZP 606 (414.12 g), respectively (Table 1). Test weight ranged from 749.17 (ZP 243) to 919.34 $kg \cdot m^{-3}$ (ZP 611k), while density varied between 1.20 (ZP 243) and 1.35 $g \cdot cm^{-3}$ (ZP 611k). Flotation index of the selected hybrids was between 0.35 (ZP 611k) and 92.70% (ZP 243). Maize with a low percentage of floaters is denser than maize with a high percentage of floaters and tends to have more vitreous endosperm, which is harder and more resistant to breakage than less dense, softer, floury endosperm.

Milling response, hard and soft endosperm content are parameters of kernel hardness which, from the aspect of maize industrial utilisation, especially starch production, represent its most important physical property (MILAŠINOVIĆ, 2005). Milling response of the investigated hybrids ranged from 9.25 (ZP 560) to 18.70s (ZP 611k). Hard endosperm content of the assayed maize hybrids varied from 51.60 (ZP 548) to 73.99% (ZP 611k), i.e. soft endosperm from 26.01 (ZP 611k) to 48.40% (ZP 548). The highest hard endosperm content was observed in hybrid ZP 611k, as expected considering that the hybrid is a popping maize type. Popcorn (popping maize), by definition, has a hard flinty endosperm that surrounds a small amount of soft moist starch in the centre (DICKERSON, 2008).

Table 1. Kernel physical properties of ZP maize hybrids

	Hybrid	1000- kernel weight (g)	Test weight ($kg \cdot m^{-3}$)	Density ($g \cdot cm^{-3}$)	Flotation index (%)	Milling response (s)	Hard endosperm (%)	Soft endosperm (%)
1.	ZP 172/8	242.30^{p}	829.68^{c}	1.27^{bc}	14.02^{ij}	14.83^{b}	60.27	39.73
2.	ZP 243	294.05^{o}	749.17^{n}	1.20^{d}	92.70^{a}	10.25^{l}	57.14	42.86
3.	ZP 341	352.49^{ij}	786.03^{ghijk}	1.24^{cd}	40.53^{de}	9.95^{lm}	58.46	41.54
4.	ZP 362	362.90^{gh}	776.55^{ijkl}	1.25^{bcd}	51.92^{c}	10.98^{ijk}	58.98	41.02
5.	ZP 377	332.22^{l}	776.66^{ijkl}	1.28^{bc}	35.02^{efg}	13.43^{def}	57.58	42.42
6.	ZP 434	386.44^{c}	781.55^{hijkl}	1.26^{bcd}	39.77^{def}	11.83^{gh}	56.69	43.31
7.	ZP 444	376.73^{de}	798.45^{efg}	1.30^{abc}	$8.94j^{k}$	11.25^{hi}	59.59	40.41
8.	ZP 484	337.87^{kl}	824.78^{c}	1.31^{ab}	5.35^{kl}	11.43^{ghi}	60.76	39.24
9.	ZP 505	317.36^{mn}	846.22^{b}	1.30^{abc}	0.97^{l}	13.78^{cd}	64.45	35.55
10.	ZP 548	340.45^{kl}	781.41^{hijkl}	1.24^{cd}	69.41^{b}	9.40^{m}	51.60	48.40
11.	ZP 560	382.85^{cd}	808.35^{de}	1.29^{abc}	8.02^{jkl}	9.25^{m}	62.12	37.88
12.	ZP 574/8	364.82^{fg}	759.55^{mn}	1.27^{bc}	32.0^{fg}	11.98^{g}	61.63	38.37
13.	ZP 600	418.92^{ab}	781.49^{hijkl}	1.29^{abc}	17.30^{i}	11.48^{ghi}	59.71	40.29
14.	ZP 606	414.12^{b}	774.24^{jklm}	1.28^{bc}	34.6^{efg}	14.48^{bc}	59.21	40.79
15.	ZP 611k	153.40^{q}	919.34^{a}	1.35^{a}	0.35^{l}	18.70^{a}	73.99	26.01
16.	ZP 620b	371.24^{efg}	793.49^{efgh}	1.28^{bc}	30.51^{g}	13.13^{def}	62.85	37.15
17.	ZP 633	344.48^{jk}	803.74^{ef}	1.30^{abc}	6.86^{jkl}	12.75^{f}	64.17	35.83
18.	ZP 666	367.66^{fg}	788.33^{fghij}	1.27^{bc}	28.76^{gh}	13.00^{efi}	58.36	41.64
19.	ZP 677	351.77^{ij}	791.38^{fghi}	1.28^{bc}	20.24^{i}	11.68^{ghi}	61.09	38.91
20.	ZP 704wx	319.45^{m}	790.24^{fgij}	1.25^{bcd}	53.70^{c}	10.30^{kl}	65.69	34.31
21.	ZP 74b	334.56^{l}	776.16^{ijkl}	1.28^{bc}	6.12^{kl}	13.23^{def}	64.33	35.67
22.	ZP 747	355.96^{hi}	767.81^{lm}	1.24^{cd}	70.02^{b}	11.65^{ghi}	54.48	45.52
23.	ZP 749	371.90^{ef}	770.86^{klm}	1.26^{bcd}	43.85^{d}	11.15^{hij}	59.40	40.60
24.	ZP 789	367.97^{fg}	784.94^{ghijk}	1.28^{abcd}	21.18^{hi}	9.93^{lm}	60.34	39.66
25.	ZP 808	323.34^{m}	791.07^{fghi}	1.26^{bcd}	59.13^{c}	11.35^{khi}	58.19	41.81
26.	ZP 877	423.02^{a}	783.18^{ghijkl}	1.27^{bc}	28.22^{gh}	10.48^{jkl}	60.78	39.22
27.	ZP Rumenka	310.72^{n}	821.39^{cd}	1.25^{bcd}	29.32^{g}	13.58^{de}	60.22	39.78
	$LSD_{0.05}$	8.54	16.11	0.07	7.80	0.71		

Means in the same column with different superscripts differ ($p<0.05$)

The kernel structure of ZP maize hybrids is presented in Table 2.

The highest amount of pericarp (9.68%) and lowest amount of germ (10.06%) was determined in ZP 611k. Red kernel hybrid ZP Rumenka had the highest content of germ (14.23%) and the highest endosperm content was determined in hybrid ZP 633 (83.08%).

Chemical compositions widely differed among twenty seven selected maize hybrids, as shown in Table 3.

Maize hybrids used in this study were selected by different characteristics of their kernel endosperm and its colour. Assessment of the chemical composition showed that contents of the investigated components varied significantly among the selected twenty seven ZP maize genotypes. The starch, protein, oil, crude fibre and ash content ranged from 65.38 (ZP Rumenka) to 75.50% (ZP 74b), 8.86 (ZP 808) to 13.24% (ZP 611k), 4.76 (ZP 574/8) to 7.43% (ZP 747), 2.73

(ZP 74b) to 1.98% (ZP 548), 1.21 (ZP 74b) to 1.58% (ZP Rumenka). The range of obtained values corresponds with those previously reported for ZP maize hybrids by RADOSAVLJEVIĆ *et al.* (2012) who studied the effects of hybrid on maize grain and plant carbohydrates. According to the results presented in tables 1, 2 and 3, it can be concluded that ZP maize hybrids investigated in this study have very different physical characteristics and chemical composition which could allow various possibilities of their use.

Table 2. Kernel structure of ZP maize hybrids

	Hybrid	Pericarp (%)	Germ (%)	Endosperm (%)
1.	ZP 172/8	5.44^{k}	12.41^{ghi}	82.15^{bcd}
2.	ZP 243	5.07^{l}	12.48^{fghi}	82.45^{abcd}
3.	ZP 341	7.27^{d}	12.30^{ghijk}	80.44^{ijkl}
4.	ZP 362	5.49^{k}	12.08^{ijkl}	82.43^{abcd}
5.	ZP 377	7.00^{e}	12.25^{ghijkl}	80.75^{hijk}
6.	ZP 434	7.02^{e}	12.12^{ijkl}	80.87^{hijk}
7.	ZP 444	6.63^{f}	13.52^{bc}	79.85^{l}
8.	ZP 484	7.45^{c}	12.81^{defg}	79.75^{l}
9.	ZP 505	6.20^{h}	12.75^{efgh}	81.04^{ghd}
10.	ZP 548	5.78^{j}	13.02^{cdef}	81.20^{fghi}
11.	ZP 560	6.44^{g}	13.25^{cde}	80.31^{jkl}
12.	ZP 574/8	5.91^{ij}	12.36^{ghij}	81.73^{cdefg}
13.	ZP 600	5.03^{l}	12.81^{defg}	82.17^{bcd}
14.	ZP 606	5.82^{j}	11.36^{m}	82.82^{ab}
15.	ZP 611k	9.68^{a}	10.06^{n}	80.26^{efghi}
16.	ZP 620b	6.00^{i}	13.95^{ab}	80.06^{kl}
17.	ZP 633	5.56^{k}	11.36^{m}	83.08^{a}
18.	ZP 666	6.46^{g}	12.51^{fghi}	81.04^{ghij}
19.	ZP 677	6.20^{h}	11.80^{jklm}	82.00^{cdef}
20.	ZP 704wx	5.76^{j}	13.40^{bcd}	80.84^{hijk}
21.	ZP 74b	7.05^{e}	11.65^{lm}	81.31^{efgh}
22.	ZP 747	6.42^{g}	13.35^{bcde}	80.23^{jkl}
23.	ZP 749	6.16^{h}	12.14^{hijkl}	81.71^{defg}
24.	ZP 789	6.00^{i}	12.08^{ijkl}	81.92^{cdef}
25.	ZP 808	6.23^{h}	11.75^{klm}	82.02^{bcde}
26.	ZP 877	5.55^{k}	11.92^{ijklm}	82.53^{abc}
27.	ZP Rumenka	8.00^{b}	14.23^{a}	77.78^{m}
	$LSD_{0.05}$	0.15	0.61	0.82

Means in the same column with different superscripts differ ($p<0.05$)

Table 3. Grain chemical composition of ZP maize hybrids

	Hybrid	Starch (%)	Protein (%)	Oil (%)	Crude fibre (%)	Ash (%)
1.	ZP 172/8	72.85^{fghi}	9.35^{lmn}	7.15^{b}	2.06^{ij}	1.44^{cd}
2.	ZP 243	73.50^{def}	9.41^{lm}	6.23^{defg}	1.98^{j}	1.25^{jkd}
3.	ZP 341	70.40^{j}	9.75^{j}	6.28^{def}	2.32^{defgh}	1.34^{ghifgh}
4.	ZP 362	74.61^{b}	9.28^{no}	6.07^{fgh}	2.22^{ghi}	1.31^{hij}
5.	ZP 377	72.57^{ghi}	9.90^{gh}	6.31^{cbef}	2.34^{cdefgh}	1.42^{de}
6.	ZP 434	72.04^{i}	10.16^{ef}	6.02^{ghi}	2.42^{bcdefg}	1.40^{defg}
7.	ZP 444	72.25^{i}	9.39^{lmn}	6.56^{c}	2.28^{defghi}	1.35^{fgh}
8.	ZP 484	69.60^{j}	10.09^{f}	7.32^{ab}	2.47^{bcde}	1.44^{d}
9.	ZP 505	73.38^{efg}	9.88^{ghi}	6.38^{cd}	2.21^{ghij}	1.31^{hij}
10.	ZP 548	72.04^{i}	9.19^{op}	6.08^{efgh}	1.98^{j}	1.41^{def}
11.	ZP 560	72.39^{hi}	9.63^{k}	5.79^{ijk}	2.57^{ab}	1.35^{fgh}
12.	ZP 574/8	72.07^{i}	10.75^{c}	4.76^{s}	2.37^{bcdefgh}	1.52^{ab}
13.	ZP 600	74.42^{bc}	10.18^{ef}	5.06^{qr}	2.43^{bcdefg}	1.42^{de}
14.	ZP 606	73.16^{fgh}	10.22^{de}	5.45^{mnop}	2.14^{hij}	1.40^{defg}
15.	ZP 611k	68.57^{k}	13.24^{a}	5.36^{nop}	2.56^{abc}	1.45^{cd}
16.	ZP 620b	73.31^{efg}	9.59^{k}	5.74^{jkl}	2.26^{efghi}	1.35^{fgh}
17.	ZP 633	73.55^{def}	9.81^{hij}	6.34^{cde}	2.23^{fghi}	1.40^{defg}
18.	ZP 666	74.26^{bcd}	9.42^{l}	5.55^{klmn}	2.46^{bcdef}	1.26^{jk}
19.	ZP 677	74.67^{ab}	9.07^{q}	5.00^{rs}	2.51^{abcd}	1.36^{efgh}
20.	ZP 704wx	74.13^{bcde}	10.30^{d}	5.71^{jklm}	2.26^{efghi}	1.51^{bc}
21.	ZP 74b	75.5^{a}	9.11^{pq}	5.88^{hij}	2.73^{a}	1.21^{k}
22.	ZP 747	74.08^{bcde}	9.31^{mn}	7.43^{a}	2.39^{bcdefg}	1.36^{efgh}
23.	ZP 749	73.46^{def}	10.11^{ef}	5.52^{lmno}	2.14^{hij}	1.30^{hij}
24.	ZP 789	73.66^{cdef}	9.94^{g}	5.50^{lmnop}	2.40^{bcdefg}	1.35^{fgh}
25.	ZP 808	74.83^{ab}	8.86^{b}	5.24^{pqr}	2.33^{cdefgh}	1.28^{ij}
26.	ZP 877	74.68^{ab}	9.77^{ij}	5.26^{opq}	2.21^{ghi}	1.30^{hij}
27.	ZP Rumenka	65.38^{l}	11.53^{b}	7.08^{b}	2.22^{ghi}	1.58^{a}
	$LSD_{0.05}$	0.87	0.11	0.26	0.23	0.07

Means in the same column with different superscripts differ ($p<0.05$)

Hydrolysis and fermentation stages of the bioethanol production experiments were conducted on whole grain maize flour samples obtained from 27 selected ZP hybrids. In order to enable fermentation by yeast, starch must first be broken down into simple six carbon sugars by

enzymes during hydrolysis. Prior to hydrolysis maize kernels was ground into coarse flour through a 0.5 mm mesh screen hammer mill. Grinding allows water penetration and maximizes the accessibility of enzymes to starch molecules, and allows separation of unfermented particles from liquid at the end of the process (NICHOLS and BOTHAST, 2008). Values of the parameters important for bioethanol production determined after 24 and 48 h of fermentation of whole grain maize flour hydrolysates of investigated hybrids are presented in Table 4 and Figure 1.

Table 4. Production parameters of bioethanol

	Hybrid	24h of fermentation			48h of fermentation		
		Ethanol content (% w/w)	Percent of theoretical ethanol yield (%)	Volumetric productivity ($g\ l^{-1}\cdot h^{-1}$)	Ethanol content (% w/w)	Percent of theoretical ethanol yield (%)	Volumetric productivity ($g\ l^{-1}\cdot h^{-1}$)
1.	ZP 172/8	5.75^{abc}	55.72^{cde}	2.40^{abc}	8.18^{efghij}	79.27^{cdef}	1.70^{efgh}
2.	ZP 243	5.36^{ef}	51.49^{ghij}	2.23^{de}	8.45^{cdefg}	81.18^{bcd}	1.76^{bcdefg}
3.	ZP 341	5.99^{a}	60.08^{a}	2.50^{a}	7.97^{jk}	79.94^{bcdef}	1.66^{hi}
4.	ZP 362	5.48^{bcdef}	51.84^{fghij}	2.29^{bcde}	8.41^{cdefgh}	79.57^{cdef}	$1.75^{bcdefgh}$
5.	ZP 377	5.74^{abcd}	55.84^{bcde}	2.39^{abc}	8.75^{abc}	80.62^{bcde}	1.75^{abc}
6.	ZP 434	6.09^{a}	59.51^{ab}	2.53^{a}	8.96^{a}	87.85^{a}	1.87^{a}
7.	ZP 444	5.49^{bcdef}	53.67^{defghi}	2.29^{bcde}	$8.25^{defghij}$	80.65^{bcde}	1.72^{defgh}
8.	ZP 484	5.37^{def}	54.46^{cdefg}	2.24^{de}	7.66^{k}	77.69^{defg}	1.60^{ij}
9.	ZP 505	5.36^{ef}	51.54^{ghij}	2.27^{cde}	8.01^{ijk}	77.02^{efg}	1.67^{ghi}
10.	ZP 548	5.82^{ab}	57.06^{abcd}	2.43^{ab}	8.83^{ab}	87.07^{a}	1.84^{ab}
11.	ZP 560	5.37^{def}	52.39^{efghij}	2.24^{de}	8.09^{ghij}	78.93^{cdef}	1.69^{efghi}
12.	ZP 574/8	5.28^{ef}	51.71^{fghij}	2.20^{de}	8.15^{fghij}	79.83^{bcdef}	1.70^{efgh}
13.	ZP 600	5.42^{cdef}	51.42^{ghij}	2.26^{cde}	$8.33^{defghij}$	79.03^{cdef}	1.74^{cdefgh}
14.	ZP 606	5.30^{ef}	51.16^{ghij}	2.21^{de}	8.46^{bcdefg}	81.66^{bc}	1.77^{bcdef}
15.	ZP 611k	5.24^{efg}	53.96^{cdefgh}	2.19^{de}	7.25^{l}	74.67^{g}	1.51^{j}
16.	ZP 620b	5.60^{bcde}	53.95^{cdefgh}	2.33^{bcd}	8.18^{efghij}	78.76^{cdef}	1.70^{efgh}
17.	ZP 633	5.98^{a}	57.39^{abc}	2.50^{a}	8.17^{efghij}	78.41^{cdef}	1.71^{defgh}
18.	ZP 666	5.81^{ab}	55.23^{cdef}	2.42^{ab}	8.61^{abcd}	81.85^{bc}	1.80^{abcd}
19.	ZP 677	5.43^{cdef}	51.32^{ghij}	2.27^{cde}	8.46^{bcdefg}	79.97^{bcdef}	1.76^{bcdefg}
20.	ZP 704 wx	5.19^{fg}	49.43^{j}	2.17^{ef}	8.07^{hij}	76.86^{fg}	1.68^{fghi}
21.	ZP 74b	5.33^{ef}	50.19^{ij}	2.20^{de}	8.35^{defghi}	78.63^{cdef}	1.74^{cdefgh}
22.	ZP 747	5.27^{ef}	50.23^{ij}	2.20^{de}	8.75^{abc}	83.41^{b}	1.82^{abc}
23.	ZP 749	5.24^{efg}	50.34^{hij}	2.18^{e}	8.48^{bcdef}	81.46^{bc}	1.77^{bcdef}
24.	ZP 789	5.81^{ab}	55.70^{cde}	2.42^{ab}	8.43^{cdefgh}	80.83^{bcd}	1.76^{bcdefg}
25.	ZP 808	5.34^{ef}	50.57^{hij}	2.23^{de}	8.54^{bcde}	80.68^{bcd}	1.78^{abcde}
26.	ZP 877	5.55^{bcdef}	52.46^{efghij}	2.31^{bcde}	8.41^{cdefgh}	79.49^{cdef}	1.75^{bcdefg}
27.	ZP Rumenka	4.87^{g}	52.59^{efghij}	2.03^{f}	7.25^{l}	78.30^{cdefg}	1.51^{j}
	$LSD_{0.05}$	0.37	3.71	0.15	0.37	3.64	0.09

Means in the same column with different superscripts differ ($p<0.05$)

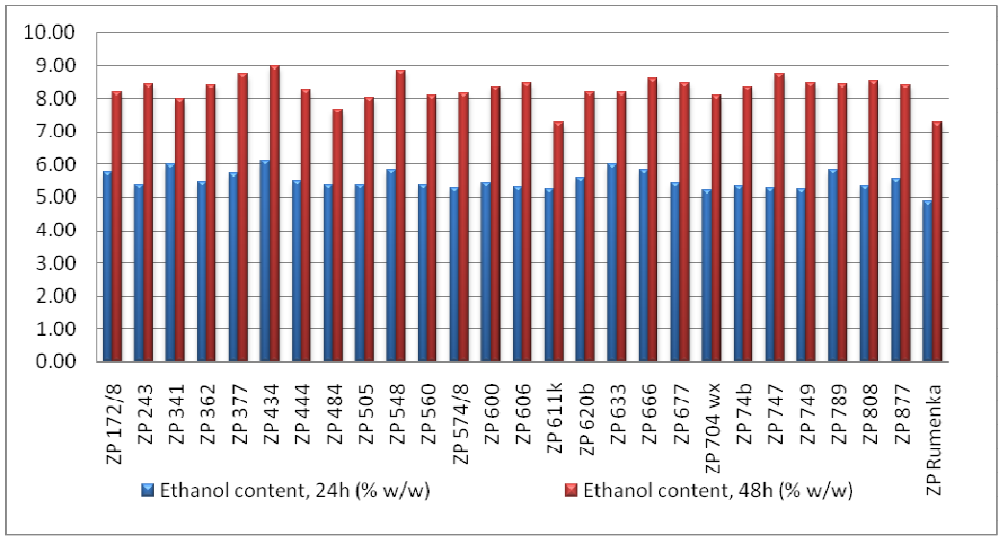

Figure 1. Bioethanol content after 24 and 48 hours of fermentation, in percents

Ethanol production by *Saccharomyces cerevisiae* is carried out via the glycolytic pathway also known as the Embden-Myerhof-Parnas or EMP pathway. In the simplest form, production of ethanol from glucose can be expressed by the following equation:

$$C_6H_{12}O_6 + 2Pi + 2ADP \rightarrow 2C_2H_5OH + 2CO_2 + 2ATP + H_2O$$

From the above equation it can be calculated that the theoretical yield is 0.511 g ethanol produced per gram glucose consumed. This yield can never be realised in practice since not all of glucose consumed is converted to ethanol but part of it is used for cell mass synthesis, cell maintenance, and production of by-products such as glycerol, acetic acid, lactic acid and succinic acid. Under ideal conditions, however 90 to 95% of the theoretical yield can be achieved. Theoretical ethanol yield is a function of the initial kernel starch content. Values of the theoretical ethanol yield of 27 selected hybrids varied between 9.26% for red kernel genotype ZP Rumenka and 10.62% for white kernel hybrid ZP 74b.

Genotype ZP 434 showed the highest bioethanol yield (8.96% w/w), volumetric productivity (1.87 $g \cdot l^{-1} \cdot h^{-1}$) and percent of theoretical bioethanol yield (87.85% w/w) (Table 4). The lowest bioethanol yield of 7.25% w/w obtained by hybrid ZP 611k after 48 h of fermentation (Table 4) can be attributed to the high percentage of hard endosperm (Table 2). The amount of hard (vitreous) and soft (floury) endosperm in kernel is considered one of the most important factors that can influence the overall bioethanol yield. The surfaces in the soft endosperm are rough and have more pores compared to hard endosperm. More exposed starch granules and rough surfaces produced from soft endosperm create more surface area which benefits to the solid phase hydrolysis. Results of a study conducted by WANG *et al*. (2010) indicate that soft endosperm resulted in higher final bioethanol concentrations compared to ground corn and hard endosperm. Larger amount of soft fraction of the endosperm enables easier decomposition of the starch granules during enzymatic hydrolysis, leading to a higher bioethanol yield. The lowest amount of

hard fraction, and therefore the highest amount of soft endosperm fraction, was determined in hybrid ZP 548, followed by ZP 747 and ZP 434 (Table 2). As expected, these hybrids did show good fermentative characteristics (Table 4, Figure 1). Statistical assessment of the dependence between bioethanol yield after 48h (Table 4) and the amount of soft endosperm in kernel (Table 1) of the investigated ZP maize hybrids (r=0.66) points out to a very significant positive correlation between these two parameters (Table 5).

Table 5. Correlation Coefficients between Kernel Properties and Fermentative Characteristics of ZP Maize Genotypes

	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	-0,68**	-0,39**	0,03	-0,63**	-069**	-0,17	0,65**	0,41**	-0,50**	-0,21	0,18	-0,31*	-0,16	0,18	0,19	-0,31*	0,20	0,67**	0,17	0,67**
2		-0,11	0,15	0,59**	0,65**	-0,24	-0,23	-0,48**	0,62**	0,45**	-0,30*	0,59**	0,60**	-0,60**	-0,31*	0,04	-0,32*	-0,65**	-0,38**	-0,65**
3			-0,20	0,16	0,18	0,40**	-0,48**	-0,25	0,16	-0,09	0,03	0,05	-0,25	0,23	0,02	0,22	0,04	-0,18	0,03	-0,18
4				-0,10	0,46	-0,18	-0,20	0,01	0,19	0,45	-0,47	0,14	0,37**	0,36**	0,02	0,02	0,01	-0,16	-0,23	-0,16
5					0,24	0,23	-0,35**	-0,22	0,28	0,18	-0,12	0,22	0,16	-0,14	-0,19	0,12	-0,20	-0,42**	-0,13	-0,43**
6						-0,20	-0,56**	-0,52**	0,64**	0,47**	-0,36**	0,52**	0,43**	-0,42**	-0,16	0,20	-0,17	-0,53**	-0,23	-0,53**
7							-0,68**	0,29*	-0,24	-0,32*	-0,19	-0,40**	-0,35**	0,36**	-0,18	-0,09	-0,17	-0,04	0,11	-0,04
8								0,10	-0,23	-0,06	0,10	-0,02	0,03	-0,03	0,25	-0,09	0,25	0,40**	0,72	0,40**
9									-0,69**	-0,22	0,09	-0,60**	-0,48**	0,49**	0,17	-0,05	0,16	0,50**	0,38**	0,50**
10										0,71**	-0,59**	0,66**	0,66**	-0,68**	-0,14	0,12	-012	-0,67**	-0,51**	-0,67**
11											-0,86**	0,55**	0,64**	-0,65**	-0,02	0,09	0,00	-0,42**	-0,43**	-0,42**
12												-0,44**	-0,61**	0,61**	-0,02	-0,11	-0,03	0,46**	0,49**	0,46**
13													0,56**	-0,56**	-0,17	-0,01	-0,16	-0,39**	-0,36**	-0,39**
14														-0,88**	-0,25	-0,16	-0,25	-0,62**	-0,69**	-0,62**
15															0,29*	0,20	0,28	0,66**	0,76**	0,66**
16																0,87**	0,99**	0,40**	0,37**	0,40**
17																	0,86**	0,05	0,28*	0,05
18																		0,39**	0,36**	0,39**
19																			0,78**	0,99**
20																				0,78**

* and ** significance at 0.05 and 0.01 probability levels, respectively.

1. Starch (%); 2. protein (%); 3. oil (%); 4. cellulose (%); 5. ash (%); 6. pericarp (%); 7. germ ; (%) 8. endosperm (%); 9. 1000-kernel weight (g); 10. test weight ($kg \cdot m^{-3}$); 11. density ($g \cdot cm^{-3}$); 12. flotation index (%); 13. milling response (s); 14. hard endosperm (%); 15. soft endosperm (%); 16. bioethanol yield, 24h (%); 17. percentage of the theoretical bioethanol yield, 24h (%); 18. volumetric productivity ($g \cdot l^{-1} h^{-1}$), 24h; 19. bioethanol yield, 48h (%); 20. percentage of the theoretical bioethanol yield, 48h (%); 21. volumetric productivity, 48h ($g \cdot l^{-1} h^{-1}$)

Similarly, the correlation between the bioethanol yield after 48h and hard endosperm content was very significant, however, negative (r=-0.62) (Table 5).

A very significant positive correlation was determined between kernel starch content and the bioethanol yield after 48h of fermentation, as well as volumetric productivity (48h) (r=0.67). Given that kernel starch is the key substance that is being primarily hydrolyzed, subsequently converted into bioethanol by fermentation, an obvious expectation would be that the starch content determines bioethanol yield. A limited number of investigators (DIEN *et al.*, 2002; HAEFELE *et al.,* 2002; SINGH *et al.,* 2005) reported the influence of maize hybrid selection on bioethanol production in a laboratory-scale process (~ 300 ml volume) and concluded that the yields were not dependent exclusively on starch content. Studies concerning bioethanol production carried out on wheat by SWANSTON *et al*. (2007) and AWOLE *et al*. (2012) led to the same conclusion; i.e. that there is no direct correlation between bioethanol yield and starch content of the grain. Therefore, the relationship between bioethanol yield and kernel starch content is not 100% reliable and starch content cannot be a sole predictor of bioethanol yield. Likewise, the results of our study presented

in this paper impose the conclusion that starch content is not the only factor influencing bioethanol yield.

A very significant negative correlation was determined between kernel protein content and the bioethanol yield after 48h of fermentation, as well as volumetric productivity (48h) (r=-0.65). Researchers like SRICHUWONG *et al.* (2009) and WU *et al.* (2006) reported that in the conventional dry-grind ethanol process, large ethanol yields have been produced from maize kernels containing large starch and small protein and lipid contents. Thus, the kernel composition and starch content were important factors determining the bioethanol yield.

A very significant negative correlation was determined between kernel pericarp content and the bioethanol yield after 48h of fermentation, as well as volumetric productivity (48h) (r=-0.53). A very significant negative correlation was determined between kernel test weight and the bioethanol yield after 48h of fermentation, as well as volumetric productivity (48h) (r=-0.67).

The results obtained in our study showed that percentage of floating kernels correlates with hardness (hard endosperm content) more precisely than does test weight; as presented in table 5, the correlation coefficient between flotation index and hard endosperm content (r=-0.61) has a slightly stronger statistical significance than the correlation coefficient between flotation index and test weight (r=-0.59).

CONCLUSION

According to the results of the study presented in this paper it can be concluded that all investigated ZP maize genotypes have very different physical characteristics and chemical composition which could allow various possibilities of their use.

Genotype ZP 434 showed the highest bioethanol yield (8.96% w/w), volumetric productivity (1.87 $g \cdot l^{-1} \cdot h^{-1}$) and percent of theoretical bioethanol yield (87.85% w/w). The hybrid ZP 434 was selected as the most promising ethanol producer. This property may be attributed to the highest level of the soft endosperm fraction, which is more susceptible to starch hydrolyzing enzymes. High yield potential per hectare makes it the best candidate for the commercial bioethanol production because land requirements are minimal. The lowest bioethanol yield of 7.25% w/w obtained by hybrid ZP 611k after 48 h of fermentation can be attributed to the high percentage of hard endosperm. Statistical assessment showed a very significant positive correlation between kernel starch content and the bioethanol yield after 48h of fermentation, as well as volumetric productivity (48h) (r=0.67). Between bioethanol yield after 48h of fermentation and soft endosperm content in kernel of the investigated ZP maize hybrids a very significant positive correlation was assessed (r=0.66).

Higher overall bioethanol yields have been obtained from genotypes containing higher starch and lower protein and lipid contents, which leads to an important conclusion that genetic base does influence the utilization of maize grain in bioethanol production. Obtained results are of an exceptional importance for the selection of potentially most suitable hybrids for this alternative fuel production because they indicate that genotype influences bioethanol yield to a great extent.

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GENETIČKA OSNOVA PRIMENE ZRNA KUKURUZA KAO VREDNE OBNOVLJIVE SIROVINE ZA PROIZVODNJU BIOETANOLA

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Izvod

Kukuruz (*Zea mays* L.) je jedna od najznačajnijih žitarica, a svrstava se i u veoma važne obnovljive ugljenohidratne sirovone za proizvodnju energije i mnogobrojnih proizvoda različite namene. Brojna istraživanja su pokazala da na sastav zrna i strukturu kukuruznog skroba u velikoj meri utiče i genetička osnova kukuruza. Kukuruzno zrno u proseku sadrži oko 70% skroba zbog čega je veoma pogodna sirovina za proizvodnju bioetanola. Cilj ovog istraživanja bio je da se što bolje razjasni na koji način genetičko poreklo utiče na prinos bioetanola i druge fermentativne karakteristike odabranih genotipova kukuruza u procesu proizvodnje bioetanola. Dvadeset sedam hibrida kukuruza različite genetičke osnove Instituta za kukuruz „Zemun Polje“ korišćeno je u ovom istraživanju. Najniži prinos bioetanola nakon 48h fermentacije (7.25% w/w) ostvario je hibrid ZP 611k a najviši ZP 434 (8.96% w/w). Utvrđena je veoma značajna pozitivna korelacija između sadržaja skroba u zrnu i prinosa bioetanola nakon 48h fermentacije (r=0.67). Između sadržaja brašnastog endosperma i prinosa bioetanola takođe je ustanovljena veoma značajna pozitivna korelacija (r=0.66). Visok prinos bioetanola ostvaren je na genotipovima kukuruza koji sadrže više skroba kao i niži udeo proteina i ulja u zrnu.

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